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In Vitro Models for Photosynthetic Energy Conversion

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Abstract

A summary is presented of recent work on the photochemistry of chlorophyll in solution. It is shown that reactions occur which are close counterparts of *in vivo* photoprocesses. These are (a) photoproduction of chlorophyll cation radical (analog of photosystem I reaction centre primary photoprocess), (b) one-electron phototransfer from bacteriochlorophyll to quinone (analog of bacterial reaction centre primary photoprocess), (c) chlorophyll photosensitized one-electron transfer from hydroxylic compounds to quinone (analog of photosystem II reaction centre photoprocess). The mechanisms of these reactions and their implications for photosynthetic energy conversion are discussed.

Introduction

Conversion of energy from one form to another is involved in an enormous variety of physical, chemical and biological systems. Virtually all of man's technology, and indeed his very existence, is absolutely dependent upon the occurrence of energy conversion processes. One need only point out that the sole source of energy for all of the life forms which exist on the earth is electromagnetic radiation from the sun, and that in order for this to be available to do biological work it must first be converted into chemical bond energy. This type of conversion lies at the heart of the photosynthetic process in higher plants, algae and bacteria. Furthermore, as a by-product of energy capture in photosynthesis, at least in green plants, molecular oxygen is generated

from water. Oxygen also plays an important role in bioenergetics as a consequence of its participation in ATP formation during respiration. It is clear from these considerations that an understanding of the molecular-electronic processes which are involved in photosynthetic energy conversion and oxygen formation constitutes an important intellectual goal for science. Furthermore, it has long been recognized that solar energy represents a potentially enormous technological resource, if efficient means could be found to convert it into usable forms. In this context the photobiological systems could provide an important input, inasmuch as the biotechnology represented therein has evolved over a period of millions of years to an extremely high level of sophistication and efficiency [1]. Thus, if one could obtain a clear understanding of how these systems operate and could learn to duplicate their performance in simple photochemical models, it is quite possible that new and superior technologies for solar energy utilization would be suggested. The goals of the research to be described in this article are to provide such inderstanding and to develop such model systems.

General Consideration of Photosynthetic Energy Conversion

In view of the enormous literature which exists in the field of photosynthesis, it would be futile to attempt to provide an historical review of all of the information which is relevant to this article. Thus, it seems reasonable to state, without detailed documentation, a number of generally accepted viewpoints which form the basis of our experimental approach, and to reserve a more in-depth consideration of the literature to those aspects which bear directly on specific investigations which we have done. Excellent sources of material dealing with the more general concepts in photosynthesis are the following: R. K. Clayton, "Molecular Physics in Photosynthesis", Blaisdell, New York, 1965; R. K. Clayton, "Light and Matter, Vol. 2: The Biological Part", McGraw-Hill, New York, 1971.

In an overall sense, photosynthesis represents a photopotentiated transfer of electrons against a thermodynamic energy gradient from a donor (water in the case of green plants, sulfide or reduced carbon compounds in the case of bacteria) to an acceptor (generally pyridine nucleotide). A variety of electron carriers participate as intermediates in this process (eg cytochromes, plastocyanin (a copper containing protein), non-heme iron proteins, flavoproteins). ATP synthesis has been shown to be coupled to this electron transport system. Light energy enters the system at two points in green plants. One of these is located on the water oxidation side (Photosystem II) and the other on the pyridine nucleotide reduction side (Photosystem I). Specialized forms of chlorophyll [2] participate in the energy conversion processes occurring at the so-called reaction centres in these two photosystems. These are distinguishable

from the bulk of the chlorophyll (which serves a light harvesting function and feeds energy into the reaction centres by non-radiative transfer) mainly on the basis of absorption spectral properties, although recent developments have permitted fractionation of chloroplast materials so as to provide a structural as well as functional separation. The reaction centre chlorophyll of Photosystem II (PSII) is usually designated P_{680}, and that of Photosystem I (PSI) as P_{700}, based on the wavelengths at which spectral changes can be observed which correlate with the functioning of these species. In the most commonly accepted scheme of photosynthesis, these two light reactions operate in series and are connected by a chain of electron carriers. The chemical nature of the immediate electron donors and acceptors which are involved with P_{680} are unknown. The immediate electron donor to P_{700} is probably a c-type cytochrome or plastocyanin; the electron acceptor is uncertain, although it may be a non-heme iron protein. It has been clearly established, however, that the primary electron transfer in both photosystems involves only a single electron and that the photochemistry of PSII generates a strong oxidant and a weak reductant, while that of PSI produces a weak oxidant and a strong reductant.

The situation in the photosynthetic bacteria is somewhat simpler than in the green plants. Here only a single photosystem is most likely operative (although some workers have suggested that more than one photoreaction may actually occur) which resembles PSI in its properties. The reaction centre chlorophyll in the bacteria has been designated P_{870}. The electron donor to P_{870} is a c-type cytochrome; the acceptor is either ubiquinone or a non-heme iron protein (or perhaps a complex containing both of these species).

It is clear from the above that the basic phenomenon underlying energy conversion in photosynthesis involves the coupling of photon absorption to one-electron transfer. Thus, a critical question is: what are the molecular-electronic mechanisms by which this coupling is achieved in the two photosystems? As will be documented below, a partial answer to this question can be given on the basis of our own work and that of others. A second question which arises out of these considerations is the following: what is the mechanism by which oxygen is produced from water? We will also demonstrate below that some insights into this problem have been obtained as a consequence of our work.

Experimental Approach

In order to attempt to elucidate the molecular-electronic mechanisms by which chlorophyll functions in the energy conversion processes of photosynthesis, we have chosen to utilize model systems. Implicit in such an approach is the assumption that one can in fact devise models

which are relevant to chlorophyll function in the biological environment. As will be demonstrated below, we feel that this requirement has been reasonably met. It is also important to point out that, in view of our current state of relative ignorance concerning the complex ultrastructure of the photosynthetic apparatus, it is unlikely that a simple model would be able to duplicate all of the *in vivo* properties of chlorophyll or its detailed environment and physical state. However, it should be possible to formulate general principles concerning chlorophyll photophysical and photochemical properties and to utilize these to interpret and explain the results obtained from *in vivo* studies.

The specific systems we have studied have been solutions of chlorophyll, bacteriochlorophyll or other porphyrin analogs either alone or in the presence of electron acceptors or donors. The latter have most often been quinones and hydroquinones. Quinones have long been considered as likely candidates for the role of primary electron acceptor, and recent results (see below) have provided a firmer experimental basis for this postulate, at least in bacterial photosynthesis.

The techniques which we have utilized in this research have been principally flash photolysis [3] and electron spin resonance (ESR) spectroscopy [4]. In the flash photolysis method, a sample is subjected to a short (several nanoseconds to several microseconds, depending upon the light source), bright flash of light which converts an appreciable fraction of the light-absorbing molecules into an electronically excited state. The subsequent transformations which occur within the system as a function of time are then followed by conventional absorption spectrophotometry. In ESR spectroscopy, molecules containing unpaired electrons can be detected by virtue of transitions between spin states which are induced by microwave irradiation of the sample in a strong magnetic field. Thus, free radical species which might be produced by light absorption are observable.

Photoproduction of Chlorophyll and Bacteriochlorophyll Cation Radicals: Models for PSI.

Experiments by Commoner *et al.* [5] have shown that two electron spin resonance signals can be generated in green plants and algae upon illumination. These have been designated as signals I and II, and are respectively associated with the two photosystems of green plant photosynthesis (PSI and PSII). Signal II is a broad asymmetric resonance with partially resolved hyperfine interactions ($\Delta H_{pp} \simeq 19$ gauss and $g \simeq 2.0045$) which decays slowly (many minutes) upon the termination of illumination [6]. Signal I, on the other hand, is a symmetrical resonance with $\Delta H_{pp} \simeq 7$ gauss and $g \simeq 2.0025$, and decays rapidly when the light is extinguished. Intact photosynthetic bacteria give rise to only one ESR signal, which is very similar to signal I except that ΔH_{pp} is several gauss wider [7]. Recently, however, a second ESR signal has been

discovered in bacterial photoreceptor subunit preparations which have been specially treated to remove iron [8, 9, 10, 11]. It has been suggested that this signal is due to a ubiquinone radical. It has generally been assumed that the precursor to signal II is a quinone, most likely a plastoquinone [6]. However, more recently other alternatives have been suggested, eg, a pteridine radical [12, 13] or a plastochromanoxyl radical [14].

The greatest success with regard to the identification of the species giving rise to these signals has come from studies of signal I and its counterpart in bacteria. It has been shown that in some photosynthetic bacteria (eg, *Rhodopseudomonas spheroides)* the appearance of the ESR signal corresponds exactly to a bleaching of P_{870} [15, 16, 17]. Recently, Warden and Bolton [18], using simultaneous optical and ESR detection, have demonstrated an exact correspondence between the generation of signal I and the bleaching of P_{700} in green plants and, more significantly, between the regeneration of P_{700} and the decay of signal I. Hence, there seems to be little doubt that the precursors of the radicals which display these ESR signals are the reaction centre components P_{700} and P_{870}. The work of Borg *et al.* [19], who carried out an extensive study of the cation radical of chlorophyll *in vitro,* showed that a reasonable correspondence exists between the ESR parameters of $Chl^{+\cdot}$ ($\Delta H_{pp} \simeq 9$ gauss and $g \simeq 2.0025$) and those of signal I. On the basis of this they identified signal I as the cation radical of a reaction centre chlorophyll molecule (ie $P^{+\cdot}_{700}$). Furthermore, an analogous study [17] has demonstrated that the ESR parameters for bacteriochlorophyll cation radical *in vitro* correspond in a similar manner with the *in vivo* results, which suggests that oxidized P_{870} is the ESR active species. Additional chemical evidence has been obtained which demonstrates that the formation of signal I is the result of a one-electron oxidation [20]. The experiments described above can be considered to provide strong support for the concept that the primary photochemical event in the PSI and bacterial reaction centres involves the generation of a chlorophyll cation radical.

It is important to note that prior to our work the *in vitro* formation of $Chl^{+\cdot}$ had been achieved only through chemical or electrolytic oxidation. Thus, the question arose as to whether or not chlorophyll *in vitro* could undergo photo-oxidation to produce this species. We have recently shown [21] that illumination ($\lambda > 550$ nm; Corning CS 3-66 filter) of solutions of chlorophyll in various solvents (alcohols, dichloromethane, tetrahydrofuran, etc.) at low temperatures (−50 to −150°C) produces a paramagnetic species whose ESR spectrum has no resolvable hyperfine structure and is characterized by $g = 2.0025 \pm 0.0003$ and $\Delta H_{pp} \simeq 7.5$ gauss. Furthermore, bacteriochlorophyll will also generate a single line ESR signal upon illumination at low temperatures ($\Delta H_{pp} \simeq 12.5$ gauss). Comparison of these ESR spectra with those obtained upon I_2 oxidation

and upon use of deuterated chlorophyll [22] has led to the conclusion that the paramagnetic species being produced was the cation radical of the chlorophyll molecule. Quenching experiments utilizing β-carotene and O_2 suggested that the chlorophyll excited singlet state was the precursor of this radical [23]. Pheophytin (chlorophyll minus the chelated magnesium) will not generate a cation radical upon illumination.

When chlorophyll and benzoquinone were illuminated with red light in dry acetone at low temperatures, a greatly enhanced $Chl^{+\cdot}$ signal was obtained. Thus, quinone can act to facilitate the photoejection of an electron from excited chlorophyll without the formation of quinone anion radical as a final product. This could involve coordination with the central magnesium atom inasmuch as pheophytin is not able to undergo this reaction. It is noteworthy that bacteriochlorophyll in dry acetone behaves differently from chlorophyll and upon illumination transfers an electron to benzoquinone with the generation of $BChl^{+\cdot}$ and $BQ^{-\cdot}$ (see below for details).

The photochemical generation of $Chl^{+\cdot}$ *in vitro* can be considered to be analogous to the photoreactions of PSI and of bacterial photosynthesis. Thus, it appears that the one-electron photo-oxidation of chlorophyll is an inherent property of the molecule rather than a capability which results from its specific environment in the chloroplast. The requirement for the presence of Mg for this reaction suggests that a complex involving the Mg and a Lewis-base type ligand (solvent molecules or quinone molecules in the present case; *in vivo* this could correspond to the primary acceptor) may be present in the PSI and bacterial reaction centres. This could act to facilitate ejection of the electron by utilization of the ligand as a pathway for removal. If the appropriate electron acceptor were present (eg, a non-heme iron protein), presumably the ejected electron could be trapped. In fact, recent experiments in our laboratory have shown that the presence of Fe(III) in chlorophyll-quinone solutions results in an increased efficiency of cation radical photoproduction.

Photosensitization by Chlorophyll of One-Electron Transfer: Models for PSII

Studies in our laboratory of the mechanism of photochemical reduction reactions involving chlorophyll have provided new insights into the processes occurring in PSII of green plants. Much of our work has utilized quinones as electron acceptors. The basic observation is that red light illumination of deoxygenated solutions of chlorophyll and quinone leads to the formation of semiquinone radicals, ie, the one-electron reduction product of the quinone. Our earlier work (see ref. 24 for summary and pertinent references) established the following facts:

1. radical formation is completely reversible, ie, there is no net chemical change occurring;
2. the quantum yield is high (0.1-1.0 depending upon the temperature);
3. the lowest triplet state of chlorophyll is involved in sensitization;
4. no ESR signal due to a chlorophyll radical is observable at temperatures above $-90°C$;
5. radical formation does not occur in hydrocarbon or aprotic solvents;
6. quinone radical decay is by disproportionation (ie, $2Q^{\cdot-} + 2H^{+} \rightarrow Q + H_2Q$);
7. a variety of quinones will function as electron acceptors.

Flash photolysis studies (24, 25) indicated that prior complex formation between quinone and chlorophyll was involved and that the source of electrons for quinone reduction possibly was the solvent. More recently (26, 27), we have obtained ESR evidence for solvent (ethanol) oxidation sensitized by chlorophyll in the presence of quinone (bacteriochlorophyll and pheophytin were shown to behave similarly). Fig. 1a shows some of the spectra (obtained with pheophytin as sensitizer) upon which this conclusion was based. Note that the spectral resolution is markedly affected by the presence and number of deuterium atoms in the solvent molecules. Furthermore, replacement of hydrogen by deuterium in the quinone also changes the spectral shape. Varying the microwave power levels in the ESR spectrometer (Fig. 1b) shows that the inner and outer regions of the spectrum respond differently. This latter result is good evidence for the presence of two radical species whose spin states exhibit different relaxation properties. One of these is undoubtedly the quinone anion radical (as shown by the deuterium substitution) and the other was suggested to be a solvent radical (probably $EtOH^{+}$). Computer simulation studies have provided further support for this interpretation. It is significant that blue light irradiation of quinone alone in ethanol produces an identical set of ESR spectra (we will come back to this point below).

Measurements of the decay kinetics of these radical signals demonstrate that first order behaviour obtains and that the decay rate is influenced by the nature of the sensitizer molecule used (Fig. 2). This shows that the two radical species and the sensitizer are not kinetically independent of one another, ie, that complex formation is occurring.

When chlorophyll (or bacteriochlorophyll) was used as the sensitizer, a cation radical ESR signal was produced in addition to the signals shown in Fig. 1. Quenching experiments indicated that the formation of this species proceeded by an independent photoprocess, and thus is presumably the result of the same reaction described in the preceding section.

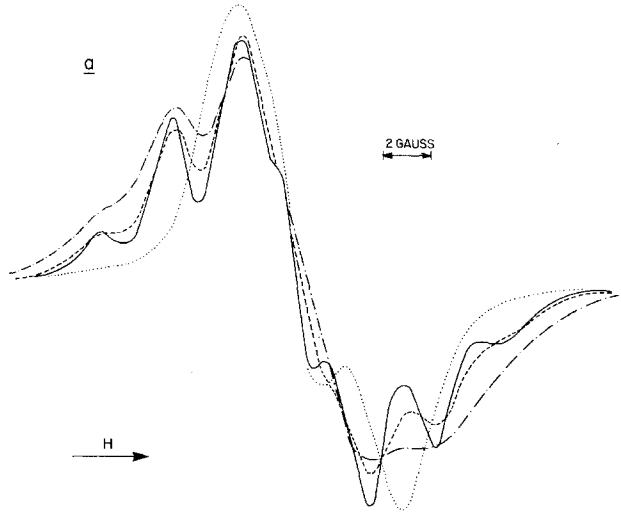

Figure 1a. Comparison of ESR spectra obtained upon irradiation with red light at −137° C of degassed solutions of pheophytin (10^{-5} M) and benzoquinone (10^{-2} M) in ethanol with varying degrees of deuteration

—•— Ethanol + Benzoquinone
--------- Ethanol-OD + Benzoquinone
——— Ethanol-d_6 + Benzoquinone
• • • • Ethanol-d_6 + Benzoquinone-d_4

The following mechanism was proposed to account for our results:

1. $Chl + h\nu \rightarrow Chl_S \rightarrow Chl^{+\cdot} + e^-$
2. $Chl_S \rightarrow Chl_T$
3. $Chl_T + Q{---}EtOH \rightarrow (Chl_T{---}Q{---}EtOH)^{exciplex}$
4. $(Chl_T{---}Q{---}EtOH)^{exciplex} \rightarrow (Chl{---}Q^{-\cdot}{---}EtOH^{+\cdot})$
5. $(Chl{---}Q^{-\cdot}{---}EtOH^{+\cdot}) \rightarrow (Chl{---}Q{---}EtOH)$ (low temperatures)
6. $(Chl{---}Q^{-\cdot}{---}EtOH^{+\cdot}) \rightarrow Chl + Q^{-\cdot} + EtOH^{+\cdot}$ (high temperatures)
7. $Q^{-\cdot} + Q^{-\cdot} + 2H^+ \rightarrow Q + H_2Q$
8. $EtOH^{+\cdot} + EtOH^{+\cdot} \rightarrow EtO\text{-}OEt + 2H^+$
9. $H_2Q + EtO\text{-}OEt \rightarrow Q + 2EtOH$

Equations [3], [4] and [5] represent the formation of a ternary complex between chlorophyll triplet and a solvated quinone molecule which undergoes an electron transfer process. The resulting radical complex is stabilized at low temperatures and decays by a first order

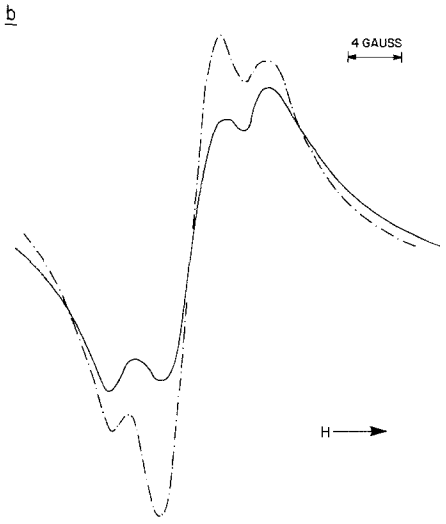

Figure 1b. Microwave power dependence of ESR signal at −160° C; pheophytin (10^{-5} M), benzoquinone (10^{-2} M) in ethanol-OD

——— 20 mW
—•— 1 mW

process (reverse electron transfer) back to a neutral state. As the temperature is raised, the complex gains sufficient thermal energy to break apart into its constituent components (equation 6). Once this has occurred, $Q^{\cdot-}$ and $EtOH^{\cdot+}$ are free to disproportionate and dimerize (equations 7 and 8). The $EtOH^{\cdot+}$ species must be sufficiently unstable so as to yield a steady-state concentration which is undetectable by ESR in order to be consistent with the fact that no solvent radical signal is observed in the liquid phase. On the other hand, $Q^{\cdot-}$ decay is slow enough so that it accumulates to a measurable extent under these conditions. Equations [8] and [9] are presented as a likely means by which the reversibility of the system may be maintained, and have no direct experimental justification. The mechanism is consistent with the fact that no $Chl^{\cdot+}$ can be observed as a direct consequence of quinone radical formation (i.e., it is formed independently at low temperatures via reaction [1]).

Since we observe the same ESR signals upon illumination with blue light of benzoquinone alone in ethanol, it is likely that a similar diradical complex is being generated, ie ($Q^{\cdot+}$---$EtOH^{\cdot+}$). Thus, if chlorophyll could transfer sufficient energy to benzoquinone, it could effect formation of a diradical complex. However, the energy levels of these molecules prevents this type of energy transfer. How, then, can one account for

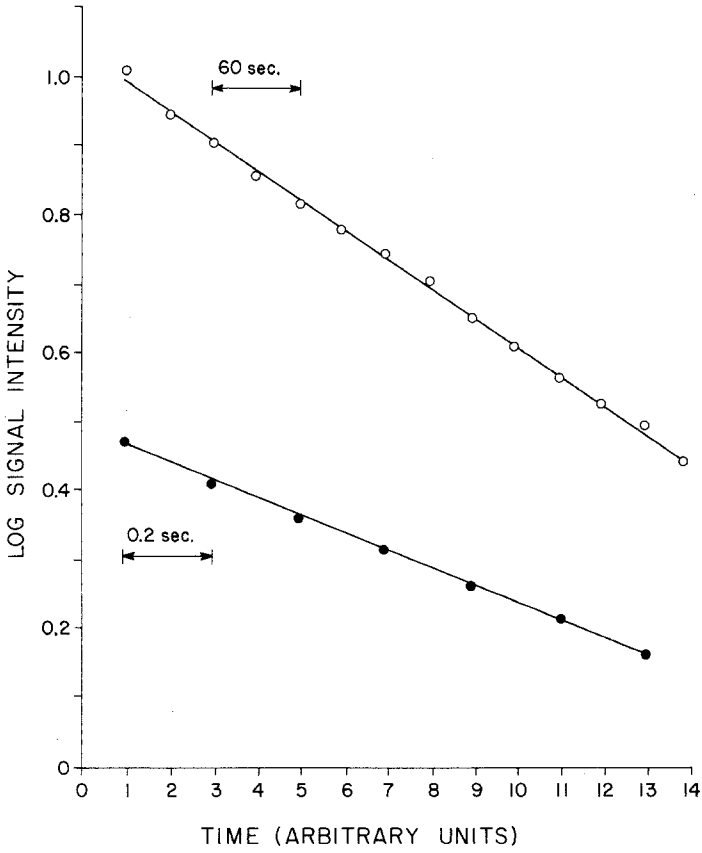

Figure 2. First order plots of decay kinetics of ESR signals obtained upon irradiation with red light at −140° C of degassed solutions of chlorophyll or pheophytin (10^{-5} M) and benzoquinone (10^{-2} M) in ethanol. Note difference in time scales. The decay rate of signal obtained with benzoquinone alone is slightly greater than that obtained with pheophytin sensitization.

○——○ Phe plus BQ
●——● Chl plus BQ

sensitized radical production? This apparent dilemma can be clarified somewhat if we consider the nature of the precursor to the ternary complex (see equation 3). Once an exciplex is formed, the energy levels of the separate species are perturbed, and this is accompanied by a change in the quantum mechanical wave function which describes the system. It is not unreasonable to suppose that this new wave function permits energy delocalization into the quinone component so that the electron transfer can occur, resulting finally in the formation of the

ternary radical complex. Thus, according to this view, it is exciplex formation which allows sensitization to occur. A second possible way of interpreting this is that internal conversion processes within the exciplex lead to the formation of a vibrationally excited ground state of the solvated quinone molecule. The electron transfer reaction, providing it is rapid enough to compete with further non-radiative deactivation, could thus obtain activation energy by coupling to one or more of these vibrational modes. It may be significant in this regard that we have evidence that electron transfer between alcohols and quinones can proceed in the dark in basic solutions utilizing a thermally-activated pathway. Although further studies of the mechanism of this photo-process are required, it is apparent that this system provides a promising avenue for obtaining insight into the fundamental details of photochemical energy conversion by chlorophyll.

The indication that ethanol oxidation could be achieved by chlorophyll raised the possibility that water itself might also be oxidizable. Recent ESR studies [28] have in fact provided evidence for this. As mentioned above, illumination with red light of degassed solutions of benzoquinone and pheophytin in dry acetone resulted in no observable ESR spectra at any temperature. Hence, no apparent quinone reduction (to yield the semiquinone radical) or cation radical formation occurred in this system, at least as could be detected by steady-state observation. This can be interpreted in terms of our ethanol studies to imply that acetone cannot function as an electron donor, particularly in view of the fact that other studies in our laboratory have shown that the semiquinone radical can be readily formed in dry acetone. We have already noted that chlorophyll plus benzoquinone in dry acetone yields Chl^+ upon illumination. However, when small amounts of ethanol or water (5-20% v/v) were added to the dry acetone [29], excitation of pheophytin at temperatures at which the solution was still fluid resulted in a large ESR signal due to the quinone anion radical. This can be interpreted in terms of providing a suitable electron donor to the system. It is also of importance to point out that sensitization by direct visible light excitation of quinone (in the absence of pheophytin) gave identical results in the acetone system, as was the case in pure ethanol.

In a series of studies analogous to those carried out with pure ethanol, in which microwave power was varied and deuterium substitutions made, evidence was obtained for the formation of two radical species in the acetone-H_2O mixtures. One of these was the quinone anion radical and the other was presumed to be a water-derived radical. Some of the data are shown in Fig. 3. Note that increasing microwave power levels act to selectively suppress the central portion of the ESR spectrum (this is again evidence for the existence of two radical species) and that replacement of H_2O by D_2O narrows this region of the spectrum.

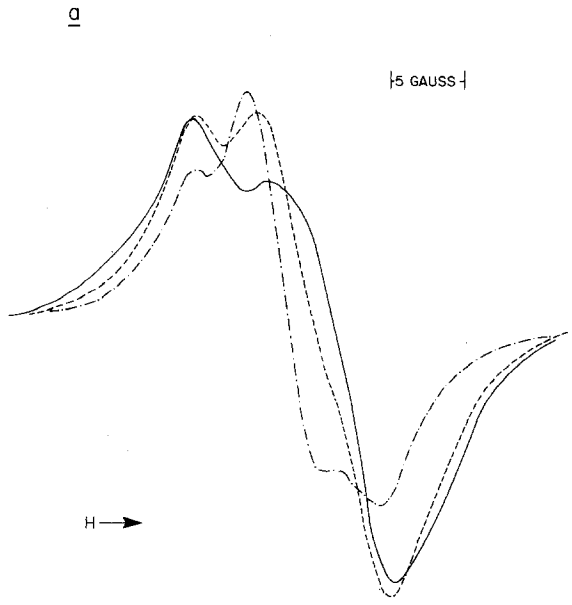

Figure 3a. ESR spectra obtained upon illumination and rapid freezing of a degassed solution of pheophytin (10^{-5} M) and benzoquinone (10^{-2} M) in acetone-H_2O (90% v/v) at −140° C.

——— 20 mW
--------- 5 mW
—•— 1 mW

Furthermore, deuteration of the BQ narrows the outer portion of the spectrum.

The actual identity of the water-derived radical suggested as being formed in these photoreactions must be considered uncertain, inasmuch as there is no correspondence between the known ESR parameters for various water radicals and the present data. Although a likely candidate is $H_2O^{\cdot+}$, an ESR spectrum for this radical has not been reported in the literature. However, the $NH_2^{\cdot}$ radical, which is isoelectronic with $H_2O^{\cdot+}$, has been observed [30] and has $a_H = 23$ gauss. This is much broader than the ESR signal which we have observed, although the spin distribution in $NH_2^{\cdot}$ is known to be highly dependent upon its environment. An alternative could involve a cluster of H_2O molecules functioning as an electron donor, such that upon oxidation the radical species formed could be represented as $(H_2O)_n^{\cdot+}$. Delocalization of the unpaired spin throughout such a cluster could account for the narrow line width which we observe and the relatively small effect of deuteration.

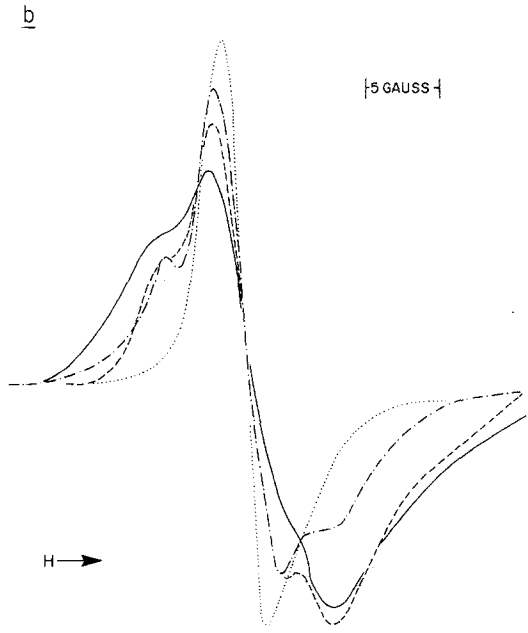

Figure 3b. ESR spectra obtained after illumination and rapid freezing of a degassed solution of pheophytin (10^{-5} M) and benzoquinone (10^{-2} M) in acetone-D_2O (90% v/v) at −140° C.

——— 20 mW, benzoquinone
--------- 5 mW, benzoquinone
—•— 1 mW, benzoquinone
• • • • 5 mW, benzoquinone-d_4

If H_2O or D_2O (5-10% v/v) was added to a solution of chlorophyll and benzoquinone in dry acetone, the spectrum shown in Fig. 4 was obtained at low temperatures. The spectrum obtained in dry acetone is shown for comparison. The appearance of two new peaks at low magnetic field (high g-value) is clearly evident. These lines are exactly coincident with the low field portion of the spectrum shown in Fig. 3, and the effects of microwave power variation were identical. This result can be interpreted as evidence that chlorophyll is also sensitizing the same electron transfer as does pheophytin (or quinone upon direct excitation), in parallel with cation radical formation. The results of these experiments can be interpreted in terms of the following mechanism (by analogy to the ethanol system):

1. $Chl \xrightarrow{h\nu} Chl_S \rightarrow Chl_T$
2. $Chl_T + BQ + H_2O \rightarrow [Chl\text{-}BQ\text{-}H_2O]^*$
3. $[Chl\text{-}BQ\text{-}H_2O]^* \rightarrow [Chl\text{-}BQ^{\cdot-}\text{-}H_2O^{\cdot+}]$ (low temperatures)
4. $[Chl\text{-}BQ^{\cdot-}\text{-}H_2O^{\cdot+}] \rightarrow BQ^{\cdot-} + H_2O^{\cdot+}$ (high temperatures)
5. $BQ^{\cdot-} + BQ^{\cdot-} + 2H^+ \rightarrow BQ + H_2Q$
6. $2H_2O^{\cdot+} \rightarrow H_2O_2 + 2H^+$
7. $H_2Q + H_2O_2 \rightarrow Q + 2H_2O$

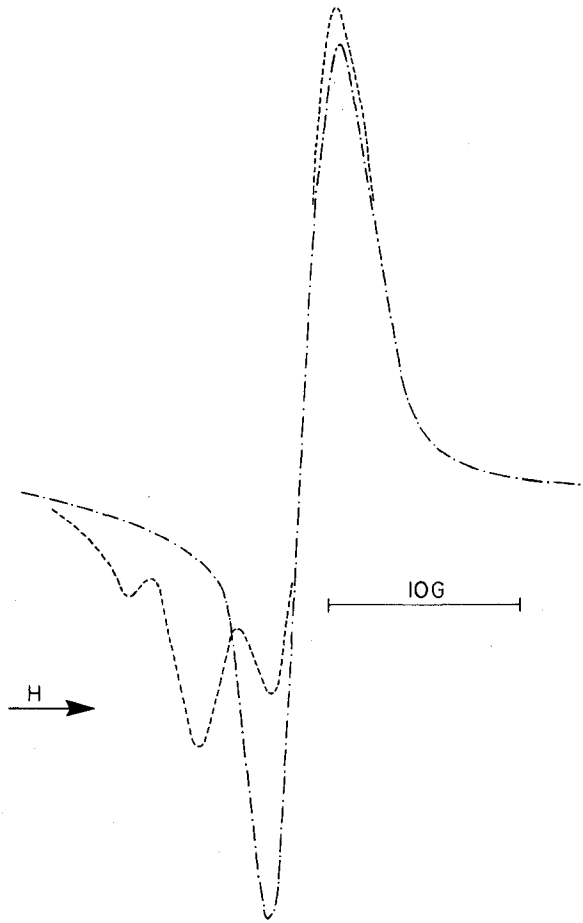

Figure 4. ESR spectra obtained after illumination with red light of deuterated chlorophyll-benzoquinone solutions at −140° C. Microwave power = 1 mW.

—•— Dry acetone
--------- Acetone D_2O (90% v/v)

[Chl–BQ–H_2O]* represents an excited complex (exciplex) and [Chl–$BQ^{\cdot-}$–$H_2O^{\cdot+}$] the biradical complex. Reactions 4-7 summarize the liquid phase mechanism; reactions 6 and 7 are included as a likely pathway for the decay of the water radical. The fact that direct benzoquinone excitation produces identical radical species is significant inasmuch as it implies that the electron necessary for quinone reduction is available in the absence of chlorophyll and thus suggests that chlorophyll electrons are not directly involved in electron transfer to produce radicals.

Our results are in accord with the work of Soma [31], in which it was found that H_2O and ethanol were effective liquid electrodes for photoinjection of electrons into quinone crystals and that chlorophyll dissolved in the ethanol could sensitize this process. In addition, we have provided evidence that chlorophyll can photosensitize a one-electron transfer from H_2O to quinone (since Soma used pure H_2O he could not utilize a chlorophyll solution as an electrode as in the case of ethanol). The results also suggest a possible route of photosynthetic oxygen formation and thus can be considered as providing a model for the photochemistry of PSII. Specifically, the $H_2O^{\cdot+}$ species, through proton loss and dimerization, can form hydrogen peroxide. Oxygen can easily be produced from peroxide via a catalase-type of enzyme reaction. It should be noted that four such $H_2O^{\cdot+}$ radicals would be required to produce one oxygen molecule, which is in accord with current concepts of PSII function.

One-Electron Phototransfer between Bacteriochlorophyll and Quinone: Model for Bacterial Photosynthesis

If a degassed solution of bacteriochlorophyll in dry acetone is irradiated (Corning CS3-66 filter; $\lambda > 550$ nm) in the temperature range $-100°$ to $-150°C$, only a small ESR signal due to $BChl^{\cdot+}$ is generated (see above). However, in the presence of benzoquinone illumination under identical conditions results in the generation of large ESR signals [32]. The signal obtained at $-140°C$ is shown in Fig. 5 at two different microwave power levels. It can be seen from these spectra that two radical species of differing power saturation characteristics were produced. The outer resonance, with ΔH_{pp} of 12-13 gauss, most likely is due to $BChl^{\cdot+}$.

In order to determine if the central resonance signal was due to a quinone radical, we utilized fully-deuterated benzoquinone ($BQ\text{-}d_4$) in place of the normal species. The narrowing of ΔH_{pp} in the central region of the spectrum and the corresponding increase in intensity which occurred when $BQ\text{-}d_4$ was used demonstrated that the central ESR signal was indeed due to a quinone radical. The fact that the quinone signal occurred at lower magnetic field (ie, higher g-value) than the outer

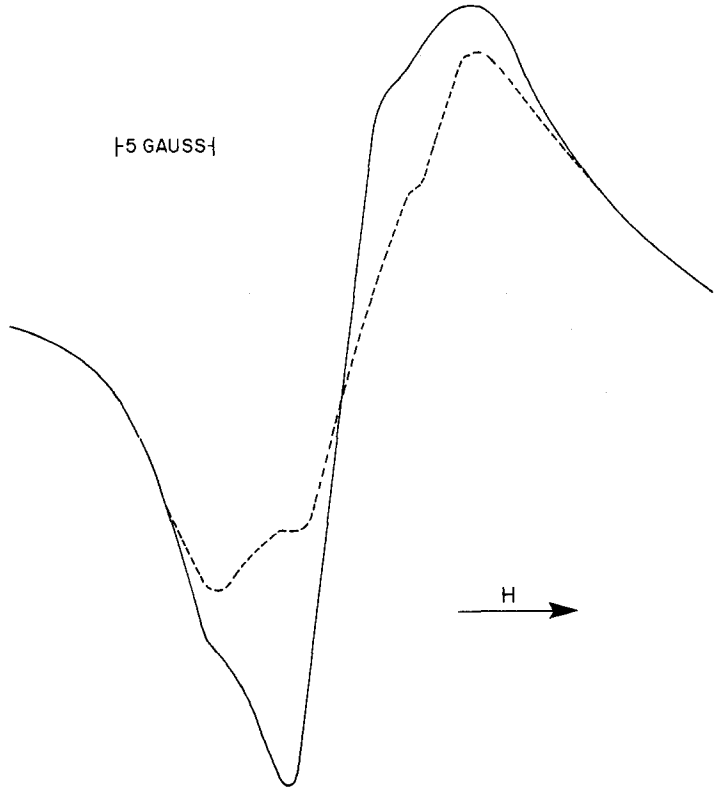

Figure 5. ESR signals obtained at −140° C upon illumination with red light of a degassed solution of bacteriochlorophyll (10^{-5} M) and benzoquinone (10^{-2} M) in dry acetone.

——— 1 mW
--------- 20 mW

resonance was consistent with expectation. These observations provide strong evidence that a direct light-induced electron transfer occurred between the components leading to the formation of $BChl^{+}$ and $BQ\cdot^{-}$. Similar results were obtained using ubiquinone in place of benzoquinone. This is directly analogous to observations made with iron-free reaction centre preparations from photosynthetic bacteria [8, 9, 10, 11], in which two ESR signals are produced by light and are identified as being due to oxidized bacteriochlorophyll and reduced ubiquinone.

Summary and Conclusions

The data presented in the above sections has provided evidence for the occurrence of several types of photoreactions which mimic the primary events in green plant and bacterial photosynthesis. These are:

1. Cation radical photoproduction from chlorophyll (analog of photosystem I reaction centre primary photoprocess);
2. One-electron phototransfer from bacteriochlorophyll to quinone (analog of bacterial reaction centre primary photoprocess);
3. Chlorophyll photosensitized one-electron transfer from hydroxylic compounds to quinone (analog of photosystem II reaction centre photoprocess).

Although further study of these photochemical processes from a basic mechanistic point of view is required, it seems clear that we are now in a position to obtain new insights into the fundamental molecular-electronic events associated with chlorophyll photosensitized electron transfer reactions. Regardless of whether or not the specific mechanisms found to be operative in these systems are totally transferable to the photosynthetic apparatus, it is certainly significant that chlorophyll and bacteriochlorophyll in solution are able to undergo photoreactions which are close counterparts of what is believed to be happening *in vivo*. This may have evolutionary significance in terms of the choice of a porphyrin derivative as the sensitizing agent in photosynthesis. Although structural modifications and elaborations have occurred in living systems as a result of selective pressures, it may well be that the fundamental electronic events, which are inherent properties of the porphyrin molecule, have remained unchanged. According to such a view, the complex structures found in present-day photosynthetic organisms would serve to increase efficiency, channel reactions along the desired pathways and prevent energy-wasting back reactions.

References and Notes

1. Assuming a quantum requirement of eight for CO_2 fixation and oxygen evolution, the overall efficiency of the photosynthetic process is about 38%. The efficiency of the primary light energy conversion reactions is probably considerably higher than this, perhaps as much as 70-75%.
2. Although all of the details of the environmental and structural factors which distinguish these chlorophylls from the bulk pigment are not known at present, recent evidence suggests that chlorophyll dimers may be the photoactive species in the reaction centres (cf. for example J. J. Katz and J. R. Norris, Jr.) in *Current Topics in Bioenergetics*, (D. R. Sanadi and L. Packer, eds.), Vol. 5, Academic Press, New York, (1973) 41.
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22. Because the deuteron has a smaller nuclear magnetic moment than the proton, ESR hyperfine lines which arise from interactions between the unpaired electron and hydrogen atoms in the radical species will be narrowed by a factor of approximately 0.15.
23. No ESR signal for a reduced species has as yet been found in these systems. It is possible that a trapped electron is being generated whose ESR signal is destroyed by the visible light irradiation (cf. ref. **4**, 357).
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33. This work was supported in part by the U.S. Atomic Energy Commission. (Contract AT(11-1)908.)