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PRIMARY EVENTS AND THE TRAPPING OF ENERGY

Kenneth Sauer

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Bioenergetics of Photosynthesis

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Chapter 3

Primary Events and the Trapping of Energy

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1. Introduction

The conversion of light energy to chemical potential in photosynthesis is a form of energy transduction. It begins with the absorption of incident photons by chlorophyll or by various accessory pigments and reaches its goal with the biosynthesis of the carbohydrates, proteins, lipids, etc. that make up the complex biological organism. This chapter will treat the very earliest stages in this highly complex process--just through the point at which the first chemical intermediates appear. It will necessarily treat both the dynamics of the process and the chemical nature and arrangement of the molecules involved.

The first stage of photosynthetic energy conversion is commonly known as the "physical" part. This is by contrast with the later stages, where the influences of "chemistry" are dominant. The most penetrating experimental methods of investigating the primary processes of photosynthesis involve techniques of the physicist or physical chemist. These approaches often appear remote and mysterious to the student of biology, who may feel uncomfortable upon encountering the formalism and theoretical underpinnings that the physicist assumes as basic tools. As a result there often arises a language barrier or communication gap between the two groups. It will be a major objective of the present chapter to interpret the physical phenomena and concepts in a way that will provide insight for the biologist without being technical beyond comprehension.

The physicist approaches his task by making measurements, formulating models based on the observations, and then developing mathematical expressions to interpret the models in terms of fundamental physical laws. Here we will describe the same measurements and models, but we will use primarily diagrams and word pictures for their interpretation. The apparent

complexity of the mathematical equations of the physicist should not deceive the uninitiated into overestimating their sophistication, for it is generally the quality of the model that determines how closely the physicist is able to approximate the truth.

The primary events of photosynthesis will be divided into four sequential steps and will be examined more or less in this order:

- (1) The absorption of light and the formation of electronic excited states of the pigment molecules;
- (2) the transfer of electronic excitation among the molecules in the pigment array (see also Chapter 4);
- (3) trapping of the excitation at a particular location known as the reaction center;
- (4) the initiation of chemistry via the transfer of electrons from donors to acceptors.

In addition to examining the dynamics of these steps, we will also treat the energetics of the processes and the nature of the molecular complexes in which they take place.

In order to develop a general picture in which the fundamental processes are not clouded by a host of often controversial details, it will be necessary to be selective from the vast literature on the subject. For this reason emphasis will be given to current views and a statement of outstanding problems. For comprehensive literature surveys and the historical background the reader will be referred to the many monographs and review articles on the subject. Among the most valuable of the general references which deal extensively with the primary processes are the monographs by Clayton (1965; 1970; 1971) and by Rabinowitch and Govindjee

(1969). Clayton (1972) has also written an excellent summary of the current status and outstanding problems in the realm of physical processes of photosynthesis. For a comprehensive treatment of work prior to 1956, the volumes of Rabinowitch (1945, 1951, 1956) are invaluable.

2. Chlorophyll Spectroscopy

2.1 Chlorophylls--The Essential Pigments of Photosynthesis

The central light-absorbing pigments of photosynthesis are the chlorophylls: chlorophyll a (Chl a) in plants and algae, bacteriochlorophylls a (BChl a) or b in photosynthetic bacteria. The molecular structures of Chl a and BChl a are shown in Fig. 1. No organism is known to carry out photosynthesis without one or another of these essential pigments. In addition, there may be one or more accessory pigments that absorb light and make the resulting excitation available to the photosynthetic light reactions. Such accessory pigments include chlorophylls b, c and d, chlorobium chlorophyll, carotenes, xanthophylls and phycobilins. Despite the wide occurrence of these accessory pigments and the doubtless beneficial effect of their presence in the organism for optimal performance in the natural environment, each of them is expendable as far as the essential photosynthetic energy conversion is concerned.

2.2 The Absorption of Light

Photosynthetic energy conversion is initiated by the absorption of light, either by a chlorophyll or by one of the accessory pigments. In order to understand the nature of this process we will need to examine some important aspects of photon absorption by molecules. A very readable and comprehensive account of the subject is given in the small monograph by Clayton, Light and Living Matter, vol. 1: The Physical Part (1970).

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Fig. 1. Molecular structures of chlorophyll a and bacteriochlorophyll a. The porphyrin π -electrons are indicated by the shading. Absolute configurations are shown for the asymmetrically substituted porphyrin carbon atoms and for phytol. The esterifying group (phytol or trans, trans-farnesol) of BChl a varies with the bacterial source. Chl b differs from Chl a in the replacement of $-\text{CH}_3$ on ring II by $-\text{C}(=\text{O})\text{H}$. The convention for x and y directions within the plane of the porphyrin ring is indicated by the arrows.

The following is a simplified survey of absorption, circular dichroism and emission spectroscopy, emphasizing those elements of particular importance in photosynthesis. The properties of the chlorophylls will be used to illustrate the general principles. In this regard we will consider spectroscopic properties that yield information about molecular interactions and associations and that can give important clues concerning the local environment. We will be especially interested in those spectroscopic states that participate in the light reactions of photosynthesis.

The absorption or capture of an incident photon by a molecule leads to a rearrangement of the distribution of electrons within its nuclear framework. The new state of the molecule is known as an excited electronic state and its energy is greater than that of the initial or ground state. The extra energy comes from the absorbed photon and is governed by the Planck relationship $\Delta\epsilon = \epsilon_1 - \epsilon_0 = h\nu = \frac{hc}{\lambda}$, where ν is the frequency of the absorbed radiation, λ is its wavelength, h is Planck's constant of proportionality, c is the velocity of light, and ϵ_1 and ϵ_0 are the energies of the molecule in the excited (final) and ground (initial) states, respectively. The absorption process is alternatively described by an equation

There is actually a set of such excited electronic states, each with its own characteristic energy and electron distribution. Incident radiation is absorbed only if it has the correct energy to achieve one of these "allowed" states from the ground state of the molecule; photons with intermediate energies are transmitted by the compound and leave it unaffected. The absorption process is illustrated by an energy-level diagram (Fig. 2A).

Fig. 2. Absorption and fluorescence of chlorophylls. The illustration is scaled for BChl a, but applies qualitatively to Chl a and Chl b as well. (A) Energy level diagram, showing spectral transitions (vertical arrows). The energy levels are broadened (shading) by vibrational sublevels that are not usually resolved in solution spectra. (B) Absorption spectrum corresponding to energy levels of part A. This spectrum is turned 90° from the usual orientation in order to show the relationship to the energy levels. Conventions for designating the spectral transitions (Q_y , Q_x , B_x , B_y , where x and y refer to the axes shown in Fig. 1) are shown. (C) Diagram showing radiationless relaxation (dashed arrows) and fluorescence (shaded arrow). (D) Fluorescence emission spectrum corresponding to part C. Note the red shift (shorter arrow) of the fluorescence compared with the corresponding Q_y absorption illustrated in parts A and B. This Stokes shift owes to vibrational relaxation prior to fluorescence emission.

An absorption spectrum, which can be determined experimentally, is a graph of the probability or intensity of radiation absorption versus energy (usually plotted versus wavelength, λ , of the radiation). For molecules like chlorophyll, the energy levels are rather broad because of the possibility of exciting molecular vibrations as well as the electrons themselves. This is seen in the absorption spectrum of BChl a shown in Fig. 2B. (In Fig. 2B the absorption spectrum is turned by 90 degrees from its conventional orientation in order to relate it to the energy level diagram.)

The electronic absorption spectra of the chlorophylls consist of a set of reasonably well defined bands extending through the ultraviolet and visible regions of the spectrum, terminating with a long wavelength (lowest energy) band that lies between 600 and 1000 nm in the red or near infrared. An important property of an absorption band is its strength or intensity. It can be characterized by an oscillator strength (on a scale that goes from 0 to 1 for simple, non-degenerate transitions), by an absorption cross-section (i.e., the target size for an incoming photon), by a transition dipole moment, or by various other related measures. The interrelationships among these quantities are described in books on electronic absorption spectroscopy (Hanna, 1969; Sandorfy, 1964; Orchin and Jaffé, 1971). The electronic absorption bands of the chlorophylls are comparatively intense, indicating that the corresponding transitions from the ground to the excited states are strongly allowed in the presence of a flux of photons. A 1 cm cube of a solution containing only 10^{-5} M of a chlorophyll is intensely colored. This intense absorption is, of course, of great value in providing for the efficient capture of photons for photosynthesis. The oscillator strengths are typically in the range

0.1 to 1.0, corresponding to absorption cross sections of 10 to 100 Å². Another way to appreciate the strength of the absorption is to compare these absorption cross sections with the cross sectional area of the porphyrin ring, roughly 150 Å². We can understand from this comparison that most of the photons of the appropriate energy that are aimed at the molecule are absorbed.

A second important property of the absorption band is its characteristic energy, Δε (alternatively, the wavelength, λ, or frequency, ν), of the absorption maximum. These quantities are related by the Planck formula. In the case of chlorophyll a, the lowest energy or longest wavelength transition occurs at about 660 nm in solution. The corresponding energy is 1.9 electron volts per molecule or 43 kcal per mole. Energies of this magnitude are equivalent to those required to dissociate weak covalent chemical bonds, such as that in the I₂ molecule. No bond dissociation occurs at these wavelengths for the chlorophylls, however. The excitation process results only in a change in the electron distribution within the nuclear framework. (The nuclear positions are only slightly different in the excited states from those of the ground state.) Typically, the redistribution of electron density is confined mostly to one or a few of the most labile electrons. In chlorophylls these are π-electrons from the conjugated porphyrin ring system (Fig. 1, shading). As we shall see later, these electrons are much more weakly bound in the excited molecule, and in the presence of a molecule, A, with a strong affinity for electrons (a strong electron acceptor), an electron from the excited molecule may be transferred entirely, resulting in an ion-pair

This does not usually occur in simple solutions, however.

The qualitative picture of electronic absorption described above is greatly simplified. The process can be treated (approximated, at best) using the formalism of quantum mechanics. In the quantum mechanical picture, the properties of the electron distribution in the ground state are described by a wavefunction, ψ_0 . The excited state is described by a different wavefunction, ψ_1 . Each wavefunction, which varies in amplitude with the position (x,y,z) within the molecule, has the property that its square, ψ^2 , gives the electron density or probability of finding the electron at each point in the molecule for the respective state.

The absorption transition itself is represented by an operation that describes how the molecule couples to the oscillating electromagnetic field associated with the incident light. The relevant quantity is the transition dipole moment, μ_{01} ,

$$\mu_{01} = \iiint \psi_0 \mu \psi_1 dx dy dz \quad [2-3]$$

where μ is the dipole moment operator and the integration is carried out over the entire space where the wavefunctions are significantly greater than zero. μ_{01}^2 is the transition dipole strength, which is a direct measure of the intensity of the transition and can be related to the area under an absorption band. This is one of the many ways in which the predictions of quantum mechanical theory can be tested using experimentally observed properties. Even from this cursory view of a process which is much more complex when examined in detail, one can appreciate two important aspects of the absorption process:

(1) The strength of the absorption depends in a very direct and sensitive way upon the wavefunctions of both the initial and final states, hence on the distribution of their constitutive electrons.

(2) The transition has a vectorial character, because the dipole moment is a vectorial quantity and imparts a directionality (with respect to the molecular axis system) to the transition. A collection of molecules which are ordered, as in a crystal or two-dimensional monolayer, will preferentially absorb incident light that is plane polarized in the direction of the transition moments. This is the phenomenon known as linear dichroism. Solutions of molecules with random orientations do not exhibit linear dichroism.

The properties of two of the absorption transitions of BChl a are shown pictorially in Fig. 3. At each of the porphyrin atoms where there is appreciable π -electron density, a circle is drawn to show the magnitude of the change in charge density accompanying the transition. This may be either an increase or a decrease in charge density, as indicated by the two kinds of shading. (There is no change in the zero net charge of this neutral molecule, only in the charge distribution.) Following the transition, certain regions of the molecule have changed their charge densities more than others. Careful analysis of the redistribution of charge permits one to establish the direction and intensity of the absorption transition. It is important to appreciate that the pictures shown in Fig. 3 are the result of theoretical calculations using molecular orbital theory. There is no good experimental way as yet to measure the details of the redistributions of charge associated with such transitions.

2.3 Circular Dichroism

Molecules which are asymmetric and, therefore, not superimposable with their mirror images possess a property known as chirality or handedness. This is the property that distinguishes a screw cut with

Fig. 3. Transition monopole representation of the Q_y and Q_x absorptions of BChl a. The different shadings represent an increase or decrease, respectively, of the electron density at the various atoms. (The transition dipole is a response to the oscillating electromagnetic field of the incident light. Consequently, there is no absolute sign associated with the transition moment.) Note that the Q_y transition is nearly symmetric across the y-axis, and that the principal charge displacement occurs in the y direction; just the opposite is true for the Q_x transition. The magnitudes of the changes in electronic charge are represented by the areas of the circles. Furthermore, because each contribution to a dipole moment is the product of charge times distance, charge changes farther from the center of the molecule make a correspondingly greater contribution to the transition moment than do those closer in. Taking into account the cancellation produced by adjacent increases and decreases, the reader should be able to discern that the magnitude or intensity of the Q_y transition is greater than that of the Q_x transition, in agreement with the experimental result shown in Fig. 2. The diagrams shown in Fig. 3 represent the results of theoretical calculations carried out by Dr. C. Weiss, Jr., as described by Philipson, Tsai and Sauer (1971).

a left-hand thread from one which is cut with a right-hand thread. The distinction is intrinsic to the screws and is not lost even if their orientations are scrambled. In a similar way the electron density distributions or wavefunctions of chiral molecules, such as the chlorophylls, have aspects of handedness associated with them. Chlorophyll a, for example, has five asymmetrically substituted carbon atoms--three associated with the porphyrin ring part and two in the phytyl ester. These are shown in the molecular structure depicted in Fig. 1. If you were to examine a contour map of the electron density throughout Chl a, then you would easily be able to distinguish it from the map of its mirror-image molecule. (The latter molecule does not actually occur in nature, of course.) By using circularly polarized light, whose electric vector sweeps out a helix as it passes through space, to measure the absorption spectrum of chiral molecules, one can detect small differences in the absorption of left (handed) circularly polarized light from the absorption of right circularly polarized light. This difference, $\Delta A = A_L - A_R$ is known as the circular dichroism or CD. It is a property that results from the fundamental asymmetries of the wavefunctions. Circular dichroism is typically small (10^{-5} to 10^{-2}) compared with the absorption itself and is correspondingly difficult to measure. Nevertheless, sensitive CD spectrometers exist and numerous reports of CD spectra are appearing in the literature.

The CD spectrum of BChl a in ether solution is shown together with the normal absorption spectrum in Fig. 4. In general there will be a CD feature associated with each of the major absorption components. Notice that the CD bands can be either + or -. This property of the CD can be

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Fig. 4. Absorption and CD spectra of BChl a in ether at 10^{-5} m⁻¹ concentration. In this solvent and at this concentration the BChl a is monomeric. Spectra taken from Philipson and Sauer (1972).

of practical advantage in enabling one to decompose or separate transitions that overlap strongly in the absorption spectrum.

By contrast with linear dichroism, even a solution of randomly oriented asymmetric molecules will exhibit circular dichroism in the absorption bands. This is because the chirality is intrinsic to the molecules and not a function of their orientation in space. For surveys of this subject the reader is referred to the monographs by Crabbe (1972) and by Velluz, Legrand and Grosjean (1965). The applications to photosynthesis have been reviewed recently (Sauer, 1972).

2.4 Fluorescence

The fluorescence of the chlorophylls is the subject of several chapters in this volume. It has special significance as an indicator of processes occurring very near the primary steps of energy conversion. It is important, therefore, to understand its origin and the manner in which alterations of the environment can change its characteristics.

Fluorescence is the process by which a molecule in an excited state undergoes a spontaneous decay and returns to the ground state with the simultaneous emission of radiation. It can be seen in the energy level diagram (Fig. 2C,D), and it can be represented by the equation

The fluorescence of Chl a is typical of large aromatic molecules in the following respects:

- (1) The fluorescence occurs only from the lowest excited state reached via absorption. Higher states, such as 2,3,4, etc. in Fig. 1A, undergo a very rapid relaxation to state 1 without the emission of radiation.

(2) Because of thermal relaxation that occurs in the excited states, the maximum in the fluorescence emission spectrum typically lies a few nanometers to longer wavelength (lower energy) from the absorption maximum. This is known as the Stokes shift.

(3) The measured fluorescence lifetime, τ , of Chl a in "good" solvents (see section 2-9) is very short--only about 5 nsec (5×10^{-9} sec) (Brody and Rabinowitch, 1957; Dimitrievsky, Ermolaev and Terenin, 1957).

(4) The proportion of excitation that results in fluorescence is about 30% for Chl a in solution (Latimer, Bannister and Rabinowitch, 1956; Weber and Teale, 1957). Fluorescence quantum yields less than 100% indicate the presence of competing processes for deactivation of the excited state.

(5) The origin of the fluorescence emission process is governed by the same kind of relationships among the wavefunctions as govern absorption. For this reason, fluorescence has essentially the same polarization as the long wavelength absorption band.

Each of these five properties can give useful information about the excited state and its various decay processes.

Fluorescence is not the only way in which the excited state can decay.

Some important competing processes are shown in the following equations:

There may be additional processes under special circumstances, e.g., very high excitation intensities, high concentrations, photochemical reactants present, electron donors or acceptors present, etc. All of the reactions listed above are kinetically first order in Chl^* concentration. The lifetime of Chl^* is determined by the rate constants for all of the reactions involved. A common way of treating this is in terms of a quantum yield. The quantum yield for fluorescence, ϕ_F , is given by the ratio of the fluorescence rate to the sum of the rates of all deactivation processes. When the processes are all first order, the concentration of Chl^* cancels out, leaving

$$\phi_F = \frac{k_F}{k_F + k_I + k_T + k_Q[Q] + \dots} \quad [2-8]$$

The constant k_F is considered to be an intrinsic property of the molecule, governed by the quantum mechanical properties of the ground and excited states. Its reciprocal $\tau_0 = \frac{1}{k_F}$ is known as the "natural" lifetime of the excited state. This is simply the lifetime that the excited state would have in the absence of any processes competing with fluorescence. The natural lifetime can be calculated from directly measured quantities, such as the oscillator strength of the transition. Because the denominator of Eq. 2-8 above is just the reciprocal of the actual (observed) lifetime, τ , we can rewrite the equation as

$$\phi_F = \frac{\tau}{\tau_0} \quad [2-9]$$

On the basis of the observed values of ϕ_F and τ given above for Chl a in solution, the reader may readily confirm that τ_0 for Chl a is about 15 nsec. This agrees closely with the value 15.2 nsec calculated from the absorption band intensity (Brody and Rabinowitch, 1957). The observed lifetime, τ , will always be less than this value in the presence of competing

deactivation processes, thereby providing a very useful measure of the contribution of these competing processes. As we shall see shortly, the fluorescence yield and observed lifetimes of chlorophylls in their in vivo situations vary over a wide range, depending on environmental circumstances.

2.5 Triplets and Phosphorescence

The excited states that are reached directly via absorption of radiation by ground state chlorophylls are known as singlet states. The term singlet refers to the fact that the electron spin quantum numbers of electrons in the excited state correlate completely with those of the ground state, where all of the electrons are paired. There exists an additional important set of states, known as triplets, where two of the electrons have become unpaired. These triplet states are normally not produced via direct absorption of radiation by the ground state molecules; for quantum mechanical reasons, the oscillator strengths for these transitions are usually less than 10^{-7} and the transitions are observed only under very special circumstances. The triplet states can be reached by intersystem crossing from the excited singlet states, so long as the process is energetically favorable (i.e., not uphill). This is the process represented by Eq. 2-6 above.

Triplets can be observed by their characteristic emission spectrum (phosphorescence), which has both a much longer lifetime (reflecting the very low oscillator strengths for the corresponding absorption process) and a different wavelength dependence than does fluorescence. The triplet absorption spectra (excitation to higher triplet states) can be measured using the techniques of flash spectroscopy suited to short-lived species. The triplets of Chl a and Chl b were first observed using flash absorption

spectroscopy by Livingston, Porter and Windsor (1954). The observed lifetimes depend on the solvent, etc., but values of about 10^{-3} sec are commonly observed (Livingston, 1960). Bowers and Porter (1967) made quantitative measurements of the quantum yield of triplet formation, ϕ_T . In ether solutions, the values were 0.64 for Chl a and 0.88 for Chl b. Since the quantum yields for fluorescence in the same solvent are 0.32 and 0.12, respectively, it is apparent that these two processes together account for over 95% of the excited singlet state decay for the chlorophylls in simple solutions.

Phosphorescence of chlorophylls is difficult to observe because of a very low quantum efficiency. Apparently the triplets return to the ground state predominantly by radiationless intersystem crossing or by quenching reactions. Emissions with decay times of the order of 10^{-3} sec have been reported for Chl a at 755 nm and for Chl b at 733 nm (Fernandez and Becker, 1959). [Longer wavelength emissions are also observed (Singh and Becker, 1960).] Because these wavelengths are greater than the corresponding fluorescence maxima, it suggests that the lowest triplet energy level lies somewhat below the first excited singlet state shown in Fig. 1. As a consequence, the long-lived triplet state has been a persistent dark-horse candidate for the process of trapping (extending the lifetime by decreasing the energy) of the excitation in photosynthesis (see section 4-2).

2.6 Dichroism and Fluorescence Polarization

The orientations of the electronic transition moments of different excited states of a molecule can be determined on a relative basis by fluorescence polarization studies. In these measurements the exciting

light wavelength is varied throughout the region of absorption and the polarization of the fluorescence, which always comes from the first excited state, is measured. It is important that the molecules not undergo rotation during the excited state lifetimes; hence, viscous solvents are used. The measurements can be carried out on an ordinary solution containing randomly oriented molecules so long as they do not undergo rapid rotation. The information achieved is limited, however; one can only determine the angle between the absorption and the emission oscillators, and not the absolute orientation of either with respect to the molecular framework.

In order to make absolute polarization measurements one needs to use an oriented sample, such as a crystal or monolayer. In this case either polarized absorption (linear dichroism) or emission can be used. Because of the very strong absorption properties of the chlorophylls, it has so far not been possible to measure spectra of single crystals. Too little light gets through to permit reliable measurements.

The most extensive studies of polarization of the chlorophyll spectra were carried out by Goedheer (1957, 1966). He studied not only fluorescence polarizations, but also the dichroism of chlorophylls incorporated into two-dimensional myelin figures. With the aid of some theoretical insight into the origins of the transitions, he was able to assign them to either the y or the x direction within the porphyrin plane. (See Fig. 1). These band assignments, which are indicated in Fig. 5, have been confirmed by later studies (Gouterman, 1961; Gouterman and Stryer, 1962).

With this brief introduction to spectroscopy, we will now proceed to examine some of the properties of different chlorophylls and of the significant influences of the environment on the various spectra.

Fig. 5. Absorption spectrum of methyl chlorophyllide a (lower curve) and polarization spectrum of fluorescence excitation (points, and upper curve). The highest (most positive) polarization values indicate absorption oscillators parallel to the emission oscillator (e.g., B_y at 415 nm and Q_y at 660 nm) and the lowest polarizations are for perpendicular absorption and emission oscillators (e.g., B_x at 430 nm and Q_x at 580 nm). Figure taken from Gouterman and Stryer (1962).

2.7 Comparison of the Spectra of Different Chlorophylls

Any influence which affects the electronic distribution of either the ground or the excited state of the molecule will, in general, be reflected in the absorption band intensity, wavelength position, circular dichroism strength, fluorescence efficiency, etc. In this and the following sections we will explore the consequences of some of the more important of these influences in order to uncover systematic effects. For a comprehensive survey of the spectra of the chlorophylls, the reader is referred to the following sources: Smith and Benitez (1955); Strain and Svec (1966); Goedheer (1966); and Gurinovich, Sevchenko and Solov'ev (1968).

Differences in chemical structure of the chlorophylls produce spectroscopic effects that can be treated systematically. For example, the molecules Chl a and BChl a differ in two respects: (1) Ring II of BChl a is saturated, with hydrogens on the peripheral carbon atoms, but ring II is unsaturated in Chl a; and (2) the acetyl substituent on ring I of BChl a is a vinyl group in Chl a (See Fig. 1). The absorption spectra of these molecules differ appreciably in the wavelengths and intensities of the bands (Fig. 6). From systematic studies of related molecules (e.g., Chl a with acetyl instead of vinyl on ring I), it can be demonstrated that the difference in the absorption spectra of Chl a and BChl a arises almost entirely from the saturation of ring II in the latter compound. Such spectral differences can be rationalized in terms of the electronic wavefunctions using molecular orbital theory, and we are now able to make reasonably reliable predictions of the spectral effects arising from molecular structure changes.

Fig. 6. Absorption spectra of BChl a and Chl a in ether solutions, compared in terms of equal molar concentrations. Millimolar absorptivities are plotted along the ordinate. Band assignments are based on polarization measurements and theoretical evaluations. The origin of the differences in intensity and wavelengths of the maxima is discussed in the text.

The most important influences are (1) the extent of saturation of the porphyrin ring system, which increases from protochlorophyll to Chl a or Chl b to BChl a; (2) the presence of magnesium or other atoms at the center of the porphyrin system; and (3) the occurrence of the cyclopentenone ring V in the molecule. Of lesser significance is the specific nature of the groups substituted peripherally around the ring; groups such as vinyl or acetyl which can conjugate with the porphyrin π -electron system have more influence than do alkyl substituents. Certain changes have very little, if any, detectable effect on the absorption spectrum. Examples are (1) the replacement of the phytyl esterifying group with methyl or ethyl and (2) epimerization (reversal) of the asymmetry at the saturated carbon of ring V. As might be expected, this last change does have a readily detectable effect on the CD spectrum. In fact, systematic studies have shown that each of the asymmetric centers associated with the porphyrin ring exerts a largely independent influence on the CD. The contributions of the individual asymmetric substitution sites are, therefore, additive (Philipson, Tsai and Sauer, 1971).

2.8 Solvation

For a chlorophyll with a particular chemical structure, the environment of the molecule has effect on the spectra which can be highly informative. It is usual in spectroscopy of small molecules to use as a reference point the absorption in the absence of environmental influences, i.e., the situation in the gas phase at low pressure. This kind of isolation has not yet been achieved for the chlorophylls, because of their negligible vapor pressures at moderate temperatures and their tendency to decompose at higher temperatures. When a substance is dissolved in a

"good" solvent, there is a favorable interaction between the solvent and the solute which generally results in a lowering of its energy in the ground state. This may result from strong dipole-dipole interactions in the case of polar solutes in a polar solvent, or from weaker London dispersion forces in the case of non-polar components. Other interactions, such as hydrogen bonding, can also contribute. For the same reasons the energy of the excited electronic state is lower for the molecule in solution relative to the same excited state in a vacuum. However, the electrical properties of the excited state are different from those of the ground state, as we have seen above. Strongly allowed transitions usually result in an excited state which has a dipole moment appreciably different (either greater or less) from that of the ground state. In addition, the excited state electrons are more polarizable (more loosely bound) than those of the ground state. Because both the dipole moment and the polarizability can contribute to the interaction with a solvent in solution, in general the excited state energy will be influenced to a different extent by solvation than will the ground state. As a result, the absorption bands will exhibit a red shift if the ground and excited state energies are brought closer together, and a blue shift if they separate.

Solvent effects on chlorophylls have been widely observed, the most extensive examination being that of Seely and Jensen (1965) for Chl a. For the red band the range of positions is from about 660 to 675 nm for a set of forty common solvents. While several influences clearly contribute, there is a readily discernable trend of increasing red shift with increasing dielectric constant of the solvent. This is the expected result if the excited state has a larger dipole moment than does the ground state.

Because of the electric dipole origin of these effects, which fall off rapidly with increasing separation, those solvent molecules which are in the immediate environment of the chlorophyll will make the overwhelming contribution to the absorption shifts. Certain molecules, such as the strongly polar or nucleophilic compounds water, pyridine, acetone, etc., have a very strong tendency to associate with chlorophylls, probably binding at the fifth and sixth coordination positions (normal to the porphyrin ring plane) of the magnesium. In "poor" solvents (saturated hydrocarbons, benzene, carbon tetrachloride) the addition of only millimolar concentrations of a nucleophilic ligand to chlorophyll in solution will produce a solvent shift essentially as large as if the chlorophyll were in the nucleophilic solvent alone. This ability of spectroscopic properties to signal the immediate environment of the molecule is of great value in deducing the nature of the surroundings in vivo.

2.9 Aggregation

Attempts to remove the last traces of nucleophilic ligands from chlorophylls in non-polar solvents usually results in aggregation of the chlorophylls, leading eventually to precipitation. Detailed studies of this phenomenon have been carried out by Katz and coworkers (Katz, Dougherty and Boucher, 1966; Ballschmiter, Truesdell and Katz, 1969; Ballschmiter, 1969). It appears that, in the absence of other nucleophilic compounds, one chlorophyll may serve as ligand for a second molecule, resulting in the formation of a dimer or, with increasing aggregation, of a precipitate. Katz, Ballschmiter et al. (1968) have summarized the evidence that water is commonly present as a linking molecule in these aggregates.

A new effect, known as exciton splitting, occurs in the absorption spectra of aggregated chlorophylls. This results from the coupling via coulomb interaction of the transition dipole moment of one molecule with the corresponding transition moment of one or more identical (or very similar) molecules. Exciton coupling amongst small and large sets of closely arrayed chromophores has been described in reviews by Kasha (1963), Tinoco (1963) and Hochstrasser and Kasha (1964). The result of the exciton coupling is that each excited electronic state is split into a set of N exciton states, where N is the number of coupled molecules in the array. What one typically observes is the formation of multiple absorption bands in regions where there was only one for the monomer. These bands are often incompletely resolved, for the energies of interaction (band splittings) are seldom much greater than the intrinsic band widths. Resolution may be appreciably improved by making measurements at liquid nitrogen temperature or lower (see section 3.5 and Fig. 11). The intensities of the individual exciton components and their spectral location is not invariant, but depends sensitively on the geometry--the relative positions and orientations--of the interacting molecules. For asymmetric chromophores or for symmetric chromophores asymmetrically arranged (as for the bases in a helical nucleic acid molecule) each exciton component appears also in the CD spectrum. Both the magnitude and sign of each CD exciton component depends intimately on the geometry of the array. It is this dependence on geometry that is potentially of great value in using exciton splittings to interpret molecular arrangements and interactions in biological systems.

There is a classical analogy that may help in appreciating the origin of this exciton interaction. This is the coupled pendulum problem. If

one mounts two identical pendulums near one another and sets them independently in motion, each will swing with a period or frequency determined only by the distance of its center of mass from the point of attachment. The periods of the two identical pendulums will be precisely identical, and each will swing in a constant fashion whether the second is also in motion or not. In this condition they are said to be "isolated" from one another. If one now attaches a weak elastic band between the two pendulum arms, they are observed to interact with one another to an extent determined by the strength of the coupling provided by the elastic band. (Other coupling mechanisms serve this purpose, including motion imparted to the air surrounding the pendulums or insufficient rigidity of the mount from which they are suspended.) In the coupled situation, one pendulum will be observed to swing slightly faster and the other slightly slower than the period of the previous, independent (uncoupled) case. The effect is seen very dramatically, for the pendulums will pass smoothly from swinging in phase with one another, to swinging completely out of phase, then back to in phase, etc., indefinitely (or until they finally come to a stop). The coupled oscillators (pendulums) are characterized by a pair of new frequencies that are a property of both oscillators (including the coupling interaction). In the exciton case the molecular absorption oscillators (transition moments) are the analogs of the individual pendulums, and the coulomb interaction is the origin of the coupling. It is important to recognize that the new exciton states are properties of the entire array; we cannot assign one state to one molecule, another to the second molecule, etc. (This may not seem obvious from the pendulum analogy, for we arbitrarily chose to

focus attention on the different periods of each of the coupled pendulums. We could have as well described the motion of the system by the average period and the time between successive in-phase motion, i.e., the period of beating. In this case neither number is separately representative of the motion of one or the other pendulum.)

For certain geometries (e.g., perpendicular orientation or parallel orientation of the transition moments) all of the absorption or CD intensity of a set of N molecules may appear in only one transition component and no intensity in any other. Although a number of such arrangements may exist for large numbers of coupled oscillators, these arrangements are still relatively few in comparison with the very many other possibilities (angles other than 0° and 90°) where each exciton component is present and observable. Nevertheless, counting the number of exciton components can provide us with only the minimum number of coupled molecules in the array. In practice, especially as it may be difficult to resolve small splittings or detect components with small amplitude, it is necessary to exercise caution and be conservative in drawing conclusions about the molecular origins of these spectroscopic effects.

It is also important to be aware that the exciton interaction is appreciable (i.e., measurable) over only relatively short distances. These are typically 20 Å or less for molecules like the chlorophylls; i.e., only three times the thickness of the porphyrin ring and less than twice its diameter. By contrast, the Förster (1965) resonance interaction (see Chapter 4) may be observed out to distances as great as 100 Å. This distinction becomes significant in connection with the analysis of mechanisms of delocalization of electronic excitation (see section 3.7 of this chapter, and Chapter 4).

Chlorophyll molecules provide excellent examples of the usefulness of the analysis of exciton interactions, particularly with the ability to observe them in CD as well as in absorption spectra. In suitable solvents, such as carbon tetrachloride, it is possible to prepare solutions that contain a high proportion of dimers of chlorophylls. The assignment of these species as dimers is confirmed by measurements of equilibrium (Sauer, Lindsay Smith and Schultz, 1966), apparent molecular weights, and IR and NMR spectra (Katz, Closs et al., 1963; Closs, Katz et al., 1963). Absorption spectra of these solutions of (bacterio)chlorophyll confirm the presence of dimers in the appearance of two exciton components (one, a long wavelength shoulder). Furthermore, CD spectra clearly show the presence of two bands of opposite sign where only one is present in monomeric chlorophyll solutions (Dratz, Schultz and Sauer, 1967). Fig. 7 shows the absorption and CD spectra of a solution of BChl a in carbon tetrachloride, under conditions where the BChl a molecules occur largely as dimers. The consequences of the exciton interaction can be observed as a splitting of the absorption bands (note the new "shoulder" on the long wavelength or Q_y band) and, much more dramatically, in the multiplicity of the CD bands. Compare the spectra of Fig. 7 with those in Fig. 4 for BChl a in ether, a solvent where the monomer predominates. Inspection of the CD spectra will demonstrate that the amplitudes of CD exciton components of the dimeric BChl a are several times larger than the CD bands of the monomeric form of this molecule. The effect of the exciton interaction on the energy levels of some of the electronic states of BChl a is illustrated schematically in Fig. 8.

Chlorophyll crystals provide an interesting example of the usefulness

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Fig. 7. Absorption and CD spectra of BChl a in CCl_4 at 10^{-3} m^{-1} concentration. Under these conditions the BChl a is largely dimeric. Spectra taken from Dratz, Schultz and Sauer (1966). CD amplitude has been multiplied by 0.5 at wavelengths longer than 700 nm. Relative to the absorption, the CD amplitudes of the dimers are several fold greater than those for the monomers, shown in Fig. 4.

Fig. 8. Energy level diagram for BChl a, showing the splitting of excited state levels resulting from exciton interaction in the dimers. The various energy levels are characterized by their frequencies, in cm^{-1} . This diagram illustrates the origin of the differences between the spectra shown in Fig. 4 and in Fig. 7.

of the CD spectra as a supplement to absorption. Microcrystalline suspensions of Chl a in saturated hydrocarbon solvents exhibit a very sharp absorption band, strongly red shifted out to about 740 nm. This band remains unresolved even at liquid nitrogen temperatures. The CD spectrum in this region exhibits two large components of opposite sign (Dratz, Schultz and Sauer, 1966). This indicates the presence of at least two exciton states, albeit nearly degenerate, for Chl a crystals. Even so, why should there be only two components in a spectrum of crystals where there is potentially a very large number of interacting molecules? Analysis of this problem shows that for a periodic array of molecules, as in a molecular crystal, the maximum number of allowed transition components is equal to the number of molecules in the unit cell (Hochstrasser and Kasha, 1964). The detailed X-ray crystallography of Chl a has not been determined; however, it is known to have a similar crystal habit and unit cell dimensions to that of methyl pheophorbide a (Chl a minus Mg and with phytol replaced by methyl). Recent analysis of the single-crystal X-ray diffraction pattern of methyl pheophorbide a shows it to contain two molecules per unit cell (Fischer, Templeton et al., 1972). It is apparently the predominant interaction between two molecules in the unit cell that we observe in the CD spectrum of the Chl a microcrystals.

The origin of the very pronounced red shift of the long wavelength absorption band in the crystals is obscure. Although the effect of an environment of other molecules of Chl a cannot be duplicated by a colorless solvent, nothing we know from solution spectra would lead us to expect such a large absorption band shift. It would appear that there are effects operating in the crystal that have no counterpart in solutions of either the monomeric or aggregated pigment.

The fluorescence properties of the chlorophylls are affected even more dramatically by aggregation. Watson and Livingston (1950) observed a strong quenching of Chl a fluorescence with increasing concentration in ether, and Weber (1960) has shown that the quenching behavior is consistent with a model in which the excitation is transferred to non-fluorescent dimers. A similar quenching occurs as a function of surface concentration of chlorophylls in a two-dimensional monolayer at an air-water interface (Tweet, Bellamy and Gaines, 1964).

2.10 The Usefulness of Spectroscopic Measurements

Before going on to examine the spectra of the chlorophylls in vivo, it will be worthwhile to present a brief summary of the different spectroscopic parameters and the kinds of information that can be deduced from them. The following abbreviated list is drawn from the more detailed descriptions just completed.

(1) Wavelength shifts of absorption maxima provide information about the electrical characteristics of the surrounding matrix. The inclusion of chlorophyll crystals, for example, in photosynthetic membranes would be readily apparent from the extreme red shift of the long wavelength absorption maximum.

(2) Multiple bands that disappear upon extraction into a "good" solvent are indicative of plural molecular sites or of coupling between closely associated chlorophylls. CD spectra provide even more dramatic evidence than do absorption spectra.

(3) Polarization of absorption (dichroism) or fluorescence may be indicative of net molecular orientations within the surrounding matrix.

(4) Decreased fluorescence lifetimes or efficiencies can occur as a result of chlorophyll aggregation, the presence of specific excited state quenchers or competition from photochemistry.

(5) Transient species, such as the triplet states, give rise to their own characteristic absorption or emission spectra.

(6) Bleaching or disappearance of the absorption of pigments provides direct information about the photochemical fates of the responsible molecules. As we shall see in the next section, this has been a most valuable tool in elucidating the primary photochemical processes of photosynthesis.

These are but a sampling of the many ways in which electronic spectroscopy can furnish useful information about photochemical kinetics and molecular structure. Many new techniques are emerging and give promise of expanding the horizons of our search. Some of the most exciting of these are X-ray or electron-induced electron emission spectroscopy, resonance Raman spectroscopy, double resonance methods and high-intensity, ultrashort pulse laser spectroscopy. The imaginative application of these approaches to photosynthesis research can be expected in the near future.

3. Chlorophylls in Photosynthetic Membranes

3.1 Spectra of Chlorophylls in the Biological Environment

Measurements of in vivo absorption and fluorescence spectra of entire photosynthetic organisms (leaves, algae, bacteria, etc.), of organelles, membrane preparations, and of subunits have been made with increasing sophistication during the past 40 years. The following general observations are noteworthy:

(1) Chlorophyll absorption bands are much more complex in vivo than they are in solution. This is most readily apparent for BChl in the photosynthetic bacteria, where a number of well resolved components are observed in the near infrared (Clayton, 1963a). In algae and higher plants the presence of as many as 8 to 10 spectral components has been reported in in vivo absorption spectra, especially at low temperatures (Brown and French, 1959; Frei, 1962; Butler, 1966; Butler and Hopkins, 1970a,b).

(2) Fluorescence spectra also exhibit composite character, although generally of lesser complexity. In some cases (especially for higher plants and algae), new components appear only at very low temperatures (Brody, 1958; Goedheer, 1961; Butler, 1961).

(3) Extraction of the photosynthetic membranes with organic solvents invariably leads to a complete collapse of the structure of both the absorption and fluorescence spectra. There is no good evidence that the pigments in vivo are chemically any different from the Chl a, Chl b, BChl, etc. molecules found in the extracts (Clayton, 1966a).

(4) The wavelength maxima are generally appreciably red shifted from those in solution, but not nearly so much as for the crystals. There is no evidence to support the earlier notion that chlorophyll in the membranes is in a crystalline environment.

(5) Neither absorption nor fluorescence is strongly polarized, supporting the idea that the pigments are not ordered in a simple periodic lattice (Goedheer, 1957; Olson, Butler and Jennings, 1961, 1962). The polarization evidence is not entirely negative, however.

There is some indication from these studies of an underlying order of a more complex character (Morita and Miyazaki, 1971; Breton and Roux, 1971).

(6) Both the absorption and fluorescence are known to be kinetically heterogeneous. Small portions of the absorption may disappear or shift in wavelength upon photoexcitation (Duysens, 1952; Kok, 1956). A large portion of the fluorescence exhibits a change in its efficiency upon photoexcitation ("live" fluorescence), but another portion does not ("dead" fluorescence) (Clayton, 1969). See section 4.3 for a fuller description of these forms of fluorescence. The shapes of absorption envelopes can be altered appreciably by varying the growth conditions or in response to genetic mutation. Such changes occur dramatically in the course of the greening of seedlings germinated in the dark and then transferred to the light (Shibata, 1957).

These observations support a picture in which the chlorophylls occur in vivo in a variety of environments. Much of the work during the past fifteen years has been aimed at determining the number and nature of the different environments, what role is played by the pigments in each environment, and how the individual components are coupled together in the natural photosynthetic membrane.

3.2 Photosynthetic Membranes--Structure and Organization

Photosynthetic bacteria and chloroplasts from higher plants and algae are filled with internal membranes or vesicles. These can be readily visualized by electron microscopy (Park, 1966; Branton and Park, 1967; see also Chapter 2 of this volume), and they are known to contain the

chlorophyll pigments and the sites of photochemical activity (Schachman, Pardee and Stanier, 1952; Park and Pon, 1961; Lintilhac and Park, 1966). Preparations of these isolated membranes and their fragments from broken cells have been subjected to chemical and spectrophotometric analysis. In addition to the chlorophyll and carotenoid pigments, the membranes contain a variety of cytochromes, quinones, non-heme iron (including bound ferredoxin, in the case of chloroplast membranes), proteins and colorless lipids (Park and Biggins, 1964; Oelze and Drews, 1972). The membranes are typically of the order of 5-6% by weight of (bacterio)chlorophyll, which corresponds to an average concentration greater than 0.05 M. For such a complex and heterogeneous system it must be recognized that the physical and chemical properties of the chlorophylls in vivo may bear little relationship to those of chlorophylls in dilute solutions, in homogeneous thin films or in pure crystals.

Numerous early, and even recent, models for photosynthetic membranes incorporate pigment monolayers, linear arrays, quasi-crystalline aggregates, homogeneous solid solutions or other readily visualized arrangements. Although such arrangements may be convenient for purposes of carrying out theoretical calculations, it is the purpose of this section to present evidence that such models are quite unrealistic. The picture that emerges from recent investigations is one in which the membrane is a composite of individual macromolecular subunits arranged in a regular, ordered fashion (Branton and Park, 1967). The major subunits include reaction center complexes, small chlorophyll-proteins, electron transport proteins such as the cytochromes and ferredoxin, and the components responsible for coupling of phosphorylation. The overall structure also

includes the carotenoids, quinones, colorless lipids and proteins and, in the case of higher plants, the apparatus involved in oxygen evolution. The manner in which these individual components are associated in functional photosynthetic membranes is one of the most active and challenging areas of current investigation.

3.3 Reaction Centers

Ever since the pioneering and highly imaginative studies of Emerson and Arnold (1932a,b), it has been known that the concentration of sites of photochemical activity in chloroplasts corresponds to only a small fraction (typically less than 1% in chloroplasts; of the order of 1% in bacteria) of the chlorophyll present. The initial studies described the maximum photochemical yield of O_2 evolved or CO_2 fixed resulting from a brief (μ sec) saturating flash of actinic light. This picture was subsequently amply confirmed when active membrane components were identified on the basis of photoinduced absorption changes (Duyens, 1952; Kok, 1956), chemical analysis of the content of cytochromes, quinones, ferredoxin, manganese, etc. As soon became apparent, key components in the light reactions were observed in the photoinduced reversible absorption changes in bacteria of a small portion of the BChl known as P870 (Vredenberg and Duyens, 1963) and a similar small portion of Chl a known as P700 in the case of higher plants (Kok and Hoch, 1961). (The numbers refer to the wavelengths of maximum absorption change and are somewhat variable, depending on the organism, method of sample preparation, etc.) The photoinduced bleaching of these components is due to the reversible oxidation of a small portion of the (bacterio)chlorophyll.

A most important advance in our ability to examine the details of the bacterial membrane organization occurred when it was discovered that chemical treatments (e.g., strong oxidants, detergents) or illumination at high intensity of a carotenoidless mutant results in the exposure of the photochemically active sites. These are now generally known as reaction centers and are free of the overburden of chemically inactive, light absorbing pigments, called the antenna or light-harvesting pigments (Clayton, 1963b; Loach, Andrees et al., 1963; Kuntz, Loach and Calvin, 1964; Clayton, 1966a). The reaction center preparations exhibit photoinduced absorption changes and unpaired electron (EPR signal) production characteristic of the intact chromatophores or bacteria. The absorption spectra and the photoinduced absorption changes characteristic of one of the earliest and most widely studied of these reaction center preparations are shown in Fig. 9.

The isolation and purification of a reaction center subunit was first reported by Reed and Clayton (1968). They treated chromatophores of Rps. spheroides, R-26 (carotenoidless) mutant with Triton X-100, a non-ionic detergent, followed by sedimentation in a sucrose density gradient and polyacrylamide gel filtration. The reaction center subunit was shown by Reed (1969) to have a molecular weight of about 650,000 daltons, relatively large amounts of ubiquinone, iron and copper, together with P870, P800 and cytochromes (see Table I). Recently procedural variations have produced an active reaction center particle with lower molecular weight (Clayton and Wang, 1971). In addition to the photochemically active bacteriochlorophyll absorption changes, the reaction center particles exhibit an EPR signal at $g = 2.0025$ that is stoichiometric with and kinetically similar to $P870^+$ (Bolton, Clayton and Reed, 1969).

Fig. 9. Reaction center complexes from *Rps. spheroides*, R-26 mutant.

(A) Absorption spectra taken in the "dark" (solid curve) and under cross-illumination by a beam of 800 nm actinic "light" (dashed curve). (B) Difference spectrum, "light" minus "dark", between the illuminated and unilluminated samples. Figure is taken from Reed (1969).

Table I. Comparison of Properties of Isolated Photosynthetic Bacterial Reaction Centers
Free of Antenna Bacteriochlorophyll

Organism	Detergent	MW (kdaltons)	<u>Cyt 562</u> <u>P865</u>	<u>Cyt 552</u> <u>P865</u>	<u>UQ</u> <u>P865</u>	<u>Fe</u> <u>P865</u>	Reference
<u>Rps. spheroides</u> (R-26)	Triton (1%)	650	2	1	13	16	} Reed (1969)
	Triton (2.3%)		0.8	0	7.4	5.4	
	LDAO	70				3.2	} Feher (1971); Feher, Okamura and McElroy (1972)
	LDAO+EDTA				1	0.8	
	SDS+LDAO	37			1	0.2-0.3	
	LDAO	~100	0	0	<0.1		Clayton and Yau (1972)
<u>Rps. spheroides</u> (wild type)	SDS	200	<0.02	0.90	3.3	20	} Slooten (1972)
	SDS/AUT	120	<0.02	0.25	1.2		
<u>Rps. spheroides</u> (strain Y)	CTAB	150		1.5	8	0.5 (non-heme)	Reiss-Husson and Jolchine (1972)
			<u>Cyt 442</u> <u>P870</u>		<u>UQ</u> <u>P870</u>		
<u>R. rubrum</u> (G-9)	Triton		0.5				Gingras and Jolchine (1969)
<u>R. rubrum</u> (wild type)	SDS	80-100	≥1.0		1.7		Smith, Sybesma and Dus (1972)
			<u>Cyt 558</u> <u>P960</u>	<u>Cyt 553</u> <u>P960</u>			
<u>Rps. viridis</u>	SDS	110	2	5			
							{ Thornber, Olson et al. (1969) Thornber and Olson (1971)

By using the zwitterionic detergent lauryl dimethyl amine oxide (LDAO), Feher (1971) was able to isolate an active reaction center particle with a molecular weight under 100,000 daltons. Each reaction center P870 was associated with about 1 molecule of ubiquinone and 1 non-heme iron. Clayton and Yau (1972) reported the absence of cytochromes from a similar LDAO preparation and, by contrast with the previous report, the presence of only 0.1 molecule of ubiquinone per P870. Further treatment of the LDAO particles with the anionic detergent sodium dodecyl sulfate (SDS) split them into two subunits; one with a molecular weight of 37,000 retained the P870 activity (Feher, Okamura et al., 1971). This is the smallest active subunit so far reported. We shall return to discuss its properties below.

Following the initial report by Reed and Clayton, a number of other organisms were investigated. A summary of some of the properties of reaction centers from several of these preparations is given in Table I. Reaction centers have now been isolated from several species of photosynthetic bacteria, including some wild-type species with the normal complement of carotenoids. Various detergents have been used and a wide range of molecular weights is seen. Similarly, a considerable variation in the content of demonstrated or potential electron transport cofactors (cytochromes, ubiquinone, non-heme iron) is found. In each case a few molecules of pheophytinized BChl are associated with the reaction center; however, their role in the primary photochemistry is not yet clear. In each of the cases described in Table I, the bulk or antenna BChl is completely absent in the reaction center preparation. This results in a decrease of about 30 fold in the overall BChl content compared with the intact chromatophores.

Organisms other than the purple photosynthetic bacteria cited in Table I have thus far resisted such a dramatic enrichment in reaction center pigments relative to the antenna. Detergent solubilized preparations from Chromatium D exhibit molecular weights of about 500,000 daltons, but still contain about 40 antenna BChl molecules per active center (Garcia, Vernon and Mollenhauer, 1966; Thornber, 1970). Still larger photoactive particles, with molecular weights in excess of 1.5×10^6 daltons, have been prepared without the use of detergents from the green bacteria Cps. ethylica (Fowler, Nugent and Fuller, 1971) and Chlorobium thiosulfatophilum (Fowler, Nugent and Fuller, 1972; Olson, Philipson and Sauer, 1972). Particles of much smaller molecular weight were obtained using simultaneous treatment at high (alkaline) pH with urea and Triton X-100 (AUT) from Rps. spheroides (MW 160,000 daltons) (Loach, Sekura et al., 1970) and from R. rubrum (MW 100,000 daltons) (Loach, Hadsell et al., 1970). Nearly all of the antenna pigments, bacteriochlorophyll and carotenoids, is retained in these preparations together with the photochemical reaction centers. In an interesting extension of these studies, Loach and Hall (1972) report that column electrophoresis removes most of the iron from these preparations, leaving a ratio $\text{Fe/P865} = 0.2$ to 0.3 . This is of special relevance with regard to the possibility that the primary electron acceptor contains non-heme iron (see section 4.5). Feher, Okamura and McElroy (1972) report a similar low value for the iron content of the 37,000-dalton particle from Rps. spheroides, R-26 mutant, obtained by combined LDAO/SDS treatment.

Attempts to prepare exposed reaction centers of P700 from higher plants have been frustrated by the inability to remove all of the antenna pigment, although enrichments of 10 to 20 fold have been reported. Yamamoto and Vernon (1969; Vernon, Yamamoto and Ogawa, 1969) obtained a preparation

(HP700) of particles with a Chl/P700 ratio of 30:1 using extraction with organic solvents (acetone:hexane) followed by suspension with Triton. Sane and Park (1970) have used low temperature acetone extraction to achieve a preparation with a 15:1 ratio of Chl to P700. Other particles have been prepared from chloroplasts and from a blue-green alga by Thornber and coworkers using SDS treatment followed by hydroxylapatite chromatography (Thornber and Olson, 1971; Dietrich and Thornber, 1971); however, these have larger contents of antenna Chl a. Vernon, Shaw et al. (1971) used Triton to achieve an enriched particle with photosystem 2 activity from chloroplasts.

We shall return to consider the photochemical properties of these reaction centers in section 4.

3.4 Chlorophylls in the Reaction Centers

A major feature of photoinduced absorption changes in purple photosynthetic bacteria, chromatophores and reaction centers is the bleaching of absorption around 870, 600 and 375 nm, associated with the major BChl absorption bands, and accompanying increases in absorption at 1250, 1140, 980 and 420-450 nm (Clayton, 1962a). These are now thought to result from the one-electron oxidation of a reaction center BChl molecule (see section 4.4). At the same time there occurs a blue shift of 3-4 nm in the position of a second absorption peak at 800 nm. Clayton (1966a) and Clayton and Sistrom (1966) made extensive studies of the relationship of these components and reached the conclusion that the absorption at 800 and at 870 nm results from BChl components of the same reaction center. Because the absorption bands occur in the ratio 2:1 in a variety of bacterial reaction centers and because oxidative bleaching resulted in a loss of at most one third of the total absorption residing in the two components, the view was put

forward that P870 represents a reactive oxidizable BChl molecule, and that P800 represents two other BChl molecules that participate in the reaction center but do not become oxidized. Not only is the light absorbed at 800 nm transferred efficiently to the reaction center (Clayton and Sistrom, 1966), but fluorescence from the reaction center is distinctly less polarized when excited at 800 than at 870 nm (Ebrey and Clayton, 1969). This evidence supports the idea that the excitation at 800 nm produces electronic states that are distinct from but closely coupled to those reached directly via absorption at 870 nm.

Circular dichroism spectra provide support for strong coupling among several associated BChl molecules in the reaction centers. Sauer, Dratz and Coyne (1968) observed evidence of exciton interaction in the CD spectra of reaction center particles from the R-26 mutant of Rps. spheroides. The CD spectrum indicated the presence of at least two energy levels (states) in the 800 nm absorption band and one more at 865 nm for the reduced reaction center preparation. Upon chemical oxidation or illumination with actinic light, the CD spectrum changed in a way consistent with a smaller number of interacting molecules. The absorption and CD changes are illustrated in Fig. 10. This evidence supports the idea that at least three BChl molecules are strongly coupled in the (reduced) reaction center particles, and the resulting exciton interaction gives rise to three states in the absorption and CD spectra (see section 2.9). Because each state giving rise to an absorption or CD component results from the coupling of all three molecules, it is not appropriate in this model to assign one molecule to each absorption band. Upon oxidation, the three coupled molecules lose one electron to the acceptor, and an altered exciton interaction is left among the remaining two BChl molecules.

R. spheroides (R-26 mutant)

REACTION CENTERS

XBL 685-4216 A

Fig. 10. Reaction center complexes from Rps. spheroides, R-26 mutant.

Absorption spectra (top) and CD spectra (bottom) for samples either in the dark (solid curves) or under cross-illumination by an actinic beam. (Sauer, Dratz and Coyne, 1968).

Examination of reaction center preparations from R. rubrum and Rps. viridis shows them to exhibit CD properties similar to those of Rps. spheroides (Philipson and Sauer, 1972b). Even the particles from Chromatium (Thornber's Fraction A) containing substantial antenna BChl, exhibit similar changes in the CD spectrum upon illumination. In each case oxidation of the reaction center particles results in bleaching of only one of the exciton components; the others merely shift in wavelength and exhibit altered exciton coupling. Is this simply a fortuitous circumstance of the exciton states for the particular configuration of BChl molecules in the reaction centers from at least four different bacteria, or is Clayton's original picture involving a P870 molecule and two P800 molecules indeed closer to the truth? Perhaps one of the BChl molecules is sufficiently uniquely sited so as to give rise to the 870 nm absorption band independently. The remaining P800 molecules simply indicate or sense at a distance the redistribution of electronic charge upon oxidation of the reaction centers.

Several recent studies support the exciton model involving strong coupling between several BChl molecules in the reaction centers. EPR spectra of both protonated and deuterated algae and bacteria show characteristic EPR line narrowing for the signal at 2.0025 in comparison with the EPR of chemically oxidized Chl or BChl in solution (Norris, Uphaus et al., 1971). The explanation put forward for this line narrowing is that the positive charge of the oxidized pigment in vivo is delocalized, probably over two molecules. Similar conclusions were reached by McElroy, Feher and Mauzerall (1972). This is clearly at variance with a picture in which a single P870 molecule becomes oxidized in the absence of strong coupling with at least one other molecule.

In the case of photosystem I from higher plants, recent studies of the HP700 preparation of Yamamoto and Vernon (1969) using CD spectroscopy (Philipson, Sato and Sauer, 1972) confirm the earlier observation of Döring, Bailey et al. (1968), Murata and Takamiya (1969) and Vernon, Yamamoto and Ogawa (1969) that the P700 absorption change is coupled to a weaker photo-bleaching at about 680 nm. Photoinduced CD changes of about equal magnitudes but of opposite sign occur at the two wavelengths (Philipson, Sato and Sauer, 1972). Although the P700 oxidation has been shown to correspond to a one-electron transition, the decrease in absorption intensity is apparently distributed, albeit unequally, between two components of an exciton pair. In this case it seems clear that there is no "P700 molecule" per se.

In principle, it should be possible to count the number of coupled BChl molecules in purified reaction center preparations from the number of components in the absorption or CD spectra. In practice it is possible to determine only a minimum number, as mentioned in section 2.3, because some of the transitions may be degenerate and unresolved, or the strengths of one or another of the transitions may be fortuitously very small owing to the particular molecular configuration. For reaction centers from Rps. spheroides, R. rubrum and for Chromatium, Fraction A, we estimate a minimum of three interacting BChl molecules in the reduced state (Sauer, Dratz and Coyne, 1968; Philipson and Sauer, 1972b). Other estimates range from 2 to 5. Clayton (1966a) originally concluded that the number was 3, on the basis of observed extinction coefficients in reaction center preparations and because nearly one-third of the absorption at 800 and 870 nm was lost upon light or chemical oxidation. Furthermore, alcohol

extracts of reduced and of oxidized reaction centers recovered BChl in the ratio 3:2. A similar stoichiometry was proposed for reaction centers prepared from Rps. spheroides, strain Y (Jolchine, Reiss-Husson and Kamen, 1969), R. rubrum (G-9 mutant) (Gingras and Jolchine, 1969) and R. viridis (Thornber and Olsen, 1971; Thornber, 1971).

Recently, several reports have presented evidence that supports different stoichiometries. Feher (1971) analyzed the metal content of highly purified LDAO reaction centers from Rps. spheroides and found a ratio of 5:1 for Mg/Fe. The possibility that this was also the ratio of BChl to the primary acceptor led Feher to suggest that the previously accepted values be reexamined. Clayton, Fleming and Szuts (1972) analyze the case for 3, 4 or 5 BChl per reaction center complex on the basis that BPheo is an integral part of the reaction center complex (Yau, 1971) and quoting unpublished reports of Mauzerall and of Straley that the ratio of BPheo/BChl is 1:2. Recently Reed and Peters (1971) and Reed and Ke (1972) presented reports favoring 2 BChl per reaction center. The evidence available suggests that these workers are not simply dealing with different materials, for there seems to be general agreement on the absorbance ratios P760:P800:P870. Values greater than 3 BChl per reaction center inevitably lead to quantum yields greater than unity for certain substrate oxidations and to a loss in the correspondence between oxidized P870 and the number of unpaired electrons as measured by EPR. On the other hand, numbers less than 3 are very difficult to reconcile with the absorption and CD spectra. Attempts are currently underway to resolve these discrepancies.

3.5 Bacteriochlorophyll- and Chlorophyll-Proteins

In addition to the various particles containing active reaction centers with or without accompanying antenna pigments, a variety of (bacterio)chlorophyll proteins without any measurable photochemical activity have been isolated. These fall into two distinct classes: (a) water-soluble pigment-proteins that are obtained by sonication or other methods of cell rupture followed by fractionation of the cellular debris, and (b) pigment complexes obtainable only through the use of detergents, which are required both to excise the complexes from the internal membranes and to provide them with sufficient hydrophilic character to become water soluble.

A prime example of the first type is the water soluble BChl protein isolable from sonicates of the green bacteria Cps. ethylica or Chlorobium thiosulfatophilum (Olson and Romano, 1962; Olson, 1971). This substance has been highly purified and is readily crystallizable. It has a molecular weight of 152,000 daltons and consists of four equivalent subunits, each containing five BChl molecules (Thornber and Olson, 1968). The BChl are apparently well covered by a protein coat and are in close proximity to one another within the subunits, giving rise to pronounced exciton splittings in low temperature absorption and CD spectra (Fig. 11). The spectroscopic properties of the BChl-protein do not, however, closely resemble those of the membrane-bound antenna BChl associated with green bacterial reaction center particles (Fowler, Nugent and Fuller, 1971; Olson, Philipson and Sauer, 1972). Despite the conclusion that it is not an integral part of the photochemically active membranes of the green bacteria, the water soluble BChl-protein is able readily to transfer electronic excitation to

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Fig. 11. BChl-protein complex from *Cps. ethylica*; absorption and CD spectra taken for the same sample at room temperature (dashed curves) and at 77°K (solid curves). Both an increase in resolution of the components and a considerable enhancement of the CD features are evident at the lower temperature. Curves taken from Philipson and Sauer (1972a).

the photosynthetic reaction centers in order to drive the light reactions.

Several interesting water-soluble Chl-proteins have been isolated from higher plants. One obtained from Chenopodium album or from cauliflower has a molecular weight of 78,000 daltons and contains 6 Chl a and 1 or 2 Chl b (Yakushiji, Uchino et al., 1963; Takamiya, 1971; Murata, Toda et al., 1971). Upon illumination the pigments of the Chl-protein from Chenopodium undergo an irreversible photooxidation. The Chl-protein from cauliflower is light-insensitive. Other small Chl-proteins have been isolated from Lepidium; however, their function in these organisms is not known (Takamiya, 1971; Murata and Murata, 1971). Frozen, aged and thawed spinach chloroplasts release two small water-soluble chlorophyll proteins that contain plastoquinones and carotenoids as well (Tachiki, Parlette and Pon, 1969).

The second class of inactive pigment-proteins requires solubilizing detergents for their isolation. Two BChl-proteins obtained from Chromatium D, fractions B and C, using SDS have molecular weights of about 100,000 and contain about 10 BChl molecules together with carotenoids (Thornber, 1970). A much smaller pigment protein with molecular weight about 14,000 and containing one molecule of BPheo (presumably converted from BChl during an acid-extraction step) was obtained from Rps. spheroides using SDS (Fraker and Kaplan, 1971; 1972). A protein of similar size associated with the antenna BChl has been isolated by Clayton and Haselkorn (1972). This is the smallest of the pigment-proteins isolated to date.

Chlorophyll-proteins isolated from higher plant chloroplasts incubated with SDS are of two types (Kung and Thornber, 1971). One has a molecular weight of 100,000 and a Chl a/b ratio 5:1; the other

has a molecular weight of 35,000 and a Chl a/b ratio 1:1. These are thought to derive from photosystems 1 and 2, respectively.

It is tempting to postulate that these detergent solubilized (bacterio)chlorophyll proteins without reaction centers represent subunits of the antenna pigment systems. In several cases they are obtained in yields corresponding to more than half the (bacterio)chlorophyll of the membranes prior to detergent treatment. Nevertheless, in most cases it remains to be determined that the pigment-proteins exist in a similar configuration within the active membranes and that the detergents have not caused a major rearrangement of the constituents. This note of caution applies equally to the reaction center preparations described in section 3.4.

3.6 Models of Photosynthetic Lamellae

The preparation and elucidation of an almost bewildering variety of reaction center particles, reaction centers with associated bulk pigments and inactive (bacterio)chlorophyll proteins has led to the formulation of various models for the photosynthetic lamellae. Thornber (1970) has envisioned a set of basic components or building blocks which are suitably arranged to give both the light-harvesting properties and the photochemical activity of the photosynthetic units. The building blocks consist of a set of reaction centers containing the primary electron donor and acceptor molecules, cytochromes serving as secondary donors, and (bacterio)chlorophyll proteins serving as the antenna. The basic units of the latter may contain between 2 and 20 pigment molecules encased in protein. These basic units are presumed to be held together by lipid components that are extracted by the solubilizing detergents.

The two-dimensional photosynthetic lamellae of higher plants are thought to contain repetitions of the photosynthetic units in a closely packed array (Branton and Park, 1967). Similar models for bacterial membranes or vesicles are envisioned by Reed, Raveed and Israel (1970) and by Slooten (1972). The picture that emerges from these ideas is akin to the tessellated or pebble mosaic floors characteristic of ancient pavements uncovered in the Near East. Imagine a characteristic and well defined unit pattern compounded from a set of differently colored or shaped pebbles, and then imagine this compound unit repeated extensively in each direction on the surface. Mosaic models with varying degrees of rigidity have been presented for a wide variety of subcellular membranes (Green, Allman et al., 1967; Singer and Nicholson, 1972). This pebble mosaic model, illustrated in a conjectural fashion in Fig. 12, has features which should command the attention of those who seek to understand the essential properties of active photosynthetic membranes. We will examine some of its consequences for the phenomenon of electronic excitation transfer in section 3.7.

Other models with lesser degrees of internal organization have been postulated by Kreutz (1970) and by Weier and Benson (1967). These differ in placing all or part of the pigments in a kind of two-dimensional layer or sheet with interleaving carotenoids or colorless lipids, and do not emphasize the packaging of the chlorophylls in individual small protein subunits. Numerous earlier models exist, but they are even more speculative because they did not profit from the recent studies of membrane substructure.

Support for rather specific interactions of particular proteins with reaction centers or light harvesting pigments comes from studies of

Fig. 12. A highly schematic illustration of the pebble mosaic model.

At the top is an expanded view of a single unit, consisting of an integrated array of different "pebbles": the electron transport cofactors (Fd, Cyt f, PCy, A, Q, etc.), reaction centers (RC 1 and RC 2) and specific pigment-protein subunits (Chl a - proteins and Chl a + b proteins). Carotenes and uncharged colorless lipids may be interspersed between components as a kind of glue; charged sulfo- and phospholipids may occur at the peripheral surface of the unit in contact with the aqueous surroundings. The model omits such additional features as phosphorylation coupling factors, for which the stoichiometry and placement are even less well known. At the bottom is pictured a portion of a single thylakoid membrane, which consists of a two-dimensional mosaic of the individual units. Electron micrographs show that the arrays are not always so regular as indicated here. This variability may control the extent of electronic excitation energy transfer from one unit to its neighbors.

protein composition following extensive detergent treatment (Remy, 1971; Clayton and Haselkorn, 1972). Mutants which are missing the light reaction or reaction center activities are found to be deficient also in specific proteins (Gregory, Raps and Bertsch, 1971; Segen and Gibson, 1971; Clayton and Haselkorn, 1972).

The membranes or thylakoids of chloroplasts are enclosed vesicles, for which the inside and outside surfaces are distinguishable using the electron microscope (Branton and Park, 1967). In addition, labeling studies using diazonium compounds (Dilley, Peters and Shaw, 1972) or antibodies (Briantais and Picaud, 1972) indicate that these faces differ in their activity, with access to photosystem 1 on the outside and photosystem 2 on the inside of the chloroplast thylakoids.

3.7 Excitation Transfer and Trapping

Within a few picoseconds ($1 \text{ psec} = 10^{-12} \text{ sec}$) following the absorption of a photon by a chlorophyll anywhere in an antenna of up to 500 molecules, the resulting electronic excitation arrives at the photochemical trap (see section 4.3). This rapid transfer of excitation places severe demands on the properties of such an array. The mechanisms invoked for the excitation transfer range from the diffusion of an exciton wave throughout a more or less homogeneous array of closely spaced chlorophylls, to a migration via a Förster mechanism (resonance transfer) involving the hopping of the excitation to neighboring molecules more or less at random (random walk). The purpose of this section is to point out aspects of this problem which have not been given sufficient consideration in any theoretical model to date. A detailed description of the models that have been studied and of their predictions is given by Knox in Chapter 4.

Most of the models of excitation transfer have used a simple picture of a regular repeating lattice (in 1, 2 or 3 dimensions) of identical pigment molecules (Duysens, 1964; Bay and Pearlstein, 1963; Robinson, 1966; Knox, 1968). The photochemical traps are assumed to be dispersed more or less uniformly in this array, although some models consider only isolated photosynthetic units containing a single trap. Evidence gathered from studies of fluorescence yields as a function of the fraction of reaction centers in the oxidized condition (and presumably not capable of acting as traps) suggest that excitation can pass relatively easily from one such unit to another in photosynthetic bacteria and photosystem 2 of algae (Clayton, 1966c, 1967; Duysens, 1966a; Borisov, 1967; Tumerman and Sorokin, 1967).

Several models have considered heterogeneous arrays in which molecules which are closer to the traps have somewhat lower energy excited states than do those molecules farther away (Duysens, 1966b; Seely, 1971). In certain arrangements calculations suggest that the speed of trapping can be increased in this way as much as 10 fold over that of equivalent homogeneous arrays (Borisov and Fetisova, 1971).

A different kind of picture is suggested by the pebble mosaic model described in section 3.6 and illustrated in Fig. 12. The isolation of pigment-protein complexes containing a small number (2 to 7) of strongly coupled (bacterio)chlorophyll molecules suggests that the antenna may consist of a repeating network of such pigment proteins. This model is spatially heterogeneous, in the sense that the antenna array consists of repeating units of groups of closely spaced chlorophylls in chlorophyll-protein subunits. The consequences of this model cannot be appreciated

by simply re-ordering the lattice points in the previous studies, however, for there is ample evidence that the underlying mechanism of molecular interaction is quite different for the closely grouped molecules within each protein subunit from what it is for interaction between adjacent subunit groups.

The absorption and CD spectra of Olson's BChl-protein from green bacteria (Philipson and Sauer, 1972a) and of both a Chl a - Chl b-protein and a Chl a-protein from higher plants (Murata and Murata, 1971; Murata, Philipson and Sauer, unpublished results) suggest that the (bacterio)chlorophyll molecules within these proteins are relatively strongly coupled. The resulting exciton states are properties of the molecular group. In these exciton states it is appropriate to consider the electronic excitation to be spread over all of the chlorophylls in the group, probably in a time shorter than the 10^{-13} sec characteristic of thermal relaxation in such molecules. Furthermore, there is probably a similarly rapid transition (intersystem crossing) to the lowest energy state(s) in the manifold of exciton levels. Evidence to support this view comes from the low temperature fluorescence spectrum of the BChl-protein (Olson, 1971), where it is seen that the fluorescence spectrum is the mirror image not of the entire absorption manifold, but only of the long wavelength exciton component. Although the fluorescence lifetime undoubtedly is much longer (ca. 10^{-9} sec) than the relaxation lifetimes that we are concerned with above, the fluorescence result emphasizes the conclusion that we should not consider these closely coupled pigment molecules to act as individual entities for purposes of excitation transfer calculations within or between photosynthetic units.

It is reasonable to suppose and consistent with the meager experimental data available that excitation transfer between adjacent BChl-protein or Chl-protein molecules in the antennas occurs via the very weak coupling typically invoked in the Förster mechanism. The rate of such transfer varies inversely as the sixth power of the distance between centers and is proportional to the overlap between the emission spectrum of the donor group and the absorption spectrum of the acceptor group.

While an arrangement such as that described above has not been explored by quantitative calculations, we can predict some of its features relative to that of a homogeneous array of regularly spaced, individual chlorophylls. Because intergroup transfer will be much slower than the intragroup delocalization, the effect of grouping the pigments is to reduce the number of Förster steps required to reach the trap by a factor N_g , where N_g is the number of pigment molecules in each closely coupled group. The time required to reach the trap is therefore shortened by a similar factor. On the other hand, the overlap between the fluorescence and absorption envelope may be reduced for the groups as compared with the separated molecules (monomers). The fluorescence comes only from the lowest state(s) of the manifold, whereas the absorption is distributed (albeit unequally) among all of the states. The relevant properties of the groups need to be known in greater detail, however. Emissive transition probabilities for the groups and their orientation with respect to adjacent groups play a strong role in the transfer probability (Knox and Chang, 1971). The forces of interaction between the protein sheaths of adjacent chlorophyll-proteins provide a plausible rationale for supposing that the antenna consists of a "regular" array of these subunits with some well-defined, as opposed to a random, mutual orientation.

It will be a challenge for the future both to theoreticians and to experimentalists to explore the properties and consequences of the pebble mosaic model of photosynthetic membranes.

3.8 Synopsis of Photosynthetic Membrane Organization

Before going on to discuss the photochemistry of the reaction centers, let us first summarize some of the contributions that electronic spectroscopy has made to our knowledge of the function and arrangement of the chlorophyll pigments in photosynthesis.

(1) The chlorophylls in vivo are bound into lipoprotein membranes and occur there in several distinct spectroscopic and functional forms.

(2) Antenna chlorophyll molecules, typically 99% of the total, serve to absorb incident photons and to transfer the resulting electronic excitation to the reaction centers.

(3) Reaction center chlorophylls, roughly 1% of the total, participate directly in the primary photochemistry. The reaction centers utilize the excitation energy to pump electrons from donors to acceptors in the electron transport chain.

(4) Small BChl-containing proteins with the general characteristics of either the antenna or the reaction center BChl have been isolated from photosynthetic bacteria. These proteins have a small number (2 to 7) of BChl per molecule.

(5) Photosynthetic membranes probably consist of assemblies of antenna chlorophyll proteins, reaction center complexes, electron transport proteins and other smaller molecules packaged into integrated units that are repeated in a more or less regular array distributed over the membrane surface.

(6) In the pebble mosaic model, photon absorption results in electronic excitation that is very rapidly delocalized via strong exciton interaction among several pigment molecules within each antenna chlorophyll protein. The excitation is then transferred by a slower hopping process to other antenna chlorophyll protein molecules until it eventually reaches the reaction center.

4. Primary Photochemistry of Photosynthesis

4.1 Definition of Photosynthetic Energy Trapping

For the purposes of this section we shall define photosynthetic energy trapping as the process that results in the disappearance of (singlet) electronic excitation. The onset of the "chemical" stages occurs upon the appearance of the first of a series of oxidized and reduced intermediates. It is important to recognize that there is an unexplored region lying between the trapping of electronic excitation and the initial electron transfer reactions. Speculations have populated this region with triplet states and other metastable species, but there is little evidence at present to support their direct participation in the energy conversion process. The process of trapping is, therefore, defined operationally in terms of certain initial and final states. The precise boundary between the physics and chemistry of the process may prove difficult to locate, however, unless the model is carefully specified.

An alternative way of looking at the trapping process is to consider the motion of electrons and holes (missing electrons) in the activated system. The absorption of radiation by the photosynthetic pigments

results in the promotion or excitation of a bound electron to a higher energy level and leaves a hole behind in the original level. During excitation migration this electron and its associated hole move together among the molecular arrays (e.g., between adjacent Chl-proteins) in a correlated fashion. One feature that is characteristic of this correlation is that the excited electron always has the opposite spin to that of the remaining electron in the orbital containing the hole. When trapping occurs this spin correlation is lost. The electron and the hole eventually move independently of one another, either in conduction bands or amongst a set of donor and acceptor molecules. The free energy of the electronic excited states has then become converted into stored chemical potential, and we have entered the region of chemistry.

Recent reviews emphasizing various aspects of the photochemical trapping have been written by Fork and Ames (1970), by Witt (1971) and by Clayton (1972). Tributsch (1971) has presented a formulation of the trapping process in electrochemical terminology.

4.2 Primary Photochemical Processes

The experimental evidence suggests that the first events consequent upon trapping of the electronic excitation are oxidation-reduction (electron transfer) reactions. The evidence varies in detail, depending on whether photosynthetic bacteria or higher plants are studied. It is worthwhile starting with some generalizations to provide a framework for interpreting the detailed observations.

The earliest detectable event appears to be the transfer of an electron from a chlorophyll or bacteriochlorophyll in the reaction center complex to a primary acceptor, according to the equation

This process is very rapid (< 20 nsec) and proceeds with a quantum efficiency of the order of unity. It is temperature independent and occurs even at 1°K . The reaction can be observed by characteristic changes in the absorption spectrum (P870 or P700) and by the distinctive EPR signal of the oxidized pigments.

Reaction 4-1 competes very favorably with the other mechanisms of singlet excited state decay listed in section 2.4. As a consequence, photosynthetically active reaction centers exhibit a low yield of fluorescence, short fluorescence decay times, low yields of triplet formation, etc. relative to chlorophylls in solution. We will see in section 4.3 how measurements of these quantities can be used to deduce information about the kinetics of the primary photochemistry.

Following the primary event, several secondary processes may occur, either separately or together.

(a) The primary acceptor may pass the electron to one or more secondary acceptors

(b) An electron may be transferred from a donor associated with the reaction center complex

(c) The process may reverse to regenerate the pigment excited state, leading to delayed fluorescence

(d) The reduced and oxidized species may be coupled via an external (cyclic) electron flow pathway

The processes represented by steps (a) and (b) are extended through other electron carriers in non-cyclic electron transport. The ultimate electron donor in higher plant photosynthesis is H_2O and the electron acceptor is CO_2 via ferredoxin and $NADP^+$. Process (d) may also result in the production of stored chemical potential via a cyclic phosphorylation step.

While the events described above have been extensively documented, several additional intermediate stages have been proposed but have not yet been convincingly demonstrated. For example, the triplet state of chlorophyll has been invoked as an intermediate in the trapping process prior to the initial electron transfer step (Robinson, 1963, 1966; Franck and Rosenberg, 1964). Although evidence is available supporting the occurrence of the Chl triplet in photosynthetic organelles or membranes, the triplet state is observed only when the primary electron transfer step is blocked (Moraw and Witt, 1961; Rikhireva, Sibel'dina *et al.*, 1968; Dutton, Leigh and Seibert, 1972; Borisov, Godik and Chibisov, 1970). We cannot, however, rule out the possibility that triplets do participate in the normal course of the photosynthetic energy conversion but that their lifetimes are too short or concentrations too low to have been observed.

An alternative mechanism for photochemical trapping invokes the separation of electrons and holes into conduction bands characteristic of large molecular arrays (Calvin, 1958; Arnold, 1965). Such a delocalization of charges over a large matrix would provide a favorable entropy barrier against their wasteful recombination. The hole and electron

would productively be captured by an electron donor and an acceptor, respectively, associated with the molecular array. Subsequent events would occur much as in the simpler mechanism presented previously.

Photoconductivity and light-induced dielectric changes have been observed for dried films of chloroplasts (Arnold and Sherwood, 1957) or chromatophores (Arnold and Clayton, 1960) in DC experiments. Recently, high frequency (10^{10} Hz) photoconduction has been observed using an electrodeless (microwave cavity) technique (Blyumenfeld, Kafaliev et al., 1970; Bogomolni, 1972). Bogomolni made measurements of the Hall effect of this photoconductivity. He obtained evidence for both positive and negative carriers in both chromatophores and chloroplasts, but the quantum yield for their formation was less than 10^{-2} .

Reaction center preparations do not contain the antenna BChl molecules, and there is only a very small number of molecular sites over which charge delocalization can occur. From the ability of these reaction center preparations to carry out normal photochemical trapping it would appear that electron conduction bands typical of organic semiconductors are not necessary for photosynthetic energy conversion.

4.3 Trapping Times Deduced from Fluorescence Measurements

Once a reaction center becomes activated, it will not be able to utilize additional excitation until C^+ and A_1^- [Eq. 4-1] are returned to their dark states. During this dead time no further photochemistry is possible, and the additional absorbed energy is lost via heat or emitted radiation. The refractory period lasts until the secondary donor, D_1 , transfers an electron to C^+ and the reduced primary acceptor, A_1^- , is able to transfer an electron to a secondary acceptor, A_2 .

The available evidence (sections 4-4 and 4-5) suggests that there is only one oxidizable (bacterio)chlorophyll and one primary acceptor molecule in each reaction center complex. However, the secondary donors and acceptors can, in many cases, transfer or accept more than one electron upon successive flashes or continuing illumination (Parson, 1969b; Case and Parson, 1971). Once these secondary pools are emptied or filled, additional refractory periods or kinetic rate-limiting steps will be observed,

As the primary trapping sites become progressively filled there is a concomitantly greater probability that absorbed photons will be released as fluorescence. As a consequence, an increase in fluorescence efficiency (i.e., the ratio of emitted to absorbed light intensities) is observed with increasing intensities of actinic light (Duysens and Sweers, 1963; Vredenberg and Duysens, 1963). Fluorescence, thus, becomes a relatively more important pathway for excitation loss as the chemical steps saturate. We expect, on the basis of Eq. 2-9, that an increased fluorescence efficiency will be accompanied by an increased fluorescence lifetime. Studies by Tumerman and Sorokin (1967), and by Müller, Lumry and Walker (1969) of the fluorescence lifetime of Chl a in Chlorella did indeed show a strong intensity dependence. The lifetime increased from a value of 0.35 nsec at low intensity to 1.9 nsec at saturation. (This observation appears to resolve earlier discrepancies in lifetime values [Brody and Rabinowitch, 1957; Tomita and Rabinowitch, 1962; Butler and Norris, 1963; Nicholson and Fortoul, 1967], assuming that the different experiments were carried out at different exciting light intensities.) Mutants with non-functional reaction centers did not show any significant variation in fluorescence lifetime over the same intensity range (Müller, Lumry and Walker, 1969).

Two significant properties of this in vivo fluorescence are worth noting. The minimum fluorescence efficiency, extrapolated to zero actinic intensity, is distinctly greater than zero, and the saturation value is distinctly less than that for the Chl a or BChl in solution. From the observed lifetime of 0.35 nsec at low intensity (Müller, Lumry and Walker, 1969) and the intrinsic lifetime of 15 nsec for Chl a, we can calculate (Eq. 2) an expected quantum efficiency of 0.023. This is in excellent agreement with the observed value of 0.024 for Chlorella at low intensities (Latimer, Bannister and Rabinowitch, 1956). Clayton (1969) has interpreted this as "dead" fluorescence which is insensitive to the condition of the photochemical traps. The variable fluorescence is designated "live" fluorescence, by contrast. Clayton attributed these two forms to different Chl pigments or Chl in different situations in the lamellae.

The live fluorescence has been extensively studied, both in higher plants and in bacteria, as an indication of the condition of the photochemical traps. Clayton (1967) has provided a detailed analysis of the quantitative aspects of this correspondence. The live fluorescence of higher plants appears to originate in photosystem 2, and entirely from the Chl a antenna pigments. Fluorescence from PS 1 can be observed only at low temperatures. No live fluorescence of PS 1 has been reported. The absence of both live and dead fluorescence from PS 1 at room temperature is not understood at present, but it suggests a rather different reaction center configuration from those of PS 2 or photosynthetic bacteria.

Zankel, Reed and Clayton (1968) have studied reaction centers isolated from Rps. spheroides and have observed a "live" fluorescence that appears

to originate from the reaction center pigments themselves. On the basis of the very low quantum yield, approximately 4×10^{-4} , one can calculate a lifetime of 7 psec (7×10^{-12} sec) for the electronic excited state of the BChl of the reaction centers. The lifetime of the live fluorescence was investigated for intact bacteria using a sensitive phase fluorometer by Borisov and Godik (1970). They concluded that excitation reaches the open traps within 30-50 psec, which was at the limit imposed by their instrument. At the other extreme, the dead fluorescence from the bacteria when the traps were closed had a lifetime of 1.35 nsec. Since the quantum yield for photochemistry at low actinic intensities is of the order of unity in these preparations, the photochemical trapping must occur within a few picoseconds following absorption of a photon.

4.4 P870 and P700 - Primary Electron Donors

The first experimental evidence for the direct participation of bacteriochlorophyll or chlorophyll in the primary photochemistry was the observation by Duysens (1952) of a photoinduced, reversible bleaching of a small portion (ca. 1%) of the absorption spectrum of bacteriochlorophyll in intact bacterial cells or chromatophores. Although the difference spectrum varies somewhat from one organism to another, we shall refer to this species as P870, after the long-wavelength component of the bleaching. Shortly thereafter, Kok (1956; Kok and Hoch, 1961) reported an analogous species, P700, characteristic of higher plants and algae. P700 is now known to be associated with photosystem 1 (PS 1). In a search for a corresponding species associated with PS 2 in oxygen-evolving organisms, Döring and coworkers (Döring, Stiehl and Witt, 1967; Döring, Bailey et al., 1968; Döring, Renger et al., 1969) uncovered a rapidly reversible bleaching

at 680 nm; however, they concluded that its role may be an indirect one and not reflect the oxidation of a PS 2 reaction center chlorophyll (Witt, 1971). Floyd, Chance and De Vault (1971) showed that a portion of the P680 decays with kinetics corresponding to the oxidation of cyt b_{559} at low temperature. They favored placing P680 as an active component of the PS 2 reaction center. Butler (1972) has written a detailed analysis of this question, concluding that there is no good evidence at present to rule out P680 as the primary electron donor of PS 2.

At about the same time as the photoinduced absorption changes P870 and P700 were first observed, studies using EPR techniques uncovered the photoproduction of unpaired electrons in chloroplasts and in photosynthetic bacteria. Sharp, structureless EPR signals were observed to appear upon illumination and to decay in the dark (Commoner, Heise and Townsend, 1956; Calvin and Sogo, 1957). At first it proved difficult to establish the relationship between the absorption changes and the EPR signals; however, extensive studies carried out in several laboratories during the past few years have established a common origin for them. We are now reasonably confident that the bleaching of P870 or P700 and the appearance of the EPR signal (signal 1, in the case of higher plants) both witness the one-electron oxidation of (bacterio)chlorophyll in the reaction center. Kohl (1972) has written a comprehensive review of the properties of the EPR signals associated with the photosynthetic reactions.

Both P870⁺ and P700⁺ and the corresponding EPR signals in chromatophores and chloroplasts can be produced chemically in the dark through the use of an oxidant such as ferricyanide. Each active pigment undergoes a one-electron oxidation, and the reversible midpoint potentials are +0.44 V

for P870/P870⁺ (Kuntz, Loach and Calvin, 1964) and +0.43 V for P700/P700⁺ (Kok, 1961). The light-induced changes disappear progressively as the pigments are oxidized chemically, as shown in Fig. 13A. In Chromatium a somewhat higher potential of 0.49 V for P870 has been reported by Cusanovich, Bartsch and Kamen (1968).

Loach and Sekura (1967) showed that the decay kinetics of the photo-induced absorption changes owing to P870 are identical to those of the EPR signal. Further kinetic and stoichiometric evidence supporting the identification of P870⁺ as oxidized BChl and responsible for the EPR signal was reported by Bolton, Clayton and Reed (1969) and by McElroy, Feher and Mauzerall (1969). Fig. 14 illustrates this similar kinetic behavior for reaction centers from Rps. spheroides. Bolton, Clayton and Reed (1969) showed, furthermore, that the quantum yield for the formation of P870⁺ and of the EPR signal are both unity. In the case of higher plants, studies of Weaver (1958) and of Warden and Bolton (1972) demonstrated the identity of the kinetics of P700 bleaching and EPR signal 1, at the same time resolving some apparent earlier discrepancies for this species.

Under high intensity, laser-pulse illumination the onset times of the P870 and P700 absorption changes are too rapid to measure using currently available techniques of sensitive flash absorption spectrometry. The experiments show that they are faster than 50 nsec for P870 (Seibert, Dutton and De Vault, 1971) and faster than 20 nsec for P700 (Witt and Wolff, 1970). These limits are still very much longer than the trapping time of 7 psec (7×10^{-12} sec) estimated by Zankel, Reed and Clayton (1968) on the basis of low fluorescence yields in reaction center preparations. New techniques perhaps based on the mode-locked laser, will be required

Fig. 13. Oxidation reduction titrations of the light-induced P870 absorption change in chromatophores of R. rubrum. The two figures are taken from Kuntz, Loach and Calvin (1964). (A) Oxidation of P870 by added ferricyanide (open symbols), followed by a reductive titration with sodium dithionite (solid symbols). Measurements were made at three different wavelengths characteristic of the primary photochemical oxidation. The solid line is the theoretical curve for a one-electron oxidation with a midpoint potential of +0.439 V. The dashed line is the theoretical curve for a two-electron oxidation. (B) Titration of P870 at low potentials, presumably the reduction of the electron acceptor. The experimental procedure was varied for the different symbols (see original reference for details). The solid line is the theoretical curve for a one-electron reduction with a midpoint potential of -0.044 V; dashed line is for a two-electron reduction.

MU-30802 A

MU 30803 A

Fig. 14. Kinetics of the EPR and absorption changes at 795 nm for reaction centers of Rps. spheroides, R-26 mutant, upon actinic illumination. The same sample was used for both experiments. Rise times at 77°K, which vary with incident intensity from 14 ± 3 msec (at 10^5 erg-cm⁻²-sec⁻¹) to 30 ± 5 msec (at 10^4 erg-cm⁻²-sec⁻¹), agree within $\pm 10\%$ for the two measurements. Decay times are 30 ± 3 msec for both measurements at 77°K, and exhibit no significant change down to 1.7°K. The figure and the numerical results are taken from McElroy, Feher and Mauzerall (1969).

to investigate the nature of intermediates present between 10^{-11} and 10^{-8} sec.

Further evidence supporting the primacy of both the absorption and EPR signals comes from their characteristically high quantum yields and virtual independence of temperature. For bacterial systems both the EPR signal (Sogo, Jost and Calvin, 1959) and the P870 absorption change (Arnold and Clayton, 1960) can be induced rapidly by light at low temperatures, even down to 1°K. At room temperature the quantum yields are close to unity for both the P870 change (Loach and Sekura, 1968; Parson, 1968; Beugling, 1968; Bolton et al., 1969; Clayton, Fleming and Szuts, 1972; Clayton, Szuts and Fleming, 1972; Slooten, 1972) and for the EPR signal (Loach and Walsh, 1969; Weaver and Weaver, 1969; Loach and Hall, 1972) for chromatophores and reaction center preparations. The quantum yield of the P870 bleaching is changed hardly at all at 1°K (Clayton, 1962b).

The wide range of evidence cited above provides strong support for the proposal that the primary electron donor associated with the photosynthetic light reactions is (bacterio)chlorophyll in a special environment. In addition to the other chlorophylls present in the reaction centers (section 3.4), one important feature of this special environment is the close proximity of a good electron acceptor.

4.5 Primary Electron Acceptors

The primary electron acceptor is defined as the first component to receive an electron from the primary donor, now presumed to be P870 or P700. It must be recognized at the outset that the primary acceptor may prove to be more than one molecule (e.g., a pool of acceptors), or

there may be several different primary acceptors for a given donor (parallel pathways). Furthermore, the specific primary acceptor(s) may change depending on reaction conditions (e.g., electrochemical potential) or other chemical treatments which the photosynthetic materials have undergone.

A variety of compounds have been proposed for the primary electron acceptor in reaction centers. These include quinones, non-heme iron proteins, pteridines, cytochromes, a second chlorophyll molecule, and other components identified only in terms of absorbance changes or EPR signals. This is one of the most active areas of current research.

The difference between the electrochemical potentials of oxidized (bacterio)chlorophyll and the reduced primary acceptor defines the chemical potential produced from the absorbed photon. Ross and Calvin (1967) present arguments based on the second law of thermodynamics that limit this difference to about 1.2 eV for organisms containing Chl a and about 0.8 eV for photosynthetic bacteria. Since the oxidation of P700 and of P870 each exhibits a midpoint potential of about +0.44 V, this places lower limits for the potentials of the primary acceptors of about -0.76 V and -0.36 V in plants (PS 1) and bacteria, respectively.

If an external chemical reducing agent added to the photosynthetic materials is able to reduce the primary acceptor

then P870 or P700 are no longer able to transfer electrons upon illumination and the associated absorption and EPR changes disappear. The state $C \cdot A^-$ is inactive photochemically and exhibits a high fluorescence efficiency (Clayton, 1966b). Kuntz, Loach and Calvin (1964) found a midpoint

potential of -0.04 V for a reversible, one-electron reductive titration of P800 (coupled to P870) in the bacterium R. rubrum (Fig. 13B). A similar value, -0.03 V, was found by Nicholson and Clayton (1969) for isolated reaction centers from Rps. spheroides. Both of these investigations used steady illumination. Using flash spectroscopy following brief (10 nsec) laser pulses, Dutton (1971) and Seibert and De Vault (1971) found values near -0.14 V for Chromatium D and for Rps. gelatinosa, and obtained evidence that a small portion of the light reactions was operative down to -0.35 V. Although these potentials are measured for different organisms and, therefore, need not be the same, it is important to recognize that measurements made using steady illumination may differ from those using brief flashes even for the same organism. The origin of this difference is kinetic rather than thermodynamic, and we shall return to consider the underlying reasons shortly.

In the case of chloroplasts from higher plants, a series of studies using added weak electron acceptors (Kok, Rurainski and Owens, 1965; Zweig and Avron, 1965) point to a potential of about -0.50 V for the primary acceptor. In some cases added electron acceptors with midpoint potentials below -0.60 V could be reduced to some extent by chloroplasts (Kok, Rurainski and Owens, 1965; Black, 1966). These studies were all carried out using steady illumination; however, they did not involve reversible titrations of an endogenous chloroplast electron acceptor. It is possible for an electron donor to reduce an acceptor with a more negative midpoint potential if the concentration ratios of oxidized and reduced forms of the respective molecules favor the transfer. For one-electron carriers, in accordance with the Nernst equation, each order of

magnitude change in the concentration ratio adds (or subtracts) 0.06 V to its effective potential. As a consequence, one must be cautious in interpreting the results of such studies.

In none of these cases does the outside limit for the potential of the primary acceptors appear to challenge the thermodynamic constraints. As more definite information becomes available, it will be interesting to learn what is the actual thermodynamic efficiency of photosynthetic energy conversion.

Attempts have been made to isolate and characterize the primary acceptor of chloroplasts. Ferredoxin has been proposed to serve this role, particularly by Arnon (1969). The midpoint potential of soluble ferredoxin, which is -0.45 V, would seem to make it too weak a reductant to account for the photo-reduction of the viologen or dipyridyl dyes (Kok, Rurainski and Owens, 1965; Black, 1966). A ferredoxin-reducing substance (FRS) has been reported by Yocum and San Pietro (1969; 1970), but it has proved elusive in other laboratories and has so far defied a clear definition.

The primary acceptor is membrane-bound both in plants and in bacteria; the P870 and P700 absorption changes survive cell breakage and extensive washing. Recent studies by Hiyama and Ke (1971a, 1971b, 1972) have identified an absorption change with a maximum at 430 nm in chloroplast particles enriched in photosystem I activity and having the characteristics of the primary acceptor. It couples to P700 and exhibits a one-electron reduction with a midpoint potential at about -0.47 V. The rise time for this absorption change is 100 nsec or less following a brief laser flash. Observations have been reported only for detergent treated or sonicated membranes so far, and there remains the possibility that this acceptor is not the physiological primary one.

Membrane-bound ferredoxin has been found in chloroplasts and has been implicated as the primary acceptor in higher plants on the basis of low temperature EPR spectroscopy by Malkin and Bearden (1971; Bearden and Malkin, 1972). In this case whole chloroplasts were studied, but it was necessary to cool the samples to 25°K in order to assay the reduced acceptor. The evidence consists of several EPR features, typical of those observed for the non-heme iron, labile sulfur proteins of which ferredoxin is an example. A comparison of these EPR signals is shown in Fig. 15. The EPR spectrum appears upon illumination of chloroplasts at 77°K or room temperature (Leigh and Dutton, 1972; Evans, Telfer and Lord, 1972), or from chemical reduction using dithionite.* Yang and Blumberg (1972) report that amount of ferredoxin signal measured at 1.5°K is only 10-20% of the P700 EPR signal amplitude; however, Bearden and Malkin (1973) find a ratio close to unity in a set of carefully documented quantitative measurements at 20°K.

Neither kinetic nor redox titration studies have been possible using the low temperature EPR assay in order to establish the role of this component under physiological conditions. Because of the apparent requirement for a primary acceptor with a midpoint potential of -0.50 V, the membrane-bound "ferredoxin" may have a somewhat lower potential than does the soluble ferredoxin or it may be a chemically distinct species with the observed EPR spectrum. Such a modification or chemical distinction would be required to reconcile the EPR results with the P430 of Hiyama and Ke (1971b), for soluble ferredoxin does not exhibit the absorption difference spectrum reported for P430 (Fig. 16).

*Dithionite is commonly used as a "strong" reductant; however, its effective potential is difficult to deduce because of a complex dependence on pH and because of the instability of the species in solution.

Fig. 15. EPR spectra of species obtained from spinach chloroplasts. (A) Soluble spinach ferredoxin, chemically reduced with sodium dithionite and measured at 20°K (Malkin and Bearden, 1971). (B) Whole spinach chloroplasts, illuminated using 715 nm light and measured at 25°K. The spectrum of an unilluminated (dark) sample has been subtracted (Bearden and Malkin, 1973). The large signal at about 3320 gauss is the $g = 2.0025$ signal of $P700^+$. The features at $g = 1.86$, 1.94 and 2.05 are to be compared with the EPR spectrum of reduced ferredoxin in part (A).

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Fig. 16. Comparison of the dark minus light difference spectrum of the P430 of Hiyama and Ke (1971) with the oxidized-minus-reduced difference spectrum of spinach ferredoxin. The two curves have been arbitrarily normalized near 430 nm for purposes of comparison; however, Hiyama and Ke estimate that $\Delta\epsilon_{430}$ (light-dark) for P430 is $12 \pm 3 \text{ mM}^{-1}\text{cm}^{-1}$, whereas the $\Delta\epsilon_{430}$ (oxidized-reduced) for ferredoxin is only about $3 \text{ mM}^{-1}\text{cm}^{-1}$.

The search for the primary acceptor in photosynthetic bacteria has resulted in similar complexities. Feher (1971) reported an EPR spectrum of three broad lines that accompanies the sharp signal of BChl^+ of Rps. spheroides reaction centers at 1.4°K (Fig. 17). The broad lines, which are generally reminiscent of non-heme iron, are produced in the light and they decay in the dark with the 30 msec time constant of the BChl^+ EPR signal. The preparation of reaction centers used in this particular study by Feher contained one iron per P870. A similar low temperature EPR signal has been observed for subchromatophore particles from Chromatium (Leigh and Dutton, 1972).

Subsequently Loach and Hall (1972), working with a preparation of photoreceptor subunits from R. rubrum containing active P870 but only 0.30 equivalents of Fe per reaction center, observed a rather narrow EPR signal at $g = 2.0050$ which was kinetically similar to the BChl^+ signal at $g = 2.0025$. This observation was corroborated by Feher, Okamura and McElroy (1972) using a new low-iron preparation of active reaction centers from Rps. spheroides obtained by more extensive detergent treatment of their previous preparation (Fig. 18). By measuring this EPR spectrum also at higher microwave frequencies, these workers were able to demonstrate a striking similarity to the EPR signal of the radical anion of ubiquinone. They postulate that ubiquinone is not the physiological primary acceptor, but has adopted this role by virtue of the removal of the iron-containing species. Ke (1969) had previously examined absorbance changes at 280 nm attributable to ubiquinone in untreated Chromatium chromatophores. On the basis of the disappearance of these changes in the presence of reducing agents that did not abolish the P890 changes, he concluded that it behaved

Fig. 17. The light induced EPR signal at 1.4°K for Rps. spheroides (R-26 mutant) reaction centers using either actinic light (upper trace) or infrared radiation that is not actinic (lower trace), taken from Feher (1971). Note, in comparison with the traces shown in Figs. 15 and 18, that this scan is over a much wider range of magnetic field, and that the spectrum is shown in the absorption mode rather than in the more conventional first derivative mode.

Reaction Centers Rps. Spheroides R-26

X⁻ Radical

Ubiquinone Radical

Fig. 18. The light induced EPR signal for a subunit of *Rps. spheroides* (R-26 mutant) reaction centers with low iron content (spectra at the left) and the EPR spectra of the radical of ubiquinone Q-50 (spectra at the right) ($T = 1.3^\circ K$). Curves shown at the top and bottom in each case were measured at different microwave frequencies. The higher microwave frequency provides a much better resolution of the components of the g-tensor. Figures are reproduced from Feher, Okamura and McElroy (1972).

like a secondary rather than a primary electron acceptor from BChl. Reed, Zankel and Clayton (1969) came to a similar conclusion for reaction centers from Rps. spheroides. Clayton, Fleming and Szuts (1972) presented evidence for a one-electron acceptor prior to ubiquinone in reaction centers from Rps. spheroides, confirming the conclusion of Ke and of Reed, Zankel and Clayton. A new spectroscopic component undergoing absorption changes at 450 nm has been studied by Clayton and Straley (1970) and proposed as a candidate for the primary electron acceptor.

With such a plethora of possible primary acceptors, it is refreshing that some previous candidates can now apparently be ruled out. Reed and Mayne (1971) have looked for pteridines in chromatophores and reaction centers prepared from the R-26 mutant of Rps. spheroides and have found a convincingly insufficient amount present for it to remain a serious candidate.

Rather than leave wide open the question of why there should be such apparently contradictory evidence concerning the nature of the primary acceptor, it is worthwhile first to examine some possible limitations inherent in the experimental findings. The distinction between primary and secondary electron acceptors is important to appreciate. Primacy is determined by which of several acceptors receives the electron first from the chlorophyll. If the electron is transferred quickly to the secondary acceptor (see eq. 4-2) or if there is rapid equilibration between the primary and secondary acceptors, then both of them will appear to change with the kinetics of the $C \rightarrow C^+$ reaction. There are still several ways in which the distinction could be made, however. By using very short, high intensity light pulses the primary process (eq. 4-1) may be speeded up sufficiently to separate it from the secondary $A_1^- + A_2 \rightarrow A_1 + A_2^-$

reaction, as in the studies of the 280 nm absorption change by Ke (1969). Alternatively, an inhibitor of this last reaction that still permits electrons to be transferred to A_1 , can serve to distinguish between them. Clayton, Szuts and Fleming (1972) report that o-phenanthroline serves further to characterize ubiquinone as a secondary acceptor in Rps. spheroides reaction centers. However, in this approach, as with the extensive detergent treatment, there remains the possibility that the normal pathways are altered and non-physiological ones are exposed by the chemical treatment.

A second complication arises in the event of rapid equilibration of primary and secondary electron acceptors. Chemical titrations or photochemical processes occurring on a long time scale will sense only some average property--e.g., midpoint potential--of the heterogeneous pool. Even when the addition of substances accelerates or retards the rates of the coupled reactions to the same extent, one cannot be certain that the primary and secondary acceptors do not equilibrate at a still more rapid rate. Reductive titrations, such as the disappearance of the P870 change, will then appear to involve two (or more) electrons, as was observed for whole cells and chromatophores of R. rubrum by Loach (1966). For the bacterial chromatophores of Rps. gelatinosum, but not for those of Chromatium, Dutton (1971) found titration evidence of two primary acceptors. One has a midpoint potential of -0.14 V, but the other is still active at -0.35 V. He considered the possibility that these participate in two different reaction centers in Rps. gelatinosum.

The primary reactants of photosystem 2 of higher plants are even less well understood. The case for the fast absorption change of P680 reflecting

the primary electron donor was described in section 4.4. A component that appears to control the variable fluorescence of PS 2, designated Q by Duysens and Sweers (1963), has been implicated as the primary acceptor. It behaves identically to a component, C-550, that is seen in difference absorption spectra (Knaff and Arnon, 1969; Erixon and Butler, 1971) and may be the same as the species responsible for the EPR signal associated with PS 2, which is probably a plastoquinone radical or closely similar species (Kohl and Wood, 1969; Kohl, 1972).

Recent experiments of Mauzerall (1972) showed, however, that the fluorescence rise controlled by Q is delayed following a brief laser flash. The delay is 25 nsec following the first flash after prolonged darkness, but it increases to 3 μ sec for subsequent flashes in a closely spaced series. These delays are sufficiently long to provide evidence against Q being the primary acceptor of PS 2. Because the studies of Erixon and Butler (1971) did not involve fast kinetic measurements, there remains the possibility that C-550 acts prior to Q and is an earlier acceptor. The two components could be oxidatively coupled in a pseudo-reversible fashion on the slower time scale. Support for a component between the primary donor and Q comes from experiments of Govindjee, Döring and Govindjee (1970). In studies on lyophilized chloroplasts extracted with "wet" heptane, the characteristic absorption changes of P680 are still present. The constant, high fluorescence yield of this preparation is an indication that Q has been removed, or at least inactivated. Butler (1972) discusses the consequences of these experiments in some detail (but without knowledge of the result of Mauzerall) as well as making a case for P680 as the primary electron donor.

Because of the many and sometimes conflicting reports of the nature of the primary process in photosynthetic energy conversion, it is useful

to outline a current picture of some of the important features discussed in the preceding paragraphs. This picture must be considered provisional, owing to the uncertainties of some of the conclusions.

(1) The immediate consequence of excitation trapping is a one-electron transfer reaction that occurs with a quantum yield near unity.

(2) The primary electron transfer occurs in a time less than 20 to 50 nsec and is temperature independent down to 1°K.

(3) The primary electron donor is a chlorophyll or bacteriochlorophyll molecule that shares its (photo)oxidized state with one or two additional (bacterio)chlorophylls in the reaction center complex. The midpoint potential for this oxidation is about +0.44 V.

(4) The identity of the primary electron acceptor molecule(s) is uncertain, but ferredoxin-like non-heme iron proteins or quinones are popular candidates. The reduction of the primary acceptor occurs with a midpoint potential of -0.03 V or less in photosynthetic bacteria and -0.5 V or less in PS 1 of higher plants.

4.6 --- And Beyond

With this discussion of the nature of primary electron donors and acceptors we have reached the limit of the territory that we set out to explore. We can see pathways leading off in various directions; in many cases their topography is described in other chapters in this volume. For example, we have said relatively little here about the processes associated with oxygen evolution in higher plants and algae, which is central to the discussions of Chapters 5, 7 and 11.

New insights will help to open fresh avenues of approach to photosynthetic energy conversion. To cite just one example, recent studies

of Parson and coworkers (Case and Parson, 1971; Callis, Parson and Gouterman, 1972) show that the storage of free energy in the primary process in photosynthetic bacteria is primarily an entropy effect, and has little if any contribution from enthalpy or chemical bond energy sources. One way to interpret this finding is to view the primary process in terms of a photoinduced rearrangement of the electron distribution of the molecules in the reaction center complex, in a way that does not result in an increase in the enthalpy of the system. The chemical potential associated with this charge redistribution may arise in a fashion analogous to that for solid-state photovoltaic devices (solar cells). The EPR evidence that indicates the delocalization of the unpaired electron over more than one chlorophyll molecule in photooxidized reaction centers (section 3.4) may be a manifestation of this entropy contribution. On the basis of absorption and CD spectroscopy, all reaction centers studied so far appear to contain more than one (bacterio)chlorophyll molecule.

In most of the work that has been described in this chapter, more questions have been raised than have been answered. Although we have come a long way in the past 40 years, the next generation need have no fear that the major outstanding problems are all but wrapped up. We still are very far from knowing nature's secret for converting solar energy into useful chemical potential on such an enormous scale. At the same time there is reason to hope that we can improve on the present utilization of sunlight. Photosynthesis makes use of only a fraction of 1% of the intensity at suitable wavelengths falling on the surface of the earth. This is an important direction that we must explore in order to conserve our dwindling resources of conventional (including fissionable) fuels.

5. Summary

In the preceding pages a broad map has been sketched, covering the processes of absorption of radiation, excitation transfer and photochemical trapping. Although much detailed information is still missing, it is worthwhile presenting a brief summary of those areas that we feel confident about and those which are largely unknown.

(1) The excitation of molecules of chlorophyll (Chl a, BChl a, etc.) is prerequisite to photosynthetic energy conversion. While other molecules may serve for the initial absorption of radiation, excitation which is not transferred to one of the principal chlorophyll pigments is not available for photochemistry.

(2) The sites at which the photochemical trapping occurs are located in lipo-protein membranes. In these membranes they occur at a relatively low concentration (1% or less) in comparison with the total chlorophyll of the antennas.

(3) Quantum yields for photon utilization can approach 100%, which demonstrates that excitation can move from the many antenna molecules to the few traps with very little loss.

(4) The isolation of chlorophyll-proteins, reaction center particles and electron transport proteins in good yields has led to the formulation of a membrane model--the pebble mosaic model--in which these and other components constitute subunits of a photosynthetic unit. The membranes are visualized as larger arrays formed by repetition of these basic subunits in an interacting network.

(5) Excitation transfer occurs via two distinct mechanisms: exciton delocalization within small closely coupled groups of chlorophylls in the

chlorophyll proteins, and resonance or Förster transfer between the chlorophyll proteins.

(6) Trapping occurs when the electronic excitation has been converted to separate oxidized and reduced species in the reaction centers. The molecule oxidized is a special reaction center chlorophyll; the specific nature of molecule reduced is uncertain, but non-heme iron proteins or quinones are current popular candidates.

(7) There is a span of time, between 10^{-13} and 10^{-8} sec following photon absorption about which we know almost nothing. There may be essential steps in this interval involving conduction electrons, triplet states, or other entities that will only be confirmed by a new generation of experiments.

(8) Finally, nothing that we are reasonably certain of in the current body of photosynthetic knowledge leads us to require processes outside our current experience in physics or chemistry. Quantum yields are comfortably at or less than unity; neither the first nor the second law of thermodynamics is under attack; tested mechanisms of excitation transfer are adequate to account for the efficiency of trapping; and the chemical properties of the primary donors and acceptors are reasonable ones for the molecules proposed.

In other words, we need not look for totally new phenomena to be at the heart of this fundamental biological process. It appears that the best set of tools for elucidating photosynthetic energy conversion will include solid foundations in chemistry and physics--and an adventurous mind.

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- Arnold, W. (1965). J. Phys. Chem. 69, 788.
- Arnold, W. and Clayton, R. K. (1960). Proc. Nat. Acad. Sci. U.S.A. 46, 769.
- Arnold, W. and Sherwood, H. K. (1957). Proc. Nat. Acad. Sci. U.S.A. 43, 105.
- Arnon, D. I. (1969). Naturwissenschaften 56, 295.
- Ballschmiter, K. (1969). Angew. Chem., Int. Ed. 8, 617.
- Ballschmiter, K., Truesdell, K. and Katz, J. J. (1969). Biochim. Biophys. Acta 184, 604.
- Bay, Z. and Pearlstein, R. M. (1963). Proc. Nat. Acad. Sci. U.S.A. 50, 962.
- Bearden, A. J. and Malkin, R. (1972). Biochem. Biophys. Res. Commun. 46, 1299.
- Bearden, A. J. and Malkin, R. (1973). Biochim. Biophys. Acta, in press.
- Black, Jr., C. C. (1966). Biochim. Biophys. Acta 120, 332.
- Blyumenfel'd, L. A., Kafalieva, D. N., Livshits, V. A., Solov'ev, I. S. and Chetverikov, A. G. (1970). Dokl. Akad. Nauk SSSR 193, 700.
- Bogomolni, R. A. (1972). Ph.D. Thesis, University of California, Berkeley, Lawrence Berkeley Laboratory Report LBL-1036.
- Bolton, J. R., Clayton, R. K. and Reed, D. W. (1969). Photochem. Photobiol. 9, 209.
- Borisov, A. Yu. (1967). Dokl. Akad. Nauk SSSR 173, 208.
- Borisov, A. Yu. and Fetisova, Z. G. (1971). Molekul. Biol. 5, 509.
- Borisov, A. Yu. and Godik, V. I. (1970). Biochim. Biophys. Acta 223, 441.
- Borisov, A. Yu., Godik, V. I. and Chibisov, A. K. (1970). Molekul. Biol. 4, 500.

- Bowers, P. G. and Porter, G. (1967). Proc. Roy. Soc. A296, 435.
- Branton, D. and Park, R. B. (1967). J. Ultrastructure Res. 19, 283.
- Breton, J. and Roux, E. (1971). Biochem. Biophys. Res. Commun. 45, 557.
- Briantais, J.-M. and Picaud, M. (1972). FEBS Letters 20, 100.
- Brody, S. S. (1958). Science 128, 838.
- Brody, S. S. and Rabinowitch, E. (1957). Science 125, 555.
- Brown, J. S. and French, C. S. (1959). Plant Physiol. 34, 305.
- Butler, W. L. (1961). Arch. Biochem. Biophys. 93, 413.
- Butler, W. L. (1966). In "The Chlorophylls" (L. P. Vernon and G. R. Seely, eds.), pp. 343-379. Academic Press, New York.
- Butler, W. L. (1972). Biophys. J. 12, 851.
- Butler, W. L. and Hopkins, D. W. (1970a). Photochem. Photobiol. 12, 439.
- Butler, W. L. and Hopkins, D. W. (1970b). Photochem. Photobiol. 12, 451.
- Butler, W. L. and Norris, K. H. (1963). Biochim. Biophys. Acta 66, 72.
- Callis, J. B., Parson, W. W. and Gouterman, M. (1972). Biochim. Biophys. Acta 267, 348.
- Calvin, M. (1958). Brookhaven Symp. Biol. 11, 160.
- Calvin, M. and Sogo, P. B. (1957). Science 125, 499.
- Case, G. D. and Parson, W. W. (1971). Biochim. Biophys. Acta 253, 187.
- Clayton, R. K. (1962a). Photochem. Photobiol. 1, 201.
- Clayton, R. K. (1962b). Photochem. Photobiol. 1, 305.
- Clayton, R. K. (1963a). In "Bacterial Photosynthesis" (H. Gest, A. San Pietro and L. P. Vernon, eds.), p. 495. Antioch Press, Yellow Springs, Ohio.
- Clayton, R. K. (1963b). Biochim. Biophys. Acta 75, 312.
- Clayton, R. K. (1965). "Molecular Physics in Photosynthesis." Blaisdell Publ., New York.

- Clayton, R. K. (1966a). Photochem. Photobiol. 5, 669.
- Clayton, R. K. (1966b). Photochem. Photobiol. 5, 679.
- Clayton, R. K. (1966c). Photochem. Photobiol. 5, 807.
- Clayton, R. K. (1967). J. Theoret. Biol. 14, 173.
- Clayton, R. K. (1969). Biophys. J. 9, 60.
- Clayton, R. K. (1970). "Light and Living Matter," Vol. 1, The Physical Part. McGraw-Hill, New York.
- Clayton, R. K. (1971). "Light and Living Matter," Vol. 2, The Biological Part. McGraw-Hill, New York.
- Clayton, R. K. (1972). Proc. Nat. Acad. Sci. U.S.A. 69, 44.
- Clayton, R. K., Fleming, H. and Szuts, E. Z. (1972). Biophys. J. 12, 46.
- Clayton, R. K. and Haselkorn, R. (1972). J. Mol. Biol. 68, 97.
- Clayton, R. K. and Siström, W. R. (1966). Photochem. Photobiol. 5, 661.
- Clayton, R. K. and Straley, S. C. (1970). Biochem. Biophys. Res. Commun. 39, 1114.
- Clayton, R. K., Szuts, E. Z. and Fleming, H. (1972). Biophys. J. 12, 64.
- Clayton, R. K. and Wang, R. T. (1971). Methods in Enzymology 23, 696.
- Clayton, R. K. and Yau, H. F. (1972). Biophys. J. 12, 867.
- Closs, G. L., Katz, J. J., Pennington, F. C., Thomas, M. R. and Strain, H. H. (1963). J. Amer. Chem. Soc. 85, 3809.
- Commoner, B., Heise, J. J. and Townsend, J. (1956). Proc. Nat. Acad. Sci. U.S.A. 42, 710.
- Crabbé, P. (1972). "ORD and CD in Chemistry and Biochemistry." Academic Press, New York.
- Cusanovich, M. A., Bartsch, R. G. and Kamen, M. D. (1968). Biochim. Biophys. Acta 153, 397.

- Dietrich, Jr., W. E. and Thornber, J. P. (1971). Biochim. Biophys. Acta 245, 482.
- Dilley, R. A., Peters, G. A. and Shaw, E. R. (1972). J. Membrane Biol. 8, 163.
- Dimitrievsky, O. D., Ermolaev, V. L. and Terenin, A. N. (1957). Dokl. Akad. Nauk SSSR 114, 751.
- Döring, G., Bailey, J. L., Kreutz, W., Weikard, J. and Witt, H. T. (1968). Naturwissenschaften 55, 219.
- Döring, G., Renger, G., Vater, J. and Witt, H. T. (1969). Z. Naturforsch. 24b, 1139.
- Döring, G., Stiehl, H. H. and Witt, H. T. (1967). Z. Naturforsch. 22b, 639.
- Dratz, E. A., Schultz, A. J. and Sauer, K. (1966). Brookhaven Symp. Biol. 19, 303.
- Dutton, P. L. (1971). Biochim. Biophys. Acta 226, 63.
- Dutton, P. L., Leigh, J. S. and Seibert, M. (1972). Biochem. Biophys. Res. Commun. 46, 406.
- Duysens, L. N. M. (1952). Thesis, University of Utrecht.
- Duysens, L. N. M. (1964). Progress in Biophysics and Molecular Biology 14, 1.
- Duysens, L. N. M. (1966a). Brookhaven Symp. Biol. 19, 71.
- Duysens, L. N. M. (1966b). In "Currents in Photosynthesis" (J. B. Thomas and J. C. Goedheer, eds.), pp. 263-269. Donker, Rotterdam.
- Duysens, L. N. M. and Sweers, H. E. (1963). In "Studies on Microalgae and Photosynthetic Bacteria," pp. 353-372. University of Tokyo Press, Tokyo.
- Ebrey, T. G. and Clayton, R. K. (1969). Photochem. Photobiol. 10, 109.

- Emerson, R. and Arnold, W. (1932a). J. Gen. Physiol. 15, 391.
- Emerson, R. and Arnold, W. (1932b). J. Gen. Physiol. 16, 191.
- Erixon, K. and Butler, W. L. (1971). Biochim. Biophys. Acta 234, 381.
- Evans, M. C. W., Telfer, A. and Lord, A. V. (1972). Biochim. Biophys. Acta 267, 530.
- Feher, G. (1971). Photochem. Photobiol. 14, 373.
- Feher, G., Okamura, M. Y. and McElroy, J. D. (1972). Biochim. Biophys. Acta 267, 222.
- Feher, G., Okamura, M. Y., Raymond, J. A. and Steiner, L. A. (1971). Biophys. Soc. Abstr., New Orleans, 11, 38a.
- Fernandez, J. and Becker, R. S. (1959). J. Chem. Phys. 31, 467.
- Fischer, M. S., Templeton, D. H., Zalkin, A. and Calvin, M. (1972). J. Amer. Chem. Soc. 94, 3613.
- Floyd, R. A., Chance, B. and De Vault, D. (1971). Biochim. Biophys. Acta 226, 103.
- Förster, Th. (1965). In "Modern Quantum Chemistry" (O. Sinanoğlu, ed.), p. 93-137. Academic Press, New York.
- Fowler, C. F., Nugent, N. A. and Fuller, R. C. (1971). Proc. Nat. Acad. Sci. U.S.A. 68, 2278.
- Fowler, C. F., Nugent, N. A. and Fuller, R. C. (1972). Biophys. Soc. Abstr., Toronto, 12, 87a.
- Fraker, P. J. and Kaplan, S. (1972). J. Biol. Chem. 247, 2732.
- Franck, J. and Rosenberg, J. L. (1964). J. Theoret. Biol. 7, 276.
- Frei, Y. F. (1962). Biochim. Biophys. Acta 57, 82.
- Garcia, A., Vernon, L. P. and Mollenhauer, H. (1966). Biochemistry 5, 2399.

- Gingras, G. and Jolchine, G. (1969). In "Progress in Photosynthesis Research," Vol. I (H. Metzner, ed.), pp. 209-216. H. Laupp, Tübingen.
- Goedheer, J. C. (1957). Thesis, University of Utrecht.
- Goedheer, J. C. (1961). Biochim. Biophys. Acta 53, 420.
- Goedheer, J. C. (1966). In "The Chlorophylls (L. P. Vernon and G. R. Seely, eds.), pp. 147-184. Academic Press, New York.
- Gouterman, M. and Stryer, L. (1962). J. Chem. Phys. 37, 2260.
- Govindjee, Döring, G. and Govindjee, R. (1970). Biochim. Biophys. Acta 205, 303.
- Green, D., Allmann, D., Bachman, E., Baum, H., Kopaczyk, K., Korman, E., Lipton, S., MacLennan, D., McConnell, D., Perdue, J., Rieske, J. and Tzagoloff, A. (1967). Arch. Biochem. Biophys. 119, 312.
- Gregory, R. P. F., Raps, S. and Bertsch, W. (1971). Biochim. Biophys. Acta 234, 330.
- Gurinovich, G. P., Sevchenko, A. N. and Solov'ev, K. N. (1968). "Spectroscopy of Chlorophyll and Related Compounds," Izdatel'stvo Nauka i Tekhnika, Minsk (English Translation, U. S. Atomic Energy Commission, AEC-tr-7199, 1971).
- Hanna, M. W. (1969). "Quantum Mechanics in Chemistry," 2nd Ed., Chapter 4. W. A. Benjamin, New York.
- Hiyama, T. and Ke, B. (1971a). Proc. Nat. Acad. Sci. U.S.A. 68, 1010.
- Hiyama, T. and Ke, B. (1971b). Arch. Biochem. Biophys. 147, 99.
- Hoch, G. and Knox, R. S. (1968). In "Photophysiology" (A. Giese, ed.), Vol. 3, pp. 225-51. Academic Press, New York.
- Hochstrasser, R. M. and Kasha, M. (1964). Photochem. Photobiol. 3, 317.
- Holt, A. S. and Clayton, R. K. (1965). Photochem. Photobiol. 4, 829.

- Jolchine, G., Reiss-Husson, F. and Kamen, M. D. (1969). Proc. Nat. Acad. Sci. U.S.A. 64, 650.
- Kasha, M. (1963). Rad. Res. 20, 55.
- Katz, J. J., Ballschmiter, K., Garcia-Morin, M., Strain, H. H. and Uphaus, R. A. (1968). Proc. Nat. Acad. Sci. U.S.A. 60, 100.
- Katz, J. J., Closs, G. L., Pennington, F. C., Thomas, M. R. and Strain, H. H. (1963). J. Amer. Chem. Soc. 85, 3801.
- Katz, J. J., Dougherty, R. C. and Boucher, L. J. (1966). In "The Chlorophylls" (L. P. Vernon and G. R. Seely, eds.), pp. 185-251. Academic Press, New York.
- Ke, B. (1969). Biochim. Biophys. Acta 172, 583.
- Ke, B. (1972). Arch. Biochem. Biophys., in press.
- Knaff, D. B. and Arnon, D. I. (1969). Proc. Nat. Acad. Sci. U.S.A. 63, 963.
- Knox, R. S. (1968). J. Theoret. Biol. 21, 244.
- Knox, R. S. and Chang, J. C. (1971). Abstracts, Conf. on Primary Photochemistry of Photosynthesis, Argonne, Ill., p. 13.
- Kohl, D. H. (1972). In "Biological Applications of Electron Spin Resonance" (H. M. Swartz, J. R. Bolton and D. C. Borg, eds.), pp. 213-264. Academic Press, New York.
- Kohl, D. H. and Wood, P. M. (1969). Plant Physiol. 44, 1439.
- Kok, B. (1956). Biochim. Biophys. Acta 22, 399.
- Kok, B. (1961). Biochim. Biophys. Acta 48, 527.
- Kok, B. and Hoch, G. (1961). In "Light and Life" (W. D. McElroy and B. Glass, eds.), pp. 397-416. Johns Hopkins Press, Baltimore.
- Kok, B., Rurainski, H. J. and Owens, O. von H. (1965). Biochim. Biophys. Acta 109, 347.

- Kreutz, W. (1970). Adv. Bot. Res. 3, 53.
- Kung, S. D. and Thornber, J. P. (1971). Biochim. Biophys. Acta 253, 285.
- Kuntz, Jr., I. D., Loach, P. A. and Calvin, M. (1964). Biophys. J. 4, 227.
- Latimer, P., Bannister, T. T. and Rabinowitch, E. I. (1956). Science 124, 585.
- Leigh, Jr., J. S. and Dutton, P. L. (1972). Biochem. Biophys. Res. Commun. 46, 414.
- Lintilhac, P. M. and Park, R. B. (1966). J. Cell Biol. 28, 582.
- Livingston, R. (1960). Rad. Res., Suppl. 2, 196.
- Livingston, R., Porter, G. and Windsor, M. (1954). Nature 173, 485.
- Loach, P. A. (1966). Biochemistry 5, 592.
- Loach, P. A., Androes, G. M., Maksim, A. F. and Calvin, M. (1963). Photochem. Photobiol. 2, 443.
- Loach, P. A., Hadsell, R. M., Sekura, D. L. and Stemer, A. (1970). Biochemistry 9, 3127.
- Loach, P. A. and Hall, R. L. (1972). Proc. Nat. Acad. Sci. 69, 786.
- Loach, P. A. and Sekura, D. L. (1967). Photochem. Photobiol. 6, 381.
- Loach, P. A. and Sekura, D. L. (1968). Biochemistry 7, 2642.
- Loach, P. A., Sekura, D. L., Hadsell, R. M. and Stemer, A. (1970). Biochemistry 9, 724.
- Loach, P. A. and Walsh, K. (1969). Biochemistry 8, 1908.
- Malkin, R. and Bearden, A. J. (1971). Proc. Nat. Acad. Sci. U.S.A. 68, 16.
- Mauzerall, D. (1972). Proc. Nat. Acad. Sci. U.S.A. 69, 1358.
- McElroy, J. D., Feher, G. and Mauzerall, D. C. (1969). Biochim. Biophys. Acta 172, 180.
- McElroy, J. D., Feher, G. and Mauzerall, D. C. (1972). Biochim. Biophys. Acta 267, 363.

- Moraw, R. and Witt, H. T. (1961). Z. Physikal. Chem. (Frankfurt) 29, 25.
- Morita, S. and Miyazaki, T. (1971). Biochim. Biophys. Acta 245, 151.
- Müller, A., Lumry, R. and Walker, M. S. (1969). Photochem. Photobiol. 9, 113.
- Murata, T. and Murata, N. (1971). Carnegie Inst. Wash. Yearbook 70, 504.
- Murata, N. and Takamiya, A. (1969). Plant & Cell Physiol. 10, 193.
- Murata, T., Toda, F., Uchino, K. and Yakushiji, E. (1971). Biochim. Biophys. Acta 245, 208.
- Nicolson, G. L. and Clayton, R. K. (1969). Photochem. Photobiol. 9, 395.
- Nicholson, W. J. and Fortoul, J. I. (1967). Biochim. Biophys. Acta 143, 577.
- Norris, J. R., Uphaus, R. A., Crespi, H. L. and Katz, J. J. (1971). Proc. Nat. Acad. Sci. U.S.A. 68, 625.
- Oelze, J. and Drews, G. (1972). Biochim. Biophys. Acta 265, 209.
- Olson, J. M. (1971). Methods in Enzymology 23, 636.
- Olson, J. M., Philipson, K. D. and Sauer, K. (1972), Biochim. Biophys. Acta, in press.
- Olson, J. M. and Romano, C. A. (1962). Biochim. Biophys. Acta 59, 726.
- Olson, R. A., Butler, W. L. and Jennings, W. H. (1961). Biochim. Biophys. Acta 54, 615.
- Olson, R. A., Butler, W. L. and Jennings, W. H. (1962). Biochim. Biophys. Acta 58, 144.
- Orchin, M. and Jaffé, H. H. (1971). "Symmetry, Orbitals and Spectra," Chapter 8. Wiley-Interscience, New York.
- Park, R. B. (1966). In "The Chlorophylls" (L. P. Vernon and G. R. Seely, eds.), pp. 283-311. Academic Press, New York.
- Park, R. B. and Biggins, J. (1964). Science 144, 1009.

- Park, R. B. and Pon, N. G. (1961). J. Mol. Biol. 3, 1.
- Parson, W. W. (1968). Biochim. Biophys. Acta 153, 248.
- Parson, W. W. (1969a). Biochim. Biophys. Acta 189, 384.
- Parson, W. W. (1969b). Biochim. Biophys. Acta 189, 397.
- Philipson, K. D., Sato, V. L. and Sauer, K. (1972). Biochemistry, in press.
- Philipson, K. D. and Sauer, K. (1972a). Biochemistry 11, 1880.
- Philipson, K. D. and Sauer, K. (1972b). Submitted.
- Philipson, K. D., Tsai, S. C. and Sauer, K. (1971). J. Phys. Chem. 75, 1440.
- Rabinowitch, E. I. (1956). "Photosynthesis," Vol. II, Part 2. Interscience, New York.
- Rabinowitch, E. and Govindjee (1969). "Photosynthesis." J. Wiley, New York.
- Reed, D. W. (1969). J. Biol. Chem. 244, 4936.
- Reed, D. W. and Clayton, R. K. (1968). Biochem. Biophys. Res. Commun. 30, 471.
- Reed, D. W. and Ke, B. (1972). Biophys. Soc. Abstr., Toronto, 12, 89a.
- Reed, D. W. and Mayne, B. C. (1971). Biochim. Biophys. Acta 226, 477.
- Reed, D. W. and Peters, G. A. (1971). Abstract, Conf. on Primary Photochemistry of Photosynthesis, Argonne, Ill., p. 11.
- Reed, D. W., Raveed, D. and Israel, H. W. (1970). Biochim. Biophys. Acta 223, 281.
- Reed, D. W., Zankel, K. L. and Clayton, R. K. (1969). Proc. Nat. Acad. Sci. U.S.A. 63, 42.
- Reiss-Husson, F. and Jolchine, G. (1972). Biochim. Biophys. Acta 256, 440.
- Remy, R. (1971). FEBS Letters 13, 313.

- Rikhireya, G. I., Sibel'dina, L. A., Gribova, Z. P., Marinov, B. S.,
Kayushin, L. P. and Krasnovskii, A. A. (1968). Dokl. Akad. Nauk
SSSR 181, 1485.
- Robinson, G. W. (1963). Proc. Nat. Acad. Sci. U.S.A. 49, 521.
- Robinson, G. W. (1966). Brookhaven Symp. Biol. 19, 16.
- Ross, R. T. and Calvin, M. (1967). Biophys. J. 7, 595.
- Sandorfy, C. (1964). "Electronic Spectra and Quantum Chemistry," Chapter 5.
Prentice-Hall, Englewood Cliffs, N. J.
- Sane, P. V. and Park, R. B. (1970). Biochem. Biophys. Res. Commun. 41, 206.
- Sauer, K. (1972). Methods in Enzymology 24, 206.
- Sauer, K., Dratz, E. A. and Coyne, L. (1968). Proc. Nat. Acad. Sci. U.S.A.
61, 17.
- Sauer, K., Lindsay Smith, J. R. and Schultz, A. J. (1966). J. Amer. Chem.
Soc. 88, 2681.
- Schachman, H. K., Pardee, A. B. and Stanier, R. Y. (1952). Arch. Biochem.
Biophys. 38, 245.
- Seely, G. R. (1971). Abstract, Conf. on Primary Photochemistry of Photo-
synthesis, Argonne, Ill, p. 12.
- Seely, G. R. and Jensen, R. G. (1965). Spectrochim. Acta 21, 1835.
- Segen, B. J. and Gibson, K. D. (1971). J. Bacteriol. 105, 701.
- Seibert, M. and De Vault, D. (1971). Biochim. Biophys. Acta 253, 396.
- Seibert, M., Dutton, P. L. and De Vault, D. (1971). Biochim. Biophys.
Acta 226, 189.
- Shibata, K. (1957). J. Biochem. 44, 147.
- Singer, S. J. and Nicholson, G. L. (1972). Science 175, 720.
- Singh, I. S. and Becker, R. S. (1960). J. Amer. Chem. Soc. 82, 2083.
- Slooten, L. (1972). Biochim. Biophys. Acta 256, 452.

- Smith, J. H. C. and Benitez, A. (1955). In "Modern Methods of Plant Analysis" (K. Paech and M. V. Tracey, eds.), Vol. IV, pp. 142-96. Springer Verlag, Berlin.
- Smith, Jr., W. R., Sybesma, C. and Dus, K. (1972). Biochim. Biophys. Acta 267, 609.
- Sogo, P. B., Jost, M. and Calvin, M. (1959). Radiation Research, Suppl. 1, 511.
- Strain, H. H. and Svec, W. A. (1966). In "The Chlorophylls" (L. P. Vernon and G. R. Seely, eds.), pp. 21-66. Academic Press, New York.
- Tachiki, K. H., Parlette, P. R. and Pon, N. G. (1969). Biochem. Biophys. Res. Commun. 34, 162.
- Takamiya, A. (1971). Methods in Enzymology 23, 603.
- Thornber, J. P. (1970). Biochemistry 9, 2688.
- Thornber, J. P. (1971). Methods in Enzymology 23, 688.
- Thornber, J. P. and Olson, J. M. (1968). Biochemistry 7, 2242.
- Thornber, J. P. and Olson, J. M. (1971). Photochem. Photobiol. 14, 329.
- Thornber, J. P., Olson, J. M., Williams, D. M. and Clayton, M. L. (1969). Biochim. Biophys. Acta 172, 351.
- Tinoco, Jr., I. (1963). Radiation Res. 20, 133.
- Tomita, G. and Rabinowitch, E. (1962). Biophys. J. 2, 483.
- Tributsch, H. (1971). Bioenergetics 2, 249.
- Tumerman, L. A. and Sorokin, E. M. (1967). Molekul. Biol. 1, 628.
- Tweet, A. G., Bellamy, W. D. and Gaines, Jr., G. L. (1964). J. Chem. Phys. 41, 2068.
- Velluz, L., Legrand, M. and Grosjean, M. (1965). "Optical Circular Dichroism," Academic Press, New York.

- Vernon, L. P., Shaw, E. R., Ogawa, T. and Rayeed, D. (1971). Photochem. Photobiol. 14, 343.
- Vernon, L. P., Yamamoto, H. Y. and Ogawa, T. (1969). Proc. Nat. Acad. Sci. 63, 911.
- Vredenberg, W. J. and Duysens, L. N. M. (1963). Nature 197, 355.
- Warden, J. T. and Bolton, J. R. (1972). J. Amer. Chem. Soc. 94, 4351.
- Watson, A. and Livingston, R. L. (1950). J. Chem. Phys. 18, 802.
- Weaver, E. C. (1968). Photochem. Photobiol. 7, 93.
- Weaver, E. C. and Weaver, H. E. (1969). Science 165, 906.
- Weber, G. (1960). In "Symposium on Comparative Biology" (M. B. Allen, ed.), pp. 393-411. Academic Press, New York.
- Weber, G. and Teale, F. W. J. (1957). Trans. Faraday Soc. 53, 646.
- Weier, T. E. and Benson, A. A. (1967). Amer. J. Bot. 54, 389.
- Witt, H. T. (1971). Quart. Revs. Biophys. 4, 365.
- Witt, K. and Wolff, Ch. (1970). Z. Naturforsch. 25b, 387.
- Yakushiji, E., Uchino, K., Sugimura, Y., Shiratori, I. and Takamiya, F. (1963). Biochim. Biophys. Acta 75, 293.
- Yamamoto, H. Y. and Vernon, L. P. (1969). Biochemistry 8, 4131.
- Yang, C. S. and Blumberg, W. E. (1972). Biochem. Biophys. Res. Commun. 46, 422.
- Yau, H. F. (1971). Photochem. Photobiol. 14, 475.
- Yocum, C. F. and San Pietro, A. (1969). Biochem. Biophys. Res. Commun. 36, 614.
- Yocum, C. F. and San Pietro, A. (1970). Arch. Biochem. Biophys. 140, 152.
- Zankel, K. L., Reed, D. W. and Clayton, R. K. (1968). Proc. Nat. Acad. Sci. U.S.A. 61, 1243.
- Zweig, G. and Avron, M. (1965). Biochem. Biophys. Res. Commun. 19, 397.

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