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PHOTOSYNTHESIS - THE LIGHT REACTIONS

Kenneth Sauer

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Annual Review of Physical Chemistry, 1979

PHOTOSYNTHESIS - THE LIGHT REACTIONS

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LIST OF ABBREVIATIONS

Chl	chlorophyll
BChl	bacteriochlorophyll
(B)Chl	bacteriochlorophyll and/or chlorophyll
Ph	pheophytin
BPh	bacteriopheophytin
CIDEP	chemically induced dynamic electron polarization
LIMP	light induced magnetic polarization
ENDOR	electron nuclear double resonance
ODMR	optically detected magnetic resonance
CD	circular dichroism

PHOTOSYNTHESIS - THE LIGHT REACTIONS

Kenneth Sauer

INTRODUCTION

During the past three or four years there has been a substantial advance in our knowledge of the light reactions in photosynthesis. This comes at a time of heightened interest in sunlight as a source of energy for our daily needs. The exploration of potential practical applications in this area will be helped by identifying the common features of the light reactions in higher plants and algae and in the simpler photosynthetic bacteria. These include the processes of light absorption by an antenna of chlorophyll (Chl) and other pigment molecules, the transfer of excitation to a photochemical reaction center and the initiation of the electron transfer reactions - all within a few picoseconds of the arrival of the photon.

Because the field of this review is broad (the author has identified over 550 relevant articles published during 1976-78), major developments will be emphasized and mention will be made of numerous other review articles that cover substantial portions of the subject matter.

Light harvesting by a pigment antenna The light-collecting pigment molecules include (bacterio)chlorophyll, carotenoids and, in some organisms, phycobilins, whose absorption spectra collectively span the ultraviolet, visible and near infrared spectra to about 1.06 μm . The chromophores are

located largely in well-defined complexes with protein, and these are closely associated with or intrinsic to internal membranes of the cells or organelles. Light absorption results in electronic excitation that is rapidly transferred within this pigment bed, during times in the psec or sub-psec range. Normally the target of this excitation transfer is the photochemical reaction center, and the process must be rapid because it competes with alternative excitation decay modes, such as fluorescence, that can be very efficient for many of these pigment molecules.

[Reviewed in (1, 2, 3, 4)].

Charge separation in a reaction center A special complex of chlorophylls and protein with other electron donors and acceptors is the site of initiation of the oxidation-reduction reactions. The primary donor, P870 or P700, consists of (bacterio)chlorophyll molecules in a dimer or "special pair" that has unique optical absorption, circular dichroism and EPR properties. Usually the long wavelength absorption band of the reaction center (B)Chl lies at lower energy than the corresponding light-harvesting chlorophylls; bacteria containing BChl b are a notable exception. Complexes enriched in reaction centers contain intermediate electron carriers, I, low-potential electron acceptors (A,Q,X) and sometimes secondary electron donors. [Reviewed in (5, 6, 7, 8, 9, 10, 11, 12).] A general equation describing the primary

electron transfer can be written

The events shown occur typically in the sub-nanosecond region and have aspects that are temperature independent to 4°K. [Reviewed in (13)].

Charge migration in an electron transport chain A series of donors and acceptors on either side of the reaction center complex serves to stabilize the initial charge separation against otherwise rapid back reactions. At the same time an electric field appears across the membrane as a consequence of the preferential accumulation of positive and negative charges on opposite surfaces. The surface carriers not only communicate with cytoplasmic oxidants and reductants involved in metabolism, but they also are involved in binding or releasing protons in a fashion that results in H^+ and other ion gradients to appear rapidly between the inside and outside spaces. The resulting electrochemical potential is the thermodynamic origin of phosphorylation coupling. [Reviewed in (5,14)].

PHOTOSYNTHETIC BACTERIA

The light reactions of the photosynthetic bacteria have been the most extensively studied and are the best known at present. [Reviewed in (5,12,13,15,16,17)]. Each bacterium

contains one type of reaction center, by contrast with higher plants where two different light reactions operate in series. An exhaustive compendium of information is available in The Photosynthetic Bacteria, edited by Clayton & Sistrom (18).

Reaction Centers

Reaction center complexes have been isolated from a large number of photosynthetic bacteria. Those isolated from a particular carotenoidless mutant, R-26, of Rhodopseudomonas sphaeroides, first by Reed & Clayton, have been described in the most detail (19,20). They contain 4 BChl a, 2 BPh, 1 or 2 quinones (usually ubiquinone), Fe and peptides of 21, 24 and 28 kdaltons. Preparations differ in whether they also contain cytochromes or light-harvesting BChl or both (16). Treatments that remove the Fe and the 28 kdalton peptide still retain a measure of reaction center activity; however, extraction of the quinones causes a (reversible) loss of activity (21).

Upon illumination an active reaction center complex undergoes an electron transfer that results in bleaching of absorption associated with a portion of the BChl, commonly designated P870, and the appearance of absorption in the near infrared (at 1250 nm) and elsewhere attributable to P870⁺. Within a few psec the electron appears on an intermediate carrier I that appears to be one of the BPh molecules in the reaction center complex. The radical pair

that results, $P870^+I^-$, lasts for about 200 psec before the electron is transferred to a quinone (ubiquinone or menaquinone) complexed with Fe. Subsequently, the electron moves to a second quinone that is associated with the reaction center in vivo. The $P870^+$ is rereduced on the microsecond time scale by cytochromes, the electron transport chain components that donate electrons to the reaction center BChl. Absorption of a second photon can then lead to the transfer of a second electron to the $Q_1[Fe]Q_2$ complex, which has been shown to occur in both one electron- and two electron-reduced states. The overall process is summarized in Figure 1.

Figure 1

Most bacterial reaction center preparations also contain a large (ca 20 molecules) pool of quinone which can accumulate electrons transferred pairwise from Q_2^{2-} ; however, this pool is readily removed by extraction without blocking the primary reaction center activity. In active bacterial intracytoplasmic membranes there are, in addition, light-harvesting BChl-protein complexes, additional carriers for electron transport, and the coupling factor complex required for photophosphorylation (2).

Reaction centers isolated from different organisms contain the following principal distinctions: Rps. sphaeroides, Strain 2.4.1, retains about one molecule of the carotenoid sphaeroidene per P870 (22); those from Rps. gelatinosa contain only two distinguishable polypeptides (23); functional reaction centers from Rhodospirillum rubrum with only 0.5

molecule of ubiquinone per P870 have been reported (16,24); organisms such as Rps. viridis contain the pigment variant BChl b, an altered pattern of polypeptides, and menaquinone instead of ubiquinone (25).

Primary Electron Donor, BChl₂ (P870)

The primary photoprocess involves the transfer of one electron per reaction center P870 and occurs with a quantum yield reported to be 1.02 ± 0.04 (26) and largely independent of temperature (27). The electrochemical midpoint potential for this step is + 470 mV (15). The formation of P870⁺ results in the appearance of an EPR signal with a linewidth narrower by about $\sqrt{2}$ than that of monomeric BChl a⁺ in organic solvents. The optical, EPR and ENDOR spectra of P870⁺ have been interpreted in terms of delocalization of the unpaired electron between the two BChl molecules of a "special pair", BChl₂. [Reviewed in (11,28)].

The optical changes of Rps. sphaeroides are representative for those of BChl a-containing organisms. The most prominent effects are bleaching at 870 and 590 nm, an apparent band shift toward the blue at 800 nm, and an increase in absorption at long wavelength, 1250 nm (19). The set of 4 BChl and 2 BPh are closely coupled in the reaction center (29); evidence based on circular dichroism (CD) spectra implicates molecular exciton interactions among these molecules (30). Low temperature absorption (31,32) and CD spectral studies help to resolve 4 exciton components associated with the BChl molecules and 2 more with the BPh in the long wavelength region.

The assignment of the 870 nm band to the oxidizable BChl₂ is based on its total bleaching upon illumination; the coupling of this transition to a much weaker absorption component at 810 nm, as proposed by Vermeiglio & Clayton (32), is supported by studies of linear dichroism (33), polarized photoselection (34) and circular dichroism (30,35). [A similar assignment has been proposed for coupling between the 960 and 850 nm bands of the BChl b-containing bacteria Rps. viridis and Thiocapsa pfennigii (36,37,38,39)]. The other two BChl molecules in the reaction center appear to be spectators, in the sense that they do not have a known direct role in the electron transfer process. The spectroscopic assignment presented here is not uniformly accepted (40,41).

Additional information about the arrangement of the BChl associated with P870 has come from studies of triplets formed in the reaction centers when the photochemical reactions are blocked, usually by chemical reduction of the electron acceptors (42). Analysis of the zero-field splitting parameters and spin polarization was presented in terms of the BChl₂ "special pair" by Uphaus, Norris and Katz (43). Transitions among the triplet sublevels at zero magnetic field using optical detection (44) have been interpreted in terms of reaction center geometry (45,46). Unfortunately there is disagreement among reported values of the decay constants (45,47,48), and questions have been raised about

whether the exciton model is adequate for calculating the molecular geometry (48,49). The role of triplets in photosynthesis has been extensively reviewed (28,50).

The kinetics of bleaching of the reaction center BChl_2 of Rps. sphaeroides was studied using picosecond spectroscopy (51), and a rise time of 7 ± 2 psec was observed. The transient state formed has a lifetime of 150 psec (52) to 250 psec (53), whereupon it decays to a second state in which the BChl_2 absorption bands remain bleached. The appearance of the absorption of BChl_2^+ at 1250 nm within 10 psec following a brief flash demonstrates that the first product detected contains oxidized BChl_2 and is not just an electronically excited state (54). Once oxidized, the BChl_2^+ lasts for microseconds or longer unless it undergoes charge recombination by a back reaction. The 200 psec absorption changes result from transfer among the electron acceptors, which are discussed below.

Intermediate Electron Carrier, BPh (I)

The first carrier to serve as an acceptor of the electron from the BChl₂ (P870) is an intermediate, commonly designated I. The charge-separated state $P870^+I^-$ was designated P^F when it was first observed in *Rps. sphaeroides* by Parson, Cogdell and coworkers (15,55,56) using nano-second flash spectroscopy. P^F was seen when the subsequent electron acceptors Q[Fe] were reduced chemically prior to the flash illumination. The difference absorption spectrum, in addition to the bleaching of BChl₂, showed an absorption decrease near 540 nm that suggested the involvement of BPh (15,53). The assignment of BPh as the intermediate carrier was confirmed by Fajer, et al (57) who demonstrated a close resemblance between the absorption difference spectrum of P^F and that expected from the appearance of $BChl^+BPh^-$ following an electron transfer. Of the two absorption components attributable to BPh at 532 and 544 nm seen in low temperature difference spectra, it is the longer wavelength one that contributes to the formation of P^F (27).

The decay of P^F in the presence of reducing agents occurs in 10 nsec at 293°K following a flash (56); even at 25°K it occurs in 20 nsec (55). Under reducing conditions the decay of P^F coincides with the appearance of the state P^R , which has been identified with the reaction center BChl triplet (17,49).

Studies on the psec time scale using mode-locked laser pulse excitation revealed a state corresponding to P^F even when the secondary acceptors were not reduced (52,53). Under these conditions the initial charge-separated state appears within 10 psec of the light pulse, and it decays subsequently in 200 ± 50 psec to a state in which $BChl_2^+$ is still present, but there is no longer a bleaching of BPh at 540 nm (5,58). In conjunction with these experiments it was found that extraction of quinone serves to prevent secondary electron transfer in much the same way as chemical reduction with dithionite.

The primary electron transfer steps are essentially temperature independent from 300 to 4.2°K, both the transfer within 10 psec from $BChl_2$ to BPh and the subsequent 200 psec transfer from BPh^- to Q_1 (59). The processes, therefore, have the character of electron tunneling, in which there is no thermal activation requirement. Detailed analyses of the light reactions of photosynthesis in terms of electron tunneling have been presented by Hopfield (60) and by Jortner (61).

Several reports in the literature suggest that the interpretation of charge separation events presented above is oversimplified. The observation of a 40% attenuation of the maximum absorbance change at 1242 nm (BChl_2^+ formation) produced by psec pulses compared with that observed upon continuous illumination led Moskowitz and Malley (62) to propose that stimulated emission at the high intensities of the mode-locked laser pulses competes as a de-excitation path. Evidence that the reduction of BChl, involving bleaching at 800 nm for a duration of 35 psec, precedes the reduction of BPh has been reported for Rds. rubrum by Shuvalov, et al (63). In addition, one interpretation of the doublet EPR spectrum of I^- in Chromatium vinosum (see below) invokes a delocalization of the electron between BPh and BChl (64). The earlier studies on Rps. sphaeroides did not show evidence for an acceptor preceding BPh in that organism, however (52,53).

The rapid reversal of the $\text{P870}^+\text{I}^-$ reaction, even at low temperatures, made studies of its spectral properties difficult. Shuvalov, Klimov and coworkers (65,66) demonstrated that the lifetime of I^- can be prolonged to several seconds in reaction center complexes from Chromatium minutissimum with dithionite present as reductant. Under these conditions a prominent absorption increase occurs between 620 and 690 nm and is assignable to BPh^- . Spectrophotometric titrations showed

that BPh^- can be seen upon illumination when the potential is more negative than -60 mV at pH 7.5, and that it continues to be formed at potentials as low as -620 mV, at pH 10.5 (66). The difference in the kinetic behavior between Rps. sphaeroides and C. minutissimum (also C. vinosum) results from the presence of coupled cytochrome electron donation to BChl_2^+ in the latter organisms (65,67). Under reducing conditions and prolonged illumination, BPh becomes reduced by electron transfer from the associated cytochrome, leading to the state $\text{Cyt}^+ \text{BChl}_2 \text{BPh}^-$. At the same time there is a disappearance of recombination luminescence, of the EPR spectrum of the reaction center BChl triplet state and of carotenoid triplet formation that are normally the consequence of the $\text{BChl}_2^+ \text{BPh}^-$ back reaction (65). From an analysis of the difference spectrum in the region around 800 nm, van Grondelle et al (68) concluded that the electron on I^- is located on BPh and is not shared with a BChl.

The formation of I^- at low potentials (-430 mV) in Chromatium is accompanied by the appearance of an EPR signal that, at room temperature, is a single line ($g = 2.0025$, $\Delta H_{\text{pp}} 12.5$ G) with a decay time (30 sec) corresponding to that of BPh_{760}^- (65). At low temperatures a similar signal ($g = 2.003$, $\Delta H_{\text{pp}} 15$ G) is seen along with a second signal ($g = 2.003$, double line split by 60 G) that has a different temperature and microwave power dependence (5, 60, 67). This complex low temperature EPR appears at potentials below 0 to -100 mV (5). It is formed upon illumination by a one-electron

transfer from cytochrome c_{553} (64,67). The origins of the two signals and of the splitting of the second one are unknown; an analogous signal split by 100 G has been seen in Rps. viridis (69). It has been proposed that they arise from interactions between the electron on the BPh^- and the nearby Fe or Q[Fe] of the secondary acceptor complex, or perhaps from delocalization to one of the reaction center BChl (5,6,64,67).

A component of the fluorescence emission near 900 nm in reaction centers from Rps. sphaeroides is increased about 3-fold in intensity when the centers are "closed" by chemical reduction (70). Under the reduced conditions, where the fluorescence quantum yield is about 10^{-3} , Clayton (71) has found it to be temperature independent between 40 and 180°K. If it were known what fraction of this fluorescence results from charge recombination, then it would be possible to reach some conclusions concerning the energy of the charge separated state relative to that of the fluorescing excited singlet state of the reaction center. In principle, this can be determined from fluorescence lifetimes, but the relevant information is not at hand.

The fluorescence lifetime, τ , of a homogeneous population of excited molecules is the product of the fluorescence yield, ϕ , times the natural lifetime, τ_0 . The value of τ_0 for BChl has been estimated to be 20 nsec (70). Thus one expects observed lifetimes of 20 psec for closed reaction centers,

where $\phi = 10^{-3}$, and about 6 psec for open ones. Such lifetimes are very difficult to measure, especially at the long wavelengths where this fluorescence occurs. Using a mode-locked ruby laser pulse incident on reaction centers of Rps. sphaeroides under unspecified reduced conditions, Knox, Adamova et al (72) found a prominent 15 psec decay component in the 850-950 nm region. The rise time of 10 psec was within the instrument resolution, and the rapid decay component disappeared upon chemical oxidation of the $BChl_2$. A second and slower decay component of 250 psec duration was seen both in the long wavelength region and at shorter wavelengths (710-820 nm) where the 15 psec decay component was not observed. Probably the slower component arises from pigments not directly in the reaction center. Intact cells of this organism exhibit single-exponential fluorescence decay in 200 psec (73). Isolated chromatophores (intracytoplasmic membranes) exhibit a 300 psec decay component, which increases to 550 psec as the reaction centers are closed by chemical oxidation or by background illumination (74).

In studies of chromatophores from four strains of Rps. sphaeroides, Campillo, Hyer et al (75) used picosecond flashes and a streak camera and obtained decay times of 100 psec in the wild type (strain 2.4.1) and 300 psec in the R-26 mutant. Their measurements at higher flash intensities (above 10^{14} photons cm^{-2}) demonstrated the contribution of

exciton annihilation in decreasing the yield and, thus, the lifetime. It is not apparent, however, that annihilation accounts for the difference between the low intensity limiting lifetime of Campillo, Hyer et al, and that of Rubin & Rubin (74) who reported values of 300-550 psec using an intensity of 5×10^{12} photons cm^{-2} . Perhaps the different Rps. sphaeroides strain, 1760-I, is the cause. The presence and, presumably, the nature of the carotenoids in the membranes does appear to have a significant influence on the fluorescence lifetime (75). Fluorescence lifetime studies are reviewed in (76).

Radical Pair Mechanism of Charge Separation

It is presently believed that the primary photo-induced charge separation occurs by the radical pair mechanism. In this picture, direct absorption of a photon or excitation transfer to the reaction center BChl produces an excited singlet state. When one of the electrons of the excited BChl_2^* is transferred to the electron acceptor, I, the radical pair formed is initially in the singlet configuration, in the sense that the two separated electrons have their spins correlated and opposite to one another. The rapid kinetics (< 10 psec) associated with the charge separation process is a strong argument against the initial formation of a triplet, $^3\text{BChl}_2$, in the reaction center. Because the odd electron on the oxidized donor, BChl_2^+ , and that on the reduced acceptor, I^- , have different local magnetic environments,

singlet-triplet mixing occurs on the sub-nanosecond time scale, and the radical pair develops triplet character by a process described as

If there is a proper amount of magnetic interaction during this period, then electron spin polarization [also called Chemically-Induced Dynamic Electron Polarization, CIDEP, or Light Induced Magnetic Polarization, LIMP (28)] may develop. This is detected in EPR studies as stimulated emission or super-absorption of microwave radiation. As the electrons of the radical pair become dephased, usually by spin-lattice relaxation or coupling with other paramagnetic species present, they lose the memory of their initial correlation and the spin polarization disappears. Meanwhile, the electrons (holes) may transfer to secondary acceptors (donors) which alters the local magnetic environment.

As this charge separation continues to develop, it results in the electron transport process normally associated with photosynthetic energy conversion. It may reverse, however, resulting in a recombination of the charges that were separated in the radical pair. In this case it may produce either a singlet $^1(\text{BChl}_2)^*$ or triplet $^3(\text{BChl}_2)^*$ excited state in the reaction center. The singlet will have a probability of

producing delayed fluorescence, where the delay or unusually long decay profile is a measure of the lifetime of the radical pair states. Often this decay process is complex, indicative of the variety of electron donor and acceptor species that can participate in the radical pairs. The triplet $^3(\text{BChl}_2)^*$ that is formed by radical pair recombination can be detected by its optical absorption changes (i.e. the state P^R of Parson and coworkers) or by using EPR or optically-detected magnetic resonance, ODMR. Again, the radical pair origin produces a characteristic spin polarization, or non-thermal initial population distribution among the triplet spin sublevels. In addition to spin lattice relaxation processes, the triplet spin sublevel distribution will change in time because of different rates of relaxation to the ground state. When carotenoids are present in the reaction centers, this relaxation process results in the formation of triplet carotenoids (17).

Reaction center BChl triplets in bacteria were reported by conventional EPR methods by Dutton, Leigh and coworkers (42). The apparent discrepancies between the lifetimes of triplets observed using optical absorption (15,17) and EPR (42) appear to have been resolved (17,44,48). Based on comparisons of triplet spin polarization in vivo and in vitro (43), Thurnauer et al (77) proposed the radical pair origin of these triplet species. ODMR studies were reported by

Clarke et al (44) at about this time, and detailed studies of the population of the triplet sublevels by Hoff (47,49) have been interpreted in terms of a radical pair origin (48,78). The radical pair mechanism also serves to account for the observed magnetic-field dependent decrease in triplet formation (79,80,81) and fluorescence intensity (82) in reduced reaction centers. Although electron spin polarization has not yet been detected in intact bacteria or chromatophores (83,84) it has been observed in reaction centers where the iron is removed from the iron-quinone complex (84). Complex spin polarization decay kinetics are attributed to contributions from both partners in the radical pair.

Quinone[Fe] Electron Acceptors, Q[Fe]

The acceptor that receives the electron from the intermediate, I, is a complex of quinone (ubiquinone or menaquinone) and iron. Early experiments associated this reduction with absorption changes in the ultraviolet and visible (16,85) regions. These changes occur reversibly, even at temperatures of 100°K (86). A broad (600 G) EPR signal at $g = 1.82$ associated with this acceptor is seen at low temperatures (31,87). Upon removal of the Fe by extraction techniques, a new EPR signal associated with reduced quinone is inducible by light (16,88). The EPR signal at $g = 1.82$ [at 1.87 in Rds. rubrum (89)] has been interpreted in terms of a $[\text{Fe}]\text{UQ}^{\cdot-}$ complex in which the unpaired electron and, hence, the reducing equivalent is located mainly on the ubiquinone (20). Electrochemical titrations at various pH values have not demonstrated a clear correlation between the $g = 1.82$ EPR signal and the state of Q[Fe], however (6).

There are several identifiable pools of quinone associated with the electron transport path in photosynthetic bacteria. Differential extraction techniques can be used to identify two molecules, designated Q_1 and Q_2 , that are closely associated with the reaction centers. Okamura, et al (21) showed that if complete extraction is carried out leading to loss of photochemical activity, then reconstitution can be achieved by the addition of 1.0 stoichiometric equivalents of ubiquinone. By contrast, Morrison et al (24) found only 0.48 ± 0.05 UQ to be present in partially extracted reaction centers with unimpeded activity. These results and others have led Loach to formulate a duplex model for the bacterial reaction centers in which two donor complexes are associated with a single electron acceptor (16,24). Other current views assume a 1:1 stoichiometry, however (5,20,90).

Experiments in which a series of single turnover flashes transfer electrons one at a time from the donor complex show oscillations of period 2 in the spectra of the electron acceptor Q[Fe] (91,92). These are interpreted to reflect the acquisition of electrons singly by Q[Fe] on each flash, followed by the pairwise transfer to a subsequent acceptor on alternate flashes. A current model involves two associated bound ubiquinones, UQ_1 and UQ_2 , where electrons are transferred one at a time from UQ_1 to UQ_2 and pairwise from UQ_2 to the larger quinone pool (93). The two bound UQ molecules can be distinguished optically (93), kinetically (94) and by EPR (95).

An additional role of the ubiquinone reduction is in proton binding. Barouch & Clayton (96) found that proton binding occurs only on even-numbered flashes when the UQ becomes fully reduced. Excellent reviews of this area have been prepared recently (90,96).

PHOTOSYSTEM I OF PLANTS

Progress in understanding the structure of the reaction centers and the mechanism of the primary photoreactions in higher plants has lagged behind that in the photosynthetic bacteria. Partly this is because it has not yet been possible to isolate simple reaction center complexes free of associated antenna chlorophyll. [Reviewed in (1, 3)]. Commonly there are 40 or more Chl a associated with each Photosystem I reaction center, although smaller complexes have been reported (98,99,100).

A photosystem I reaction center preparation described by Bengis and Nelson (99,101,102) contains 5 or 6 peptides that are attributable to the several donor and acceptor species present. This can be broken down to a simpler complex that appears to have two copies of only a single polypeptide along with the reaction center chlorophyll (99,102).

Primary Electron Donor, Chl₂ (P700)

The electron donor of Photosystem I, designated P700, exhibits both characteristic optical changes (absorption, CD, linear dichroism) and a transient EPR signal, known as Signal I. The molecular species involved appears to be a

dimer of Chl a molecules with many properties (exciton band splittings, double CD, narrow EPR line, ENDOR) similar to those seen in the bacterial systems [reviewed in (8,11,103)]. The midpoint potential for the one-electron photooxidation of P700 has been reported to be +460 to 520 mV (104,105,106, 107,108). Two recent studies appeared to detect lower values, around +375 mV (109,110); however, it is possible that these measurements reflected the midpoint potential of cytochrome f (105) that serves as electron donor to P700⁺.

The time required for P700 to undergo bleaching has been measured to be < 60 psec at 293°K by Shuvalov et al (111) using a mode-locked ruby laser. Earlier reports indicated that Photosystem I differed from the bacterial light reaction in that P700 reversible bleaching was not observed at low temperatures (112). Recent studies, however, have demonstrated fully reversible optical changes of millisecond duration down to 4°K in P700 detergent particles (113,114) and in intact chloroplasts (115). A photo-induced reversible EPR Signal I at low temperature has also been reported (116,117).

Intermediate Electron Carrier, Chl (A₁)

Evidence for an intermediate carrier analogous to the I of bacteria has come from recent studies of flash-induced transient absorption changes (118). The electron transfer from P700 to the intermediate carrier, designated A₁, reverses

in 3 μ s at room temperature when subsequent electron acceptors, X and ferredoxin centers [A] and [B], have been reduced prior to the flash. In Photosystem I reaction center particles from which the bound iron-sulfur proteins have been removed using anionic detergent (119), a similar rapid reversal of the P700 absorption changes is seen, even at low temperatures (113). Because there is evidence for the absence of pheophytin (Ph) from the Photosystem I reaction center (1), the A_1 acceptor is thought to be Chl a. Evidence supporting this assignment comes from comparison of the PS I optical difference spectra at room temperature for SDS particles (120) or at low temperature for Triton particles (121) with those of the radical anions of $\text{Chl}^\bullet$ and $\text{Ph}^\bullet$ in solution (122). The midpoint potential for the formation of $\text{Chl}^\bullet$ in vitro is -0.88 V (122); however, the effective value in Photosystem I reaction centers is not known.

The first electron acceptor A_1 of Photosystem I is further characterized as a radical species with an isotropic g-tensor at $g \sim 2.00$, based on electron spin polarization (CIDEP) studies of the photo-induced charge separation (123, 124) and direct observation (121). These properties rule out the known iron-sulfur proteins as candidates. The spin polarization studies require that the electron normally stays on A_1 only a few hundred psec before transferring to subsequent acceptors (124). Fast optical transients associated

with $\text{Chl}^{\bar{+}}$ observed recently in the subnanosecond time regime (111) indicate that this electron transfer occurs in 200 psec.

Electron Acceptor X (A_2)

Charge separation under somewhat less reducing conditions results in electron transfer from P700 to a second electron acceptor, A_2 , that then back reacts in about 300 μs at room temperature (118). The optical difference spectrum of A_2 resembles that of a bound ferredoxin or iron-sulfur protein (107, 121). On the basis of indirect evidence it has been associated with a carrier that has a midpoint potential of -730 mV (125).

A characteristic low-temperature EPR signal associated with this low potential electron acceptor, designated X, was reported by McIntosh, Bolton, Evans and coworkers (116, 117, 126). The g-tensor components (1.78, 1.90, 2.09) are somewhat unusual for an iron-sulfur protein, and the chemical identity of X is currently unknown. Studies of oriented chloroplast membranes show that X is immobilized in the reaction center complex and in a geometry such that the $g_x = 1.78$ component of the EPR signal is roughly parallel to the membrane normal (123). Again, electron spin polarization studies require just such an orientation and high-field g-tensor for the second acceptor, A_2 (108, 124). The conclusion that X and A_2 are identical is supported by the parallel loss upon chemical treatment of both iron-sulfur proteins and of absorption changes attributed to the $\text{P700}^+ A_2^-$ back reaction (127).

In the absence of strong reducing agents, the normal lifetime of the electron on X is probably a few nanoseconds before it moves to the next acceptor(s), bound ferredoxin (124).

Bound Ferredoxin Centers [A] and [B], (P430)

The bound ferredoxin of chloroplast membranes was observed in 1971 in both optical (128) and low-temperature EPR studies (129). Two such species, designated centers [A] and [B], exhibit midpoint potentials of -540 and -585 mV, respectively. They behave like two $4\text{Fe}4\text{S}$ centers that are closely coupled to one another. [Reviewed in (7 ,10 ,130)].

Release of the labile sulfur associated with the iron-sulfur proteins of Photosystem I does not destroy the P700 (131), but the light-induced absorbance changes associated with electron transfer beyond A_1 disappear concurrently with the loss of 10-12 Fe-S pairs (127,132). This is stoichiometrically in accord with the proposal that X, Fd[B] and Fd[A] are each $4\text{Fe}4\text{S}$ centers (123).

The distinction between ferredoxin centers [A] and [B] can be made on the basis of their electrochemical potentials and their EPR spectra, but the relation of these two components is not entirely clear (7). The evidence is ambiguous regarding the immobilization of center [A], but center [B] is oriented in such a way that the y-component of its g-tensor is parallel to the membrane normal (123). If center [A] is

first reduced chemically, then illumination at low temperature produces reduction of center [B] by electron transfer from P700 (133). In addition, optical studies show that two equivalents of electrons can be transferred to electron acceptors, presumably centers [A] and [B], before a subsequent flash leads to the back reaction of $P700^+X^-$ (118). A reasonable interpretation of these results is that the electron transport components of Photosystem I occur in the linear sequence $P700 \rightarrow A_1 \rightarrow Fd[B] \rightarrow Fd[A]$; however, a parallel arrangement of some of the acceptors cannot be ruled out.

The substantial variable fluorescence seen in intact chloroplasts at room temperature is associated almost exclusively with Photosystem II. At low temperatures (80°K) a considerably enhanced emission at long wavelengths (735 nm) has been associated with PS I (134,135) however, the absence of this component in certain preparations enriched in P700 make it doubtful that the reaction center itself is directly involved. (135,136). Butler, et al (137) have attributed it to a special Photosystem I antenna Chl, C705, that transfers excitation to P700 efficiently at room temperature. A search for variable fluorescence at long wavelengths at 80°K found none (138) in intact chloroplasts; however, Photosystem I particles exhibit a variable fluorescence at 690-695 nm that correlates with the level of P700 (139,140,141).

Early studies of fluorescence lifetimes in chloroplasts using mode-locked laser pulses were complicated by multi-hit phenomena leading to singlet-singlet annihilation (142,143) and a buildup of triplet quenchers during the trains of high-intensity pulses (142,143,144). In the low intensity limit, the long wavelength fluorescence has a decay component about 100 psec in duration at room temperature, but this lengthens to several nsec at low temperatures (145,146). The kinetics of excitation transfer within the antenna chlorophylls (142,145,147) and the buildup of triplet or other long-lived quenching species (76 ,142,144) have been investigated by studying the fluorescence yield or lifetime as a function of flash pulse intensity or the number of closely-spaced pulses used.

PHOTOSYSTEM II OF PLANTS

The least well characterized light reaction is that of Photosystem II, which is associated with water splitting and oxygen evolution in higher plants. In part this deficiency is because the associated machinery is the most fragile; it does not readily survive aging, mild heating, strongly oxidizing conditions or detergents aimed at dissecting it into its components.

Although several preparations that are enriched in Photosystem II activity have been described, they appear to contain large membrane fragments from which Photosystem I activity has been removed rather than small reaction center

particles. Typically there are over 100 Chl molecules associated with each reaction center, the O_2 -evolving apparatus is either missing or severely damaged, and the photochemical kinetics are modified from the in vivo situation. As a consequence no components have been characterized which have a sufficiently high electrochemical potential (> 800 mV at pH 7) to be good candidates for participation in the water oxidation reactions.

The electron donor, P680, associated with Photosystem II is more difficult to study than that of Photosystem I or bacteria because the associated absorption and EPR signals are more difficult to detect. Partly for this reason, much more use has been made of measurements of variable fluorescence yield, delayed fluorescence, absorption changes associated with the electron acceptor Q (or X320) and selective inhibitors or treatments that permit access to sites in the electron transport chain close to the reaction center. There is an extensive literature covering this work. [Reviewed in (9, 148,149,150)].

Primary Electron Donor, P680

The reaction center of Photosystem II exhibits both optical absorption changes and an EPR signal that suggest the participation of a form of Chl a, perhaps a dimer, as the primary electron donor. The optical absorption changes, first reported by Döring, et al (151), exhibit multiple decay components in the μ sec and sub μ sec region. These reflect the donation of electrons from several donors as well as

back reactions with reduced acceptors. Van Best & Mathis (152) found a decay component of 25-45 nsec duration that accompanies single laser pulse excitation; however, evidence based on the kinetics of the rise of the fluorescence yield (153,154) and more recent absorption transient studies (155) suggests that for multiple excitation studies the rate-limiting step is slower for activations after the first one. The results of O_2 evolution and fluorescence induction studies also support the idea that the first flash illumination after prolonged darkness has enhanced "double hit" characteristics (i.e., the probability of two electrons transferred per brief flash) than do subsequent flashes in a train (154,156,157).

Earlier kinetic studies using repetitive flash sequences identified decay components at room temperature of 35 and 200 μ sec, (158) a faster one of about 4.5 μ sec (155,159), and a decay of 130-200 μ sec characteristic of a back reaction when access by the secondary donors is blocked (155,160,161). The Photosystem II light reaction occurs reversibly at low temperatures as well, but with slower decay kinetics (162, 163). Kinetics similar to those of P680 are seen not only in the rapid fluorescence rise, but also in the 20 μ sec risetime of EPR Signal II_{vf} (164), the decay of delayed fluorescence (150,161,165) and the rate-limiting steps involved in advancing the S-states leading to O_2 evolution (166,167).

A rapidly reversible photoinduced EPR signal ($g = 2.0026$, $\Delta H_{pp} \approx 8$ G) attributed to the reaction center chlorophyll of

Photosystem II is seen at 77°K under strongly oxidizing conditions (168,169,170,171) or at low pH (172). The decay time of 2-5 msec (163,173) is similar to that seen for P680 at low temperature. However, the disappearance of this EPR signal at potentials below +475 mV (169) does not correspond with the behavior of P680, which does undergo rapid reversal at low temperature even under non-oxidizing conditions (162). It may be that the electrochemical transition seen in the EPR originates from a secondary component of the reaction center that serves in its reduced form as an efficient relaxer of the electron spins.

Although there have been no ENDOR or CD studies to support the proposal that P680 is a dimer (170) or even a trimer (174) of Chl, studies of the polarization of the absorption changes of P680 indicate that it does have a definite orientation in the chloroplast membranes (175).

Intermediate Electron Carrier, Ph

The participation of pheophytin as an intermediate electron carrier in Photosystem II is suggested by the observation of photoinducible, reversible absorption changes attributable to Ph at low potentials, -50 to -400 mV, both at room temperature and at 100°K (176). Accompanying these absorption changes is a decrease in the fluorescence yield, indicating an association with the reaction center photochemistry. At potentials of -400 mV and below, the light-induced bleaching of Ph still occurs, but the reversal is much slower.

Electron Acceptor Q (X320)

The transfer of an electron from the primary donor to the electron acceptor of Photosystem II results in a several-fold increase in fluorescence yield associated with "closing" the reaction centers. Duysens and Sweers (177) equated the extent of quenching of fluorescence by "open" reaction centers with the fraction of the electron acceptor, Q, in the oxidized state. Later it was recognized that reaction centers with oxidized electron donors also have a low fluorescence yield. (178). The extensive studies of this variable fluorescence phenomenon have been reviewed in detail. (9 , 148, 149).

Absorption changes associated with reduction of the electron acceptor, designated X320, were discovered in the ultraviolet spectral region (179). The difference spectrum is characteristic of the one-electron reduction of plastoquinone to the unprotonated semi-quinone (180,181), and the identification of the X320 species with the fluorescence quencher Q is supported by kinetic and electrochemical similarities (182). Evidence supporting the molecular origin of these changes as a plastoquinone derives from experiments involving extraction with organic solvents, which abolishes the transient changes associated with the reaction center of Photosystem II. Reconstitution occurs when plastoquinone is added back (183,184). An alternative spectroscopic candidate, C550, for the electron acceptor did not become reconstituted at the same time unless β -carotene was added back as well; C550 is currently thought

to be an indirect indicator of the redox state of the acceptor Q.

A second, further reduced state of the electron acceptor participates in generating oscillations of period 2 in net substrate reduction (185), in fluorescence yield (186, 187, 188) and in absorption changes (189, 190) associated with Q (X320). Among several models proposed to account for these results, a current view holds that there are two acceptor quinones, Q_1 and Q_2 , that accumulate electrons and transfer them pairwise to a larger pool of plastoquinone, much as in the photosynthetic bacterial reaction centers.

Based on a series of electrochemical studies, beginning with those of Cramer and Butler (191), it now appears that the fluorescence yield quencher undergoes two stages of one-electron reduction (192, 193) with $E_{m7.8}$ of -45 to +25 and -247 to -270 mV, respectively, and the latter showing a 50 mV per pH unit dependence (192).

Attempts to analyze the relaxation kinetics of components associated with Photosystem II are complicated by factors arising from heterogeneity, either of the electron donors and acceptors (154, 156, 194) or of the reaction centers themselves (182, 195). At least a portion of these complications derive from double hits or other processes that occur uniquely upon the first illumination after prolonged darkness (154, 156, 157). A detailed analysis of these phenomena is beyond the scope of this review.

The variable fluorescence yield that is characteristic of Photosystem II also results in a variable fluorescence lifetime. Analysis of these observations was obscured in early studies because of the difficulty in resolving short lifetimes and multiple exponential decay curves using phase shift techniques (196). More recently, the use of high-intensity mode-locked laser pulses has produced complications from multi-photon processes leading to singlet exciton annihilation, (142,145,147,197,198,199,200) the build-up of metastable (triplet) quenchers during repetitive pulse trains, (142,143,197,198,199) and the difficulties in analyzing noisy decay curves obtained from streak camera records (146,201).

Nevertheless, it is clear that there are components of Photosystem II fluorescence whose decays are sensitive to the state of the reaction centers in chloroplasts (146,202,203,204,205,206) and Chlorella (199,201,206). Low intensity studies using either the phase shift method (205) or single-photon counting detection (146,206) measure an exponential decay for open traps of 200-500 psec, depending on the organism studied and factors such as Na^+ or Mg^{2+} concentrations. When the traps are closed by inhibitors or background illumination or both, this decay time increases severalfold (204,205,206) and an additional contribution from a longer, ca 1300 to 2000 psec decay component appears (146,199,201,202,206). The significance of these new findings is yet to be determined, but they should be helpful in revealing excitation

transfer within and among the photosynthetic units, as well as clarifying the reaction center processes themselves.

[Reviewed in (135,207)].

CONCLUSION

This overview of recent progress in the investigation of the photosynthetic light reactions reveals numerous common features. Following the absorption of a photon by chlorophylls of the light-harvesting antenna, the excitation migrates to an adjacent reaction center where it produces initially an excited singlet state in a small aggregate of reaction center chlorophylls. Within a few psec there occurs charge separation leading to radical pair formation: the electron donor is an aggregate (special pair) of (B)Chl in the reaction center and the intermediate acceptor is either a (B)Ph or Chl molecule, also apparently an integral part of the reaction center itself. Estimates of the electrochemical potential associated with this initial radical-pair formation are 1.0-1.1 eV for purple bacteria, 1.35 eV for PS I and 1.4 eV for PS II. These values are 70-77, 76 and 76%, respectively, of the energy of the reaction center excited singlet state and, hence of the longest wavelength absorbed photon that is efficiently used. The charge separation is not yet "stabilized", however, for the electron spends less than a nsec on the intermediate carrier before going on to a second electron acceptor. As Parson has pointed out (208), one cannot apply the usual thermodynamic (2nd law) limitations to these transient states that are not equilibrated with their surroundings.

The molecules involved in the next stage of electron acceptors show significant differences between bacteria (Q[Fe]), Photosystem I (iron-sulfur proteins) and Photosystem II (plastoquinone). Nevertheless, in each case there is evidence that electrons can accumulate pairwise upon subsequent flashes and then participate in two-electron reductions of components further along the chain of electron carriers. The reduction of the oxidized reaction center (B)Chl special pair by endogenous electron donors occurs after an interval of several tens of nsec to μ sec. In bacteria, cytochromes appear to be the secondary donors, but in higher plants the molecular nature of the species responsible in each light reaction remains to be identified (152,209). Once the charge-separation has become "stabilized" on the secondary donors and acceptors, the fraction of the absorbed photon energy that is stored is about 30% for purple bacteria and 50% for the higher plant light reactions.

One of the objectives of this transfer of electrons and holes along a chain of carriers is to produce charge separation between the inside and outside surfaces of the membranes in which the reaction centers are imbedded. The immobilization of the reaction center components and their orientations with respect to the membrane normal are becoming known through studies of optical polarization and dichroism and of EPR studies on oriented membranes. The spatially segregated charges produce transmembrane fields. They are the origin of

proton and other ion translocations that provide direct sources of the chemiosmotic potential used in photophosphorylation. It may be at this level of microscopic membrane photopotential that the most valuable prospect lies for practical solar energy conversion based on the photosynthetic model.

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FIGURE CAPTION

Figure 1. Generalized scheme of electron transfer in bacterial photosynthesis associated with two photons absorbed successively by the same reaction center complex.

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