Light-Induced Paramagnetism in Photosynthetic Systems

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Received January 4, 1974

The maintenance of life on earth is ultimately dependent on the series of complex chemical reactions which comprise photosynthesis. Thus solar radiant energy serves as the driving force for the creation of "negative entropy," the growth of highly structured biological organisms out of a universe tending toward chaos. The overall chemical reaction of photosynthesis can be represented as

$$CO_2 + H_2O \xrightarrow{h\nu} \tfrac{1}{6}(C_6H_{12}O_6) + O_2$$
$$\Delta H = 112 \text{ kcal mol}^{-1}$$

where $\frac{1}{6}(C_6H_{12}O_6)$ represents the basic unit of a carbohydrate molecule. This process occurs through the "photoenzyme" chlorophyll, a ubiquitous pigment of fundamental importance for light acquisition and transduction to chemical products. The incredible complexity of photosynthesis has necessitated the cooperation of physicists, chemists, and biologists to decipher the mechanisms of light absorption by the plant and the consequent transfer of energy to a chemical conversion center where chemical free energy is obtained. Additionally, the process whereby chemical free energy is utilized to form stable metabolic products (*e.g.*, glucose) remains to be elucidated fully. Although significant advances in understanding of photosynthesis have been made in the past 30 years, our knowledge is far from complete.

The purpose of this Account is to provide for chemists (and of course any others who read this journal!) an overview of some of the recent developments in photosynthesis, particularly those related to our own field of interest, namely, light-induced paramagnetism. Since our coverage cannot be exhaustive, key references will be given, especially to reviews where they exist.

General Formulation of Photosynthesis

It is advantageous and realistic to examine the photosynthetic process from the viewpoint of a redox couple (see Figure 1).

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$$4e^- + CO_2 + 4H^+ \quad h\nu \quad 2H_2O$$
$$\tfrac{1}{6}(C_6H_{12}O_6) + H_2O \qquad 4H^+ + O_2 + 4e^-$$
reduction oxidation

This oxidation–reduction reaction is localized in green plant leaves or algae (excluding the prokaryots) within small organelles called chloroplasts.[1] The essential details of the reduction half-reaction, that is, the carbon fixation pathway, have been fairly well understood[2,3] for some time. Thus, we will focus on the light-mediated oxidation half-reaction, an area of photosynthesis which is still enigmatic. In passing we will simply note that four electrons are required per CO_2 molecule reduced. This reducing potential is provided by two molecules of the intermediate NADPH (reduced nicotinamide adenine dinucleotide phosphate), and additionally, two to three molecules of ATP (adenosine triphosphate) supply the energy necessary for the assimilation of a single CO_2 molecule. Both NADPH and ATP are products of the oxidation half-reaction, the latter by a process designated photophosphorylation (Figure 1).

Photosynthesis, however, is not limited to green plants and algae. Certain species of bacteria possess the ability to fix carbon photosynthetically, although without a concomitant oxidation of water. Thus, the photosynthetic process might be represented in a more generalized form

$$CO_2 + 2H_2A \xrightarrow{h\nu} \tfrac{1}{6}(C_6H_{12}O_6) + H_2O + 2A$$

where H_2A may be hydrogen sulfide, an organic substrate (*e.g.*, 2-propanol) or even hydrogen, depending upon the species of bacteria. Of course, in green plant or algal photosynthesis H_2A is water. This general formulation for photosynthesis was first proposed by Van Niel[4] and has stimulated much of the research on photosynthetic bacteria.[5] For details on these and other aspects of photosynthesis, the reader is referred to the excellent monograph by Rabinowitch and Govindjee.[6]

Electron-Transfer Reactions in Photosynthesis

Current formulations of the photosynthetic mecha-

(1) R. B. Park, *Annu. Rev. Plant Physiol.*, **22**, 395 (1971).
(2) M. D. Hatch and C. R. Slack, *Annu. Rev. Plant Physiol.*, **21**, 141 (1970).
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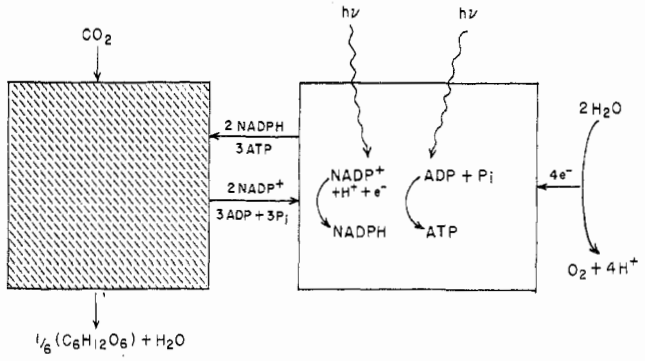

Figure 1. A diagrammatic view of photosynthesis. The light-induced oxidation half-reaction is represented by the compartment on the right, whereas the reduction half-reaction, which can proceed in the dark, is illustrated by the left-hand compartment. The net photosynthetic reaction is visualized as a flow of electrons from the upper right corner to the lower left corner of this diagram.

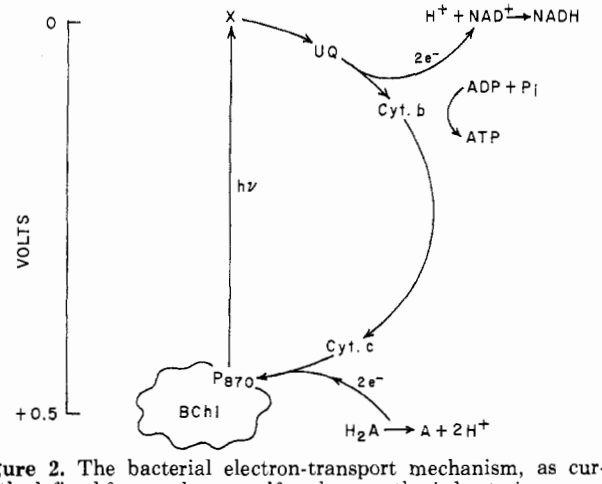

Figure 2. The bacterial electron-transport mechanism, as currently defined for purple non-sulfur photosynthetic bacteria.

nism (henceforth, to denote only the light-driven oxidation half-reaction) are based on the premise that light energy is converted into electrical energy by light-induced one-electron transfers. Subsequent dark reactions leading to storage of chemical free energy are also believed to proceed in most cases by discrete one-electron steps. All reactions comprising the oxidation half-reaction are postulated to occur within a single domain, designated the photosynthetic unit (PSU).[7] A photosynthetic membrane can be regarded as an ensemble of PSU's.

Our discussion of the electron-transport mechanism of photosynthesis will commence with the photosynthetic bacteria, since these organisms appear to be less complex than algae or green plants. A widely accepted construct for light-driven electron transport in bacteria is illustrated in Figure 2. The incident light quanta are absorbed by a "harvesting" bed of bacteriochlorophyll (or accessory pigments such as carotenoids) and are ultimately transferred as singlet excitation to a special bacteriochlorophyll species. This species, designated P870, which denotes a pigment absorbing at 870 nm (actually species dependent, varying from 850 to 980 nm), functions as an energy trap. Here the excitation energy is converted to electrical energy through a single electron transfer from P870 to a low-potential component, labeled X. Subsequently the electron is transferred from X^-

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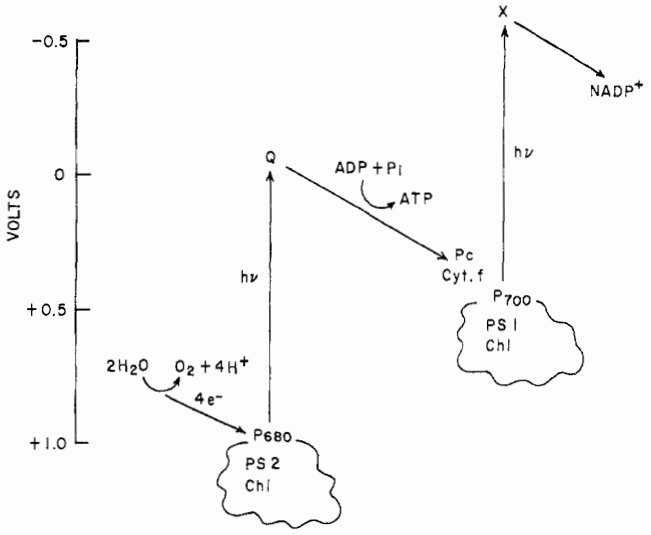

Figure 3. The Z scheme for green plant and algal photosynthetic electron transport. Net electron flow is from lower left to upper right. The components shown are identified in the text.

back to $P870^+$ *via* a cyclic electron-transport path which includes a pool of 8 to 12 ubiquinone molecules (UQ) and two or more cytochromes. The energy stored by the primary photochemical act is utilized to reduce NAD^+ (nicotinamide adenine dinucleotide) and to support photophosphorylation.

In green plants and algae the transfer of an electron from water to $NADP^+$ theoretically requires an energy equivalent of 1.2 eV. Although ~1.8 eV is available in a photon of red light, this gap cannot be bridged (with the requisite photophosphorylation) in one photochemical process and still be consistent with the second law of thermodynamics.[8,9] Hence the evolution of organisms capable of oxidizing water to molecular oxygen necessitated the development of a second photochemical system. The concerted participation of two photochemical systems in green plant and algal photosynthesis has been documented by a wide variety of experiments,[6] leading to the development[10-12] and general acceptance of the reaction scheme (commonly called the Z scheme) illustrated in Figure 3. These two photochemical systems (*e.g.*, assemblies of chlorophylls and associated pigment or redox components) have been labeled photosystem 1 (PS 1) and photosystem 2 (PS 2). Photosystem 1 is associated with the reduction of $NADP^+$ and can utilize light of wavelength longer than 680 nm to sustain electron transport. In contrast, photosystem 2 utilizes light with λ <680 nm and mediates the oxidation of water to molecular oxygen. Although there is still considerable uncertainty concerning the identity and relative positions of the intermediates in the Z scheme,[13] the overall process involving two photochemical reactions remains the basic model for current research in green plant and algal photosynthesis.

The Z scheme (like the bacterial photosynthetic scheme) postulates that each primary photochemical

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(9) R. T. Ross and M. Calvin, *Biophys. J.*, **7**, 595 (1967).
(10) R. Hill and D. Bendall, *Nature (London)*, **186**, 136 (1960).
(11) L. N. M. Duysens, J. Amesz, and B. M. Kamp, *Nature (London)*, **190**, 510 (1961).
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(13) N. I. Bishop, *Annu. Rev. Biochem.*, **40**, 197 (1971).

event results in a one-electron oxidation-reduction reaction. Subsequent electron-transfer processes couple the electrical energy to the ultimate reduction of $NADP^+$ and to two or more coupling sites for photophosphorylation.

In view of the numerous one-electron transfer mechanisms that have been proposed for bacterial, algal, and green plant photosynthesis, it is not surprising that light-induced changes in paramagnetism have been detected[14-16] in photosynthetic materials by electron spin resonance (esr) spectroscopy. We will not attempt to explain the principles of the esr technique since adequate and readable treatments are available.[17,18] In the following sections we will concentrate instead on the contributions of electron spin resonance spectroscopy to the elucidation of the identity and location of various components in the bacterial and green plant photosynthetic apparatus.

Light-Induced Electron Spin Resonance Signals

Although a number of light-induced esr signals have been detected in photosynthetic systems, only a limited number of these have been well characterized. For most resonances the analysis and identification of the physiological origin of the species responsible for the signal are incomplete and require considerably more work. In the subsequent discussion we have adopted a systems approach; that is, each of the observed light-induced esr signals will be discussed in its relationship to the physiological system being studied. In this regard we are adopting a different approach than previous reviewers of this subject.[19-21] We begin thus with the esr signals associated with the bacterial system, since our knowledge is most complete for that system. The less-characterized systems associated with green plant and algal photosynthesis, system 1 and system 2, will then be considered.

The Bacterial System. The first light-induced esr signal observed in photosynthetic bacteria was by Sogo, *et al.*, in *Rhodospirillum rubrum* in 1959.[16] A recent trace of this signal (now designated signal B1 after Kohl[19]) is given in Figure 4. The line shape is gaussian, with a first derivative peak-to-peak line width ΔH_{pp} = 9.5 G. Signal B1 lacks any observable hyperfine structure, has g = 2.0025 ± 0.0002, and saturates at moderate levels of microwave power. This g factor is characteristic of a "hydrocarbon-type" environment for the unpaired electron. Growth of photosynthetic bacteria in deuterated media results in a signal narrowing to 3–4 G,[22,23] thus indicating that a large fraction of the observed line width is due to unresolved proton hyperfine splittings. Oxidation-reduction titrations have established that signal B1 arises from a one-electron oxidation process.[24,25] Signal B1 is photoproduced at low temperatures,[26,27] even as low as 1.8 K,[23] a fact which is consistent with the view that signal B1 arises from the primary photochemical act.

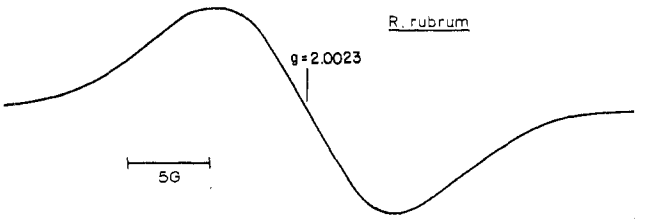

Figure 4. Signal B1 (*R. rubrum*) as observed during illumination with light, λ 800 nm.

The identity of signal B1 was the subject of much speculation and investigation during the early years of interest in the primary reactions in photosynthetic bacteria. A clue was provided by the fact that almost all bacterial systems exhibit a reversible light-induced bleaching (oxidation) in a bacteriochlorophyll band at ~870 nm which has kinetic characteristics very similar to that of signal B1 both at room temperature[28] and at 4 K.[23] In addition, a mutant of *R. spheroides* possessing the normal complement of bacteriochlorophyll but lacking the bleaching at 870 nm showed no signal B1 formation.[29] Careful quantitative work has shown that the ratio of bleached P870 entities to signal B1 entities is 1:1 within experimental error.[30,31] The quantum yield for P870 bleaching[30] and for signal B1 production[31] is essentially unity.

Comparison of the *in vivo* signal B1 with the *in vitro* bacteriochlorophyll radical cation ($BChl^+$) shows a close correspondence in esr characteristics for both protonated and deuterated systems.[23] However, the line width of the *in vivo* signal B1 is always about 30% less than the *in vitro* $BChl^+$ signal. This discrepancy remains even after hyperfine and g anisotropy line width contributions are considered[32] and has been interpreted by Norris, *et al.*,[33] in terms of a delocalization of the unpaired electron over *two* BChl molecules, which are linked by a "bifunctional" ligand, possibly water (*i.e.*, $[BChl \cdot H_2O \cdot BChl]^+$). This view has received dramatic confirmation in recent electron nuclear double resonance (endor) studies[34,35] where the proton hyperfine splittings *in vivo* are found to be exactly half of those *in vitro*. This explanation has also been in-

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(20) E. C. Weaver, *Annu. Rev. Plant Physiol.*, **19**, 283 (1968).
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(29) W. R. Sistrom and R. K. Clayton, *Biochim. Biophys. Acta*, **88**, 61 (1964).
(30) J. R. Bolton, R. K. Clayton, and D. W. Reed, *Photochem. Photobiol.*, **9**, 209 (1969).
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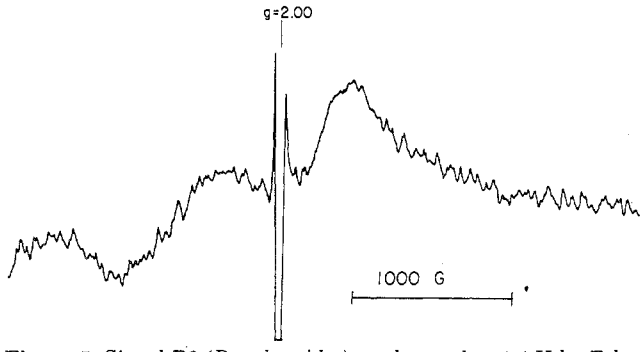

Figure 5. Signal B2 (*R. spheroides*) as observed at 1.4 K by Feher using light modulation for signal detection.[37] The signal appears here as an absorption trace, rather than the usual first derivative presentation (modulation light wavelength, 900 nm). (Reprinted with permission from ref 37.)

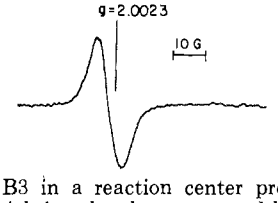

Figure 6. Signal B3 in a reaction center preparation from *R. spheroides* from which iron has been removed by SDS treatment. (Reprinted with permission from ref 40.)

voked for the line-width narrowing in plant and algal signal I (*vide infra*).

In light of the weight of evidence presented thus far there can be little doubt that signal B1 arises from a dimer cation radical of bacteriochlorophyll which results from a light-induced one-electron oxidation in the primary photochemical process of bacterial photosynthesis.

With the recent availability of well-characterized reaction center protein preparations,[36,37] some progress has been made in the identification of the primary acceptor X. At room temperature there is little evidence of an esr signal from X, even though a change in paramagnetism must occur. This paradox was first resolved by Feher;[37] he observed a very broad light-induced esr signal at 1.4 K (see Figure 5) which he ascribed to the primary acceptor. He claimed that the signal, which we will henceforth refer to as signal B2, was consistent with an Fe^{2+} species in an $S = 1$ state. A similar signal (centered at $g = 1.82$) has also been observed at 10 K by Leigh and coworkers.[38,39a] This esr species exhibits redox behavior analogous to that attributed to the primary acceptor[39b] and additionally possesses kinetic parameters consistent for the redox partner of P870.[39a] These significant investigations by Feher and Leigh and coworkers strongly attest to the involvement of non-heme iron in the primary charge separation process.

Ubiquinone may also be involved as part of the primary acceptor entity as an esr signal (which we designate signal B3) is seen in reaction-center protein preparations which have had iron removed[40] (see Figure 6). Signal B3 is also seen in iron-free photoreceptor subunit preparations from *R. rubrum*.[41] Comparison of signal B3 with the signal from an *in vitro* preparation of the ubisemiquinone leaves little doubt that signal B3 arises from the ubisemiquinone.[40] Signal B3 has also been detected by Bolton and Cost[42] using flash photolysis–electron spin resonance. They propose that iron and ubiquinone form a complex which functions as the primary acceptor. This picture is given further credence by the fact that the optical difference spectrum for $X \rightarrow X^-$ corresponds almost exactly with that of $UQ \rightarrow UQ^-$.[43]

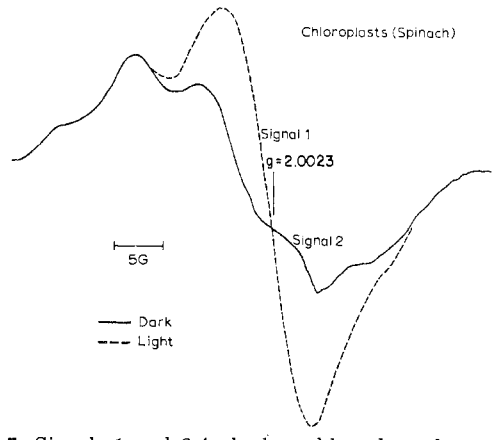

Figure 7. Signals 1 and 2 in broken chloroplasts from spinach. The two signals are superimposed during illumination, but only signal 2 is observed in the darkness following illumination.

Before leaving the bacterial system we will comment on the very unusual back reaction from X^- to $P870^+$. This dark reaction can be most easily observed at low temperature where signal B1 exhibits a first-order 1/e decay time of ~30 msec. This time is virtually *independent of temperature* from 1 to 150 K.[23] Above 150 K the decay time *increases*, until at 300 K the decay time is ~110 msec.[44] This behavior has been interpreted in terms of a quantum-mechanical process for the electron return.[23,44] The increase in decay time at higher temperatures is ascribed to a "breathing" of the protein causing $P870^+$ and X^- to be further apart. Model calculations indicate a distance of ~40 Å between P870 and X.[45]

Green Plants and Algae. The existence of two photochemical systems in the green plant and algal photosynthetic apparatus is reflected in the dichotomy of the two light-induced esr signals, designated signal 1 (after system 1) and signal 2 (after system 2), observed at room temperature (Figure 7). Assignment of the two resonances to a particular photosystem is supported by fractionation experiments[19] (chemical or physical separation of the two photosystems) and action spectra determinations.[46] System 1 most closely resembles the bacterial photosystem and thus will be dealt with first.

Photosystem 1. Although chronologically the discovery of signal 1 predated the observation of the

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(45) J. D. McElroy, Ph.D. Thesis, University of California, San Diego, 1970.
(46) L. P. Vernon, B. Ke, and E. R. Shaw, *Biochemistry*, **6**, 2210 (1967).

bacterial signal B1,[14] the analysis of signal 1 has been hampered by the lack of well-defined biological samples (*e.g.*, chromatophores or a reaction center protein preparation), the complexity of the chloroplast with its dual photochemical systems, and the lack of sensitivity and stability, which was characteristic of early esr spectrometers. The development of solid-state spectrometer systems, the computer of averaged transients (CAT), the flash photolysis technique, and techniques for separation of the two photosystems has recently sparked a rebirth in the investigation of the photosystem 1 esr signal.

Signal 1, like its bacterial analog, is a single unstructured resonance, possessing a gaussian line shape and $g = 2.0025 \pm 0.0002$ (Figure 7). In contrast to the approximate 10-G line width of signal B1, plant or algal signal 1 has a ΔH_{pp} of 7.2 ± 0.1 G. Signal 1 exhibits saturation at medium microwave power levels (~30 mW) with a saturation curve [curve amplitude *vs.* (microwave power)$^{1/2}$] typical of an inhomogeneously broadened line. Analogous to bacterial signal B1, deuteration of algae results in a narrowing of signal 1 to approximately 3 G.[22,23] Additionally, signal 1 arises from a one-electron oxidation with a midpoint potential (at pH 7.0) of ~+450 mV,[24] and can be photoproduced at liquid nitrogen temperature.[26,27]

The close similarity of signal 1 to signal B1 suggests assignment of signal 1 to oxidized P700, the system 1 counterpart of the bacterial energy trap, P870. Such a correlation of $P700^+$ and signal 1 was first proposed by Beinert, Hoch, and Kok in 1962;[47] however, a later quantitative comparison of the number of spins to the number of bleached P700 entities was not consistent with this assignment.[48] Recent kinetic and quantitative measurements in our laboratory using the simultaneous optical and electron spin resonance technique have justified the early correlation of signal 1 to oxidized P700.[49,50] Signal 1 and P700 were shown to possess similar formation and decay kinetics, and the ratio of bleached P700 moieties to unpaired spins was ~1:1. This assignment has additionally been confirmed by quantitation experiments performed during steady-state illumination of a system 1 preparation.[51]

Observation at 18–25 K of a broad light-induced resonance following irradiation at 25 or 77 K has been interpreted by a number of laboratories as evidence for participation of a non-heme iron protein in the primary electron-transfer event.[52-55a] First observed by Malkin and Bearden,[52] this signal possesses a nonaxial g tensor with g factors ($g = 1.86$, 1.94, and 2.05) and line-shape characteristic of a reduced iron–sulfur protein. Bearden and Malkin have elegantly demonstrated that this iron–sulfur resonance is associated with system 1 and is formed stoichiometrically in a 1:1 ratio to signal 1.[55c] However, the presence of multiple nonheme iron resonances[55a,b] as well as an alleged kinetic disparity between signal 1 and the nonheme iron signal[53] do not permit at this time a conclusive assignment of this species to the primary electron acceptor.

Although the primary electron-acceptor moiety cannot be observed at ambient temperature by esr, the redox interactions of the primary donor and acceptor and subsequent electron flow in system 1 have been ascertained by monitoring the transient kinetics of signal 1 following a saturating flash at room temperature.[56] These kinetic investigations generally support the conceptual framework summarized by Ke[57] and document three possible pathways of reduction for $P700^+$ in PS 1 subchloroplast preparations and in chloroplasts: (1) direct return of the electron from the primary acceptor; (2) cyclic electron transport involving PS 1 components in intact chloroplasts or an artificial electron carrier (*e.g.*, TMPD), serving both as donor and secondary acceptor, in system 1 preparations; (3) noncyclic electron flow involving a donor to $P700^+$ (*e.g.*, photosystem 2 for chloroplasts or a reduced dye for PS 1 preparations) and a different oxidant for the primary acceptor. Chloroplasts capable of assimilating CO_2 (prepared by the Jensen–Bassham method[58]) do not exhibit pathway 1; however, osmotically shocked chloroplasts display a prominent direct electron-return component.[56] Analogously, osmotically shocked chloroplasts possess an easily observable signal 1 during steady-state, far-red illumination, whereas in fresh, intact chloroplasts very little signal 1 is seen. Thus, esr additionally can serve as a monitor of the physiological state of the chloroplast.

The identity of the physiological donor to $P700^+$ has over the years remained a subject of much conjecture.[13] Recent esr investigations, however, have indicated that this donor entity in chloroplasts must transfer an electron to $P700^+$ in less than 10 μsec.[56] Additionally, chloroplasts illuminated with far-red light and subsequently frozen to 25 K reveal a resonance typical of protein bound Cu^{2+}.[59] On the basis of its spectral parameters and abundance this signal has been assigned to plastocyanin, a copper-containing protein that is known to be closely associated with system 1. These observations, combined with the additional report that another possible donor, cytochrome *f*, to PS 1 is oxidized in ~200 μsec at room temperature,[60] suggest that plastocyanin may function as the endogenous donor to system 1 at room temperature.

Photosystem 2. The only paramagnetic manifestation of photosystem 2[61] observable at room temperature, signal 2, is a broad (ΔH_{pp} ~19 G) struc-

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tured resonance (g = 2.0047 ± 0.0002) with asymmetric line shape and four or five hyperfine components (see Figure 7). With saturation behavior typical of an inhomogeneously broadened line (*i.e.*, there is no line broadening or change in line shape at high microwave powers), signal 2 saturates at ~25 mW.[62] Signal 2, as Weaver indicates,[20] is a light-induced resonance, possessing a long decay time. In spinach chloroplasts signal 2 decay follows complex kinetics with faster and slower components.[62-64] In algae similar behavior is found except that the slow decay is invariably faster ($t_{1/2}$ ~30 min).

Although signal 2 was first ascribed to plastosemiquinone,[65] Kohl and coworkers in 1969 demonstrated that the *in vitro* esr signal of immobilized plastoquinone did not resemble that of signal 2.[66] Furthermore, by isooctane extraction of plastoquinone from chloroplasts and subsequent readdition of deuterated plastoquinone, Kohl was able to show that signal 2 is formed from plastoquinone.[67] By comparison of signal 2 to immobilized α-tocopheroxyl free radical Kohl and Wood proposed a plastochromanoxyl structure for the radical.[67] Despite a tentative identification of signal 2, no functional role for this component in photosynthesis has been determined, even though two hypotheses for the mechanistic origin of signal 2 are presently in contention. Hypothesis A regards signal 2 as a reflection of the behavior of a quinoidal component situated near the reducing side of PS 2.[20,66-68] Hypothesis B, on the other hand, views signal 2 as reflecting the oxidation state(s) of components which function on the water-splitting (oxidizing) side of PS 2.[63,69] Future investigations of signal 2 are likely to resolve these conflicting hypotheses, thus clarifying the relationship of this stable radical to photosynthetic processes.

Experiments at 77 K in ferricyanide-treated chloroplast or PS 2 subchloroplast preparations have revealed the presence of a new light-induced, featureless esr signal.[70] This species has tentatively been assigned to the PS 2 reaction center chlorophyll on the basis of similarity of the esr parameters (ΔH_{pp} ~8 G, g = 2.0025) to those of signal 1. This signal is only observable at low temperatures *and* in the presence of high concentrations of ferricyanide. This chlorophyll-like resonance is generated most efficiently in red light, that is, light which supports photosystem 2 reactions. Although the esr behavior of this "photosystem 2" signal is analogous to the ferricyanide-induced fluorescence quenching reported by Okayama and Butler,[61,71] no optical confirmation has been reported to support the assignment of this new resonance to P680, the PS 2 counterpart of P700. Likewise, no esr signal corresponding to the primary electron acceptor, Q, of system 2 has been observed, even though one might be expected.

The six-lined resonance characteristic of manganese is often observed in chloroplast or algal preparations. Bound manganese has been implicated in the oxygen evolution process and is a necessary requirement for plant growth.[72] The manganese signal commonly seen in aged or hydroxylamine-treated samples is believed to result from photosystem 2 centers which have lost bound manganese. Although steady-state changes in the manganese signal intensity during photosynthesis have been reported,[73,74] no concrete evidence for *in vivo* manganese participation in system 2 has been established by esr. Examination of system 2 functional manganese by esr may be prohibitive, since this protein-complexed ion may possess a very efficient relaxation process. In addition, the extreme line width of the manganese spectrum (~600 G) renders the detection of any transient intensity change arduous. However, application of a light-modulation detection system[37] to the study of photosystem 2 may overcome this obstacle.

Other Esr Signals. Additional esr signals are often seen in photosynthetic materials; however, on the whole, these have not attracted the attention accorded to the previously discussed resonances. Often signals arising from transition metals present in growth or suspension media (*e.g.*, iron or manganese) may be observed; however, these interferents can be removed by washing or complexation, or ignored.

Although triplet excitation mechanisms have been proposed in photosynthesis, until recently there has been no esr evidence for existence of triplet intermediaries. Dutton, Leigh, and Seibert have reported the observation of a triplet species at 8 K in chromatophores unable to carry out photochemistry.[75] This triplet spectrum was somewhat unique, since some of the transitions were observed to be in emission. This phenomenon can be explained by a selective population of some of the triplet spin states due to long electron spin relaxation times inherent at cryogenic temperatures. The zero-field splitting parameter, D, determined for this biological system (0.0154 cm^{-1}) is notably less than that observed in an *in vitro* chlorophyll triplet (0.0306 cm^{-1}).[76] This difference may be attributed to the more ordered, compacted array of chlorophyll inherent in the photosynthetic unit. Alternatively, the reduced D value may result from stabilization of the two unpaired electrons on adjacent reaction center chlorophylls comprising the hypothetical dimer proposed by Norris, *et al.*[33] The participation of this triplet entity in the primary charge separation process, although postulated,[75] has not yet been established. A similar triplet resonance has also been demonstrated in spinach chloroplasts.[75]

Weaver has recently reported an additional light-induced signal in the blue-green alga, *Anacystis ni-*

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dulans.[77] This resonance is nonstructured, has a *g* factor of 2.0042, and is not readily saturated by microwave power. Formation and decay kinetics are complex for this species, and dichlorophenyldimethylurea has been demonstrated to inhibit the formation of this radical. Although Weaver has named this resonance signal III, we prefer not to apply this title until this species is demonstrated to occur in other algal species. A similar resonance has been detected by Norris, *et al.*,[78] in the thermophilic blue-green alga *Synechoccus lividus.* However, the *S. lividus* signal appears to be associated only with PS 1 and has been assigned to the flavoprotein flavodoxin.

Projections

Although a sizable number of radical entities have been observed by esr in photosynthetic organisms, future investigations must focus on those that are not presently detectable. Many of these "hidden" intermediates could provide the basis for cracking such difficult puzzles in electron transport as the oxygen evolution center, sites of photophosphorylation, and the nature of the intermediate electron-transport chain. Additionally, future research will increasingly be concerned with the formation of model systems and the reassembly of photosynthetic units from constituent parts.

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Application of such powerful techniques as stop-flow, rapid-freeze, and flash photolysis will continue to dominate photosynthetic esr investigations. Extension of transient flash photolysis esr systems into the microsecond domain should make possible the search for short-lived radical components in the intermediate electron chain (*e.g.*, plastosemiquinone). Likewise, steady-state or light-modulation experiments can be expected to increase our knowledge of pool sizes and energy migration mechanisms. Although spin labeling has been generally neglected in photosynthetic studies, specially tailored cofactors could provide a means for esr to monitor changes of transition metal species at physiological temperatures or conformational changes of protein complexes involved in energy storage (photophosphorylation). Application of cross-relaxation phenomena may likewise allow the esr practitioner to view changes in the paramagnetic state of significant ion species (*e.g.*, Mn, Fe).

This overview of the contribution of esr to our current knowledge of photosynthesis has been necessarily brief. However, it is the authors' desire that the spirit of hope and anticipation for future success in esr investigations in the biological realm will be transmitted to the reader.

This work was supported, in part, by the U. S. Atomic Energy Commission and a National Research Council grant (Canada) to J. R. Bolton.

Simple Kinetic Models from Arrhenius to the Computer

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Received January 7, 1974

"I consider induction to be that form of demonstration which upholds the sense, and closes with nature, and comes to the very brink of operation ... for hitherto the proceeding has been to fly at once from the sense and particulars up to the most general proposition, as certain fixed poles for the argument to turn upon, and to derive the rest ... Now my plan is to proceed regularly and gradually from one axiom to another, so that the most general are not reached till the last; but then, when you do come to them, you find them to be not empty notions but well defined, and such as nature would really recognize as her first principles, and such as lie at the heart and marrow of things ... Those who aspire not to guess and divine, but to discover and know, who propose not to devise mimic and fabulous worlds of their own, but to examine and dissect the nature of this very world itself, must go to facts themselves for everything."

F. Bacon, 1620

"The principles of thermodynamics occupy a special place among the laws of nature ... their validity is subject only to limitations which, though not, perhaps, of themselves negligibly small, are at any rate minimal in comparison with many other laws of nature ... there is no natural process to which they may not be applied ... considerations of molecular theory are less suited to correct established thermodynamical laws than to be themselves accommodated thereto."

W. Nernst, 1917

"The underlying physical laws necessary for the mathematical theory of a large part

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