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UNIVERSITY OF CALIFORNIA

CHEMICAL BIODYNAMICS DIVISION

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DISCLAIMER

FY 88 ANNUAL REPORT

Chemical Biodynamics Division

October 1, 1987 – September 30, 1988

Professor George C. Pimentel, Director
Professor John E. Hearst
Acting Director, July 1, 1988

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PREFACE

The Laboratory of Chemical Biodynamics (LCB) was established in 1945 as an Organized Research Unit of the Berkeley campus in the College of Chemistry to conduct basic research on the dynamics of living cells and on the interaction of radiant energy with organic matter. The Laboratory has made rich contributions to our understanding of the molecular mechanisms of photosynthesis and of the effects of environmental pollutants on plant and animal cells. Much of its funding is provided by the U.S. Department of Energy through the Lawrence Berkeley Laboratory, of which LCB is a division. Additional reseach support comes to the ORU from the National Science Foundation, the National Institute of Health, and from private and industrial grants-in-aid. Chemical Biodynamics draws its M.S. and Ph.D. candidates from over thirteen departments and groups of the University, with the single largest component being in chemistry. The Laboratory attracts research scientists from all over the world; during the current year 11 foreign postdoctoral and visiting faculty personnel from seven countries have conducted research in the Chemical Biodynamics Laboratory.

Four broad aims guide the Laboratory:

- to maintain the strong interdisciplinary character of the laboratory.
- to build upon the expertise of the existing laboratory staff and to exploit the excellent array of facilities in LCB.
- to develop coherent and contemporary research themes that are fundamental in character, forward looking in their broad direction, and that optimize the likelihood of synergistic interaction across the disciplines represented in the laboratory.
- to strengthen collaborative ties to faculty research scientists in the biological sciences.

Two new research themes that have been developed while I have been Director are consistent with these aims. Both are directed at fundamental areas of knowledge essential to the most effective utilization of solar energy. The first theme, **Photon Conversion**, pertains to the fundamental aspects of the interaction of light with matter; the absorption, migration, relaxation, transformation, and storage of energy derived from photons. It will extend from photobiology, already a strong area within the laboratory, through photochemistry to photophysics.

The second theme, **Genetics of Photosynthesis**, is the application of recombinant DNA techniques to photosynthetic organisms. The staff's experience in molecular biology, plant cell culture, and the functional mechanisms of the chloroplast are being exploited as we investigate and attempt to control the genetic material from which the photosynthetic apparatus arises.

The newest initiative within the laboratory is directed at "Structural Biology". This initiative has been principally directed by Professor John E. Hearst who will become the Acting Director of the Laboratory on July 1, 1988 as I step down to pursue my research objectives. The aim of this

new initiative is to apply the sophisticated analytical techniques that our scientists have developed to solve important structural problems in biology. This program is bringing together scientists working on NMR, X-ray Crystallography, and Molecular Biology. Examples of structures already being worked on are the oncogene product *ras*, drug interactions with DNA'es, various natural toxins, RNA and DNA structures, and proteins known for their extreme sweetness.

George C. Pimentel, Director
Laboratory of Chemical Biodynamics

OVERVIEW OF RESEARCH

The Laboratory of Chemical Biodynamics continues to base its research plan on the importance of its role in the application of sophisticated chemical sciences to problems relevant to the mission of the Department of Energy. Our Laboratory is in the unique position that it is staffed mainly by investigators trained in chemistry with an interest in applying these skills to both biological and energy science problems. Our mission is to carry out research that takes advantage of our unique skills, as well as to train young investigators in the fields we so strongly represent.

The research in the Laboratory of Chemical Biodynamics is almost entirely fundamental research. The biological research component is strongly dominated by a long term interest in two main themes which make up our Structural Biology Program. The first interest has to do with understanding the molecular dynamics of photosynthesis. The Laboratory's investigators are studying the various components that make up the photosynthetic reaction center complexes in many different organisms. This work not only involves understanding the kinetics of energy transfer and storage in plants, but also includes studies to work out how photosynthetic cells regulate the expression of genes encoding the photosynthetic apparatus. The second biological theme is a series of investigations into the relationship between structure and function in nucleic acids. Our basic mission in this program is to couple our chemical and biophysical expertise to understand how not only the primary structure of nucleic acids, but also higher levels of structure including interactions with proteins and other nucleic acids regulate the functional activity of genes.

In the chemical sciences work in the Laboratory, our investigators are increasing our understanding of the fundamental chemistry of electronically excited molecules, a critical dimension of every photosynthetic energy storage process. We are developing approaches not only toward the utilization of sophisticated chemistry to store photon energy, but also to develop systems that can emulate the photosynthetic apparatus in the trapping and transfer of photosynthetic energy.

KCO3 – CHEMICAL SCIENCES

This program encompasses research directed at a fundamental understanding of electronically excited molecules with special attention to features that relate to the storage of photon energy in the form of high free energy chemical bonds.

- One project focuses on the manganese catalytic function in the oxidation aspect of artificial photosynthesis, the photo-induced reduction of CO_2 into organic products potentially useful as fuels, and charged colloid or polyelectrolyte interfaces for increasing quantum efficiency in photosynthetic processes based on electon transfer.

- A second project spotlights the special chemistry of electronically excited molecules and atoms using infrared spectroscopy as a diagnostic tool and tuned laser excitation to map electronic reaction hypersurfaces, both for unimolecular (photochromic) and biomolecular reactions.

- The third project in this program investigates the indefinite storage of long-lived electronically excited molecules including those that can be initially prepared with near infrared photons, the spectral region in which most of the solar energy is found.

1. THE CHEMISTRY OF ELECTRONICALLY EXCITED MOLECULES – Professor George C. Pimentel

This research is directed toward fundamental understanding of the special chemistry of electronically excited molecules, which is involved in every photosynthetic photon energy storage process. An electronically excited molecule differs from the ground state in orbital occupancy, charge distribution, molecular structure, and chemical reactivities. These differences are the key to photon energy storage.

Infrared spectroscopy coupled with matrix isolation provides a powerful diagnostic technique. Absorption features are sharp and informative about molecular structures. With a tunable laser photolysis source, we are attempting to map electronic hypersurfaces. Both unimolecular (photochromic) and bimolecular reactions are under study. To increase our knowledge of matrix-induced surface crossing, we are investigating fluorescence and phosphorescence as well.

Fluorescence and Phosphorescence of Dimethyl Amino Benzonitrile

The compound dimethylamino benzonitrile (DMABN) has received a great deal of attention because in room temperature solutions it displays two fluorescent radiative relaxation paths. These two paths are strongly solvent–and temperature-dependent. The strong solvent dependence is attributed to a very large charge separation (molecular dipole moment) in the electronically excited state. This excited state is, of course, stabilized in polar solvents which affects the dual fluorescence.

To add new information and elucidate further this interesting behavior, we have investigated the fluorescence and phosphorescence of DMABN suspended in various matrices at 10°K. Both inert gas and polar matrices have been investigated. With the three inert gas matrices Ar, Kr, and Xe, both fluorescence and phosphorescence were observed. As the spin-orbit coupling constant of the inert gas increased (i.e., in the sequence Ar to Kr to Xe), the amount of fluorescence decreased and the amount of phosphorescence increased. At the same time, the sum of fluorescence plus phosphorescence increased. This shows that the most important effect of the matrix environment is to increase singlet-triplet surface crossing.

This is the first clear-cut demonstration of phosphorescence from DMABN and our spectra display interpretable vibrational fine structure that gives information about the ground state. When a polar matrix, such as ammonia (μ = 1.47D) or hydrogen bromide (μ = 0.79D) is used, the vibrational fine structure is lost but, surprisingly, the zero-zero frequencies of both the singlet and the triplet transitions are unaffected. The significance of this striking difference from the polar solvent affects observed in solutions is under study.

Column 2B Metal-Olefin Reactions

We are continuing our study of the matrix reactions between Hg, Cd, and Zn atoms excited to the ^3P state with halogenated olefins. In progress so far, direct photolytic excitation of

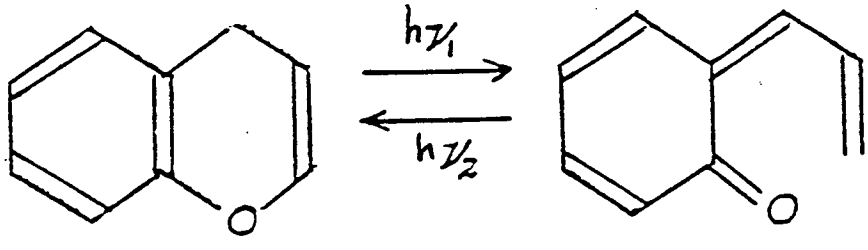
dichloroethenes results in HCl elimination whereas when Hg is added and selectively excited to the ^3P state, the reaction products change. The HCl elimination product is not observed and, instead Cl_2 elimination takes place. In addition, mercury insertion into the C–Cl bond occurs, the first demonstration of this reaction by photochemical means. We attribute the change in products to the change from chemistry on a singlet reaction surface to the different chemistry of a triplet reaction surface. To test this interpretation, we have carried out the direct photolysis experiment (in absence of mercury) in xenon matrix. Because of its strong spin-orbit coupling, xenon has been shown in our earlier work to facilitate transfer from singlet to triplet surfaces (or vice versa, of course). In agreement with our model, direct excitation of cis-1,2-dichloroethene in xenon at 10°K gave both HCl and Cl_2 elimination.

Emphasis is now being shifted toward Cd and Zn atoms because both singlet and triplet metal atom states are accessible with our tuned laser photolysis source. A Knudsen cell oven suited to controlled deposition of Cd and Zn atoms is under design and construction.

Electronically Induced Unimolecular Reactions: Photochromic Systems

We have investigated the photochromic phenylazirine ⟷ ylide system and, using IR matrix spectroscopy, established unequivocally the molecular structure of the ylide. With this starting point, we have investigated the photolytic interconversion as a function of wavelength, with the intent of searching for a double minimum potential surface in the electronically excited state. The experiment presented some ambiguities associated with overlapping absorptions of the azirine and the ylide. Nevertheless, the data provided no evidence for a threshold for interconversion that would be characteristic of a potential barrier in the excited state surface. Our tentative conclusion is that the photochromic interconversion occurs on a surface with a single potential minimum arising from surface crossing.

We are now investigating other possible photochromic systems that might involve a double minimum upper surface. The benzopyrans shown below, are typical of a number of molecules under consideration.



Electronically Induced Bimolecular Reactions

We have focussed attention on the photolysis of hydrogen halide-acetylene complexes in cryogenic matrices because the complex places the two potential reactants in close proximity and in known geometry. This permits us to investigate the photochemistry when the excited state is characteristic of the "supermolecule", the $HX \cdot C_2H_2$ complex, rather than of either of the individual reactant molecules.

The HBr-acetylene system has been well studied using broad band excitation. One possible reaction, the addition of HBr to give vinyl bromide must be considered, even though vinyl bromide does not accumulate as a product. Deposition of vinyl bromide with subsequent photolysis gives predominantly HBr elimination. In contrast, photolysis of HBr · C_2H_2 shows that DBr · C_2HD is formed, but also BrCCD + HD and BrCCH + D_2 are substantial products. These latter products plainly indicate that the main photolysis channel is not vinyl bromide formation followed by secondary photolysis. We attribute the hydrogen elimination to the photochemistry of the "supermolecule".

We have now extended this study to HI · acetylene, using tuned laser excitation. Using HI · C_2D_2, we can readily measure the relative amounts of hydrogen halide formation (through the growth of DI · C_2HD) and hydrogen formation (through the growth of C_2DI). There is plainly a wavelength dependence for the relative importance of these two reaction channels, over the range 222 to 304 nm. At short wavelengths, the favored reaction is exchange–reaction (1).

$$HI \cdot C_2D_2 \xrightarrow[222nm]{} DI \cdot C_2HD \qquad (1)$$

At longer wavelengths, hydrogen elimination occurs, reaction (2)

$$HI \cdot C_2D_2 \xrightarrow[304nm]{} HD \cdot C_2DI \qquad (2)$$

We are attempting to understand these trends in terms of the potential function of HI as perturbed by the nearby acetylene molecule, i.e., in terms of the potential function of the HI · C_2D_2 "supermolecules."

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2. CHEMISTRY WITH NEAR INFRARED PHOTONS - Dr. Heinz Frei

Extension of the wavelength response of photochemical reactions of interest in solar photochemistry into the red and near infrared range is very important for substantial improvement of the use of the sun's energy for chemical purposes. Identifying chemical reactions that can be initiated with these long wavelength quanta is the main goal of our work. We focus on reactions that may lend a basis for new concepts for chemical storage of near infrared photons, their conversion into electrical energy, and for product specific synthesis of high valued chemicals. Emphasis is on the elucidation of elementary reaction steps, since insight gained therefrom allows us to identify most readily those chemical systems most relevant to our objective. Therefore, time resolved emission and absorption spectroscopy are used for the study of redox chemistry in aqueous solution. Other reactions are initiated in an inert gas matrix in order to elucidate reaction paths by trapping chemical intermediates. FT–infrared spectroscopy is used to determine structures of intermediates and final reaction products. The wavelength dependence of the photochemistry, both in terms of reaction yield and product branching, is studied in detail by the cryogenic technique by using tuned cw dye laser radiation to initiate photolysis.

Time resolved $O_2(^1\Delta)$ redox chemistry in aqueous solution

Excited state redox reactions of metastable singlet delta $O_2(T_e = 7900\ cm^{-1})$ in aqueous solution may offer an efficient way to convert chemically stored near infrared energy into electrical energy. $O_2(^1\Delta)$ can efficiently be generated in aqueous solution by photosensitization or thermal release from a singlet O_2 storage molecule like a water soluble aromatic endoperoxide, as we have demonstrated in earlier work. Of particular interest for this purpose are regenerative systems that would allow extraction of electrical energy both in the $O_2(^1\Delta)$ redox step, and upon subsequent spontaneous regeneration of ground state O_2 from the products so formed. To establish a first system, we have studied reduction of $O_2(^1\Delta)$ by I_3^- in aqueous solution by following singlet O_2 in real time by its 1.3 micron phosphorescence, and simultaneously I_3^- product growth by transient absorption spectroscopy. The latter required development of a very sensitive visible/near infrared dual beam transient absorption spectrometer based on an Ar ion laser pumped cw dye laser with a detection limit of better than 10^{-5} absorbance units. The rate constant for quenching of $O_2(^1\Delta)$ by I^- was determined as 8.7×10^5 l mole^{-1} sec^{-1}, and the rate of the I_3^- rise was found to be equal to that of the $O_2(^1\Delta)$ decay. The most probable mechanism consistent with this result involves formation of HOOI, followed by fast reaction of this intermediate with I^- to give I_3^- and hydrogen peroxide. The $O_2(^1\Delta) + I^-$ excited state redox reaction establishes the feasibility of a key step of our concept for storage of near infrared photon energy and conversion into electricity.

Stereoselective photooxidation of alkenes with red light

Nitrogen dioxide excited at red or near infrared wavelengths to the bound $^2B_2$ state may be a sufficiently mild oxidant to permit product specific oxidation of hydrocarbons, for example alkenes. This bears special interest for controlled catalytic oxidation of hydrocarbons, a key problem in catalysis research. Namely, in cases where NO is coproduct of the oxidized hydrocarbon, photoassisted oxidation by NO_2 could be taken as the central step of a scheme of photoassisted hydrocarbon oxidation by O_2 in which NO plays the role of a catalyst. This is because NO can readily be oxidized by O_2 to regenerate NO_2, hence O_2 would be the oxidant actually consumed. We have observed very high product and stereocontrol upon photooxidation of small alkenes by NO_2 excited by red light in a cryogenic matrix.

NO_2 excited at wavelengths as long as 615 nm oxidizes trans 2–butene to trans 2–butene oxide under complete retention of stereochemistry, and no products other than epoxide and NO are formed. Reaction was induced by irradiating butene•NO_2 reactant pairs, isolated in solid Ar, with a cw dye laser, and the chemistry was monitored by FT–infrared spectroscopy. A chemical intermediate was trapped and identified by ^{18}O isotopic substitution as a butyl nitrite radical. Analysis of the photolysis wavelength dependence of the intermediate and trans 2–butene oxide (NO) growth kinetics revealed two reaction paths: Direct production of epoxide and NO along a one–photon path by elimination of NO from vibrationally hot ($\approx$ 50 kcal), electronic ground state nitrite radical intermediate. Hot radical intermediates that do not decompose are stabilized by the matrix environment and eliminate NO upon absorption of a second photon under concurrent formation of trans 2–butene oxide (two–photon path). The unusually high product specificity is attributed in part to the long wavelength of the photons used which, coupled with the small

exothermicity of the reaction, results in deposition of insufficient kinetic energy in the product for H or CH_3 group migration to occur. The observed stereospecificity implies that the coupling between excited vibrations of the butyl nitrite radical (with the CH_3 groups trans to each other), and the torsion around its central CC bond is too weak for internal rotation about that bond to compete with NO elimination.

When working at very low reactant concentrations, photooxidation of cis 2–butene by NO_2 excited at red or yellow wavelengths produces exclusively cis 2–butene oxide and NO, and cis butyl nitrite radical intermediate along the one photon path, consistent with the proposed mechanism. At higher concentration of cis 2–butene and NO_2, some trans 2–butene oxide (and trans butyl nitrite intermediate) is also formed. This points to a stronger coupling of the stretch and bending vibrations of the initially formed, hot cis butyl nitrite radical with the torsion around the central CC bond than exists in the case of the trans intermediate. It may have its origin in the steric repulsion of the adjacent CH_3 groups in the cis intermediate, which is completely absent in the trans form.

Study of the reaction of cis and trans CHD=CHD with NO_2, which exhibits a threshold at 574 nm, supports our interpretation of the stereochemical retention in terms of the dynamics of the hot nitrite radical intermediate. Trans CHD=CHD gives exclusively trans CHD-CHD-ONO, while cis CHD=CHD produces only cis CHD-CHD-ONO intermediate (no epoxide is formed along the one photon path, acetaldehyde and NO are produced instead). Hence the coupling of the stretch and bending vibrations of the hot nitrite radical intermediate is too weak for energy flow into CC torsional overtones to compete with energy flow among the stretch and bending vibrations that leads to H migration and rupture of the N-O bond. Interestingly, ethylene oxide is formed upon electronic excitation of the intermediate itself (two–photon path). Investigation of the long wavelength visible photochemistry of NO_2 with isobutylene, allene, acetylene, and dimethyl acetylene is in progress. We conclude that the unusually high degree of stereospecificity found here is primarily an intramolecular property of the hot nitrite radical intermediate formed along the reaction path. This opens the possibility of conducting the photooxidation in a higher temperature environment without concurrent loss of stereochemical integrity.

Singlet SO chemistry

In our search for new chemical reactions with low energy pathways that can be accessed by near infrared photons, we have studied photo–induced reactions in cryogenic matrices of sulfur monoxide with ethylene, allene, and acetylene. SO was generated by pyrolysis of ethylene sulfoxide in the matrix deposition line. Matrix isolated SO alkene pairs were prepared by codepositing alkene/Ar gas mixtures through a separate vacuum line. When selectively exciting $SO(^1\Delta)$ and $SO(^1\Sigma^+)$ vibronic transitions (T_e = 5800 and 10500 cm^{-1}, respectively), reaction was observed with all three alkenes. This is manifested by erosion of the SO alkene infrared pair absorption (ν(SO) at 1130 cm^{-1}), and observation of concomitant growth of infrared product bands. These reactions constitute the first observation of singlet SO chemistry in any phase. Vibrational analysis of the product spectra revealed that sulfoxides of allene and acetylene have been synthesized for the first time, demonstrating that long wavelength near infrared photons offer a way to accomplish new chemical synthesis.

3. ARTIFICIAL PHOTOSYNTHESIS – Professor Melvin Calvin and Dr. John W. Otvos

The ultimate solution to the problem of finding alternates to fossil fuels for our energy needs will be the construction of synthetic systems that will be capable of converting solar energy directly into chemical fuels. To design such systems, we are using our knowledge of the natural processes involved in photosynthesis, specifically the biophysical mechanisms that permit the photosynthetic organism to capture quanta and convert them to stored chemical energy and reductive power.

To accomplish this conversion of captured quanta to stored chemical potential requires both a phase boundary that allows for charge separation, and a set of chemical components that can utilize this charge separation, e.g., to perform redox chemistry to oxidize water and reduce a utilizable acceptor to a fuel. The overall goal of our program is to develop both the phase boundary system necessary for charge separation and to find the proper combination of catalysts and photosensitizers. In the last year we have centered our work on the latter problem and have studied various homogeneous systems that exhibited the potential of catalyzing either the oxidation of water or the reduction of carbon dioxide.

Oxidation Catalysts

The oxidation of water to molecular oxygen in photosynthesis utilizes a polynuclear manganese complex. Manganese is an ideal choice for such a reaction, having a variety of oxidation states with potentials within the physiological range. We have synthesized a variety of single and polynuclear Mn-amine macrocyclic complexes. Of particular interest are the Mn-1,4,8,11-tetraazacyclotetradecane (14-ane N_4) and the Mn-1,4,7,10- tetraazacyclododecane (12-ane N_4) complexes, which in the presence of water and dioxygen yielded Mn(III), Mn(IV) mixed valence dimers. An X-ray crystallographic analysis of the Mn-14-ane N_4 complex determined its structure to be a di-μ-oxo Mn(III), Mn(IV) species with the manganese ions having discrete levels of oxidation. This agreed with the EPR data that showed a 16-line spectrum characteristic of strongly exchange-coupled ions of Mn(III) and Mn(IV).

Electrochemical and spectroelectrochemical studies have been performed on the Mn-12-ane N_4 and the Mn-14-ane N_4 complexes. In acetonitrile, both complexes undergo reversible, one electron oxidations and quasi-reversible, one electron reductions. In the presence of water, the current for the oxidative step is dramatically increased for the Mn-14-ane N_4 complex, and the overpotential for water oxidation at the electrode surface is reduced in the presence of the Mn-12-ane N_4 complex. Thus both complexes interact with water when they are oxidized above the III, IV level. These Mn macrocylces may serve as model systems for the multiple- electron oxidation of water.

Photochemical Reduction of CO_2

We have also been studying macrocyclic amine-metal complexes as catalysts for the reduction of CO_2. We have utilized Ni-12-ane N_4 and Ni-14-ane N_4 as catalysts in an aqueous photochemical system that also contains $Ru(bpy)_3Cl_2$ as the photosensitizer and Na ascorbate, which serves both as the sacrificial electron donor and as the buffer. Both of these Ni complexes are in the II oxidation state. Irradiation of the solution at 440 nm results in the production of

CO and H_2. Radioisotopic labelling experiments with $^{14}CO_2$ established that the sole source of carbon in CO is the CO_2.

Use of the Ni-14-ane N_4 complex favors the production of CO, while use of the Ni-12-ane N_4 complex results in a large increase in H_2 production. This large increase does not occur under an argon atmosphere, indicating that hydrogen evolution is not independent of the reduction of carbon dioxide. The quantum efficiencies of these photoreduction reactions are also affected by solution pH and temperature. It is obvious that we are dealing with a complicated, multiple-event reaction, and we will continue our studies with these Ni macrocycles to devolop our understanding of the relationship between the conformation of these complexes and their selectivity and reactivity as catalysts.

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KCO6 - BIOLOGICAL ENERGY RESEARCH

The research in this program is aimed at understanding the unique features of photosynthetic organisms that allow them to collect light energy and store it in the form of chemical energy.

- One project utilizes spectroscopic techniques to map both the components as well as the kinetics of the light reactions. The focus is on excitation transfer and trapping, primary electron transfer, the composition and organization of photosynthetically active membranes.

- The aim of the second project described here is to derive functional information concerning the polypeptides which make up the PSII reaction center in *Synechococcus sp PCC 7002* using a molecular genetic approach.

- We are studying the genetics of the photosynthetic apparati of *Rhodobacter capsulatus* and *Euglena gracilis* with the ultimate aim of using DNA cloning techniques to help understand the mechanisms cells use to capture and utilize light energy.

- Our goal in a fifth project is to identify the cellular factors which regulate the tissue specific expression of plant genes.

- A complete understanding of the chemistry and stereochemistry of phycobiliproteins and phytochrome is sought to facilitate full understanding of the role of light in regulation of gene expression in green plants.

- A senventh program seeks to develop an understanding of the process involved in hydrocarbon production in plants.

1. PHOTOSYNTHESIS REACTIONS – Professor Kenneth Sauer and Dr. Melvin P. Klein

The conversion of sunlight to chemical energy in green plants provides our principal biological energy source. We are using spectroscopic and other biophysical approaches to investigate the mechanisms of photon capture, excitation transfer, and energy trapping by the photosynthetic pigments, including the earliest steps of electron transfer in the reaction centers and the mechanism of water oxidation to O_2. Fast kinetics are studied using optical and electron magnetic resonance spectroscopies, and structural features are deduced from EPR and X-ray spectroscopies. Using biochemical and molecular genetic approaches we are looking at the composition and organization of the photosynthetic complexes and their arrangement in the thylakoid membranes. We have also investigated model compounds capable of the light-induced electron transfer that lies at the heart of photosynthetic energy conversion and other compounds that are candidates for storing oxidizing equivalents in the water oxidation reaction.

Excitation Transfer in Photosynthetic Antenna Pigments

Both steady-state and time-resolved measurements of fluorescence help us to understand the exci tation transfer dynamics in photosynthetic antenna pigments. Important examples for this purpose are the phycobiliprotein compl exes that serve to absorb photons of visible light and to transfer the resulting excitation to the photosynthetic reaction centers of cyanobacteria and marine algae. The availability of X-ray crystallographic coordinates. [Schirmer, et al, J. Mol. Biol. 188, 651-675 (1986); 196, 677-695 (1987)] has enabled us to make detailed calculations of excitation transfer kinetics using th e inductive resonance transfer method of Förster [Sauer, Scheer and Sauer, Photochem. Photobiol. 46, 427-440 (1987)]. Recent re visions in the coordinates are indicative that exciton delocalization also plays a significant role in defining the excited state properties [Sauer and Scheer, Biochim. Biophys. Acta, in press]. We have extended these studies to the allophycocyanin-containing core complexes from phycobilisomes. Investigation of the excited state relaxation using time-resolved fluorescence spectroscopy allows us to characterize experimentally the paths of excitation transfer in some of these complexes that have not yet been crystallized. [Maxson, Sauer and Glazer, in *Photosynthetic Light Harvesting Systems* (Eds. H. Scheer and S. Schneider), W. de Gruyter, Berlin, 1988 pp 439-449]. The spectroscopic evidence is indicative that exciton coupling plays a major role in excitation transfer in these complexes as well. To test whether the much faster kinetics associated with the exciton mechanism can be detected, we are constructing a sub-picosecond pulsed dye laser spectrometer.

The chlorophyll pigments associated with photosynthetic reaction centers are clustered in several spectroscopically distinct protein complexes. Using steady-state as well as time-resolved fluorescence measurements, we have studied the temperature dependence of the fluorescence associated with Photosystem I reaction center complexes. A dramatic increase in fluorescence that occurs at long wavelengths at low temperatures results from increases in both the lifetime and amplitude of relatively slowly decaying (2 nsec) components. We have developed a model to interpret these results in terms of an increased photon absorption cross-section with decreasing temperature and a decreasing probability of thermally activated excitation transfer out of the low-lying excited states of the antenna pigment complexes. The occurrence of low-lying excited states in pigment complexes closely associated with reaction centers appears to be widespread

among photosynthetic organisms. These specialized pigments may play an important role in localizing the excitation in the vicinity of the reaction center, where a small amount of thermal energy may assist in the photon trapping process.

Structure and Function of Photosynthetic Reaction Centers

Photosynthetic excitation energy trapping in reaction centers results in the transfer of electrons from primary donor species to a series of primary and secondary acceptors. During the past four years since the crystallographic coordinates of the reaction center from the purple bacterium *R. viridis* have been available, detailed connections have been made between molecular constituents in these bacterial reaction centers and their spectroscopic and kinetic properties. We are extending these investigations to the structurally related reaction centers of Photosystem II of higher plants, where crystallographic coordinates are not yet available. Using time-resolved fluorescence relaxation measurements we have investigated the sub-nanosecond kinetics and mechanism of energy trapping and electron transfer. By providing a background of illumination to control the extent to which the traps are closed, we are able to monitor the several decay components of fluorescence and connect the state where all centers are open to that where all are closed. In this way we have learned that both the amplitudes and decay constants (lifetimes) are affected by trap closing. A detailed kinetic analysis has helped to clarify the sequence of events in this key energy conversion process. [Keuper and Sauer, Photosynthesis Research, in press].

Photosystem I in higher plants and cyanobacteria contains reaction centers that are dissimilar to those of PS II or purple bacteria. They contain as electron acceptors a set of three iron-sulfur proteins that operate at unusually low electrochemical potential. Using EPR and X-ray absorption spectroscopy to investigate Photosystem I reaction centers from both higher plants and cyanobacteria, we have characterized the iron-sulfur acceptors designated Centers A and B as [4Fe - 4S] centers similar to those of the corresponding soluble ferredoxins. [McDermott, Yachandra, Guiles, Britt, Dexheimer, Sauer and Klein, Biochemistry 27, 4013-4020 (1988)]. In collaboration with Prof. John Golbeck of Portland State University we have examined preparations of Photosystem I reaction centers from which Centers A and B have been removed. The remaining iron-sulfur center, designated X, also appears to be a [4Fe 4S] center. We will pursue these studies with an improved preparation from Prof. Golbeck to try to learn the structural origin of the unusually low reduction potential of this early electron acceptor of Photosystem I.

Manganese and Photosynthetic Water Oxidation

A complex containing four manganese atoms is involved in the storage of the four oxidizing equivalents needed to produce O_2 from water photosynthetically. We have pioneered the use of X-ray near edge spectroscopy and EXAFS in conjunction with low temperature EPR measurements to investigate these questions. Absorption edge energy studies on Mn are convincing that an S_0-like state, the most reduced state of the complex, has a lower oxidation state than has S_1. [Guiles, Yachandra, McDermott, Britt, Dexheimer, Sauer and Klein, in *Progress in Photosynthesis Research* (Ed. J. Biggins) M. Nijhoff Publ., Dordrecht, 1987, vol I, pp. 561-564]. The second step, from S_1 to S_2, definitely involves another Mn oxidation. [Yachandra, Guiles, McDermott,

Cole, Britt, Dexheimer, Sauer and Klein, Biochemistry 26, 5974-5981 (1987)]. The third step, from S_2 to S_3, does not appear to involve further oxidation of Mn. Presumably this electron has come from an associated ligand that does not directly involve Mn . The final step, from S_3 to S_4 and back to S_0, returns the complex to the fully reduced state. The state S_4, which should be the most oxidized but is unstable, has not yet been trapped for investigation.

A pre-edge feature, usually assigned to transitions between the 1s and 3d levels in the Mn X-ray absorption spectrum, has been characterized in several model systems as well as in Photosystem II oxygen-evolving preparations. The structure of the pre-edge region of the Mn K-edge transitions is systematically different in the Mn(III) and Mn(IV) complexes. The pre-edge region of the K-edge spectra of S_1 samples resembles those of Mn(III) complexes, while for S_2 samples this region of the spectrum resembles those of Mn(IV) complexes. These results are suggestive that in the S_1 to S_2 transition Mn(III) species are oxidized to Mn(IV). [Sauer, Guiles, McDermott, Cole, Yachandra, Zimmermann, Klein, Dexheimer and Britt, Chemica Scripta, in press].

EXAFS studies were the first to indicate the presence of a bridged binuclear Mn structure in the oxygen-evolving complex. We have obtained EXAFS spectra of photosystem II particles stabilized in the S_0, S_1, S_2 and S_3 states in order to determine the structure of the Mn complex and the changes, if any, that may be occurring during the enzymatic cycle. The salient points are that the Mn is in a cluster with a Mn-Mn distance of ~2.7 Å, with O or N ligand atoms at distances of ~1.8 and 2.0-2.2 Å, which are characteristic of oxo-bridged Mn complexes. A detailed EXAFS analysis shows that the structure is essentially unchanged in the S_1 to S_2 transition. The small differences seen in the S_2 to S_3 transition are best modelled by an increase in the spread of Mn-Mn distances. EXAFS analysis of the S_0 state indicates that a significant structural rearrangement is occurring between the S_0 and S_1 states. We suggest that the difference in the structure in the S_0 state is due to the presence of a heterogeneous mixture of formal valences of Mn including one Mn(II) which is not present in the S_1 state.

Further, comparison of the Photosystem II complexes derived from spinach and the thermophilic cyanobacterium *Synechococcus* using EPR and EXAFS show that the Mn-enzymes involved in water oxidation are very similar. However, there appear to be some differences in the position of the Mn K-edge and in the EPR spectra associated with the iron-quinone complexes on the acceptor side of Photosystem II. [McDermott, Yachandra, Guiles, Cole, Dexheimer, Britt, Sauer and Klein, Biochemistry 27, 4021-4031 (1988)]. The striking similarity of the results is suggestive that the structure of the Mn complex is largely conserved across the full evolutionary diversity of O_2 evolving photosynthetic species.

To investigate the nature of the coordinating ligands of the Mn complex we have constructed a spectrometer to perform pulsed or time-domain EPR measurements. The electron spin echo spectra exhibit modulation resulting from interactions between magnetic nuclei and the electron spin. The complex multiline EPR signal corresponding to the S_2 state has been examined with this new instrument, and coupling of the electron spin to isotopically exchangeable hydrogen atoms has been observed. [Britt, Sauer and Klein, in *Progress in Photosynthesis Research* (Ed. J. Biggins) M. Nijhoff, Publ., Dordrecht, 1987, vol I, pp. 573-576]. A nuclear envelope modulation

feature possibly attributable to nitrogen atoms in the coordination environment was investigated using cyanobacteria grown on ^{15}N as their sole nitrogen source. The fact that this envelope-modulation feature was unchanged and no new modulation appeared in the region expected for ^{15}N coupling is indicative that N-containing ligands such as histidine are not involved in the Mn coordination. We conclude that oxygen atoms, probably from glutamate or aspartate, are the most likely candidates for Mn ligation. Upon incubation of the Photosystem II complexes with NH_3, a known water analog, deep nitrogen modulation occurs, demonstrating that NH_3 ligates directly to Mn. Experiments using $^{15}NH_3$ confirm this assignment and produce the expected isotopic shift. We have demonstrated that this multiline EPR signal can be observed at temperatures of 1.5 K and shows Curie-like behavior between 4.2 K and 1.5 K. Such observations clearly contradict previous reports that the multiline signal arises from an excited S=1/2 state.

We have shown that the removal of the 33, 24 and 17 kDa extrinsic peptides does not change the X-ray near edge or EXAFS spectra indicating that these peptides do not provide any ligands to Mn. [Cole, Yachandra, McDermott, Guiles, Britt, Dexheimer, Sauer and Klein, Biochemistry, 26, 5967-5973 (1987)]. We are initiating further biochemical and molecular genetic studies of the D1 and D2 peptides to determine which protein constituents of Photosystem II are directly involved in binding the Mn complex.

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2. MOLECULAR GENETICS OF PHOTOSYNTHESIS GENES IN THE *Cyanobacterium synechococcus* sp PCC 7002 - Dr. Jeffrey C. Gingrich, Professor John E. Hearst and Professor Kenneth Sauer

The photosynthetic reaction centers perform the primary photochemistry of photosynthesis. In plants, eucaryotic algae, and in cyanobacteria one of the two reaction centers, photosystem II (PSII), utilizes light energy to drive the oxidation of water coupled to the reduction of plastoquinones. The "core" of photosystem II is made up of a small number of polypeptides. Polypeptides D_1, D_2 and cytochrome b559 make up the smallest complex capable of the primary charge separation. Associated with the core of PSII are chlorophyll-binding proteins, CP43 and CP47, which likely act as the core light-harvesting antennae. Additional proteins are bound on the oxidizing side of the membrane which help stabilize the manganese cluster involved in water oxidation. Finally, a number of low molecular weight proteins have recently been shown to be associated with the PSII reaction center. Although many of these polypeptides have been studied extensively, much remains to be learned concerning the function and interaction of the polypeptides which make up the reaction center. The major goal of this project is to derive functional information concerning the polypeptides which make up the PSII reaction center. We are attempting this by a molecular genetic approach using a transformable strain of cyanobacteria, Synechococcus sp PCC 7002, in which one can modify or delete the PSII genes.

We initially mapped the Synechococcus PSII reaction center genes using chloroplast derived PSII reaction center gene probes which have been characterized previously. Lambda phage libraries were made containing cyanobacterial DNA and the chloroplast gene probes were used to identify recombinant lambda phage which carry the PSII reaction center genes. Many of the genes have been further subcloned into pUC plasmids. The genes which we have cloned include

two of three genes which encode the D_1 polypeptide, both genes which encode the D_2 protein, and single genes which encode cytochrome b559 and the chlorophyll-binding proteins CP43 and CP47. PSII gene mapping work in the past year has focussed on additional mapping of the genes by pulse field gel electrophoresis of large cyanobacterial DNA fragments. This approach demonstrates that the PSII reaction center genes are not clustered on the cyanobacterial genome.

In the past year we have also focussed on sequencing the two genes which encode the D_2 protein. The gene sequences have been completed and were shown to encode identical polypeptides. Only eighteen differences, out of 1059 nucleotides, were noted between the coding sequences for the two D_2 proteins. Substantial DNA sequence divergance is noted however surrounding the coding sequences of the protein. Both of the genes were shown to be expressed at roughly equivalent levels by Northern hybridization experiments. Why the organism has two genes for this protein is at present unclear, particularly since the proteins are identical in primary sequence. It may be that the proteins are expressed or modified differentially under particular environmental conditions.

One of the genes encoding the D_2 protein overlaps and is co-transcribed with the single gene for the chlorophyll-binding protein CP43. This is similar to the situation in the chloroplast genome where only single copies exist for both genes. The DNA sequence of CP43 in Synechococcus suggests that this protein is encoded by a gene having a GTG start codon. This GTG codon is a different codon than that previously proposed as the chloroplast CP43 start codon. The result is a protein shorter by 15 residues. We are submitting a paper concerning the gene sequences for the D_2 and CP43 proteins along with this proposal. Particularly important in the use of a GTG start codon is that we also propose the complementary gene in the chloroplast is also expressed using a GTG start codon. This has important ramifications in consideration of chloroplast open reading frames with potential GTG start codons since such open reading frames have previously been ignored.

In order to begin analysis of the functions of the D_2 protein, we are performing site-directed mutagenesis on the D_2 protein. We initially chose amino acid residues for site-directed mutations which we anticipate will be involved in binding the manganese cluster. Mutants will be analyzed for their ability to produce oxygen while still assembling a complex capable of primary charge separation. Since the D_2 protein is encoded by two genes, we first inactivated the gene for D_2 which does not overlap the gene for CP43 by insertion of a gene which confers kanamycin resistance internal to the coding sequences for the gene. The resultant strain containing only one active D_2 gene was shown to grow at wild-type rates under a variety of growth conditions. The second step is to create site-directed mutations in the remaining copy of the D_2 gene. To achieve this, the gene encoding the second copy of D_2 was transferred to the single stranded phage M13 and site-directed mutations were created by the "Kunkel" method. Mutant genes were then returned to a pUC plasmid in which we had placed a selectable chloramphenicol gene upstream of the gene for D_2. Gene constructs containing the selectable marker and the site-directed mutations were transferred back to the cyanobacterial strain in which we had inactivated the other copy of the D_2 gene. Recombinant strains were selected on both antibiotics. Strains resistant to both antibiotics we hope have also incorporated the site-directed mutations. We are analyzing the resultant strains at the present time.

We have also continued to map, clone and sequence the genes for CP47 and cytochrome b559. In chloroplasts, these genes are co-transcribed with genes for polypeptides of less than 10kd. We are in the process of determining if these genes are also encoded and co-transcribed with the CP47 and cytb559 genes in the cyanobacteria. The function of these small molecular weight proteins is as yet unclear. Insertional inactivation of these genes would demonstrate if their presence is necessary for a functional reaction center.

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3. MOLECULAR GENETICS OF PHOTOSYNTHESIS GENES IN *Rhodobacter capsulatus* – Professor John E. Hearst

The photosynthetic bacterium *Rhodobacter capsulatus* contains a single chromosomal region, 46 kilobases (kb) in length, which codes for most of the known essential genes for photosynthesis. This cluster includes the genes for the reaction center (RC) proteins, a long wavelength light harvesting antenna (LHI), and the enzymes for the biosynthesis of bacteriochlorophyll (Bch) and carotenoid (Crt) pigments. The long term goal of our research is to elucidate the function of this photosynthesis gene cluster, including the completion of its nucleotide sequence, the identification of the open reading frames, an assignment of the enzymatic activities for the pigment biosynthesis enzymes, and an understanding of the regulation of these genes. In addition, we are engaged in physical studies of this cluster to determine the role of DNA superhelicity in gene expression.

Rhodobacter capsulatus is an ideal organism for studies of photosynthesis. In the absence of oxygen, the bacterium develops an extensive intracytoplasmic membrane system which contains the photosynthetic apparatus. It is also capable of respiratory growth in the presence of oxygen allowing photosynthetic mutants to be maintained and propogated. Thus, the effects of genetic lesions in pigment biosynthesis and in the structural proteins involved in energy transfer and electron transport can be studied. The utility of such studies has been recently demonstrated with the proposal that the D1 and D2 proteins of higher plants function as the PSII reaction center, analogous to the L and M proteins of photosynthetic bacteria (Hearst and Sauer, 1984). This proposal originated from sequencing studies of *R. capsulatus* reaction center genes and has propelled recent studies of PSII structure and function in plants.

The insights afforded by the study of photosynthetic bacteria are not limited to structural proteins. *R. capsulatus* provides an unique model system for studying the genes involved in carotenoid (Crt) biosynthesis as well. Carotenoids are highly colored pigment molecules which protect cells or tissues against photooxidative damage and serve as accessory pigments for harvesting light in photosynthetic systems. Various Crt have a wide natural distribution, and are found in both photosynthetic and non-photosynthetic organisms from bacteria to plants. A collection of transposon and point mutants accumulating characterized Crt intermediates are available for most steps in the biosynthetic pathway (Zsebo and Hearst, 1984, Scolnick, et al., 1980). In addition, the intermediates produced early in the Crt biosynthetic pathway are identical to those found in plants. Thus, *R. capsulatus* serves as a convenient stepping stone to the understanding of Crt biosynthesis in higher photosynthetic organisms.

A second biosynthetic pathway of paramount interest is that for bacteriochlorophyll (Bch) production. Bacteriochlorophyll serves as the primary photoreceptor molecule in photosynthetic bacteria and is closely related to the chlorophyll (Chl) found in algae and higher plants. In fact, the two pathways are identical until the production of chlorophyllide *a*. The difficulty of obtaining *chl* mutants in other organisms is again obviated by the metabolic versatility of the photosynthetic bacteria. A complete collection of *bch* mutants of *R. capsulatus* is available for study.

An interesting feature of the *R. capsulatus* genome is that the genes for photosynthesis are clustered on a 46 kb region of the bacterial chromosome, originally isolated by Marrs (1981) on an R-prime plasmid. A detailed restriction map has been developed, and the locations of genes for pigment biosynthesis and reaction center function have been mapped by transposon mutagenesis and complementation of point mutations (Zsebo and Hearst, 1984; Taylor *et al.*, 1983). Youvan *et al.* (1984) reported the sequence of two restriction fragments containing the subunits of the reaction center (RC) and light harvesting I (LHI) complexes which carry out the light reactions of photosynthesis. Taken together, these studies have elaborated the physical and genetic structure of the gene cluster.

The broad details of this genetic organization are simple. The highly expressed genes for the RC are located at opposite ends of the cluster and are separated by approximately 35 kb. Between these two operons are the known genes for pigment biosynthesis. Eight of the nine *crt* genes are clustered in a contiguous 10 kb stretch of DNA. This subcluster of *crt* genes is flanked on either side by genes for bacteriochlorophyll (Bch) synthesis. To understand what role, if any, this organization plays in the regulation of these genes, we are developing new methods to study bacterial genome structure. The intellectual basis for these studies is our observation that transcription of genes for photosynthesis is sensitive to inhibitors of the enzyme gyrase (Zhu and Hearst, 1988). Gyrase is responsible for maintaining the level of negative supercoiling of DNA in bacterial cells.

Characterization of Carotenoid Synthesis Genes

Despite the crucial role of carotenoids in photooxidative protection, no carotenoid gene sequences from any orgnaism have been previously reported, nor have the molecular mechanism regulating carotenogenesis been elucidated. We have recently determined the complete nucleotide sequence, 11039 bp, of the *Bam*HI-J, -M, -G, and -H fragments of pRPS404 (Armstrong *et al.*, submitted). This region contains seven of the eight previously identified *R. capsulatus* carotenoid genes (*crtA, B, C, D, E, F, and I*) (Giuliano *et al.*, 1988; Zsebo and Hearst, 1984; Taylor *et al.*, 1983). In addition we have identified a new gene located between *crtB* and *crtC* from the DNA sequence. This gene, designated *crtK*, seems to be required along with *crtC* for the conversion of neurosporene to hydroxyneurosporene. The *crt* genes form a minimum of four distinct operons, *crtA*, *crtIBK*, *crtDC* and *crtEF*, based on inversions of transcriptional orientation within the gene cluster. The 3′ end of *crtA* may overlap transcription initiation signals for a downstream gene required for bacteriochlorophyll synthesis. Possible rho-independent terminators are located 3′ to *crtI*, *B*, *K*, *C*, and *F*. *E. coli*-like σ^{70} promoter sequences are located 5′ to *crtI* and *crtD*,

suggesting for the first time that such promoters may exist in purple photosynthetic bacteria. The 5′ flanking regions of *crtA, I* and *E* contain a highly conserved palindromic sequence homologous to the consensus binding site for a variety of prokaryotic DNA-binding regulatory proteins. This putative regulatory palindrome is also found 5′ to the *puc* operon, encoding the LH-II antenna polypeptides. We have also observed two regions of exceptional amino acid homology (54% over 39 residues at the C-termini and 41% over 44 residues at the N-termini) between CrtD and CrtI, both of which are dehydrogenases. We are currently studying the regulation of the *crt* genes by light and oxygen during the shift from respiratory to anaerobic photosynthetic growth. We are also sequencing the remaining uncharacterized gene involved in carotenoid biosynthesis, *crtJ*, which is separated from the other *crt* genes by about 12 kb.

We have recently completed the high resolution mapping of Tn5.7 insertion mutations (Zsebo and Hearst, 1984) in the *crt* gene cluster (Armstrong, *et al.*, in preparation). The mapping technique uses DNA-DNA hybridization between chromosomal DNA digested with a suitable restriction enzyme, and labeled M13 probes derived from the DNA in the vicinity of the transposon insertion site. The transposon insertion has the net effect of creating a single new restriction site which can be readily detected with a suitable M13 probe. We have mapped Tn5.7 insertion mutants in *crtA, I, C, E,* and *F* to between 25 and 238 bp resolution. In addition we have found that one Tn5.7 insertion in the 5′ flanking region between *crtA* and *crtI* has no effect on carotenoid synthesis, while another Tn5.7 insertion which eliminates bacteriochlorophyll synthesis lies within the 3′ end of *crtA*. The first of these two mutants probably carries an insertion between the *crtA* and *crtI* promoters, while the phenotype of the second mutant may result from disruption of a transcript originating within the *crtA* gene, which encodes a downstream gene required for bacteriochlorophyll biosynthesis. We are using these Tn5.7 mutants to examine the operon structure of the *crt* genes in more detail. In a recent collaboration we have for the first time identified the enzymatic functions of the *crtB* and *crtE* gene products, using a ^{14}C *in vitro* incorporation assay. The label was introduced in the form of isopentenyl pyrophosphate, whcih was then converted into later carotenoid precursors. This assay shows that *crtB* likely encodes an enzyme which condenses two molecules of geranygeranyl pyrophosphate to yield prephytoene pyrophosphate, while *crtE* converts prephytoene pyrophosphate to phytoene.

Measurement of DNA Supercoiling in the Photosynthesis Gene Cluster

The expression of oxygen-regulated genes in the photosynthesis cluster of *R. capsulatus* requires DNA gyrase (Zhu and Hearst, 1988). We have shown that addition of gyrase inhibitors to photosynthetic cultures results in the rapid loss of mRNA for light harvesting and reaction center proteins, bacteriochlorophyll biosynthesis enzymes, and ribulose-bis-phosphate carboxylase, the key enzyme in carbon fixation. The loss of mRNA for these genes occurs on a time-scale comparable to the half-lives of the individual RNAs, suggesting that the inactivation of gyrase results in the immediate cessation of transcription. In contrast, the mRNA for carotenoid biosynthesis and for the subunits of the cytochrome bc_1 complex are much less sensitive to these inhibitors.

The requirement for DNA gyrase raises the possibility that photosynthesis genes may be

regulated by the level of superhelicity of the chromosome. DNA superhelicity in the region of the genes for photosynthesis may be altered by DNA gyrase, leading to repression or derepression of transcription. Since the bacterial chromosome has been shown to be composed of topologically independent domains of torsionally strained DNA (Worcel and Burgi, 1972; Sinden and Pettijohn, 1981), the photosynthesis cluster might be contained on a single domain allowing its superhelicity to be regulated independently of other parts of the chromosome. To address this model, we have developed an assay for detecting *in vivo* changes in superhelicity of small regions of the bacterial chromosome.

Our assay is based on the preferrential reactivity of trimethylpsoralen (TMP) for supercoiled versus relaxed DNA. TMP is an intercalator which can react photochemically with DNA to form cyclobutane monoadducts and interstrand crosslinks. Since intercalators bind to DNA as a function of superhelicity, the rate of TMP crosslinking to DNA can be used as a measure of superhelical density (Sinden, *et al.*, 1980). Our method allows us to determine the rate of crosslinking to any restriction fragment of the genome. In control experiments, we have demonstrated the utility of the assay in detecting *in vivo* changes in supercoiling of restriction fragments between 2 and 10 kb in size. The reactivity of individual restriction fragments decreases 1.8-fold after treatment with the gyrase inhibitor novobiocin.

We have utilized this assay to determine whether there is a change in superhelicity in the photosynthesis gene cluster upon a shift from respiratory to anaerobic photosynthetic growth conditions. We have also examined a restriction fragment containing the *fbc* operon which codes for the subunits of the cytochrome bc_1 complex. During this shift in growth conditions, the mRNAs coding for the RC and LHI complexes are induced six- to eight-fold while the amount of mRNA from the *fbc* locus remains constant. Neither the genes for photosynthesis nor those for the bc_1 complex undergo a change in superhelicity during this metabolic transition as measured by our assay.

Our results cast doubt on a current model for the control of gene expression in facultative anaerobic bacteria which proposes that a change in DNA supercoiling mediates differential transcription of genes for aerobic and anaerobic metabolism (Yamamoto and Droffner, 1985). We believe that the observed dependence on gyrase in the expression of genes for nitrogen fixation (Kranz and Haselkorn, 1986) and photosynthesis (Zhu and Hearst, 1988) in *R. capsulatus* may result from some requirement other than activation of transcription by increasing negative superhelicity of the chromosome. Our current efforts are aimed toward understanding the topological requirements for gyrase as a consequence of transcription of the photosynthesis gene cluster.

Gyrase Binding Sites and Chromosomal Organization

We are seeking evidence for spcific binding sites for the enzyme DNA gyrase in and around the photosynthesis gene cluster. Exponentially growing cells were treated with oxolinic acid and the detergent SDS. This treatment cleaves DNA at the sites of gyrase action. Isolated DNA from treated and untreated cells will be digested with restriction enzymes and hybridized with DNA

from the photosynthesis cluster. The appearance of new bands or the the disappearance of old ones will signify cleavage by DNA gyrase. This strategy has begun to reveal the distribution of sites of gyrase action in small regions of the *E. coli* chromosome (Franco and Drlica, 1988).

In order to address questions of large scale chromosome organization and the overall distribution of DNA gyrase binding sites, we have begun using pulsed field electrophoresis following the design of Chu, Vollrath, and Davis (1986). By modifying existing protocols for sample preparation, we have been able to digest the chromosome into at least 12 XbaI fragments, 11 ScaI fragments, 15 KpnI fragments, and more than 15 SstI fragments. Work is under way to assign specific genes to the corresponding bands from each of these digests. A knowledge of the local chromosome map in the region of the photosynthetic cluster will enable us to look for signs of cleavage by DNA gyrase on a larger scale than has previously been possible.

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4. LIGHT REGULATION OF NUCLEAR AND CHLOROPLAST NUCLEIC ACID SYNTHESIS IN *Euglena gracilis* – Dr. James C. Bartholomew

Our research has been directed towards the understanding of the light control of expression of the many genes encoding the photosynthetic apparatus in the eucaryotic alga *Euglena gracilis*. While the photosynthetic processes are primarily carried out in the chloroplasts of eucaryotic photosynthetic cells, the genes encoding the components are distributed into both the nuclear and the chloroplast genomes. The mechanism(s) coordinating the expression of these physically separated genomes is not known. In most eucaryotic cells the nuclear pattern of gene expression is at least parially linked to the position of the cells in the cell cycle. In *Euglena* light has a

dramatic effect on cell cycle traverse; and may therefore alter gene expression patters simply by its affects on the cell cycle. *Euglena* placed on a 12 hr light – 12 hr dark regime will become synchronized in their traverse of the cell cycle. The relationship between the cell cycle response to light and the photosynthetic gene expression as a function of light is the subject of our investigations. Is the nuclear and chloroplast gene expression program linked to traverse of the cell cycle in this photosynthetic organism, or are the photosynthetic genes regulated by light independently of cell cycle traverse. We are characterizing the cell cycle response to light of *Euglena gracilis* by flow cytometry. Wild type Euglena grown to saturation density can be stimulated by light to reenter the cell cycle. We are comparing the kinetics of reentry into the cell cycle after different periods of light exposure. Our studies have shown that *Euglena* requires at least 6 hrs of light exposure to reentry in the cell cycle. To monitor the expression of genes during the cell cycle we have used probes for the photosystem II reaction center herbicide–binding protein, *psbA*, and the large and small subunits of ribulose 1,5–bisphosphate carboxylase, *rbcL* and *rbcS*. We are also developing a probe for the light harvesting binding protein *LHCP*. All of the messanger RNA'es for these genes accumulate in a light dependent fashion with a maximum after 6 hr light exposure. Even cells at saturation density show a peak expression of these messages after 6 hrs exposure to light, however, the maximum expression of the messages is much less than cells which are traversing the cell cycle. These results indicate that these genes for photosynthetic components are regulated by light independent from the cell cycle, but that the progress of cells around the cycle can affect the message levels quantitatively.

Our studies with mutant *Euglena* lacking chloroplasts have shown that their cell cycle is also regulated, but by nutrient levels in the heterotrophic medium instead of by light. We are just beginning to study the regulation of gene expression in these mutant cells.

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5. TISSUE-SPECIFIC EXPRESSION IN *Nicotiana tabacum* – Dr. Francesca Leach and Dr. James C. Bartholomew

This project aims at identifying cellular factors which are responsible for specific gene expression in two plant tissues. Transcription studies of *Agrobacterium rhizogenes* Ri T-DNA in *Solanum tuberosum* and *Nicotiana tabacum* have led to the conclusion that expression from the Ri T-DNA genes is highly variable and tissue-specific. In particular, *Agrobacterium rhizogenes* T-DNA bears genes which are specifically expressed in leaves or in root tissues. We are studying the molecular mechanisms which are responsible for their differential expression once the bacterial T-DNA has been inserted into the plant genome. Their exact role during adventitious root formation and plant regeneration is not known: four loci, the rol loci, affect the morphology of root-formation, however with a different importance in the species tested. We are therefore working on determining what role the products of two of the rol genes have during plant development. We wish to determine how their organ-specific expression is regulated. We intend 1) to determine whether the level of regulation is transcriptional or post-transcriptional 2) to map the promoters and other regulatory sequences and 3) to follow their pattern of activity throughout plant development.

Our approach consists in fusing promoter fragments of various lengths to reporter genes such as chloramphenicol acetyltransferase (CAT) and beta-glucuronidase (GUS). Root and leaf promoter fragments containing serial 5' deletions or single base substitutions are fused to the GUS or CAT reporter genes. The chimaeric constructs are re-introduced into plant cells using a binary vector system to obtain transgenic regenerant plants. The *in vivo* analysis of their expression is on-going and is providing information on the physical characteristics of these promoter elements and on their pattern of activity throughout seedling development. The level of RNA is measured by a S1 protection assay, and GUS and CAT activities are measured by rapid enzymatic assays on small scale cell extracts.

This analysis will enable us to determine which region in the 5' upstream sequences of these promoters are responsible for tissue-specific expression.

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6. PLANT BIOCHEMISTRY – Professor H. Rapoport

The objectives of this project are to study the structure and mechanism of action of the non-chlorophyllous plant protein-pigment phytochrome. Phytochrome is the morphogenically active plant pigment that controls all developmental aspects of plant growth. It does this by undergoing a photochemical interconversion upon light excitation to a new, active form.

The specific structural features of this isomerization, as well as some other structural aspects of phytochrome, remains unknown. It is essential that these structural questions be unambiguously established in order to have a full understanding of phytochrome's function. Understanding the underlying mechanism by which phytochrome functions may allow us to control growth, flowering, and fruiting.

The specific aims of this project are:

1. To determine the stereochemistry of the pigment-protein linkage in phytochrome.

2. To determine the structural changes both in pigment and protein accompanying the change of phytochrome from the inactive P_R form to the active P_{FR} form.

3. To establish the relative and absolute stereochemistry of the bilipeptide linkages.

4. To synthesize the specific stereoisomers of S-cysteinylphytochromobilin.

5. To synthesize models of phytochrome consisting of pigment covalently attached to polypeptides of various lengths and composition in order to probe pigment-protein interactions.

Phytochrome is the morphogenically active plant chromoprotein intimately involved in all developmental aspects of plant growth. Because of its complex and sensitive nature, it has also eluded unambiguous structural assignment. However, the data obtained so far indicate it is similar to the phycobilins. We have applied new structural methodology to this extremely important

plant pigment as well and have established the structure of the phytochromobiliundecapeptide.

Phytochrome is a reversible biological switch. The action of far red light causes it to change from the inactive P_R form to the active P_{FR} form. The two most reasonable hypotheses for its mode of action in the P_{FR} form are through gene activation or by affecting membrane permeability. However, structural information on exactly what changes accompany the transformation to the P_{FR} form is totally lacking.

We plan to provide information about the P_R to P_{FR} transformation by applying our structural methodology to the P_{FR} form. We also plan to conduct the P_R P_{FR} reaction in a ^2H_2O medium and reisolate the P_R form. Detailed NMR analysis, both ^1H and ^{13}C, will then reveal if any hydrogen atoms have been exchanged in this process.

Our initial synthetic goal is S-cysteinylphytochromobilin. After this is successfully completed, the synthetic work will be extended to include some small chromopeptides. The synthetic work is essential because it will help us answer some questions about pigment conformation and function. Also, it will answer the final stereochemical question left unanswered in that it will establish the absolute stereochemistry at the thioether linkage.

Our proposed synthesis of S-cysteinylphytochromobilin begins with the protected L-vinylglycine ***1*** which we have recently prepared from methionine. With HBr in the cold this gives a mixture of about equal parts of the diasteriomeric bromides ***2*** and ***2'*** which are separable.

The synthesis now proceeds with each separate diastereomer in a displacement with a suitably N and O protected cysteine to ***3***. The α-amino group of ***3*** is now replaced by Br to give ***4*** and thence with cyanide to ***5***. Cyanoacetate ***5*** is alkylated with α-bromopropionate and the resulting ***6*** is decarboxylated to a mixture of ***7*** and ***7'*** in which the C*'s are of fixed and known stereochemistry. We now proceed individually with ***7*** and ***7'*** to ***8*** (and ***8'***. Thus in ***8*** all three centers are fixed and known, C-2 and C-3' by synthesis and C-3 by the splitting constant of its H with C-2-H.

From ***8*** we will continue the synthesis to the phytochrome bile pigment ***9*** and we can then turn to extension of the peptide chain from both the amino and carboxy ends. We shall not present the details of the peptide synthesis methodology here, since we expect to rely heavily on known methods.

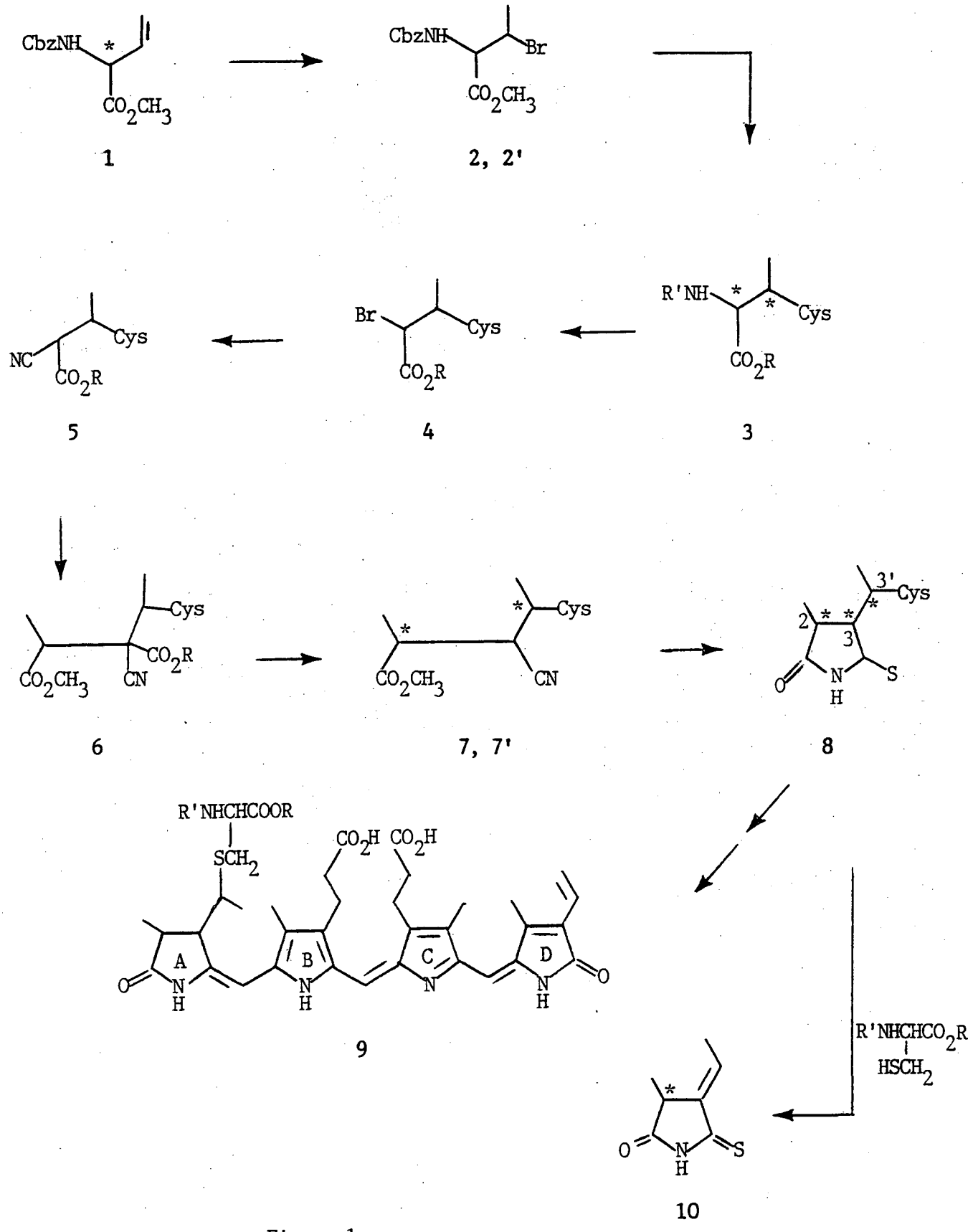
Compound ***8*** has been prepared by two different paths in an optically inactive form as a mixture of various stereoisomers. We are now evaluating and improving these processes in order to select the method best suited for preparing optically active pure compound ***8***. We have prepared the compound corresponding to the two central rings (B and C) of compound ***9***.

Preliminary synthetic experiments starting with optically active vinylglycine ***1*** have yielded promising results, in that one isomer of ***8*** seems to be the major product. If this isomer has the stereochemistry of natural phytochrome, the synthetic prospects are very promising.

At the same time, we have started investigation of an alternative and shorter path via compound ***10***, synthesized in its sterically pure form from L-alanine. Initial experiments demonstrated that suitably protected cysteine derivatives undergo 1,4-addition to ***10*** to give ***8*** as a mixture of diastereomers. Furthermore, the same addition reaction occurs with the vinyl analogue of the A-B ring dimer. In the latter case, the diastereomers have been separated and are crystalline.

During the next year we will be synthesizing optically active pure compound ***8*** and will synthesize ring D of compound ***9***. Once these compounds are available we will proceed with the synthesis of compound ***9***. Emphasis will be placed on the newly discovered route involving 1,4-addition of a protected cysteine derivative.

Following the completion of synthesis of S-cysteinylphytochromobilin, the extension of the peptide chain from both carboxyl and amino ends will be carried out.


CbzNH
CO2CH3
1
Br
2, 2'
R'NH
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CO2R
3
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CN
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7, 7'
3'
8
R'NHCHCOOR
SCH2
CO2H
A
B
C
D
N
H
O
S
9
R'NHCHCO2R
HSCH2
10

Figure 1

7. HYDROCARBONS FROM PLANTS – Professor Melvin Calvin and Dr. John W. Otvos

Hydrocarbon-producing plants are a potential alternative to fossil fuels as a source of both energy and chemical feedstocks. The focus of this program is on the biological mechanisms that control hydrocarbon production in plants, specifically the isoprenoids. Identification of these control mechanisms may permit manipulation of the plant genome to increase hydrocarbon yields.

We use latex isolated from laticifer cells of the *Euphorbia lathyris* plant for our biochemical studies. These cells provide a good model system for study, for they are the site of triterpenoid biosynthesis and storage, and the isolated latex maintains its biological activity. We have centered our studies on three problems: 1) identification of the rate-limiting steps in triterpenoid biosynthesis; 2) determination of the mechanism of cyclization of squalene to the triterpenoids; and 3) identification of the organelles found in latex, and determination of their role in triterpenoid biosynthesis.

Regulation of Triterpenoid Production

Using radiolabelled intermediates of the triterpenoid biosynthetic pathway, we have been able to determine that the conversion of β-hydroxymethyl glutaryl Coenzyme A (HMG-CoA) to mevalonic acid limits the overall rate of triterpenoid production. We have isolated and partially purified the enzyme that catalyzes this step, HMG-CoA reductase (HMGR). We have found that there are at least two isozymes of HMGR present, with different pH maxima and subcellular locations. We have also identified a second enzyme that is present in latex and will utilize HMG-CoA. This enzyme, HMG-CoA lyase, breaks down HMG-CoA and slows down triterpenoid biosynthesis. We will continue our efforts to purify and study these enzymes in order to understand better their roles in triterpenoid regulation.

Mechanism of Squalene Cyclization

The major components of *E. lathyris* latex are tetracyclic triterpenoids, produced by an epoxidation/cyclization reaction involving the acyclic triterpene, squalene. In most plant systems, the initial product of squalene cyclization is cycloartenol, and not lanosterol as found in animals and fungi. However, since lanosterol is a major component of *E. lathyris* latex, we wanted to determine if lanosterol (a) is a product of cycloartenol, (b) is produced via a separate enzyme, or (c) is a precursor of cycloartenol. Using inhibitors of both squalene cyclization and ring opening enzymes, and dual radiolabelling experiments with ^{3}H- and ^{14}C-mevalonic acid, we have concluded that the two triterpenoids are produced via separate routes.

Subcellular Organization of Triterpenoid Biosynthesis

We have fractionated latex by rate sedimentation, and have been able to separate the triterpenoid pathway into two parts; the conversion of acetate into HMG-CoA remains in the supernatant of a 5000g spin while the conversion of HMG-CoA to the triterpenoids is pelleted. Further purification of the pellet by isopycnic centrifugation showed that it consisted of at least three kinds of particle: starch grains, a triterpenoid storage structure, and an organelle capable of converting mevalonic acid into triterpenoids. Using a combination of transmission and scanning electron microscopy and marker enzyme assays, we were able to determine that this organelle was

vacuolar in nature. We also found that the earlier steps in the pathway (acetate to HMG-CoA), were also associated with a membrane fraction that was pelleted at 100,000g.

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HAO2 – ENVIRONMENTAL RESEARCH AND DEVELOPMENT

The Chemical Biodynamics programs in the Energy Research and Development section of DOE involve understanding the relationship between structure and function of biological macromolecules. The programs in the Laboratory are centered around using sophisticated new chemical analysis techniques to probe the structure of these molecules and their interactions. These techniques include such things as X-ray crystallography, NMR, ^{3}H-NMR, and fluorescence correlations spectroscopy. These analytical techniques are being used to solve fontier biological problems under investigation either principally within the Laboratory or through collaborations with investigator at the University or LBL.

1. BIOMOLECULAR STRUCTURE ANALYSIS BY NMR - Prof. David Wemmer

DNA Structure

We have collected extensive NOE data sets as a function of mixing time for a dumbell DNA containing gaps in the sugar phosphate backbone and a linear DNA of the same sequence in the duplex region, but which lacks the gaps. From these data it is clear that the structure of the linear DNA is quite near the normal B form and that there are only subtle differences with introduction of the backbone gap. We are now carrying out structure refinements using the distances determined from the NOE spectra, using a distance geometry program. These structures obtained also indicate that there are small changes in the DNA upon introduction of the gap. During the course of these calculations we have also tested the quality of distance geometry determined structures for oligonucleotides, using distances which simulate real NMR data. These calculations show that many of the major features of the DNA structure are properly reproduced. However, they have also shown that there may be significant end effects (lack of definition of the correct structure at the end of the molecule), that some of the DNA parameters are not very well reproduced by the calculations, and that there is typically a slight expansion of the structure along the helix axis. We are now working to find ways of improving the information content of the NMR measurements to minimize these computational effects. We have also begun to analyze the structure of triple strand DNA structures. Initial work has used DNA sequences which are homopurine on one strand and homopyrimidine on the other two. Imino proton work has shown that formation of these unusual structures can be followed by NMR.

Analysis of Protein Structure and Function

We are interested in the determination of protein structures in solution to understand the basis for their folding patterns, their activity, and their interactions with other molecules. We have previously assigned the resonances in several small disulfide rich toxins using the sequential approach based on 2D NMR data. We have carried through distance geometry calculations on the small bee venom peptide, apamin, and have shown that the resulting structures agree with most of the secondary structural features reported previously, but not all of them. The refined structures are clustered quite closely showing that the structure is in fact quite well determined from the NOE data. We find that there are several possible rotational orientations about the disulfide bonds which are consistent with the experimental data and which also introduce some variation of the backbone structure near the N-terminus. We have begun calculations on a 48 residue protein which was also assigned recently. These calculations show that the core segment of the molecule has a well defined structure, but that the loop containing many of the active residues has a much less well defined structure. We have also just completed assignments of the backbone protons and most of the sidechains of a related protein, which has about 60% sequence homology. Interestingly, there appears to be a rearrangement of one small section of the secondary structure, adding a few residues as a bulged loop to one of the strands in the beta sheet, relative to the first protein we studied. With more detailed structure calculations we will now analyse the extent to which this affects other regions of the protein and possibly its activity.

In order to expand the applicability of NMR to larger and more complex systems we have begun studies of two other proteins, a trypsin/chymotrypsin inhibitor from soybeans and pancreatic ribonuclease. For the inhibitor we have carried through the isolation from soybean

meal and purified the protein of interest from the mixture of isoinhibitors which are present. We have begun NMR work to assign resonances, then will determine the structure and analyse the amide proton exchange both for the free protein and in its protease complexes. The work on the very well characterized RNAse is being done to text the usefulness of computer aided methods for obtaining resonance assignments with the main chain directed approach. If this method functions well then it will greatly speed our future work on obtaining assignments for and structures of other larger proteins.

We have initiated (in collaboration with Prof. Peter Schultz) studies to determine the residues involved in substrate binding and chemical activity in catalytically active antibodies. These studies have shown that the NMR methods described previously by McConnell for studies of antibodies are not applicable in our systems due to slower binding kinetics. However we have developed methods for obtaining clean difference spectra between free Fab fragments from the catalytically active antibodies, Fabs with haptens bound and Fabs with spin labelled haptens bound in both oxidized and reduced forms. We have recently obtained antibodies grown an deuterated amino acids. Similar studies on these labelled proteins will give us unambiguous identifications of many of the residues in the active site and hence will provide important information about the mechanism of activity.

A similar approach to analysis of functional residues in a protein has been taken to determine the role of His 134 in the catalytic activity of aspartate trancarbamylase (carried out in collaboration with Prof. Howard Schachman's group). Alternate suggestions in the literature had identified this residue as a general acid to protonate substrate, or a a general base acting to activate the attacking amino group. We labelled the protein with C-13 histidine, and used a site directed mutant (alanine 134, removing one His resonance from the spectrum) to unambiguously assign the resonance of His-134. By following the resonance as a function of pH and analyzing the shift of the resonance we determined that the ring is only singly protonated over the active pH range (so it can't function as an acid), but the single proton resides on the N-3 position of the ring, which from crystallographic work can hydrogen bond, but not act as a base. Thus the role of this residue is likely the in positioning of the substrate rather than having a direct chemical rolr during the reaction.

RNA Structural Studies

We have initiated studies on a self-cleaving RNA sequence derived from a satellite RNA of tobacco ring spot virus. The sequence used, about 70 nucleotides, undergoes a self-catalyzed sequence specific cleavage. We (together with Prof. Sung-Hou Kim) have established procedures for producing milligram quantities of this RNA using T7 polymerase transcription from a plasmid DNA template. Our NMR studies have shown that these RNAs give well resolved interpretable imino proton spectra. NOE studies have been used to assign resonances and indicate that at least most of the proposed secondary structure is present in these molecules, even in the cleaved form. By counting the number of resonances we believe that there must be some tertiary interactions present as well. We have also examined a related self-cleaving sequence from Lucerne transient streak virus, with similar conclusions. A primary objective now is to compare with the uncleaved molecule. We are investigating several methods for preventing cleavage so that more direct information about the active structure can be obtained.

2. BIOPHYSICAL CHEMISTRY – Dr. Melvin P. Klein

In Vivo NMR Spectroscopy

Phosphorus-31 NMR spectroscopy is evolving into an important means for determining the *in vivo* concentrations of phosphorylated metabolites and is now entering the clinical arena. Our previous contributions to this field demonstrated the feasibility of employing implanted radio frequency coils around organs of laboratory animals to permit eliciting the NMR spectra over long periods to establish normative spectra. Using these devices and techniques we have determined phosphorus exchange reactions in rat hearts and kidney, *in situ*, and have demonstrated that there are pools of metabolic intermediates that are not directly visible in the conventional high resolution NMR spectra. Comparison of the results from NMR spectroscopy with those obtained from radiolabeling studies on chick embryo fibroblasts also showed that there are significant pools of phosphorus not visible in the P-31 NMR spectrum. Both sets of studies suggest that compartmentation occurs. The invisibility of these pools is assumed to result from the immobilization of the molecules by cellular macromolecules or organelles.

The two principle mechanisms leading to broadening of the P-31 resonances are chemical shift anisotropy and dipole-dipole coupling with protons. The former may be removed by the technique of magic angle sample spinning (MASS) while the latter is reduced by application of strong decoupling fields applied at the resonance frequency of the protons. When appropriately applied the decoupling method also leads to significant signal enhancement from the cross polarization. Were it possible to subject either an animal or tissue to the MASS experiment, the P-31 spectrum would exhibit resonances from all of the phosphorus containing molecules, even those of the nucleic acids.

To provide the reference spectra to guide such tissue experiments we have obtained the MASS spectra for a large number of biological phosphorylated molecules including many for which crystal structure data are available. The confluence of the crystal structure and chemical shift tensor data has permitted us to establish a strong correlation between the values of the shift tensor elements and bond lengths/bond angles within the phosphate moiety. These results in turn have permitted us to test the validity of some theoretical predictions for the origins of P-31 isotropic chemical shifts.

Of direct biological importance has been the determination of the chemical shielding tensors for crystalline Mg:ATP, Ca:ATP and Na:ATP. It is well established that the isotropic chemical shifts observed in solution spectra for the ATP molecules are sensitive to metal complexation and to pH. Our measurements in the solid state provide the rationale for such observations. In the Mg and Ca-ATP forms the terminal or gamma phosphates behave as mono-esters. By contrast, the terminal phosphate in Na-ATP behaves as a di-ester because it is protonated.

Technical limitations have precluded thus far the application of these techniques to intact tissue samples. We have, however, used lyophylized tissue obtained by surgical freeze-clamp excision. The P-31 MASS spectra of such tissue exhibit features assignable to the nucleic acids, to phospholipid phosphate and to ATP. That ATP is observed is confirmation that the tissue was

excised in the energized state. Not suprisingly there is a larger content of ATP in muscle tissue than in either liver or kidney. The ATP features are attributable to the Mg or Ca form rather than the free or protonated form thus resolving a long standing question in bioenergetics.

The proton spectra of the lyophylized tissue subject to MASS exhibit a rich spectrum of very narrow lines reminiscent of solution spectra. Our prior extensive work on membranes and model membrane systems provided the background to recognize that these signals originated from the fatty acid chains of the membrane phospholipids in the tissue. Resonances can be assigned to protons ranging from the glycerol group to many moieties in the acyl chain including the unsaturated carbons and the terminal methyls. The N-methyls of the choline headgroups are virtually invisible.

We are able to simulate the P-31 MASS spectra by including the solid phosphorus features of nucleic acids, phospholipids and mono- and di-esters in appropriate quantities. These simulations show that the phospholipid head groups are immobile. Simulations of the nucleic acid phosphorus demonstrate some hydration, to the extent of perhaps 10 molecules of water per base pair.

The presence of high resolution features from the fatty acid chains demonstrates that they are executing relatively rapid reorientational motions: the absence of signals from the choline N-methyls, together with the broad phospholipid phosphorus resonances, implies that the motion of the headgroups is severely limited. In summary, then, these findings are interpreted to indicate that, at the temperature of observation, the phospholipids are in the liquid crystal state characteristic of their composition and are executing the dynamics associated with their phase diagram. Normal functioning of the cellular membrane, as exemplified by the fluid-mosaic model, is assumed to require a high deree of dynamic mobility. That we observe such high resolution proton spectra in lyophylized tissue is indeed dramatic support for such a model.

The Chemical Biodynamics Division of LBL has inaugurated Tritium NMR (TMR) spectroscopy in conjnunction with the establishment of the National Tritium labeling Facility. The potential applicatiions of TMR to problems of structural biology and biophysics are very great. They promise to extend the molecular weight range of molecules that can be profitably studied with NMR by several fold, will permit the study of interactions between enzymes and bound substrates, between receptors and effectors, and between proteins and nucleic acids. This potential derives from the facts that the intrinsic sensitivity of the triton is some 7% greater than that of the proton, that there will be zero interferring background signals and because the tritium spectrum will be sparse, arising only from those tritons at the sites specifically labeled. Importantly, the abundant protons can be decoupled from the tritons thus reducing their contributions to resonance broadening.

During June of 1986 a new 300 MHz NMR spectrometer specifically configured for optimum utility with TMR was installed in the laboratory. The sensitivity anticipated and realized demonstrates that we shall be able to work with samples containing millicuries or less tritium.

In a first application of TMR to a biological problem, we have observed the conversion of glucose, tritiated at the C-1 position, to lactate by mammalian erythrocytes. During this

metabolism we observe that the two anomers of glucose are metabolized at different rates. Several intermediates are observed. The major component has been identified as 2,3 diphosphoglycerate. Upon completion, the spectrum consists of the lactate methyl resonances and some tritiated water. This water or OT resonance is at the submicromolar level and provides a method for observing directly some hitherto unobserved pathways of hydrogen in this important metabolic system. The kinetics of the individual steps in the reaction have been determined directly from these *in vivo* observations. Heretofore it was necessary to analyze extracts of cell

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3. MUTAGENESIS - FUNDAMENTAL CHEMISTRY – Professor John E. Hearst

Study of the Action Mechanism of ABC Exinuclease on Psoralen Modified DNA Substrates

ABC excinuclease is an ATP-dependent DNA repair enzyme from *E. coli* that removes nucleotides which have been modified (damaged) by a vast array of carcinogens (most recently reviewed by Sancar and Sancar, 1988). The enzyme is comprised of three subunits, UvrA, UvrB, and UvrC, and incises the damaged DNA strand at the 8th (or 9th) phosphodiester bond 5' and the 4th or 5th (or 3rd) phosphodiester bond 3' to the modified nucleotide (e.g. Sancar and Rupp, 1983; Sancar *et al.*, 1985; Beck *et al.*, 1985). Many studies utilize unique DNA sequences that have been modified randomly; however, uniquely modified DNA substrates enable more precise definition of the enzyme's incision pattern and mechanism of action. Substrates containing specific psoralen-modified nucleotides were therefore constructed and used to determine the incision pattern of ABC excinuclease with regard to HMT monoadducts, either pyrone- or furan-side, and an HMT interstrand diadduct (crosslink), as well as to study the interaction of the enzyme subunits with DNA by DNAse I footprinting (Van Houten *et al.*,1986; Van Houten *et al.*, 1987). The footprinting data showed us that binding of both the UvrA and UvrB subunits to a damaged substrate, while protecting a 19-bp region around the DNA-psoralen adduct, induces the 11th phosphodiester bond 5' to the adduct to become hypersensitive to DNAse I cleavage (Van Houten et al., 1987). Most recently, we have characterized the incision pattern of ABC excinuclease on a three-stranded substrates consisting of HMT-modified DNA oligonucleotides that were site-specifically inserted into a 2.7-kbp duplex plasmid (using the RecA protein described below) and then covalently crosslinked (Cheng et al., 1988). These three-stranded substrates served as models for an intermediate in the crosslink repair process by ABC excinuclease proposed by Van Houten *et al.* (1986). We are currently attempting to reconstitute an *in vitro* system capable of complete DNA crosslink repair using *E. coli* proteins and a site-specifically psoralen-crosslinked substrate.

Formation and Structural Analysis of Three- and Four-strand Complexes Produced by the RecA Protein

Two distinct lines of thought motivate our studies of the RecA protein and its interactions with DNA. First, such studies can contribute to the understanding of the mechanisms involved in general recombination through an understanding of RecA's actions. Secondly, a protein such as RecA may facilitate the *in vitro* hybridization of oligomeric probes to target sequences within

long (kbpr) substrates through its ability to promote homologous pairing.

The RecA protein is a multifunctional enzyme from *E. coli*. As an ATPase and helicase-recombinase, RecA will catalyze DNA-dependent hydrolysis of ATP and ATP-dependent pairing of DNA molecules, either of complementary single strands or of single-stranded (ss) with homologous double-stranded (ds) DNA. *In vivo*, RecA-mediated recombination is important in postreplication repair. This enzyme is also a protease, responsible for the ATP- and polynucleotide- dependent proteolysis of certain regulatory proteins including the LexA protein which is a multioperon repressor. The protease functions are associated with the SOS response initiated within cells following treatment that causes DNA damage. (Reviews include McEntee and Weinstock, 1981; Radding, 1982; Cox and Lehman, 1987.)

The focus of our studies is RecA-directed homologous pairing, a process which results in the formation of heteroduplex complexes. Initially, we examined the formation of three-stranded complexes by invading a single-stranded oligonucleotide into a double-stranded plasmid at the site of homology. Oligomers uniquely modified with a psoralen monoadduct were used so that irradiation to effect crosslinkage would result in fixing the heteroduplexes, preventing displacement of the inserted strand due to reannealing of the parent duplex. Various oligomer lengths and psoralen positions were tested, and the three-stranded complexes were used to test steps of a recently proposed model (Van Houten *et al.*, 1986) for crosslink repair by ABC excinuclease.

For more detailed characterization of the pairing process, an oligomeric system has been developed.

Three- and Four-Strand Complexes Formed with DNA Oligomers and RecA

We have recently found that stable three- and four-strand complexes of oligonucleotides can be formed by RecA. These complexes can be isolated by native polyacrylamide gel electrophoresis even after treatment with SDS. The formation and stability of the complexes are enhanced by the presence of a crosslink in the target double strand. We are presently engaged in studying the properties of the reaction forming these complexes. Three- and four-strand substrates with crosslinks at different positions will be probed with chemical reagents (DMS) and enzymes (DNase, restriction enzymes). We will determine whether the complexes are branch-migrated intermediates, paranemic complexes, or other structures. These complexes offer an excellent opportunity to study detailed properties of recombinational intermediates. Earlier studies (Di Capua and Müller, 1987; Leahy and Radding, 1986; Chow *et al.*, 1986) have used much larger substrates. Using crosslinked substrates also allows us to control the positions of branch points. Comparisons of crosslinked and unmodified targets may permit conclusions on the role of the RecA protein in DNA damage recognition and repair.

Stereochemistry of Psoralen Adducts in RecA-Coated DNA

In related work, we are determining the stereochemistry of psoralen adducts in RecA-coated filaments, using known HPLC and NMR methods (Kanne *et al.*, 1982). The DNA-RecA helix is dramatically extended and unwound compared to the B-form DNA helix, and information from psoralen crosslinking will offer another detailed probe of the nature of the complex. A variety of

conditions will be employed to ascertain whether the filament structure changes as a consequence of, for instance, ATP or ATP-γ-S binding or the presence of an invading third strand.

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4. RNA STRUCTURE – SELF PROCESSING RNA – Prof. John Hearst

The Chemical Approach to Structure Determination

When first transcribed many messenger RNAs contain intervening sequences, introns, which must be removed before the translation of the message into protein. While it was originally believed that all cellular catalytic activity was protein dependent, it has now been demonstrated that intron removal from a messenger RNA can be a self-catalyzed reaction which can occur in absence of any protein(Cech *et al.*, 1981) Since the message is translated as a series of ordered triples this splicing operation must retain complete fidelity for correct translation. But the homologies found near intron/exon splice junctions are far too short to provide the high accuracy based on sequence alone. Therefore the splicing must be discriminating on the basis of a 3-dimensional structure which we have yet to discover.

Other RNAs, including transfer RNAs, ribosomal RNAs, M1 RNAs and RNA viroids also fold into stable, unique structures which have important catalytic roles.

The ability of single-stranded RNA to form intramolecular hydrogen bonds gives it a much greater conformational variabililty than double-stranded DNA. This versatility combined with the size of cellular RNAs presents us with a formidable problem as we attempt to probe the form/function relationships of RNA.

RNA primary sequence data is greatly expanding thanks to rapid RNA and DNA sequencing techniques. Computer programs based on thermodynamic algorithms have achieved some success in predicting the secondary structure of small RNAs (Turner *et al.*, 1988). RNA secondary structure and some tertiary interactions can also be deduced from trans-species phylogenetic evidence.

But we need to be able to predict the three dimensional structures that RNAs will form if we are to understand how the cell works.

Probing for Structure

Through the use of photochemical crosslinking techniques we are attempting to determine the secondary and tertiary structure of specific catalytic RNAs.

The maturation of transfer RNA in eucaryotes and procaryotes involves specific cleavage of a long RNA transcript by RNase P to produce a free 5' end of transfer RNA. RNase P is a ribonucleoprotein composed of one protein and one RNA molecule (M1 RNA). Guerrier-Takada *et al.*, 1983) have shown that the essential catalytic activity of RNase P is contained within the M1 RNA and that under specific conditions M1 RNA alone can catalyze the 5' end cleavage of the immature transfer RNA transcript. Determination of the structure of M1 RNA is essential to understanding the nature of it's activity and how RNA molecules in general can be catalytic.

Recent phylogenetic studies(James *et al.*, 1988) and single and double strand specific nuclease experiments(Guerrier–Takada *et al.*, 1984) suggest certain elements of secondary and tertiary structure in M1 RNA. In order to verify the proposed structure and provide more detailed information our lab is using psoralen photochemical crosslinking techniques.

The M1 RNA is crosslinked with psoralen under conditions where it is catalytically active trapping a variety of secondary and tertiary interactions. These crosslinked species are then identified through the use of various electrophoresis and sequencing techniques.

Recently, our lab has identified nine different crosslinks in M1 RNA(Lipson *et al.*, 1988). Four of these crosslinks are small hairpins, two are long range interactions, and three are intermolecular interactions between dimers of M1 RNA. Present work in the lab is being done to more accurately locate these crosslink sites and to identify others.

Structure Prediction

We are attempting to predict the three-dimensional structure of RNA with a combination of physical data, distance geometry, and energy minimization.

The ribosome plays absolutely an crucial role in all living organisms and the ribosomal RNAs which are the major constituent of this organelle, have been extensively studied by chemical, enzymatic, and phylogenetic techniques. This large database of structural information

and the highly conserved nature of these RNAs make them the prime candidates for structure determination.

Finding the folding pattern of 16S RNA from *E. coli* is the initial goal of our work. The secondary structure posited by Harry Noller and coworkers will be used as the basis for forming three-dimensional, helical subunits (Gutell *et al.*, 1985). Since the cleavage data indicate these regions are persistent and stable we can then introduce A-form helices in these double-stranded regions as the first step in forming a three dimensional structure. Proceeding beyond this step brings us to the traditional problems of crude physical models and modeling subjectivity. The major problem with physical models is that they inadequately mimic the molecular characteristics which predetermine the final structure. Computer modeling allows us to approach the problem in a quantitative and flexible manner.

Recent developments in distance geometry algorithms have made it possible to produce objectively folded structures from 3D data(Havel and Wuethrich, 1984). Using the empirical energy modeling program, AMBER(Weiner *et al.*, 1984), we will then insure that these foldings don't violate the rules that have been deduced from smaller molecules. In effect distance geometry allows us to search conformational space broadly while energy minization will be used to locate the best 'local' conformation. The results of these analyses and the three dimensional structure of the molecule can then be displayed in stereo for visual integration and analysis by the researcher.

As our prototype, we have successfully folded the yeast phenylalanine tRNA. We used only the primary structure, the phylogenetically deduced hydrogen bonding, and five long range interactions that were known before the crystal structure was determined. The initial folding before any energy minimizations shows that this minimal data set was sufficient to determine the basic 'L' shape, revealed by X-ray crystallography.

Applying the same techniques to 16S ribosomal RNA we have been able to construct a consistent folded structure.

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5. CONTROL OF DNA SYNTHESIS IN HUMAN CELLS – Dr. James C. Bartholomew

The genome of human cells contains approximately 10^9 nucleotide pairs organized into a particular sequence. The faithful replication of this amount of information into each daughter cells is obviously a formidable task. At each round of replication not only must the sequence of the DNA be preserved, but also the number of gene copies and their organization must be maintained. The organization of the genes in the genome plays an important role in determining expression efficiency for individual genes. Very little is known about how the number of gene copies and the arrangement of genes is controlled in cells. Conceptually, the control of the organizational aspects of the genome can be maintained by limiting replication to the linear sequence within the genome. Trouble arises when individual sections of the genome overreplicate producing copies which can rearrange and/or amplify to change the phenotype of the cell.

A clue as to how the genome sequence is normally maintained in such a highly organized state during DNA replication is to learn something about the factors that destabilize the replication of the genome. Our research program centers on the hypothesis that much of the control of genome organizational stability takes place at the level of initiation of DNA replication within sections of the genome. If initiation of DNA synthesis in a section of the genome occurs more than once per cell cycle, the extra copies of this section are available for gene amplification and/or rearrangement. We are interested in understanding what cellular factors regulate this initiation, and how external environmental stresses modulates the initiation of DNA synthesis at these sites.

Because the human genome is so large, and therefore the mechanisms regulating its replication likely to be so complex, we have attempted to develop model systems which can help us understand the control of initiation of DNA synthesis. For this reason we have been studying the replication of extrachromosomal DNA elements in human cells. Extrachromosomal DNA elements have been observed by many investigators in both normal and transformed human cells. In general, these extrachromosomal elements are heterogeneous in sequence and contain a higher level of reiterated sequences than the total genome. Little is know about the function (if any) of these elements. They may be intermediates in recombinational events, genes in the process of being amplified, or residuals from the normal process of DNA replication. Many of these extrachromosomal elements maintain low copy number in the cell population. Others, like the elements we are studying, have a somewhat unstable copy number on a per cell basis with some cells in a population having low copy number and others a very high copy number. The elements we are studying are derived from SV40 virus. They arose from SV40 transformed human cells and they replicate to high copy number in some human cells, and yet maintain a stable DNA sequence without lysing the population. We are trying to understand the factors that control the replication of these extrachromosomal elements with the hope that this understanding will tell us something about how human cells normally control the replication of genomic DNA.

Our studies began with the characterization of the extrachromosomal SV40 copies in a number of SV40 transformed human cell lines. We observated that two cell lines which we obtained from the American Type Tissue Culture collection and which were transformed with wild type SV40 virus contained many copies of extrachromosomal SV40 DNA elements. The

cell lines which harbor these extrachromosomal DNA'es are GM637, a human fibroblast cell line from an apparently normal individual, and XP12RO, a line from a *Xeroderma pigmentosum* patient containing a mutation in a DNA repair gene. These extrachromosomal DNA'es are derivatives of the input SV40 virus; however, they have undergone numerous sequence changes. Although human cells are known to be semipermissive for SV40 virus production, these DNA'es are unique in that they are mutated and the mutation is preserved faithfully in high copy number.

The origin of this extrachromosomal DNA and its relationship to the input transforming DNA is not known. To understand the system better we began to characterize the DNA. We found that in an actively growing population of GM637 cells the copy number of SV40 DNA is greater than 10,000 per cell. On agarose gel electrophoresis, the extrachromosomal DNA from both the GM637 and XP12RO cell lines has a lower molecular weight than wild type DNA. Restriction enzyme digestion indicates that the lower molecular weight is the result of a deletion in the A-gene coding for the T-antigen. In both cell lines the deletion maps near the boundary of the intervening sequence and the small exon of the T-antigen and removes 323 bp. The DNA from the XP12RO cell lines also contains an insertion of 18 bp in the large exon of the A-gene. DNA sequencing indicates that the deletion has eliminated one of the splice sites for the intervening sequence and has stopped just short of the translation termination site for the small t-antigen. We have shown that the message is spliced at the normal small t-antigen splice sites, and so the gene may encode a protein of nearly the same size as the wild type large T-antigen. Its curious that two cell types that originated from separate transformation events give rise to identical deletions in this part of their genome. Other parts of these extrachromosomal DNA'es are clearly different between the two DNA'es. The similarities between the two mutated DNA'es presumably arise because of some selective pressure in the human cell hosts. We have cloned the gene for the mutated T-antigen into an adenovirus expression vector and produced enough T-antigen to isolate and characterize. The protein runs slightly faster on an SDS polyacrylamide gel indicating either a higher charge, or a smaller molecular weight relative to the wild type protein.

We have also sequenced the origin–promoter–enhancer region of the extrachromosomal DNA from the GM637 and XP12RO cells. In the DNA from both cell types, the origin region is the same as the wild type DNA origin. The enhancer region, however, in both DNA'es contains many rearrangements. Although the two DNA'es are different in this region, the deletions and duplications that occur lead to a similar overall structure. Since the enhancer region of the DNA is the site at which cellular factors interact to control the expression of the encoded genes, and in other systems, the host range of viral DNA replication, we suspect that it is these alterations in the enhancer region, possibly in conjunction with the alterations in the T-antigen caused by the deletion which gives rise to a stable long-term replication of the DNA extrachromosomally in the human cells. Since the extrachromosomal DNA contains more than one type of mutation we have separated the mutations and placed them back individually into a wild type background to test which mutations are causing the altered phenotype. These studies have shown that long-term stable replication of the DNA requires only the alterations in the promoter-enhancer. Because the promoter-enhancer region itself has numerous alterations relative to a wild type promoter-enhancer, we are now trying to engineer each of the alterations individually into a wild type promoter-enhancer to test which elements of the modified promoter-enhancer allow for stable

long-term replication in human cells. We are also beginning to do *in vitro* binding studies with wild type and mutated enhancer regions looking for differences in the way factors from the human cells bind to the different enhancer regions. It may be possible using this system to identify human cell factors responsible for the maintainance of the DNA as extrachromosomal elements.

Now that we have purified T-antigen from the wild type and mutated DNA as well as hybrid constructs of the promoter–enhancer mutations we can begin to work out the details of how these alterations affect the activities of origin–promoter–enhancer region. We have begun to setup *in vitro* assays using all of these components to test the effect of each element on the replication of the DNA *in vitro* as well as how they affect the expression of the various genes encoded by the DNA. We hope to use this system to identify various cellular factors that are required for replication of these DNA and eventually be able to set up a system using these factors to study the replication off of authentic human genome origins of replication.

It should also be noted that the extrachromosomal elements we are studying may serve as vehicles to introduced foreign DNA into human cells. The DNA appears to maintain a high copy number in the human cells from which it was derived with very little rearrangement occurring in the DNA. We are presently reintroducing this DNA into human cells that have not previously been exposed to SV40 to see if the DNA will maintain itself as an extrachromosomal element. If these experiments are successful, we will clone marker genes into our DNA to see if that can be stably introduced and expressed in human cells.

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6. STRUCTURAL BIOLOGY – Professor Sung-Hou Kim

Transforming Human *c-H-ras* Oncogene Protein: Three-Dimensional Structure and Differences from the Normal Form

Earlier studies of the *ras* oncogenes at the genetic and cellular levels proved that a point mutation in one of a few key regions of normal ras genes results in the production of ras oncoproteins with single amino acid substitution, which are capable of transforming mammalian cells. One of the most commonly found transforming ras oncogenes in human tumors has a valine codon replacing the glycine codon at position 12. The crystal structure of the transforming protein at 2.2 Å resolution reveals that the mutant protein has an enlarged loop that binds the β-phosphate of the guanine nucleotide. Such a change in the "catalytic site" conformation can explain reduced GTPase activity of the protein, thus keeping the protein in GTP bound state, which is thought to signal cell proliferation. Structural comparison of normal and transforming *ras* oncoproteins provides a basis for understanding cell transformation at the structural level.

The *ras* oncogene is one of the most commonly found oncogenes in human cancer cells and is thought to belong to a unique class of oncogenes, the signal transducer genes. The most widely accepted mechanism (for a review see Reference 1) of action of the *ras* gene product is that the extracellular signal for cell proliferation or differentiation perceived by the appropriate receptors is transmitted to the *ras* protein which in turn initiates the signal cascade inside the cell.

The *ras* protein complexed with GTP is considered to be a "signal on" state and that complexed with GDP a "signal off" state. Normal *ras* proteins have an intrinsic GTPase activity, but most of the *in vivo* and *in vitro* transforming *ras* oncoproteins have either reduced or no intrinsic GTPase activity, so that the transforming *ras* proteins stay in the "signal on" state continuously stimulating cell proliferation. The difference between the normal and transforming *ras* oncogenes is usually a single point mutation resulting in single amino acid substitution in one of a few key regions of the gene product, p21. The recent determination of the crystal structure of human normal c-H-*ras* protein (2) revealed that the structure consists of six β-strands, four α-helices, and nine connecting loops (Fig. 1), and the key regions are all in the loops clustered around the bound GDP molecule.

One of the most commonly found transforming mutations in human tumors is due to a substitution of glycine at position 12 by valine (3,4,5). The transforming mutant has drastically reduced GTPase activity (6,7,8). To understand the structural reasons for cell transformation by this single amino acid substitution, we have determined the crystal structure of the transforming mutant, p21(Val-12), which lacks the C-terminal 18 residues. We report here the overall structure at 2.2 Å resolution and compare it with the structure of normal c-H-*ras* protein at the same resolution.

The cloning and expression (9) of the synthetic gene coding for human c-H-*ras* oncoproteins as well as the crystallization conditions have been described (10). Briefly, the synthetic gene (using *E. coli* preferred codons) for amino acid residues 1-171 was cloned and expressed in *E. coli*. We have deleted the codons corresponding to the C-terminal 18 residues because there is reasonable evidence to suspect that the region is flexible (11,12) and that its flexibility may be detrimental in obtaining good quality crystals. Only the soluble fraction of the protein was purified for crystallization. The presence of calcium helped to form high quality crystals. The GTPase activity of p21 (Val 12) was less than 10% of normal protein. High quality single crystals were obtained from solutions containing 10 mg/ml protein, 0.1 M in $CaCl_2$, 0.075 M in Hepes buffer at pH 7.5, 0.5 mM EDTA, 0.5 mM DTT and 0.005% n-octyl glucoside equilibrated to 30% PEG 400. The crystals were isomorphous to those of the normal protein, and have the space group $P6_522$ with cell parameters a = b = 83.2 Å and c = 105.1 Å.

The diffraction data for p21 (Val-12) was collected on an Enraf Nonius rotation camera installed on the 8-pole wiggler line at the Stanford Synchrotron Radiation Laboratory, Palo Alto, California. The x-ray wavelength used for data collection was 1.08 Å, the crystal-to-film distance was 85mm, and 2° rotation was used for each exposure. One set of data was collected from one crystal, at 4°C, on a total of 32 films (16 film packs). The films were digitized on a drum scanner (P-1000, Optronics Corp.), and processed with a program originally written by Rossmann (13). The reflection data from different films were then merged using the PROTEIN program package (14), giving an overall R(merge) on intensities of 8% for 10500 reflections to 2.2 Å resolution.

The crystallographic refinement, using the TNT program package (15), used the model for the normal protein (2) as a starting point. During the course of the refinement, omit maps were calculated by removing residues 9-18 and 55-64 corresponding to loops L2 and L4 in Fig. 1, where the major structural differences between normal and transforming proteins were found.

Examples of such omit maps are shown in Fig. 2. The current R factor for data of 10-2.2 Å resolution is 23.7%. A detailed description of the refinements and the refined structures will be reported elsewhere.

The overall structure (Fig. 3) of the transforming p21 (Val-12) is almost identical to that of the normal protein, but there are major differences in loops L1 and L4 (Fig. 1). In the current stage of the refinement, the biggest structural difference between the normal p21 and the transforming p21 (Val-12) is located in loop L1, corresponding to the residues 9 through 18, which wraps around the β-phosphate of the bound GDP molecule (Fig. 4). This loop would have straddled the phosphodiester bond between the β- and γ-phosphates of GTP. We have suggested that this loop therefore is the prime candidate to be the catalytic site for GTP hydrolysis in the normal p21 protein (2). The simplest description of the structural difference between the normal and the transforming p21 (Val-12) is that the size of loop L1 in p21 (Val-12) is much larger than that of the normal protein (Fig. 4), and two hydrogen bonds (from the backbone NH groups of residues 12 and 13) to the β-phosphate are lost compared to the normal p21 protein. The loss of two hydrogen bonds may alter the orientation of the β-phosphate either presented to the attacking group (possibly a water molecule coordinated to the bound metal ion), or as a leaving group after γ-phosphate is attacked, thus changing the GTP hydrolysis rate. The next largest difference is located in loop L4 (Fig. 3), corresponding to residues 59 to 76, with the biggest difference being residue 59 which, interestingly, is threonine in the viral *ras* p21.

The refinement results of the normal protein as well as the transforming form reveal that there are several unusual aspects about the "catalytic" site (Fig. 4). First, there appear to be no side chains involved either in binding to the phosphate or as a nucleophilic attacking group for the hydrolysis reaction. Lys-16 is the only residue whose side chain (located below β-phosphate) forms hydrogen bonds to the β-phosphate. Second, the amide groups of the backbone are pointing toward the β-phosphate, making hydrogen bonds to the phosphate oxygens and thus fixing the orientation of the β-phosphate, which is presumably important for GTPase activity. Third, there is a metal ion (probably Mg^{++}) at a coordination distance from the oxygens of the β-phosphate. The position of this metal ion is such that it is a reasonable candidate providing the attacking group (probably one of the water molecules coordinated to the metal ion) to the γ-phosphate perhaps for the on-line displacement of the γ-phosphate. Alternatively, the cation may be simply participating in presenting the β-phosphate by fixing it in collaboration with backbone NH groups, with an as yet unidentified water molecule attacking the β-phosphate. Another possible hydrolysis mechanism, similar to that proposed for elongation factor Tu (16), is that the γ-phosphate (which is not present in our structure) is attacked by a water molecule or a hydroxyl group of threonine 59 in case of viral *ras* protein, from an opposite direction, making GDP the leaving group.

Another interesting feature is that both structures appear to consist of two recognizable domains: The N-terminal domain contains the first 70 residues (including the first three β-strands and one α-helix), and is the "phosphate binding domain," while the C-terminal domain is the "guanine binding domain" consisting of the remaining residues (containing the last three β-strands and three α-helices) (see Fig. 1). This separation of domains is further manifested by the distribution of the temperature factors of residues in each domain. The residues in the

N-terminal domain have higher temperature factors (an average of 42 Å), and thus are more mobile than those in the C-terminal domain (an average of 35Å^2). This high mobility of the N-terminal domain may have a functional significance in that it is in this domain where the catalytic site of GTP hydrolysis, the putative effector region (17,18) and the GTPase activating protein binding (10,20) region (residues 30-40) are located.

In summary, the crystal structure of transforming c-H-*ras* (Val-12) protein provides a simple explanation for the loss of GTPase activity of the protein, prolonging the time the protein stays in its GTP bound state, which is thought to be the "signal on" state for cell proliferation. An effort to obtain p21 crystals with bound non-hydrolizable GTP is in progress.

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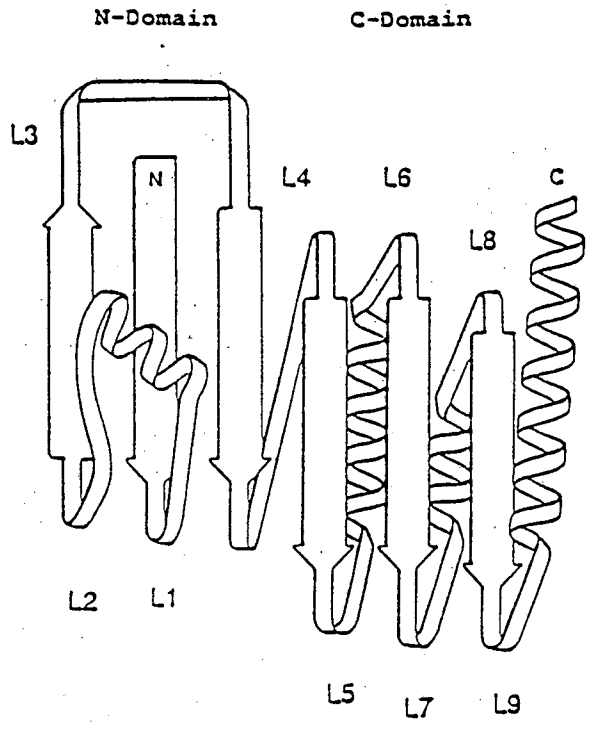


Figure 1.

"Topological structure" of human c-H-*ras* protein. Each β-strand, α-helix and loop is numbered starting from the N-terminus. The N-domain, which covers three β-strands and one α-helix, is barely hydrogen-bonded to the C-domain. The former has much higher thermal motion than the latter, suggesting that the flexibility of the N-domain is greater. The catalytic site for GTP hydrolysis is localized in the N-domain and the recognition for guanine base is in the C-domain.

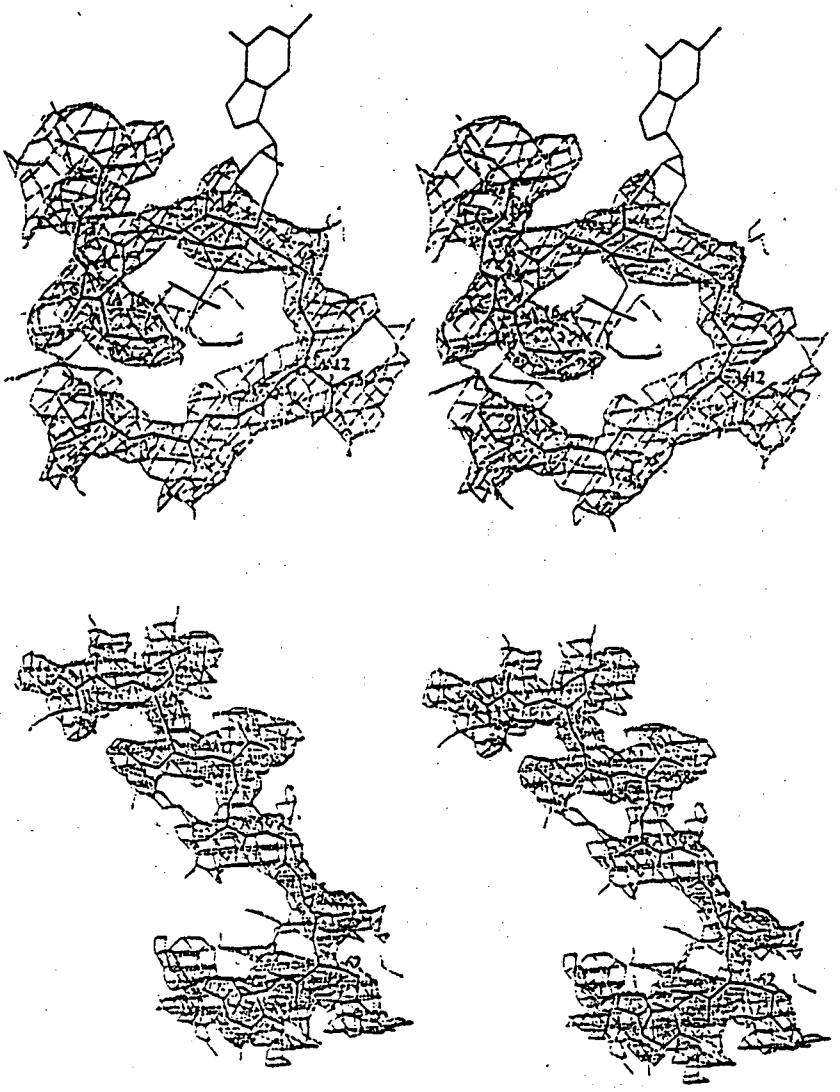


Figure 2.

Electron density maps around the loops L1 and L4 with model fitting. These portions of electron density have been calculated with phases obtained from all atoms in the molecule, omitting those belonging to residues 9-18 and 55-64. The conformation of both regions in this mutant is different from that of normal p21.

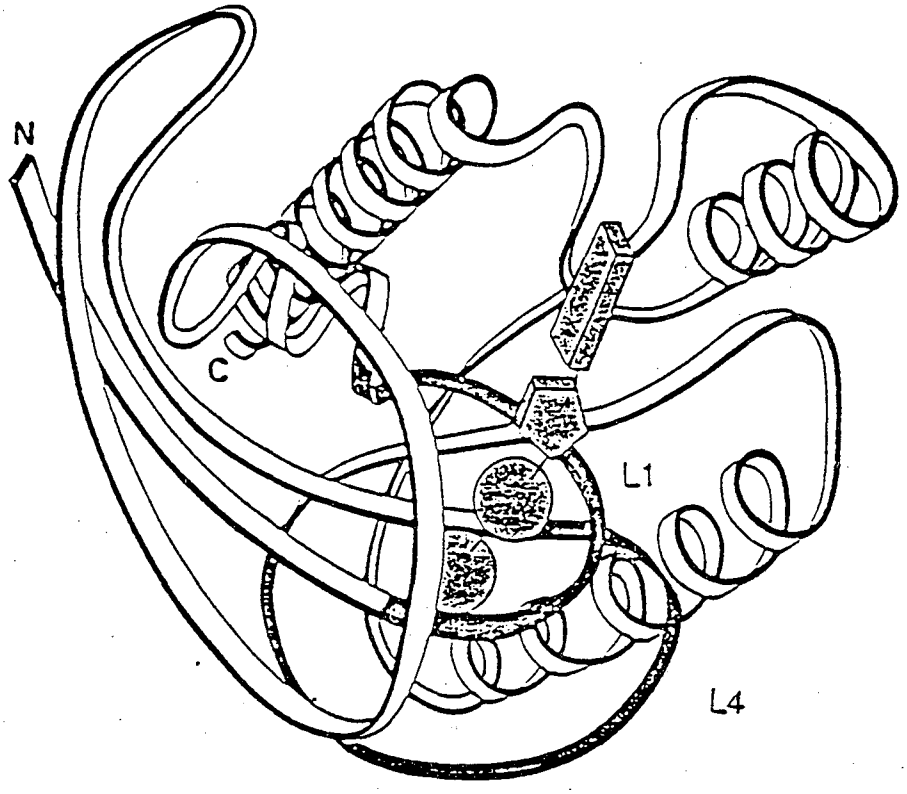


Figure 3.

An artist's drawing, looking down the "guanine nucleotide binding pocket," of transforming human c-H-*ras* oncoprotein (Val-12) crystal structure. The flow of the backbone is represented by a long flat strip, and GDP is identified by stippled shapes. Loops 1 and 4 (see Fig. 1), which have different conformation than those of normal protein (Gly-12), are shown in black.

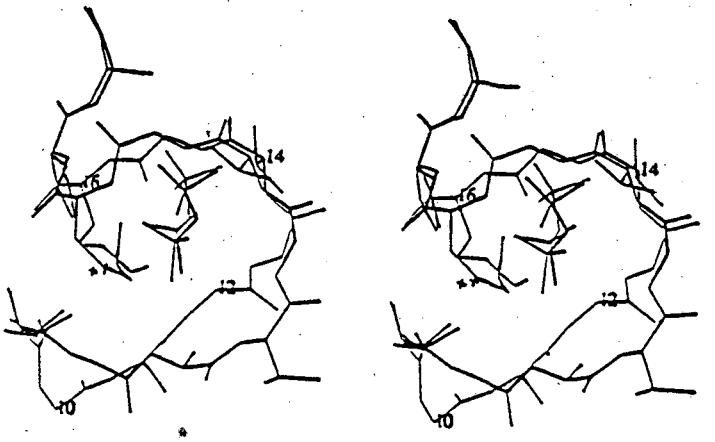


Figure 4.

A stereo view showing the conformation of loop L1. The thick line is the conformation of loop L1 in p21 (Val-12), and the thin line represents that from normal p21 protein. The small cross represents the positions of the metal ions, and two phosphate groups are also shown. Notice that the loop conformation around the β-phosphate in normal p21 protein is much tighter than that of p21.

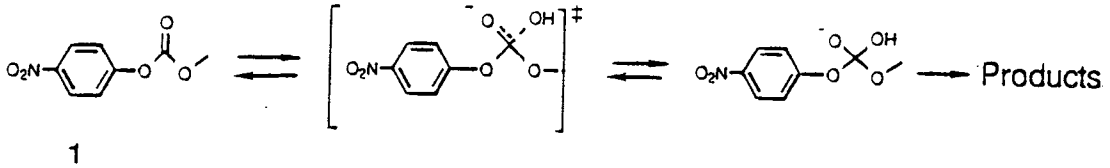
7. Artificial Enzymes - Professor Peter Schultz

The ability to design catalysts with predetermined specificities for reactions such as the selective cleavage, condensation, or modification of natural and synthetic polymers would be of great importance for drug design, molecular biology, and materials chemistry. The synthesis and characterization of these catalysts might provide additional insight into ligand receptor-recognition and catalysis. We are pursuing a new approach toward this goal, the design and synthesis of catalytic antibodies

Key to the design of selective catalysts is the generation of highly selective binding sites. With the advent of monoclonal antibodies, homogeneous ligand-binding sites with enzyme-like affinities and specificities can be generated for macromolecules and small synthetic molecules. Antibodies have been selectively generated against biopolymers such as nucleic acids, proteins, and polysaccharides; smaller multifunctional molecules such as steroids and prostaglandins; and synthetic polymers such as polypropylene. Antibodies bind ligands ranging in size from about 6 Å to 34 Å with association constants in the range of 10^4 to $10^{10}M^{-1}$ and as high as $10^{12}M^{-1}$. The development of viable strategies for introducing catalytic activity into antibody combining sites might, therefore, afford a general route to enzyme-like catalysts with tailored specificities.

A number of strategies can be envisioned for generating catalytic antibodies. Antibody specificity could be used to selectively stabilize transition state configurations or to overcome entropic barriers involved in orienting reaction partners. Catalytic groups, either amino acid residues or synthetic catalysts, might be introduced into antibody combining sites, or genetic selection might be used to generate catalytic antibodies.

An antibody generated to a haptenic group resembling the transition state configuration of a given reaction should lower the free energy of activation of reaction by stabilizing the corresponding transition state relative to reactants or products. Our initial studies focused on immunoglobulins specific for tetrahedral, charged phosphonate, and phosphate transition state analogs for the hydrolysis of carbonates and esters. We chose these well-characterized reactions in order to simplify the mechanistic studies of ligand binding and catalysis. We have succeeded in characterizing antibodies that selectively catalyze two hydrolytic reactions: the hydrolysis of p-nitrophenyl N, N, N- trimethylammonioethyl carbonate 2 and the hydrolysis of methyl p-nitrophenyl carbonate. These antibody-catalyzed reactions are competitively inhibited by the corresponding transition state analogue, show high substrate specificity, and accelerate the reactions 10^4-10^6- fold over the background rate.



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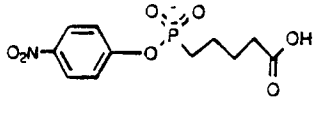

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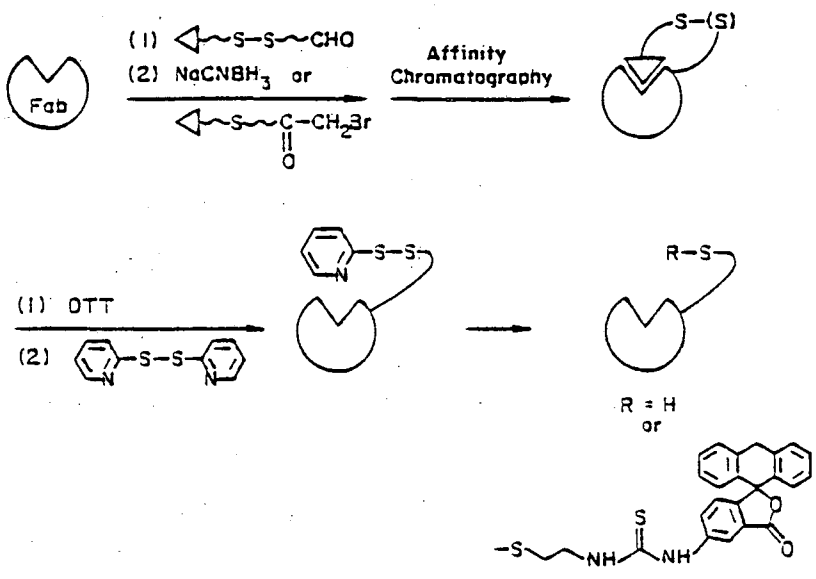
If we again exploit the notions of enzymic catalysis, it should be possible to use antibody binding affinity to increase reaction rates by overcoming entropic barriers to reaction. The binding energy in this case might play the important role of reducing translational and rotational motions for reaction by properly orienting the reactants in the antibody combining site. We have used these ideas to guide us in the generation of antibodies which carry out the formal Claisen rearrangement of chorismic acid to prephenic acid. This thermal 3,3-sigmatropic rearrangement has been demonstrated to occur through an asymmetric chair-like transition state in which the carbon-oxygen bond is substantially broken while carbon-carbon bond formation has not occurred to any appreciable extent. The unimolecular rearrangement is catalyzed approximately 10^6-fold by the enzyme chorismate mutase at the branch point in the biosynthesis of aromatic amino acids in bacteria and plants. We have characterized a catalytic properties of antibodies specific to the chorismate mutase inhibitor with a K_i = 0.15 mM. To the extent that the bicyclic diacid accurately simulates the conformationally restricted activated complex involved in the rearrangement of chorismic acid, antibodies specific for the inhibitor should lower the entropic (and enthalpic) barrier to reaction. An IgG has been identified that selectively catalyzes the Claisen rearrangement of chorismate to prephenate. The antibody-catalyzed reaction demonstrates saturation kinetics (k_{cat} = 2.7 min^{-1}, Km = 260 mM; 10°C) and is inhibited by the bicyclic transition state analog 10 (K_i = 9 mM). The antibody accelerates the rearrangement by a factor of 10^4 (k_{cat}/k_{uncat}) over the thermal reaction. Characterization of the activation parameters and mechanism of this reaction will provide additional insight into the role rotational motion may play as a driving force in this antibody-catalyzed reaction.

A third strategy whereby catalytic antibodies might be generated involves the site-specific introduction of catalytic residues into immunoglobulin combining sites. One might accomplish this by either genetic methods, including site-directed mutagenesis and selection, by selective chemical modification, or by exploiting the notion of antibody- hapten complementarity. We have successfully applied the latter approach to the generation of antibodies which catalyze the photocleavage of thymine dimers.

Thymine dimers are the major photolesions which result from irradiation of DNA with UV light. Organisms have evolved a number of systems to repair thymine dimers, including light dependent photoreactivating enzymes. Although the mechanism of the enzymatic reaction remains poorly understood, it has been demonstrated in model systems that a number of sensitizers including indoles, quinones, and deazaflavins can photosensitize dimer cleavage. One might, therefore, imagine that an antibody combining site, specific for thymine dimers and containing an appropriately positioned sensitizer, might act as a selective photoreactivating enzyme. The question arises whether we might expect antibodies generated against the p system of a thymine dimer to contain a complementary tryptophan residue, a photosensitizer for dimer cleavage. We have isolated and characterized six monoclonal antibodies specific for thymine dimers, five of which efficiently cleave thymine dimers when irradiated with > 300 nm light. The f_R = 0.6 and k_{cat} = 1 min^{-1}. The kinetics of the reaction are consistent with the Michaelis-Menten rate expression 1. Mechanistic studies suggest the participation of a tryptophan residue in the cleavage reaction. Chemical modification might also be used to introduce synthetic catalytic groups, such as a redox-active metals, nucleophilic groups, and cofactors into antibody combining

sites.



The key to this approach is the development of new mild methods for selectively functionalizing large, complex proteins with residues of unique reactivity, such as thiols, that can be subsequently derivatized with catalytic groups. We have synthesized cleavable affinity labels which allow us to introduce a nucleophilic thiol into the combining site of the IgA MOPC 315, thereby generating an antibody which accelerates ester cleavage 60,000-fold over background. Moreover, the thiol provides a handle whereby additional chemical functionality-enzymatic cofactors, metal-ligand complexes, or spectroscopic probes can be selectively introduced into the antibody combining site. For example, derivatization of the thiolated MOPC 315 with the fluorophore, N-fluoresceinthioureido- 2-mercaptoethylamine, has made possible a direct spectroscopic assay of antibody-ligand complexation.

Research Progress

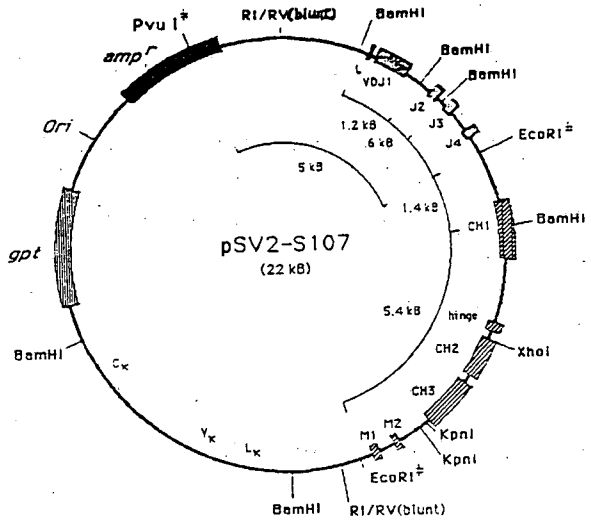
Alternatively, it should be possible to introduce catalytic residues into antibodies by site-directed mutagenesis. General bases and acids, and nucleophiles (His, Cys, Asp, Glu) can be introduced into combining sites or a cysteine can be introduced and selectively derivatized as described above. These studies will be carried out with the phosphorylcholine binding antibody S-107. Recently it was found in our lab that several phosphorylcholine binding antibodies, T-15, M-603, and M-167, have the ability to catalytically hydrolyze p-nitrophenylcarbonate esters. M-603 has been crystallized and the x-ray structure shows the presence of a tyrosine at position 33 and an arginine at position 52 in the variable region of the heavy chain binding domain. It can be seen from the crystal structure that tyrosine 33 and arginine 52 interact with the anionic phosphate moiety of phosphoryl choline by hydrogen bonding and electrostatic interactions. These residues are thought to stabilize the negatively charged tetrahedral transition state geometry generated by attack of a binding site-accessible water molecule on the substrate carbonate moiety. We will

probe the roles these residues play in catalysis and in addition introduce new catalytic residues by site-directed mutagenesis.

Because the gene coding for M-603 was not available, we chose another phosphorylcholine binding antibody, which is highly homologous to both T15 and M-603, for mutagenesis studies. Tyrosine 33 and arginine 52 are conserved in all three antibodies. Most of the contact residues in the active site of M-603 are located on the heavy chain. Tyrosine 33 has been changed to: phenylalanine to determine the importance of the tyrosine hydroxyl group; histidine, aspartate and glutamate to act as general bases. Arginine 52 has been changed to glutamine to determine the role of the charged side chain. Arginine 52 has also been replaced by cysteine which could potentially participate directly in catalysis or be modified with another active group via disulfide exchange. The wild type gene coding for the S-107 antibody has previously been cloned, sequenced, and expressed.

In order to carry out mutagenesis, pSV2-S107 was initially digested with the restriction enzyme BAM H1 in an attempt to directly isolate the 1.2 kilobasepair (kb) fragment containing the VDJ1 gene. Due to the multiple Bam H1 sites present in the plasmid, however, the fragment could not be cleanly isolated. To circumvent this problem, the plasmid PSV2-S107 was digested with Eco R1 and Pvu 1 and the resulting 5 kb fragment was isolated via agarose gel electrophoresis. This fragment which contains the heavy chain variable region coding sequences was subcloned into the plasmid pT7-3. Restriction mapping confirmed that the desired insert was present in the pT7-S107 DNA. Plasmid pT7-S107 was digested with Bam H1 and the 1.2 kb fragment was isolated via agarose gel electrophoresis. This fragment containing the VDJ1 gene was now reduced to a suitable size for cloning into bacteriophage M13. Clear plaques were grown on 2YT growth medium and single stranded M13-S107 DNA was isolated by a standard bacteriophage preparation. The sequence of the VDJ1 gene was determined using the dideoxy chain termination sequencing method developed by Sanger. Oligonucleotide directed mutagenesis was performed using the method developed by Eckstein. Mutants were sequenced to be sure that the desired mutations had occurred. Double stranded DNA was digested with Bam H1 and the mutant 1.2 kb fragments were inserted into PT7-S107.

The final step in the cloning strategy outlined is to isolate the mutant heavy chain gene (VDJ1) from the pT7-S107 plasmid and insert it into the parent vector pSV2. The resulting clones should possess mutant antibody genes which will be expressed in appropriate eukaryotic cells and the mutant antibodies isolated. These antibodies will be assayed for their ability to catalyze the cleavage of p-nitrophenylcarbonate esters. Their kinetic parameters and substrate specificities will also be evaluated. Potential catalytic groups will be introduced via selective derivitization of the cysteine 52 mutant.



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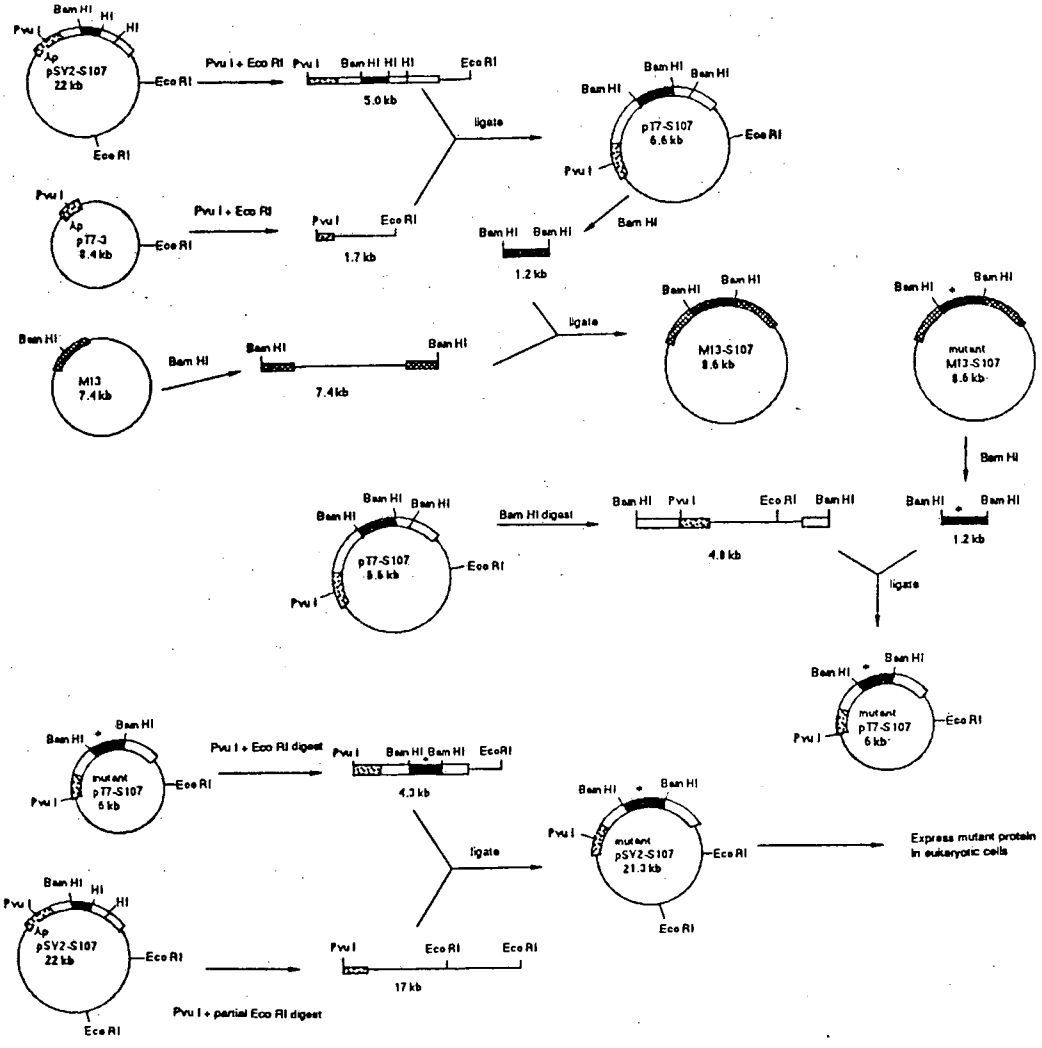
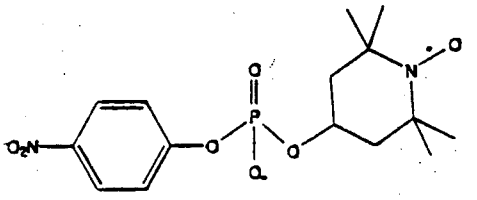
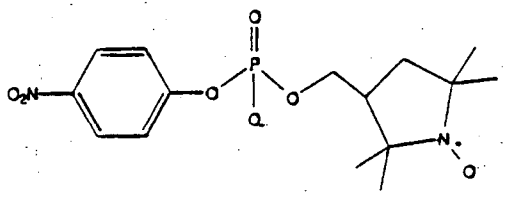
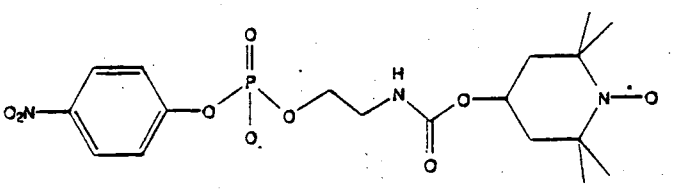
V_{S107} from pWB304

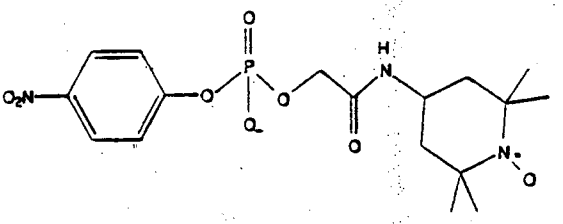


A second project being pursued involves the structural characterization of antibodies which catalyze the hydrolysis of nitrophenylcarbonates. These antibodies were generated against the corresponding nitrophenyl phosphonate and accelerate hydrolysis 10^4 - 10^5-fold over background. The following spin labels have been synthesizedand are being used to probe the Ig active sites by difference NMR of Fab fragments containing selectively deuterated side chains. Preliminary studies suggest the presence of a positively charged residue and aromatic residues in the combining site. ESR studies reveal that the spin labels are bound tightly in the Ig combining site (t = ansec) and that the combining site is 8-12 Å in depth.



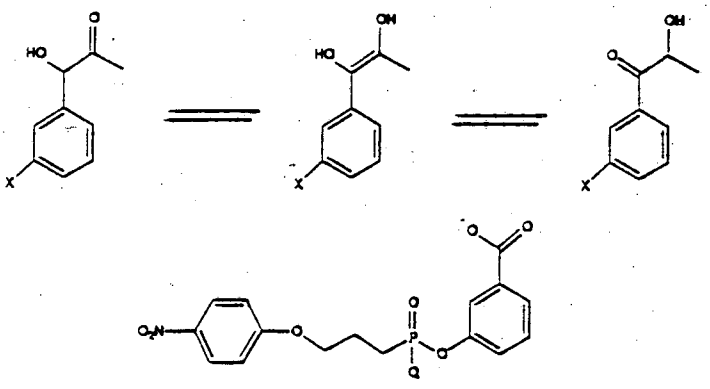






A third project being undertaken involves the generation of an antibody which catalyzes the

isomerization of an α-hydroxyketone.



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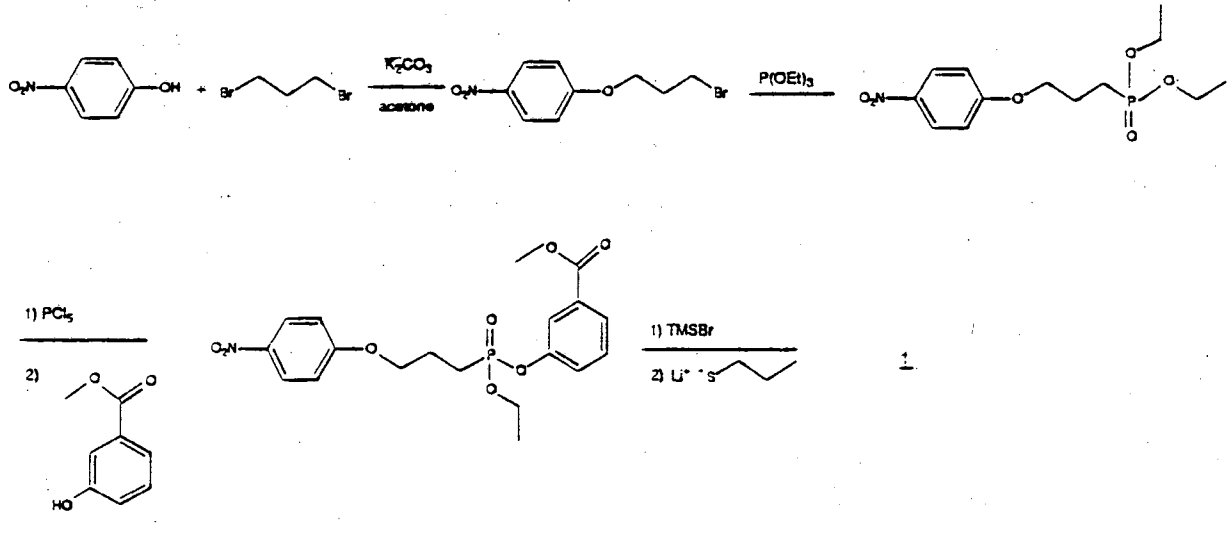
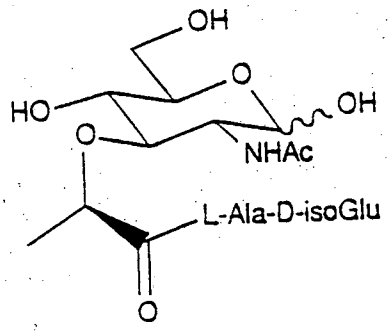
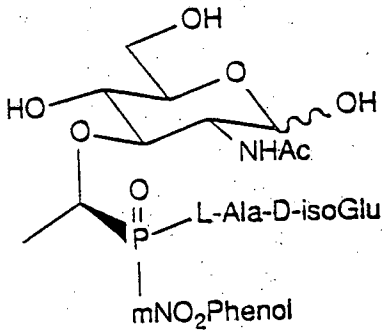
This reaction is analogous to the isomerization of dihydroxyacetone phosphate catalyzed by the well-studied enzyme triose-P-isomerase. This is a first step toward the generation of antibodies capable of catalyzing carbon-carbon bond forming aldol reactions. Hapten 1, a transition state analogue for the enediolate, has been synthesized by the route illustrated below and monoclonal antibodies are currently being produced.



Finally, in a collaborative effort with Professor C.-H Wong, Department of Chemistry, Texas A&M, we are synthesizing a transition state analogue for the glycosylation of proteins. Our goal is to generate a family of antibodies capable of glycosylating proteins with specificity for a

specific peptide side chain and sugar moiety. The hapten has been synthesized and monoclonal antibodies are being produced.





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1. THE NATIONAL TRITIUM LABELING FACILITY - Professor Henry Rapoport

The National Tritium Labeling Facility (NTLF) was established on August 1, 1982, funded by the National Institutes of Health and administered by the U.S. Department of Energy. It is housed within the Lawrence Berkeley Laboratory (a DOE National Laboratory managed by the Regents of the University of California), and is affiliated with LBL's Chemical Biodynamics Division. Since 1982 the NTLF has assumed the position of a unique Facility, able to handle large amounts of tritium, and to enable the labeling to very high specific activity of compounds which would otherwise be unavailable. It has functioned as a venue where investigators from throughout the nation can carry out high level labeling and radiopurification procedures that were not possible in their own laboratories or in commercial facilities. The Facility has grown to a fully equipped laboratory with a full-time staff of four, and routinely provides Service for approximately twenty Users per year. The established guidelines involve review of applicants and their projects, and imposition of standard charges to offset the cost of service.

Initial core research projects at the NTLF were directed toward the development of a variety of novel labeling and purification methods. Other high priority work included detailed studies into the mechanisms of various tritiation methods, and the use of tritium as a probe to further the understanding of biological processes. Among the important new techniques and uses of tritium that NTLF Technological Research has provided to the biomedical/biochemical community over the last several years are studies of excitation labeling, application of tritium NMR spectroscopy to direct monitoring of glucose metabolism, and new reagents for [^{3}H]-N-methylations and similar reactions.

Recently there has been increased interest within the scientific community in the use of high levels of tritium for specific labeling of molecules of biological importance (e.g. peptides, proteins, DNA oligomers, pheromones, and insecticides). The highly labeled products are intended for application to the solution of particular problems in structural biology and general biochemistry. Currently, there are very few facilities in industry or academia where this type of work can be accomplished since a combination of facilities for high level tritium labeling, synthesis with such labeled molecules, and analysis for sites and levels of tritiation is required. Although the NTLF has previously had some activity in this area, more emphasis will now be placed on work with larger bio-molecules. The combination of equipment already in the Facility (DNA synthesizer, NMR and tritium probe, access to a peptide synthesizer for non- radioactive work), and the momentum of several ongoing collaborative projects, will mean that the NTLF will be in position to provide the leadership for this type of research. Particular emphasis may now be placed on the applications of pure, well-characterized, tritiated materials, and the use of these substrates with attendant technology in addressing fundamental questions in biology.

PROGRESS SUMMARY (JULY 87 - JUNE 88)

The NTLF has five major functions mandated by its function as an NIH Research Resource. These functions are: Technological Research and Development, Collaborative Research, Service, Training and Dissemination of Information. Progress in each of these areas is reported below for the period of July 1987 through June 1988 .

Technological Research and Development:

a). There has been steady progress in the evolution of general labeling methods, including exchange, microwave activation and thermal triton studies. Excitation labeling is a particularly attractive method of labeling for many of our users and collaborators, and the NTLF provides one of the few facilities (if not the only one) where researchers can try this technique. Research in this area will continue with the hope of providing a general labeling method that can be used for materials that are difficult or impossible to label any other way.

b). Particular success has been achieved amongst the synthetic labeling procedures, with the synthesis of mono and multitritio methyl iodide and specific peptide or nucleotide labeling being features.

c). Research has yielded 23 publications (11 manuscripts in press) and one Ph. D. thesis.

Collaborative Research:

Core and collaborative projects bringing together all the unique features of the NTLF have been initiated, and show great promise. These projects demonstrate the capability to query biological structure- function relationships at a molecular level, and to probe fundamental molecular and biological processes, by application of the technologies available at the NTLF. The project "Tritium NMR as a Biological Probe: Application of Tritium NMR to the Study of Glycolysis in Blood" described last year has been of particular interest because it is a demonstration of how the active metabolic reactions of an important substrate can be followed using a non-invasive technique. The project "Interaction of Maltose with Maltose Binding Protein" illustrates ways that tritiated substrates may be used to probe the kinetics of binding, and the nature of the binding sites in protein-substrate interactions.

Service:

Twenty four Users from 13 institutions have visited the NTLF 16 times, and labeled 23 compounds. Fourteen NIH grants have been served over the course of these visits, representing at least five different NIH programs. There have been 30 publications by Users and 6 theses acknowledging the Resource.

Training:

A total of 16 researchers have received training at the NTLF in high level labeling and the efficient use of tritiated substrates.

Dissemination:

A total of 29 invited talks and other conference presentations have been made in addition to the four publicity articles, one thesis and 23 scientific articles published.

FACILITY USE

A survey of the usage of the tritiation line and NMR instrument shows that both these facilities are used more than 75% of their available time (tritiation line=max. 260 days p.a., NMR=168 hrs/week). There has been a prodigious increase in the number of conference presentations and invited talks. Service visits have increased only slightly, but now stand close

to the maximum which could be safely handled at the Facility, while still permitting in-house research.

RESEARCH HIGHLIGHTS

From October 1987 through September 1988 there have been 14 Core Research Projects and 3 Collaborative Projects actively pursued at the Facility. The Core Research Projects include 2 with Professor Chin-Tzu Peng at UCSF and the Collaborative Projects include work with Professors Hearst, Wemmer and Schultz of the UCB Chemistry Department. The following projects have been chosen as highlights.

Heterogeneous Group VIII Metal-Catalyzed Hydrogen Exchange:

Studies of the use of platinum, palladium and Raney nickel as catalysts for tritium exchange into organic substrates under mild conditions were undertaken. Initial results obtained with Raney nickel as catalyst gave extensive incorporation and high specificity of label, with a number of pilot experiments yielding tritiated hydrocarbons with specific activities in the region of 1-10 Ci/mmole. NMR analyses of products showed that the mechanism of the exchange process may be deduced, that aromatic *vs* aliphatic effects may be quantified, and that the resolution of modern day NMR instruments allows simple resolution of multiply labeled species. With extension of these experiments to more diverse substrates, and with careful radio-GLC and ^3H NMR analyses, we have gained more insight into the range of application and the mechanisms of these exchange procedures.

These subsequent exchange reactions with tritium gas at R.T. over Raney nickel, heterogeneous platinum and palladium catalysts gave products with specific activities in the 0.1-15 Ci/mmole range, and many had >95% radiochemical purity.

In aromatic hydrocarbons highly specific labeling was observed, with toluene, *m*-xylene, *n*-pentylbenzene and *s*-butylbenzene labeled almost exclusively in the a-CH positions. This is in agreement with results already in the literature, and is thought to occur by way of a π-allyl mechanism. Close scrutiny of the ^3H NMR spectra for the toluene samples labeled in the presence of 100% T_2 showed that hydrogenation (to give methyl cyclohexane) is a significant reaction over platinum. In addition, inspection of the expanded methyl pattern of the spectra showed a large proportion of CT_3 species over the platinum catalyst. This suggests that the substrate is very strongly adsorbed on platinum, allowing multiple exchange reactions to predominate. On nickel and palladium catalysts the exchange patterns reflect a much higher predominance of one hydrogen replacement per visit to the catalyst. Over nickel and platinum the aromatic centers are labeled predominantly in the *meta* and *para* positions. The likely mechanism for aromatic exchange is the dissociative π-complex mechanism first described by Garnett. Over palladium catalyst the aromatic center of toluene was more heavily labeled in the *ortho* position than either *meta* or *para*. This orientation was not observed at higher temperatures for a similar reaction, and this anomaly could explain the proposal of conflicting exchange mechanisms in the literature in the 1960's, where the associative mechanism was favoured by workers studying exchange over metal films at low temperature.

Aromatic hydrocarbons containing functional groups (anisole, nitrobenzene, fluorobenzene) and naphthalene were not cleanly labeled with Raney nickel as catalyst, and a major competing

reaction appeared to be ring saturation or reduction of the functional group. This preparation of Raney nickel is known to give saturation and reduction products in good yield under only slightly more stringent conditions. In particular, at 40-50°C and 280-410kPa of hydrogen gas, β-naphthol gave 5,6,7,8- tetrahydronaphthol in 83% chemical yield, phenol gave cyclohexanol (92%), and heptaldehyde gave 1- heptanol (90%).

Alkanes (*n*-heptane, methyl cyclohexane) were labeled only slightly, and did not give high radiochemical purity. The specific activities obtained were comparable to other reported results, and mechanisms for isomerization have been proposed.

In general much higher specific activities were observed than are generally obtained through many exchange labeling processes. One advantage of the technique is the mild experimental conditions, but an apparent problem is the destruction of functional groups on several of the substrates under study. These experiments illustrate the power of using combined analytical techniques such as radio-GLC and ^{3}H NMR spectroscopy for the characterization of labeling methods. A summary of these results was presented at the Third International Symposium in Innsbruck, and will be published in the Proceedings, "Synthesis and Applications of Isotopically Labeled Compounds (Proc. Third Int. Symp., Innsbruck)".

Tritiated Methyl Iodide

A number of new strategies have evolved for the preparation of tritiated methyl iodide since the report last year. These techniques are targeted for use in radiochemistry laboratories that do not have the high level facilities available at the NTLF:

(1). A Novel Synthesis of Monotritiomethyl Iodide from Thioethers: Tritiodehalogenation of monochloromethylphenyl sulfide with tritium gas and Pd-C (30%) in ethyl acetate gave the corresponding monotritiothioether in a short time with a specific activity of 28.5 Ci/mmole. Radio-HPLC analysis of this labeled precursor showed a radiochemical purity of better than 95% and the specificity of the labeling was confirmed by tritium NMR spectroscopy. Reaction of the monotritiosulfide with benzyl iodide liberated monotritiomethyl iodide in a one pot synthesis, possibly through the intermediate formation of monotritiomethylbenzyl sulfonium iodide. Upon thermal decomposition, this sulfonium salt gave benzylphenyl sulfide (90%) and monotritiomethyl iodide. A report on this work was presented at the 196th ACS National Meeting, Los Angeles, CA, Sept. 25-30 (1988).

(2). A New Synthesis of CT_3I from Aryl ^{3}H-Alkyl Thioethers: It was expected that a similar synthetic approach to that used in the monotritiomethyl iodide project ((1)., above) could be followed in this project, and a series of preliminary reactions were carried out. After a number of precursors were investigated, a novel procedure for the synthesis of multi-tritiomethyl iodide was evolved, and this also led us to a new synthesis of the target molecule. In this method, phenyl methyl sulfide was fully chlorinated in the alkyl group to yield trichloromethyl phenyl sulfide in almost 100% yield. Subsequent hydrogenolysis of the C- Cl bonds with deuterium gas in the presence of Pd-C (10%) and an organic base afforded multideuteriomethyl phenyl sulfide. Upon heating a mixture of the latter with benzyl iodide in a special apparatus, benzyl phenyl sulfide remained as a solid and multideuteriomethyl iodide was liberated. The generated methyl iodide was then trapped in a solution of dimethylaniline in benzene and formed deuteriated trimethylphenylammonium

iodide in about 50% yield. Tritium labeling of the precursor is now underway. A preliminary description of this work was presented as a poster at the Third International Symposium on the Synthesis and Applications of Isotopically Labeled Compounds, (July 17-21, 1988), and the experimental strategy will be published in the Proceedings of that meeting.

Interaction of Maltose with Maltose Binding Protein

During this Grant period several new collaborative projects were initiated, concentrating on the use of tritiated substrates in NMR studies. Although NMR of other nuclei have contributed greatly to the general understanding of solution structure and conformation of biomolecules, tritiated substrates and tritium NMR spectroscopy have never been applied to these studies. Prof. David Wemmer and Dr. Kalle Gehring proposed and began studies on the binding of maltose to the periplasmic transport protein MBP (maltose binding protein) from *E. coli*. The proton spectra of this protein (40 kD) are obviously complex, and changes occur throughout the spectrum upon maltose binding, making it impossible to identify resonances from the bound maltose. To get at the spectrum of the bound maltose, to determine the relative binding of the alpha and beta anomers, and to analyze the binding kinetics we have used maltose (and maltose oligomers) tritium labeled at the C1 position of the reducing glucose. The tritium labeled compounds are prepared by direct exchange with T_2 gas over a Pd on $BaSO_4$ catalyst. This is a simple labeling procedure for any length oligomer, the resulting products have reasonably high specific activity (*ca.* 13 Ci/mmol) and require little purification after the exchange reaction. In theory equivalent information would be available from NMR experiments with 1-^{13}C maltose, but this requires much more extensive synthetic work, and even more synthesis for longer oligomers.

Our preliminary studies titrating MBP with maltose have shown the expected behavior for slow exchange; at first two resonances appear near 3.5 ppm for the bound forms of the alpha and beta anomers, and after addition above a 1:1 ratio new peaks appear at the expected positions (4.5 and 5.1 ppm) for the free maltose. By the large difference in chemical shift of free and bound forms of maltose, this experiment alone demonstrates interaction of the maltose with an aromatic group (probably Trp) in the bound state. In addition the ratio of the two anomers in both the bound and free states is different from that of maltose in the absence of protein. We have carried out selective saturation transfer measurements and irradiation of the free alpha anomer resonance causes saturation of the upfield of the two bound resonances. This identification shows that the alpha anomer binds preferentially to MBP, with a binding constant approximately twofold higher than the beta. We are also carrying out heteronuclear NOE experiments to try to identify the resonances from amino acids in the binding site. Preliminary experiments have shown that these NOEs are observable, but we must still optimize conditions to get maximal selectivity. To aid in this work, we have MBP mutants available with Trp residues taken out. The experiments are being conducted by Dr. Kalle Gehring, who has recently been awarded a Hollaender Fellowship from DOE to carry out postdoctoral work with the Wemmer group. This work is still in progress, but preliminary results have been reported (publications 15 and 20).

CONCLUDING REMARKS

The Technological Research and Development and Collaborative Research work carried out at the NTLF represents a co-ordinated approach to the labeling of many groups of molecules, from simple organic substrates to complex bio-polymers. If these are to be fruitfully

employed in solving any problem of a chemical, biochemical or medical nature they must be pure and well-characterized. The National Tritium Labeling Facility offers resources for the preparation, purification and analysis of high specific activity radiochemicals, and is valued by the biomedical/biochemical community for the environment it makes available.

Based on the materials produced at the NTLF, a strong collaborative program has begun, probing such fundamental problems in biology as solution structure and metabolism. The glucose project has demonstrated the power of tritium NMR spectroscopy as a non-invasive probe of an in vivo system, giving fresh insight into the well-studied glycolytic pathway. The study of maltose binding protein project shows the potential of tritium NMR spectroscopy for defining the nature of binding sites, and the kinetics of intermolecular interactions.

In the next year we expect the startup of more collaborative projects, and also expect the NTLF to become one of the most versatile and capable tritium labeling laboratories in the world. In combination with the Chemical Biodynamics Division of LBL and the Chemistry Department at U.C. Berkeley, the avenues for productive research are manifold.

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E. DOE SPONSORED UNIVERSITY CONTRACT – Professor Ignacio Tinoco, Jr.

Telomeric DNA oligonucleotides form novel intramolecular structures containing guanine•guanine base pairs

We are interested in any conformation which occurs in DNA, particularly if it has a known biological relevance. Thus, the study of simple repeating sequences which occur in telomeres was particularly appealing. Telomeres contain guanine-rich strands on the 3'-ends and these strands do not have complementary strands; they overhang the usual Watson-Crick base pairs. We synthesized the oligonucleotide $(TTGGGG)_4$ using a commercial DNA synthesizer; this sequence is the telomere for Tetrahymena. As the sequence contains no adenine or cytosine, it cannot form any Watson-Crick base pairs. However, proton and phosphorous NMR showed that the molecule exists in solution as a single compact structure at low temperature, which melts to a single strand above 40oC. The evidence for this is the observation of about 24 imino proton resonances in H_2O at low temperature, which broaden and disappear with increasing temperature. This is characteristic of paired bases with hydrogen-bonded imino groups. The phosphorous resonances show a range of chemical shifts at low temperature, which merge into one average peak at high temperature. This is consistent with the melting of a base-paired structure to a single strand. Absorbance melting and gel electrophoresis studies are also consistent with this conclusion.

Two dimensional nuclear Overhauser effect (NOESY) measurements showed that 4 to 6 of the guanine bases are in an unusual syn conformation. Normal right-handed Watson-Crick base pairs all have anti conformations of the bases relative to the sugars, but guanine•guanine base pairs can form if one of the guanines is in a syn conformation. There are 4 G•G pairs and 7 G•T pairs with the hydrogen bonding shown; one of each G•G pair is in a syn conformation. Other schemes for pairing the guanines are possible and we are doing further NMR studies to establish the correct one.

This folded structure does not require complementary base pairs to form, thus, it can exist with any number of repeats. This is necessary as telomeres are known to have a range of repeats. The folded structure can be recognized by a special terminal transferase (telomerase) which elongates the 3' end. Normal primer-dependent DNA synthesis will lead to shortening of the chromosome, but elongation of the 3' end by telomerase can ensure that shortening does not occur.

Pseudoknots: tertiary structural units in RNA

The diverse biological functions of RNA, such as enzymatic activities and regulatory roles in transcription and translation, are made possible by tertiary structure. Computer algorithms can predict secondary structure using free energy parameters for base pairing, loops and bulges. However, with the exception of tRNA, little is known about the structure and thermodynamics of interactions involved in the tertiary structure of RNA. We have begun a study of pseudoknots, a tertiary structure proposed for the 3'-ends of many plant viral RNAs and probably common

in a wide range of other RNAs. A pseudoknot is formed from the interaction of two stems and loops. The questions to be answered are: Can the structure form? What is its free energy relative to its two constituent hairpins?

We have synthesized a series of RNA oligonucleotides capable of being folded into pseudoknots. The molecules range in length from 19 to 26 nucleotides and were synthesized using T7 RNA polymerase. The DNA template strand containing the T7 promoter, and the strand complementary to the promoter portion are synthesized on a commercial DNA synthesizing machine. The T7 RNA polymerase needs a double strand to start RNA synthesis, but then can continue on the single strand template. Milligram amounts of RNA can be synthesized and the only constraint on the sequence is that the RNA must start with a guanine. We characterized the conformations of the molecules using NMR, absorbance melting curves, single-strand specific enzymes, double-strand specific enzymes and gel electrophoresis. The conclusions are: (a) The pseudoknot structure can indeed form. Nuclear Overhauser effect (NOE) measurements of imino protons indicated a continuous helix made up of the the two stems of the two hairpins. This is crucial to establishing the pseudoknot structure. NOE intensities depend on the inverse sixth power of the distance between two protons; thus the NOE data for the imino protons are a sensitive indicator of the stacking of adjacent base pairs. The double-strand and single-strand specific enzymes corroborate the pseudoknot structure and rule out a rapid equilibrium between the two hairpins that constitute the pseudoknot. For an equilibrium between two species we expect the enzyme to react with each species; this would cause some links to be cut by both enzymes. This is not found. (b) The pseudoknot is thermodynamically slightly more stable than its hairpins at low temperature, but as the temperature is raised, it melts to a hairpin first and then to the single strand. Pseudoknots are classified by the number of base pairs in each stem and by the number of unbonded bases in each loop. We have used absorbance melting to study pseudoknots with 3 to 5 pairs in the stems, and with 2 to 6 unbonded bases in the loops. Loops of 4 to 6 unbonded bases are of approximately equal stability; but the stability decreases for 3 unbonded bases, and 2 unbonded bases are barely stable. This is consistent with model building studies which show that 2 nucleotides can barely stretch across the wide groove of an RNA double strand to form a pseudoknot; steric repulsion is expected.

A complete structure for a pseudoknot can be obtained by assignment and analysis of the nonexchangeable protons and their NOEs; this is in progress. Measurements of free energy, enthalpy and entropy are complicated by the non-two-state nature of the melting of the pseudoknot. However, a combination of absorbance and NMR melting experiments should allow the thermodynamics to be determined.

Comparison between DNA melting thermodynamics and DNA polymerase kinetics

The kinetics of misincorporation of the wrong nucleotide vs. the right nucleotide by a DNA polymerase lacking repair has been measured. The kinetics of extension of a strand containing an incorrect nucleotide has also been measured. We want to learn the factors that contribute to the fidelity of replication of DNA. The results show that misincorporation occurs with a rate of about 10^{-4} of the rate of incorporation of the right nucleotide. Extension of a strand with

a wrong nucleotide occurs with a rate of about 10^{-3} of extension of the right nucleotide. In both cases the decreased rate is mainly caused by an increase in the Michaelis constant, K_M, the dissociation constant for the enzyme-substrate complex. In these experiments the enzyme is the DNA polymerase with bound template and the substrate is a nucleoside triphosphate. Thus, the kinetics tell us that the main differences contributing to fidelity are the strength of binding of the right nucleoside triphosphate compared to the binding of the wrong nucleoside triphosphate.

For the same sequences in the same buffer, we measured the thermodynamic melting of oligonucleotides containing Watson-Crick base pairs and mismatches. The order of stability was A•T ⟩ G•T ⟩ C•T ⟩ T•T; this is the same order found for the rates of incorporation. Thus the first conclusion is that the enzyme does not change the order of binding; the most stable mismatches in solution are also the most stable in the enzyme binding cleft. However, the enzyme amplifies the differences. The differences in free energies for binding right vs. wrong are much larger on the enzyme than free in solution. We conclude that the enzyme has two main effects; it provides steric constraints favoring the right nucleotide and it excludes water which competes with base-base interactions. A test of this hypothesis is to measure the temperature dependence of the kinetics to provide an enthalpy for the Michaelis constant, K_M. We think that enthalpies with and without enzyme will be closer than the free energies with and without enzyme, because the enzyme is mainly effecting the entropies of the binding.

The thermodynamic measurements are much easier to do than the kinetic measurements. If further work shows that one can predict the kinetics of misincorporation of nucleotides into different sequences using simple melting experiments, the study of DNA replication fidelity will become much easier. Our studies have been done without allowing repair or proof reading, but these mechanisms to improve fidelity can also be compared with the thermodynamics of binding.

Differential polarization microscopy reveals oriented macromolecules in single cells

We have developed a microscope that forms images that depend on small differences in extinction for different forms of polarized light. By modulating the polarization of light incident on the sample and digitally recording the difference in intensities of transmitted light, we obtain images which specifically reveal either ordered linear structures or chiral (right- or left-handed) structures. Structures with neither linear order nor chirality are essentially invisible. Thus, images based on linear dichroism, circular dichroism, and linear and circular differential scattering can be used to detect specific types of structures which may be difficult to detect by other methods.

We have studied the structure of the nucleolus (the site of extensive RNA synthesis) in live primary spermatocytes of Drosophila when they are transcriptionally active or inactive. The images were obtained on live, unstained cells at a wavelength of 430 nm, where the image depends primarily on differential scattering of the light. We studied nuclei at two readily identifiable stages in the development of the sperm of it Drosophila melanogaster. The early primary spermatocyte is characterized by high transcriptional activity and low translational activity; it contains a lampbrush chromosome. Structures not seen in the usual transmission microscope images are seen in the linear and differential images. There are specific chiral

arrangements and a linear structure which is identified as the lampbrush chromosome. The late growth phase of the primary spermatocyte is characterized by low transcriptional activity and high translational activity. The differential images reveal a bipartite nucleolus which has only been seen previously in electron microscope images; the two parts may be two chromosomes that make up the nucleolus.

We have studied over 1000 deoxygenated red blood cells from subjects with sickle cell anemia. Linear dichroism images of the hemoglobin at 415 nm were used to quantitate the polymerization and orientation of sickle hemoglobin. The images reveal different patterns of orientation of aligned hemoglobin in individual erythrocytes. The hemoglobin orients to form a domain in which the hemoglobin polymer is oriented in one direction; an adjacent domain will be oriented in a different direction. The domains are not necessarily related to the shape of the erythrocyte. The most extreme example are cells that appear as normal, biconcave disks in an absorption image, but have aligned polymer arranged circularly (concentrically) within the cell; we have named these cells homokentrocytes.

Images of 1086 cells were analyzed quantitatively to classify them into distinct categories: 0 domain cells, 1-3 domain cells, multiple domain cells, central constriction cells, and homokentrocytes. The total amount of oriented hemoglobin in all domains was also determined. These measurements provide a much more detailed method of classifying sickle cells than previous methods based solely on cell morphology. Cell morphology is completely misleading for homokentrocytes which appear normal, but contain large amounts of oriented hemoglobin polymer. The effect of speed of deoxygenation on the sickling phenomena was studied by differential imaging. Some suggested methods of treating sickle cell anemia depend on injecting drugs in the blood stream which slow the rate of sickling. It is important to know if the amount of polymerized hemoglobin is kinetically controlled, or thermodynamically controlled. Our results show that hemoglobin polymerization and erythrocyte sickling are thermodynamically controlled. The number of domains and the total amount of polymerized hemoglobin was largely independent of the speed of deoxygenation. There appear to be preformed nucleation sites within the erythrocytes from which the hemoglobin polymerizes as the oxygen pressure is reduced; the amount of polymer formed just depends on the oxygen pressure. The domain structure of the hemoglobin polymers and the identification of nucleation sites will allow a much more rigorous mechanism of polymerization in sickle cells to be developed. This may provide new ideas for better methods for clinical treatment of the disease.

Left-handed Z-RNA

The spectroscopy of Z-RNA has been studied using NMR, Raman spectroscopy and circular dichroism. NMR of aqueous solutions gives the most detailed structural information, but requires the most time and the most material. Raman spectroscopy is very characteristic and can be applied to solutions, precipitates or crystals. Circular dichroism (CD) requires an order of magnitude less material than the other two methods, and a spectrum can be obtained more quickly. It is useful for surveys of many different conditions and for studying transitions as a function of temperature or salt. Poly [r(G-C)•(G-C)] forms the left-handed Z-form in 6M $NaClO_4$ or 6M

NaBr; it is also left-handed in 4M $MgCl_2$, but there it has a different CD spectrum. Thus there are at least two left-handed forms of RNA; the one in Mg^{++} ion has a CD spectrum essentially identical to Z-DNA, the other has a spectrum which is different from the spectra of the right-handed forms, but is also different from Z-DNA. The Raman spectra of the two left-handed forms are similar, however the NMR could only be measured in the sodium salts because of lack of solubility of the RNA in the magnesium salt. The NMR of the RNA in the high concentrations of sodium salts showed that it had all the expected properties of a left-handed form: 2'-endo, anti cytidines, 3'-endo, syn guanosines, and two distinct phosphate environments. We were not able to find low-salt conditions which favored the left-handed Z-RNA, although many were tried. However, bromination of poly [r(G-C)•(G-C)] did allow the left-handed form to be stable in physiological salt concentrations. About 50% bromination at G_8 and C_6 was sufficient to allow complete conversion to the Z-form in 0.15M salt. The brominated Z-form was characterized spectroscopically and by reactivity with anti-Z-DNA antibodies. These antibodies specifically bind to the brominated Z-RNA under physiological salt concentrations. This brominated Z-RNA was injected into rabbits to induce anti-Z-RNA antibodies. The polyclonal antibodies were purified and tested against Z-RNA and Z-DNA; antibodies with two different specificities were obtained. One class of antibodies reacted with Z-RNA, but not Z-DNA. The other did cross react with Z-DNA, but with a factor of ten decrease in binding constant. Decreased levels of brominated nucleotides in the immunogen correlated with the increased cross-reactivity with Z-DNA. Appropriate controls were done to show that neither antibody reacted with right-handed A-RNA or B-DNA. We concluded that the anti-Z-RNA antibody could be used to search for Z-RNA structures in vivo. This was done in Dr. Zarling's laboratory, SRI International, with the help of Dr. Hardin. They found that the anti Z-RNA antibody reacted with material in the cytoplasm of a wide variety of fixed cells (including human cells). The reaction was inhibited by ribonucleases, but not by deoxyribonucleases or by proteases. The assumption is strong that a left-handed form of RNA exists in biological cells, or at least that a left-handed form can be induced in cells. Dr. Zarling's laboratory is pursuing this research.

Our main interest has been in what novel structures can occur in RNA. While searching for other RNA sequences which would form Z-RNA, we found that poly [r(A-C)•r(G-U)] can form a B-DNA-like structure. Our only evidence so far are CD spectra which show that the polynucleotide undergoes a transition from a conformation with a typical A-RNA spectrum to one with a B-DNA spectrum. The presumed B-RNA species occurs in either very low concentrations of tetrabutyl ammonium salts, or in high concentrations of NaBr. More work is needed to verify these results, but it will be exciting if DNA and RNA can both take up the entire range of conformations found for either molecule.

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2. C. Cheong, I. Tinoco, Jr. & A. Chollet, Thermodynamics of Base Pairing Involving 2,6-

Diaminopurine, Nucleic Acids Res., 16, 5115-5122 (1988).

3. E. Henderson, C. C. Hardin, S. K. Wolk, I. Tinoco, Jr. and E. H. Blackburn, Telomeric DNA Sequences Form Novel Intramolecular Structures Containing Guanine•Guanine Base-Pairs. Cell 51, 899-908 (1987).
4. C. C. Hardin, G. T. Walker and I. Tinoco, Jr., Binding of Ethidium Ion to Left-handed Z-RNA Induces a Cooperative Transition to Right-Handed RNA at the Intercalation Site, Biochemistry 27, 4178-4184 (1988).
5. C. C. Hardin, D. A. Zarling, J. D. Puglisi, M. O. Trulson, P. D. Davis and I. Tinoco, Jr., Stabilization of Z-RNA by Chemical Bromination and its Recognition by Anti-Z-DNA Antibodies, Biochemistry 26, 5191-5199 (1987).
6. C. C. Hardin, D. A. Zarling, S. K. Wolk, W. S. Ross and I. Tinoco, Jr., Characterization of Anti-Z-RNA Polyclonal Antibodies: Epitope Properties and Recognition of Z-DNA, Biochemistry 27, 4169-4177 (1988).
7. W. E. Mickols, J. D. Corbett, M. F. Maestre, I. Tinoco, Jr., J. Kropp and S. H. Embury, The Effect of Speed of Deoxygenation on the Percentage of Aligned Hemoglobin in Sickle Cells. Application of Differential Polarization Microscopy, J. Biol. Chem. 263, 4338-4346 (1988).
8. W. Mickols, M. F. Maestre and I. Tinoco, Jr., Differential Polarization Microscopy of Changes in *Structure in Spermatocyte Nuclei*, Nature 328, 452-454 (1987).
9. J. Petruska, M. F. Goodman, M. S. Boosalis, L. C. Sowers, C. Cheong and I. Tinoco, Jr., Comparison Between DNA Melting Thermodynamics and DNA Polymerase Fidelity, Proc. Nat'l. Acad. Sci. USA , in press.
10. J. D. Puglisi, J. R. Wyatt and I. Tinoco, Jr., A Pseudoknotted RNA Oligonucleotide, Nature 321, 283-286 (1988).
11. J. D. Puglisi and I. Tinoco, Jr., Absorbance Melting Curves of RNA, in RNA Processing, J. E. Dahlberg & J. N. Abelson, Eds., Methods in Enzymology, Academic Press, New York.
12. W. S. Ross, C. C. Hardin, I. Tinoco, Jr., S. N. Rao, D. A. Pearlman & P. A. Kollman, Effects of Nucleotide Bromination on the Stabilities of Z-RNA and Z-DNA: A Molecular Mechanics/ Thermodynamic Perturbation Study, Biopolymers, submitted.
13. I. Tinoco, Jr., P. Davis, C. C. Hardin, J. D. Puglisi, G. T. Walker and J. Wyatt, RNA Structure from A to Z, Cold Spring Harbor Symp. Quant. Biol. 52, 135-146 (1987).
14. I. Tinoco, Jr., S. Wolk, F. Arnold and F. Aboul-ela, Structure and Function in Nucleic Acids: Mutagenesis, Structure and Dynamics of Biopolymers, C. Nicolini, Ed., Martinus Nijhoff, pp. 92-111 (1987).
15. M. O. Trulson, J. D. Puglisi, P. Cruz, I. Tinoco, Jr. and R. A. Mathies, Raman Spectroscopic Study of Left-Handed Z-RNA, Biochemistry 26, 8624-8630 (1987)
16. C. Tuerk, P. Gauss, C. Thermes, D. R. Groebe, N. Guild, G. Stormo, M. Gayle, Y. d'Auberton-Carafa, O. C. Uhlenbeck, I.Tinoco, Jr., E. N. Brody and L. Gold, CUUCGG Hairpins: Extraordinarily Stable RNA Secondary Structure Associated with Various Biochemical Processes, Proc. Nat'l. Acad. Sci. USA 85, 1364-1368 (1988).

17. S.Wolk, C. C. Hardin, M. W. Germann, J. H. van de Sande & I. Tinoco, Jr., Comparison of the B- and Z-form Hairpin Loop Structures Formed by $d(CG)_5T_4(CG)_5$, Biochemistry, in press.

18. J. R. Wyatt, J. D. Puglisi and I. Tinoco, Jr., Pseudoknotted RNA Oligonucleotides, in it Molecular Biology of RNA, T. Cech, Ed., UCLA Symposia on Molecular and Cellular Biology, Vol. 94, Alan R. Liss, Inc. New York, 1988.

III. APPENDICES

A. ACTIVITIES OF THE STAFF

George C. Pimentel, Director

University Service

Member, Intellectual Property Advisory Council
Member, Chemistry Unit III Planning Committee
Member, Chemistry 145 Committee

LBL Service

Director. Laboratory of Chemical Biodynamics (Organized Research Unit, UCB)
Associate Director, Lawrence Berkeley Laboratory
Head, Chemical Biodynamics Division, LBL

Honors and Awards

Challenges in Chemistry Award, 1986
Robert A. Welch Award in Chemistry, 1986
Honorary Ph.D., University of Arizona, 1986
Maurice. F. Hasler,Award, 1986
Honorary Ph.D., Colorado School of Mines, 1987

Other Service

Chairman, Survey of Opportunities in the Chemical Sciences, NAS, 1981-1986
Member, Chemical Engineering News Advisory Board
Member, Board on Chemical Sciences & Technology, NAS, 1982-
Member, Council for Chemical Research (CCR) Governing Board, 1983-
Member, American Philosophical Society
President, American Chemical Society, 1986
Immediate Past President, American Chemical Society, 1987

James C. Bartholomew, Deputy Director

University Service

Member, Graduate Group in Comparative Biochemistry, UCB
Member, Numerous Theses Committees

LBL Service

Member, LBL Committee Management Professional Labor Relations Operation Group
Member, LBL Real Time Systems Advisory Committee
Member, LBL Toxic Substance Safety Committee
Member, LBL Space Committee
Member, LBL Quality Assurance Committee–Divisional Coordinator
Chairman, Chemical Biodynamics Safety Committee
Chairman, Chemical Biodynamics Computer Use Committee
Chairman, Chemical Biodynamics Professional Staff Committee
Deputy Division Head, Chemical Biodynamics Division

Other Service

President Elect, President, Past President, "Genetic Environmental Toxicity Association"

USERDA Balance Program Planning Committee

NIH Special Study Section

Reviewer: J. Cell Biology, Analytical Biochemistry, Proc. Natl Acad Science, Cancer Research, Cytometry, Experimental Cell Research

Melvin Calvin

University Service

Member, Graduate Groups in Comparative Biochemistry, Biophysics, Agricultural Chemistry, and Plant Physiology

Honors and Awards

Melvin Calvin Lecturer, Michigan Technological University

Other Service

Member of the Scientific Advisory Board of the following corporations: DNA Plant Technology Corp., Molecular Design Ltd., Nova Pharmaceutical Corp., NPI, Michigan Biotechnology Institute, Westbridge Research Group

Galileo Galilei Foundation, Rome, Italy, International Scientific Committee

Member of the following Editorial Boards: Bio-Organic Chemistry, BioSystems, Cancer Biochemistry & Biophysics, Interdisciplinary Science Reviews, International Journal of Energy Research, Journal of Applied Biochemistry

Heinz Frei

Other Service

Reviewer, Journal of Physical Chemistry, Chemical Physics, Chemical Physics Letters, ACS Petroleum Research Fund, DOE Office Basic Energy Sciences, Research Corporation

Assisted with organization of U.S.-Japan Cooperative Photoconversion/ Photosynthesis Research Seminar, Honolulu, HI, 1987

John E. Hearst, Acting Director

University Service

Chairman, Biological Chemistry Senior Faculty Search Committee, Chemistry Department
Member, Planning Committee, Chemistry Department
Member, Awards Committee, Chemistry Department
Director, College of Chemistry Biohazard Facility, 1981-
Chairman, Ad Hoc Review Committee for Exceptional Merit Increase, Department of Chemistry

LBL Service

Acting Associate Director, Lawrence Berkeley Laboratory, 1986-87

Other Service

Consultant, HRI Associates, Inc., Emeryville, CA
Editorial Board: Analytical Biochemistry, Archives of Biochemistry and Biophysics
Reviewer of grant applications for National Science Foundation and NIH
Reviewer: American Association for the Advancement of Science, American Cancer Society, American Chemical Society, Biochemistry, Biopolymers, Cell, Journal of Molecular Biology, Nucleic Acids Research, Proceedings of the National Academy of Science
Site Visits for DOE and Office of Naval Research, National Academy of Sciences
Editorial Review Board – Molecular Toxicology, 1986-

Special Appointments

Chairman, ACS Search Committee for Editor of New ACS Journal in Chemical Toxicology, 1986-87
Chair, Department of Biochemistry Review Committee, 1986-

Sung-Hou Kim

University Service

Member, Graduate Group in Comparative Biochemistry, Graduate Group in Biophysics
Member, Biological Sciences Review Committee (Biochemistry & Molecular Biology)
Member, Faculty Recruiting Committee, Chemistry Department
Member, Preliminary Examination Committee for Chemistry, Biochemistry, Molecular Biology, Biophysics

LBL Service

Member, Award Committee, 1986 -

Honors and Awards

Ernest O. Lawrence Award - 1987

Other Services

Editorial Board Member, Nucleic Acid Research, 1983 - 1986
Reviewer: Biochemistry, Biophysical Journal, Journal of Molecular Biology, Nature, Science,

Member, Scientific Planning Committee, National Foundation for Cancer Research 1984 -
Member, Board of Directors, Korean Community Center of the East Bay, 1983

Melvin P. Klein

University Service

Executive Committee, Graduate Group in Biophysics
Qualifying Examination and Thesis Committees in Departments of Biophysics, Chemistry and Physics

LBL Service

Divisional Computer Committee
Divisional Staff Committee
Computer Services Advisory Committee
Laboratory Staff Committee
Biological Sciences Task Force

Other Service

Chairman and member of several National Institutes of Health Special Study Sections.
Member, Advisory Committee: Biotechnology Resource of the Stanford Synchrotron Radiation Laboratory, Stanford University
Member, Advisory Committee: NMR Factility at University of California, Davis.
Member, Advisory Committee: Mid-Atlantic In Vivo NMR Facility, University of Pennsylvania, Phildelphia.
Chairman, Advisory committee: Biological NMR Facility, University of Pennsylvania, Phildelphia.
Reviewer: Biochemistry, Biophysical Journal, Journal of Biological Chemistry, Journal of Chemical Physics, BBRC, BBA, J. Mag. Resonance, Rev. Sci. Instr., and Science
Review research grant applications and proposals for the National Institutes of Health, National Science Foundation, Department of Energy, Research Corporation and Petroleum Research Fund

John Otvos (Retired 12/31/87)

LBL Service

Divisional Staff Committee, 1984-1987

Other Service

Reviewer: J. Amer. Chem. Soc., J. Phys. Chem.
Referee: Division of Chemical Sciences, Office of Basic Energy Sciences, U.S. Department of Energy
Member, Review Panel for SERI, Golden Colorado on proposals for "Cell-free H_2 Technologies", Nov. 1984
Consulting and technical assistance to Shell Oil Co. in analytical chemistry, surface science and catalysis

Henry Rapoport

University Service

Faculty Representative for Chemical Storeroom, Glass Shop, and Analytical Laoratory, College of Chemistry, UCB

Ad Hoc Promotion Committees: Energy & Resources, UCB

LBL Service

Director, National Tritium Laboratory Facility

Other Service

Editorial Board: Journal of Natural Products

Kenneth Sauer

University Service

Chemistry Department

Physical Chemistry Curriculum Revision Committee, Student Advising and Awards Committee

Library Committee

Undergraduate Advisor

Member, Graduate Group in Biophysics, Graduate Group in Comparative Biochemistry, Graduate Group in Molecular and Plant Biology

LBL Service

Task Force on the Biological Sciences

Honors and Awards

Alexander von Humboldt Foundation - Senior U.S. Scientist Award

Other Service

Reviewer for 11 journals and monograph publishers

Grant Reviewer, NSF, Petroleum Research Fund, U.S. Dept. of Energy, National Research Council of Canada, Research Corp., U.S. Dept. of Agriculture, US-Israel Binational Science Foundation, National Science Foundation

Panel Member for Competitive Research Grant Program, U.S. Department of Agriculture

Evaluations for Presidential Young Investigators Award, Searle Scholars Program, Dreyfus Award

Ignacio Tinoco, Jr.

University Service

Chemistry Department, Faculty Recruiting and Affirmative Action Committee

Search Committee for Dean of Graduate Division

Honors and Awards

Member, National Academy of Sciences, 1985

Other Service

Member, Editorial Board for Biochim. Biophys. Acta, Biopolymers, Biophysical Chemistry, Cell Biophysics

Reviewer, Arch. Biochem. Biophys., Biochemistry, Chemical Physics, Eur. J. Biochem., Macromolecules, Nature, Nucleic Acids Research, J. Biol. Chem., J. Am. Chem. Soc., J. Chem. Phys., J. Org. Chem., J. Theor. Biol., Proc. Natl. Acad. Sci. USA, Science

Review for NSF, Research Corporation, NIH, DOE and ONR Principle Investigator, San Francisco Laser Center

Special Appointments:

Chairman, Human Genome Subcommittee, Health and Environmental Research Advisory Committee

David Wemmer

University Serivce:

Graduate Affairs Committee

Graduate Student Recruiting

LBL Service

Divisional Staff Committee, LCB

Other Service

External reviewer for Lawrence Livermore Laboratory, Institutional Research Development Grants

Ad Hoc member of NIH BBCA Study Section, 6/87

Reviewer for journals: Biochemistry, Biopolymers, Journal of the American Chemical Society, Journal of Magnetic Resonance

Steering Committee Member, Colorado State Univiversity NMR Center

LABORATORY OF CHEMICAL BIODYNAMICS THESES

July 1, 1987 - June 30, 1988

1. Fareed Aboul-ela. Sequence-Dependent Structure and Thermodynamics of DNA Oligonucleotides and Polynucleotides: UV Melting and NMR Studies. January 1988. Ignacio Tinoco, Jr. Chemistry.
2. Samuel Adam Abrash. The Energy Dependence of Reactions of Hydrogen Halides with Carbon Monoxide and Acetylene in Cryogenic Matrices. July 1987. George Pimentel. Chemistry.
3. Ralph David Britt. Electron Spin Echo Spectroscopy of Photosynthesis. February 1987. Carson Jeffries. Physics.
4. James L. Cole. Photosynthetic Oxygen Evolution Studied by Electron Paramagnetic Resonance and X-Ray Absorption Spectroscopy. October 1987. Kenneth Sauer. Chemistry.
5. James Corbett. Differential Polarization Imaging Microscopy of Sickle Cells: Effects of Physical Parameters on the Nucleation and Distribution of Hemoglobin S Polymer Domains. March 1988. Ignacio Tinoco, Jr. Chemistry.
6. Ronald David Guiles. Structure and Function of the Manganese Complex Involved in Photostynthetic Oxygen Evolution Determined by X-ray Absorption Spectroscopy and Electron Paramagnetic Resonance Spectroscopy. April 1988. Kenneth Sauer. Chemistry.
7. Douglas Hawkins. Triterpenoid Biosynthesis in Euphorbia Lathyris Latex. December 1987. Melvin Calvin. Chemistry.
8. Chul Hee Kang. Structural and Biochemical Studies of Intensely Sweet Molecules. April 1988. Sung-Hou Kim. Biophysics.
9. Hiromi Komiya. The Structural Basis of Inactivity of a Mutant *E. coli* Aspartate Transcarbamylase. April 1987. Sung-Hou Kim. Biochemistry.
10. Ann E. McDermott. Structural Studies of Iron and Manganese in Photosynthetic Reaction Centers. November 1987. Kenneth Sauer. Chemistry.
11. Roberta Nancy Mulford. A Study of Three Photochemical Reactions in Rare Gas Matrices. April 1988. George Pimentel. Chemistry.
12. Craig Ogata. X-ray Crystal Structure Determination of Monellin, An intensely Sweet Protein. May 1987. Sung-Hou Kim. Chemistry.
13. Emil Scoffone. I. NMR Studies of an Oligonucleotide Containing a Bulge. II. Applications of Multiple Quantum NMR to the Study of Oligonucleotides. Ignacio Tinoco, Jr. Chemistry.
14. Yun-Bo Shi. Photochemistry of Psoralen-DNA Adducts. II. Biological Effects of Psoralen-DNA. III. Applications of Psoralen-DNA Photochemistry. March 1988. John Hearst. Chemistry.
15. Cynthia Skrukrud. Terpenoid Biosynthesis in *Euphorbia Lathyris*. August 1987. Melvin Calvin. Chemistry.
16. Sarah Tabbutt. Spectroscopic Studies of Energy Transfer in Photosynthetic Reaction Centers of Higher Plants. September 1987. Kenneth Sauer. Chemistry.

17. Sun Un. Biophysical Applications of Solid State and Tritium NMR. October 1987. Alex Pines. Chemistry.
18. Muh-Ching Yee. Light Regulation of the Cell Cycle and Gene Expression in *Euglena gracillis bacillaris*. May 1988. Kenneth Sauer. Chemistry.

C. Laboratory Space

As in the past, an ongoing major problem of the Division is the saturation of the existing laboratory space and absence of space-capacity to accommodate growth. The new program of Professor Wemmer is sharing space with other on-going research programs and therefore has compressed our activities ever further. Some additional laboratory space could be gained by renovating existing laboratories. We are presently seeking funds to carry out this work.

We are in a continuous process of reevalutating present space used to optimize research productivity. The Division's research program–its productivity and its success–warrant access to more space. On the basis of this argument, our needs have been incorporated into the planning and justification for a new building for the College of Chemistry. This planning presently includes perhaps 7000 square feet of assignable research space. If it proceeds to completion, immediate advances in research productivity can be anticipated.

D. SEMINARS

The Laboratory of Chemical Biodynamics has several seminar series throughout the year.

One seminar, which all of the various students, faculty, and postdoctoral visitors attend, is held weekly. The speaker at this weekly seminar presents a summary of his/her ongoing research to the entire laboratory. The selection of speakers and dates is made by the senior staff of the laboratory. Occasionally an outside speaker (usually a former member of the Laboratory) talks at this weekly seminar.

There are in addition weekly or biweekly group seminars in the following areas:

DNA Replication and Gene Expression

Transposon Mutagenesis of Photosynthetic Genes

Solar Energy (photovoltaic solar energy conversion, biomass problems, hydrocarbon-producing plants, etc.)

Biophysics and Biophysical Chemistry

Plant Biochemistry

X-ray Crystallography

Spectroscopy and Photochemistry

The laboratory also provides the facility for an ongoing seminar entitled "Molecular Aspects of Plant Biology" sponsored by the Group in Photosynthesis. The speakers and their topics are listed on the following page. About one-third of the speakers in this seminar, which was held in the spring semester of the year, are members of the Laboratory of Chemical Biodynamics.

E. STATISTICAL DATA

1. Postdoctoral Visitors, Laboratory of Chemical Biodynamics October 1, 1987 through September 30, 1988

Name	Source of Funding	Associate
* Ansari, Mohdaslam	UC	Henry Rapoport
Aoyagi, Kazuko	UC	Francesca Leach
* Braathen, Gier	Norwegian Research Council	George Pimentel
Brewer, Karen	LBL	Melvin Calvin
Britt, David	LBL	Mel Klein
* Chou, Pi-Tai	LBL	Heinz Frei
Chi, Dae Yoon	UC	John Hearst
Craig, Carl	LBL	Melvin Calvin
DeVos, Abraham	UC	Sung-Hou Kim
Gingrich, Jeffrey	LBL	Kenneth Sauer
* Grassian, Vicki	UC	George Pimentel
Holbrook, Libby	UC	Sung-Hou Kim
Hong, Yong-Ki	Korean Science Engineering Foundation	Sung-Hou Kim
Kallick, Deborah	UC	David Wemmer
Kang, Chul Hee	UC	Sung-Hou Kim
Keuper, Hermann	LBL	Kenneth Sauer
* Li, Zong-Qi	UC	Sung-Hou Kim
McGourty, Jacqueline	LBL	David Wemmer
Matias, Pedro	UC	Sung-Hou Kim
Nagulappali, Vasant	LBL	David Wemmer
* Orton, Edward	LBL	George Pimentel
Pandit, Jay	UC	Sung-Hou Kim
* Salama, Farid	LBL	Heinz Frei
Sandusky, Peter	UC	John Hearst
Scalletar, Bethe	UC	Melvin Klein
Thurmes, William	UC	Ignacio Tinoco, Jr.
Un, Sun	LBL	Melvin Klein
Varani, Gabrielle	UC	Ignacio Tinoco, Jr.
Yan, Xinwei	LBL	Kenneth Sauer
* Young, Mark	LBL	George Pimentel
* Zimmerman, Jean-Luc	CEN Saclay, DB/SGPh, France	Melvin Klein

* Terminated

STATISTICAL DATA - Cont.

2. Countries of Origin, Postdoctoral Visitors and Visiting Faculty* 1948–1988

Country	Total thru 1985	1986–1987	1987–1988
United States	261	5	8
Great Britain	65		
Germany	48	2	
Japan	29		2
France	19	2	
Switzerland	18		
Canada	15		
Israel	15		1
Argentina	11		
Australia	12		
Netherlands	10		
India	10	2	2
Italy	9		1
Greece	6		1
Sweden	6		
Belgium	5		
Norway	4	1	
Denmark	4		
Taiwan	4		
Poland	3		
Korea	3		2
Finland	2		
USSR	2		
Bulgaria	2		
Rwanda	2		
Spain	1		
Brazil	1		
Ireland	1		
China	1		2
Totals	563	12	18

* In residence one or more months during the specified year

STATISTICAL DATA - Cont.

3. Graduate Students, Chemical Biodynamics Division October 1, 1987 to September 30, 1988

Name	Department	Research Director
Gregory Armstrong	Chemistry	John Hearst
Enoch Baldwin	Chemistry	John Hearst
Reza Beigi	Physics	Sung-Hou Kim
John Bishop	Chemistry	Henry Rapoport
* David Britt	Physics	Melvin Klein
Donald Burke-Aguerro	Chemistry	John Hearst
Ann Caviani	Chemistry	David Wemmer
Michael Chastain	Chemistry	Ignacio Tinoco, Jr.
Chae Joon Cheong	Chemistry	Ignacio Tinoco, Jr.
Hyunho Chung	Chemistry	Sung-Hou Kim
Edgar Civitello	Chemistry	Henry Rapoport
Andrea Cochran	Chemistry	Peter Schultz
* James Cole	Chemistry	Kenneth Sauer
James Corbett	Chemistry	Ignacio Tinoco, Jr.
David Cook	Chemistry	John Hearst
Victoria DeRose	Chemistry	Kenneth Sauer
Susan Dexheimer	Physics	Melvin Klein
Marc Donsky	Chemistry	Henry Rapoport
Paul Gordon	Biophysics	Sung-Hou Kim
* Ronald Guiles	Chemistry	Melvin Klein
* Douglas Hawkins	Chemistry	Melvin Calvin
Carolyn Hoener	Chemistry	George Pimentel
Michael Howard	Chemistry	Henry Rapoport
John Hubbard	Chemistry	John Hearst
Nathan Hunt	Physics	Melvin Klein
Fan Jiang	Biophysics	Sung-Hou Kim
David Jones	Chemistry	Henry Rapoport
* Chul Hee Kang	Biophysics	Sung-Hou Kim
Jason Kahn	Chemistry	John Hearst
Gary Karp	Chemistry	Henry Rapoport
Matthew Latimer	Chemistry	Kenneth Sauer
Sandra Laursen	Chemistry	George Pimentel
William Lubell	Chemistry	Henry Rapoport
Michael Matsko	Genetics	Sung-Hou Kim
Catherina Maulbecker	Comparative Biochemistry	James Bartholomew
* Ann McDermott	Chemistry	Kenneth Sauer
Michael Milburn	Chemistry	Sung-Hou Kim

Patricia Maxson	Chemistry	Kenneth Sauer
Joseph Montforte	Chemistry	John Hearst
Meredith Morgan	Chemistry	George Pimentel
Ishita Mukerji	Chemistry	Kenneth Sauer
Niles Warren	Physics	Melvin Klein
David O'Brien	Chemistry	John Hearst
John O'Connell	Chemistry	Henry Rapoport
Joseph Pease	Chemistry	David Wemmer
Jeffrey Pelton	Chemistry	David Wemmer
Joseph Puglisi	Chemistry	Ignacio Tinoco, Jr.
Nick Pugliano	Chemisry	George Pimentel
Jongnam Rhee	Chemistry	Kenneth Sauer
Renee Roemmele	Chemistry	Henry Rapoport
William Scott	Chemistry	Sung-Hou Kim
Paul Selvin	Physics	Melvin Klein
* Karen Singmaster	Chemistry	George Pimentel
Elizabeth Snowden	Chemistry	David Wemmer
Peter Spielmann	Chemistry	John Hearst
Richard Storrs	Chemistry	David Wemmer
Liang Tong	Chemistry	Sung-Hou Kim
Thien V. Truong	Chemistry	Henry Rapoport
Andrew Van Sickle	Chemistry	Henry Rapoport
* Sun Un	Chemistry	Melvin Klein
Milton Werner	Chemistry	David Wemmer
Brent Wurfel	Chemistry	George Pimentel
* Muh-Ching Yee	Chemistry	James Bartholomew

* Terminated

STATISTICAL DATA - Cont.

4. Graduate Student Statistics : Laboratory of Chemical Biodynamics 1948–1988

Departments/(Groups) of the University represented (Ph.D. Theses):

	1986–1988	Total to September 30, 1988
Chemistry	15	150
Biophysics	1	18
Comparative Biochemistry		10
Physics	1	8
Plant Physiology		4
Psychology		3
Biochemistry	1	4
Molecular Biology		3
Agricultural Chemistry		2
Botany		2
Geology		1
Physiology		1
Mathematics		1

207 Ph.D. degrees awarded 1948–1988

11 M.S. degrees awarded 1948–1988

STATISTICAL DATA - Cont.

5. Laboratory of Chemical Biodynamics Staff October 1, 1987 – September 31, 1988

Faculty Associates (Chemistry Department)
George C. Pimentel, Director
Ed Bennett
Melvin Calvin
John E. Hearst
Sung-Hou Kim
Henry Rapoport
Kenneth Sauer
Peter Schultz
Ignacio Tinoco, Jr.
David Wemmer

Senior Scientific Staff
James C. Bartholomew
Melvin P. Klein
John W. Otvos

Divisional Fellows
Heinz Frei
Francesca Leach

Scientific and Technical staff
Marie Alberti
Duncan Beniston
Jane Colman
Al Dorsky
Phil Eggers
Stephen Holbrook
Jarmila Jancarik
Rosalind Kim
David Koh
Ethel Lefall
Marian Malone
Hiromi Morimoto
Ann Orme
Ed Orton
Lisa Schain
Gary Smith
Manouchar Saljoughian
Janet Splitter
Scott Taylor
Philip Williams
Vittal Yachandra
Hisao Yokota
Yu-Sheng Zhu*

Administrative Staff
Lois Soule, Administrator
Mary Bundy
Gloria Goldberg
Beth Klingel
Rosalyn Miles
Evangeline Peterson
Marilyn Taylor

Undergraduates
Patty Casey
Johnson Chan
John Kim
Jun Kim
Prudence Lee
Hans Peterson
Ann Williamson

Graduate Students, Postdoctoral Visitors and Faculty listed separately.
* terminated.

6. Visiting Faculty October 1,1987 – September 30, 1988

Juana Acrivos
Department of Chemistry
San Jose State University
San Jose, California
(Melvin Klein)

George A. Brooks
Department of Physical Education
University of California, Berkeley
(James Bartholomew)

Alexander Glazer
Bacteriology and Immunology Dept.
University of California, Berkeley
(Kenneth Sauer)

Dolon Konwer
Faculty of Agriculture
Chemistry and Biochemistry Section
Assam Agricultural University
Jorhat, India
(Melvin Calvin)

Munitaka Nakata
Faculty of Science
Hiroshima University
Hiroshima, Japan
(Heinz Frei)

Ning Pon
Biochemistry Department
University of California, Riverside
(Henry Rapoport)

Rafael Rafaeloff
Dept. of Pure and Applied Radiation Chemistry
Soreq Nuclear Research Center
Ravine, Israel
(Melvin Calvin)

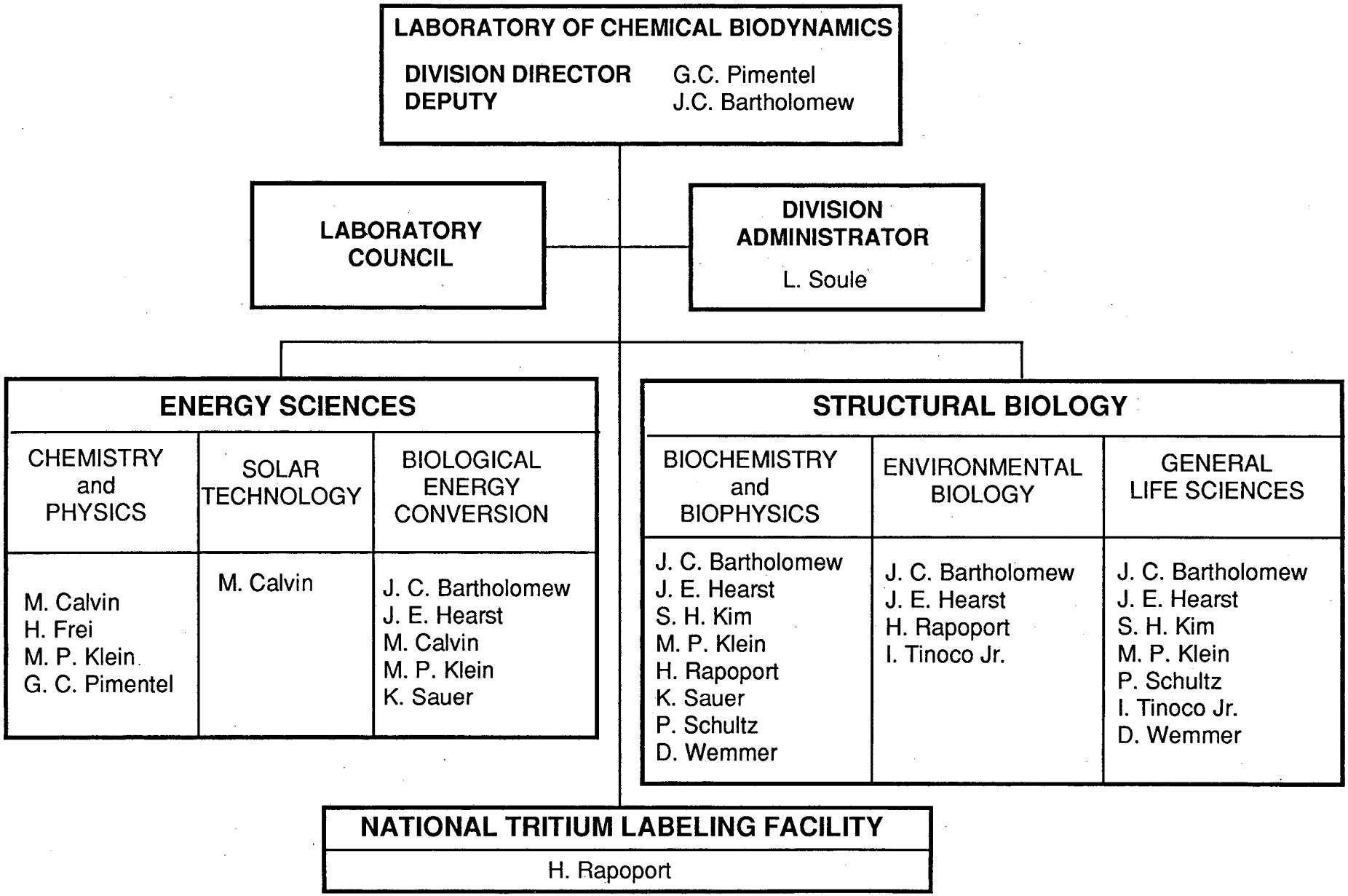
Michael Saxton
Plant Growth Laboratory
University of California, Davis
(Melvin Klein)

Lawrence Spreer
Department of Chemistry
University of Pacific, Stockton
(John Otvos)

Stavros Voliotis
Department of Chemistry
University of Patros
Patros, Greece
(Sung-Hou Kim)

UNIVERSITY OF CALIFORNIA

LAWRENCE BERKELEY LABORATORY



LAWRENCE BERKELEY LABORATORY
TECHNICAL INFORMATION DEPARTMENT
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720