Chlorophyll Organization and Energy Transfer in Photosynthesis

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Chairman's opening remarks

SIR GEORGE PORTER

The Royal Institution, London

At this meeting we are going to look at the primary processes of the most important biological application of photochemistry. The meeting was conceived so as to give chemists, physicists and biologists the opportunity of talking to each other about this important developing area of science —chlorophyll organization and energy transfer in photosynthesis—because it is a subject with a language which is obscure to many chemists and physicists. Although most people here are working full time in the field of photosynthesis, many have come into the field from other disciplines. I hope that at the end of the symposium we shall leave with a clearer understanding of the photosynthetic apparatus and how it does its wonderful chemistry. In so far as there are gaps in the picture, as there must be at this stage, we want to know what they are and to what extent there is agreement about the areas which seem to have been well mapped out.

Our theme is both timely and important. It is timely because it is developing so rapidly that it seems possible that a fairly complete understanding of the structure and function of the photosynthetic unit may be obtained in just a few more years. It is timely also because many people from outside the privileged cabal of photosynthetic research have become interested in it since the new techniques that they use (for example picosecond laser-pulse spectroscopy) are so admirably matched to studies of the primary processes. These processes, with rates that fall so tantalizingly within their time range will probably never be resolved and fully understood without direct studies on that time scale. That explains the increasing attention which is being given to mechanistic and kinetic aspects by direct picosecond studies.

On the organizational side of the process this symposium is equally timely because rapid advances have been made in the past few years in the isolation and characterization of chlorophyll–protein complexes. One has been

crystallized and its structure has been determined. The hope in our minds is that complex particles of the green leaf will be subjected to similar precise characterization. Even without this, the grosser structure of the photosynthetic unit as a whole, and the part that these complexes play in it, will figure greatly in our discussions, as will the smaller scale organization of the chlorophyll molecules and their oligomers.

I also said that the subject is important. It is; not only because it is one of the basic processes in nature and in which nature can teach the photochemist and photophysicist many skills as yet impossible *in vitro*, but also because over the past few years the importance of photosynthesis to the survival of man and his modern technology has become increasingly apparent. To live and even to survive in the modern world man needs principally two things: food and fuel. Both of these depend entirely on the process of photosynthesis. It is our hope that by understanding better the organization and mechanism of the natural photosynthetic process we may be better able to improve on it in agriculture and perhaps even to adapt it specifically to some of man's energy needs, by replacing the fossil fuels when the wells run dry at the end of the century. We shall not discuss this here but what we shall discuss is certainly not irrelevant to these practical purposes.

The subjects in the papers are interrelated in such a way that we shall inevitably oscillate between green plants and bacteria, between theory and experiment, and between the organization and the kinetics of energy transfer in the photosynthetic unit. We shall start with structure, and then discuss how this structure operates in the first steps of energy transfer in photosynthesis. We shall go no further than this; we shall stop our considerations after about the first nanosecond, when the chemistry begins. In the organization part, some of the questions to which I should like to know answers are the following: what is the state of the chlorophyll molecule (i.e. when is it a monomer and when a dimer) in the reaction centres of photosystems I and II and in the light-harvesting unit? Secondly, how do we account for the many different apparent states which are observed in the absorption spectrum? Can we account for them in terms of the dimer and of solvation differences? Thirdly, how is chlorophyll incorporated into the membrane, into the lipid, and into the protein complexes? Is it partly exposed to the lipid? What size are the basic protein complexes? How many chlorophylls are there in each unit? How are these individual units arranged with respect to each other and in the membrane? How do the chlorophyll molecules in these units manage to overcome the concentration quenching which occurs *in vitro*? Fourthly, how do the chlorophyll–protein complexes, arranged in the way we shall have discussed, transfer energy between themselves (i.e. the units as

opposed to the molecules within the complex) and at what rate? Finally how are the whole systems dispersed in the membrane and how does our picture of this account for the electron-microscope photographs of the membrane and the particles which we see in it?

If we can formulate answers to those questions before we pass on to discussion of the kinetics of energy transfer within these structures, we shall already have made great progress.

In the kinetics section there are more questions to be answered. First, what are the experimentally determined laws of fluorescence decay of chlorophyll in the chloroplast? Is the rate-determining process one of energy transfer or of trapping? In the latter case an exponential decay is expected; in the former the decay would be non-exponential and—if Förster-type kinetics are followed—an $\exp(-kt^{\frac{1}{2}})$ dependence of fluorescence on time might be expected. Second, now that the fluorescence of the different light-harvesting pigments can be time-resolved, can the results be reconciled with what is known of the structural arrangement in particles such as phycobilisomes, for example? Do they now allow us to distinguish between 'lake' and 'puddle' models of the photosynthetic unit? And, again, we have to ask how the kinetics *in vivo* can be reconciled with those *in vitro* where fluorescence lifetimes, at comparable concentrations, are so much shorter.

All these things happen in the first nanosecond of photosynthesis and if we can understand them in the three days available to us, we shall have done very well indeed.

Structure and function of photoreaction-centre chlorophyll

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Abstract Evidence from electron paramagnetic resonance (e.p.r.) studies suggests that the unpaired spin in oxidized $P700^{+\cdot}$ or $P865^{+\cdot}$ is shared by two special chlorophyll *a* (Chl *a*) or bacteriochlorophyll *a* (Bchl *a*) molecules respectively. Three classes of models have been proposed for special pair reaction centre chlorophyll: asymmetric, in which one Chl *a* (or Bchl *a*) acts as electron donor to a second acting as acceptor; models with translational symmetry only; and models with C_2 symmetry. Models with C_2 symmetry have been synthesized *in vitro* with two chlorophyll macrocycles tied together by a covalent link. The singlet and triplet states of the *in vitro* models have been characterized by e.p.r., nuclear magnetic resonance, and optical studies involving absorption, emission, and lasing behaviour. The fact that lasing occurs only from the folded configuration of the linked dimers suggests the availability of a highly effective non-radiative decay path from the S_1 state of the excited open dimer. A radical-pair mechanism that accounts for the unusual spin polarization of the special pair triplet is proposed for the primary photochemistry in the reaction centre.

THE PHOTOSYNTHETIC UNIT

Green plants and certain bacteria can carry out the process of photosynthesis in which the energy of sunlight is converted into chemical energy. The input of chemical energy makes it possible for photosynthetic organisms to do chemical reactions that otherwise would not proceed spontaneously. Crucial to the ability of photosynthetic organisms to use light energy for chemical purposes are the chlorophylls, a small group of closely related compounds (Fig. 1) that are deeply implicated in all aspects of the primary act of light conversion. Chlorophylls are the primary photoacceptors; they are the principal energy-transfer agents; they form the energy trap and they are the primary electron donor in photosynthesis. Almost 50 years ago, Emerson & Arnold

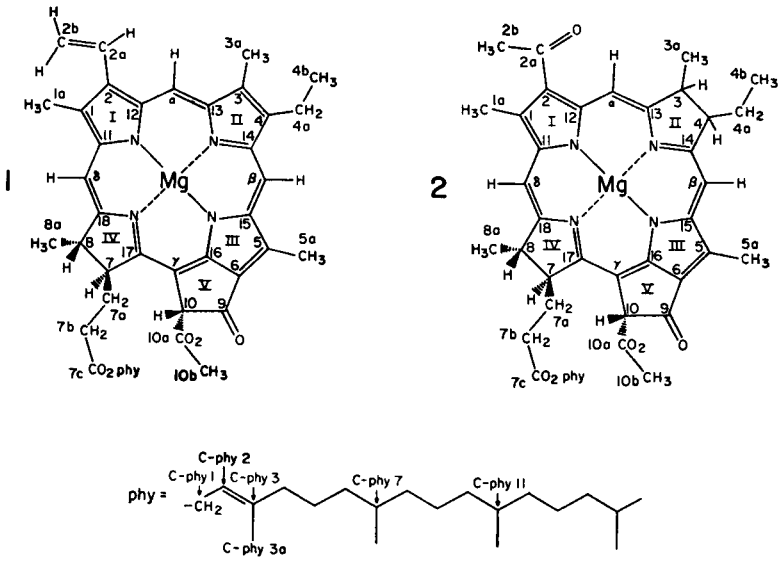

FIG. 1. Structures and numbering system of (1) chlorophyll *a* and (2) bacteriochlorophyll *a*.

(1931, 1932) proposed that chlorophyll function in photosynthesis is a cooperative phenomenon. Many chlorophyll molecules are involved in the conversion of a single photon into an electron (a reducing agent) and a 'positive hole' (an oxidizing agent). Nearly all chlorophyll molecules in the photosynthetic apparatus have a light-gathering or antenna function; these chlorophyll molecules act as the primary photoacceptors of electromagnetic radiation. The (electronic) excitation energy of a particular chlorophyll in the antenna array caused by absorption of a photon is then transferred to a few chlorophyll molecules in a photoreaction centre where energy is trapped and conversion occurs. The antenna and photoreaction-centre chlorophyll, together with auxiliary pigments and electron-transport chains, comprise a photosynthetic unit. *In vivo* antenna and photoreaction-centre chlorophylls have different physical properties, and differ from each other and from an *in vitro* solution of chlorophyll in a polar solvent in such important respects as visible absorption maxima (electronic transition) and fluorescence. Nevertheless, chlorophyll of the same molecular structure may be used to construct both the antenna and the photoreaction centre. A central problem in photosynthetic research, then, has been to provide a structural (or environmental) basis for the various species of chlorophyll that occur in the photosynthetic unit that rationalizes the anomalous properties of chlorophyll *in vivo*. The magnitude of the anomaly

can be judged from the fact that solutions of chlorophyll *a* (Chl *a*) and bacteriochlorophyll *a* (Bchl *a*) in polar solvents absorb light in the red region of the spectrum at about 665 nm and about 770 nm, respectively, and the solutions are intensely fluorescent, whereas *in vivo* chlorophylls have their red absorption maxima substantially shifted to the red and are only feebly fluorescent.

Here we shall be concerned only with the photoreaction centres I (PS I) in green plants and with bacterial photoreaction centres containing bacteriochlorophyll *a*. It is convenient to discuss PS I and bacterial reaction centres together. Although there are fundamental differences between green plant and bacterial photosynthesis, the essential features of PS I and bacterial photoreaction centres appear to be similar if not identical.

Progress in the elucidation of the structure and function of reaction centres has been greatly accelerated by the successful procedures developed by Clayton (1963), Clayton & Wang (1971), Loach & Sekura (1967), and Feher (1971) for the isolation of reaction centres from photosynthetic bacteria. These preparations of bacterial reaction centres are functional entities of relatively simple composition. They are free of antenna Bchl *a* and have the optical and redox properties of photoreaction centre Bchl *a* present in intact photosynthetic bacteria. Isolated bacterial reaction centres contain several Bchl *a* molecules as well as some bacteriopheophytin *a* (Bpheo *a*) (the Mg-free derivative of Bchl *a*). For reasons discussed below, it appears that not all the Bchl *a* in the isolated reaction centre is involved in the production of electrons in the primary light-conversion event. With respect to the primary electron-production event, however, there appears to be a great deal of similarity in both structure and function between PS I in green plants and the bacterial reaction centre. As many aspects of reaction-centre behaviour can as yet be studied only in reaction-centre preparations, experiments with bacterial reaction centres make an important contribution to studies on green plants. Progress in the preparation of reaction centres from green plants has so far been slower and most of what we shall say about green plant PS I centres is based on observations in intact photosynthetic organisms.

There are obviously many different vantage points from which photosynthetic reaction centres can be viewed, and many different levels at which interpretation can be attempted. Our objective is to provide an interpretation *on the molecular level* of the structure and function of photoreaction centres in terms of the molecular structure and physical properties of their constituent chlorophyll.

E.p.r. and optical properties of photoreaction-centre chlorophyll

It will facilitate our subsequent discussion to summarize some of the salient

e.p.r. and optical properties of the reaction centres of green plants and bacteria. Commoner *et al.* (1956) made the important discovery that free radicals (paramagnetic entities with an unpaired electron) are produced in the light-energy conversion step of photosynthesis. Because of the great sensitivity of e.p.r. spectroscopy, the photo-e.p.r. signal is readily detected and serves as the most informative experimental probe of photoreaction-centre activity now available. The e.p.r. signal is composite. Its most prominent component (generally called Signal I) is rapidly reversible and has a g-value of 2.0025, indicative of an unpaired electron delocalized over a large π-system. The line-shape of e.p.r. Signal I is Gaussian and has a peak-to-peak line-width of about 7.0 G. The corresponding e.p.r. signal in photosynthetic bacteria is also reversible, Gaussian, and has a peak-to-peak line-width of about 9.5 G (Androes *et al.* 1962). In fully deuteriated algae, Signal I is narrowed to about 3 G (Kohl *et al.* 1965) and in fully deuteriated bacteria to 3–4 G (Kohl *et al.* 1965; McElroy *et al.* 1969). In all cases, the photo-e.p.r. signal has no observable hyperfine structure. The availability of fully deuteriated photosynthetic organisms, as well as of organisms highly enriched in ^{13}C, ^{15}N and ^{25}Mg, has considerably enhanced the applicability of e.p.r. to the study of photosynthesis.

The origin of Signal I in green plants and the photo-e.p.r. signal in photosynthetic bacteria has been established by correlation of the kinetics of formation and decay of the photo-e.p.r. signal with optical transients that can also be associated with the light-conversion event, and by comparison of the *in vivo* e.p.r. signals with those of chlorophyll free radicals produced in the laboratory in defined systems. Kok (1956, 1957) observed that a decrease in the intensity of light absorption (photobleaching) occurs at 702–705 nm during active photosynthesis, and that the photobleaching is reversed in the dark, and Duysens (1952; Duysens *et al.* 1956) observed reversible photobleaching in photosynthetic bacteria at about 870 nm. From these optical transients it was deduced that the photoreaction centres in green plants and in photosynthetic bacteria absorb light at about 700 nm and about 865 nm, respectively, and the photoreaction-centre chlorophylls were assigned the symbols P700 and P865. The photobleaching was interpreted as a photooxidation. The paramagnetic (free radical) species produced in the photoreaction centres are then $P700^{+\cdot}$ and $P865^{+\cdot}$, formed by ejection of an electron during the light-conversion event. The conclusion that the photobleaching is an oxidation is reinforced by the observation that the optical changes and the concomitant e.p.r. signal produced by the chemical oxidant potassium ferricyanide are similar to those produced *in vivo* by light. Evidence that the chlorophyll free radicals produced in the conversion step are cationic free radicals is derived from the important *in vitro* studies of Fuhrhop & Mauzerall (1969) on porphyrins and by Borg *et al.*

(1970) on chlorophyll. (Although the conclusion that the *in vitro* chlorophyll free radicals are cationic free radicals is conclusive, no similar evidence establishes that the free radicals $P700^{+\cdot}$ and $P865^{+\cdot}$ are charged species. To be sure, $P700^{+\cdot}$ and $P865^{+\cdot}$ are doublet states, but they could be neutral species. Nevertheless we shall follow the usual convention and use the symbols $P700^{+\cdot}$ and $P865^{+\cdot}$ to indicate the paramagnetic photooxidized photoreaction centres.)

The assignment of Signal I to $P700^{+\cdot}$ is supported by studies that show the kinetics of the e.p.r. signal and of the photobleaching are similar (Warden & Bolton 1972, 1973). In photosynthetic bacteria, the kinetics of photobleaching of $P865^{+\cdot}$ at both 4 K and room temperature are similar to the kinetics of the e.p.r. signal (McElroy *et al.* 1974). The identity of the kinetics of the photochemistry and the e.p.r. signal is evident on even the fastest time scale on which relevant observations can be made.

Careful quantitative comparisons of the quantum yield for free-radical formation have shown that the ratio of light-induced spins in e.p.r. Signal I to bleached P700 in green plants is within experimental error 1 : 1 (Warden & Bolton 1972, 1973). Similar quantitative experiments on reaction-centre preparations from photosynthetic bacteria also show that the ratio of photobleached P865 centres to the number of spins is essentially 1 : 1 (Bolton *et al.* 1969; Loach & Sekura 1967; Wraight & Clayton 1973). The experimental evidence is thus convincing that the quantum yield for free-radical formation in both green plant chloroplasts and in isolated bacterial reaction centres is close to unity, that is, for each photon trapped in the reaction centre one electron is ejected leaving the reaction centre with one unpaired spin.

The characteristics of the e.p.r. signals from the photooxidized reaction centres are consistent with the oxidation of a large aromatic molecule. The Gaussian line-shape and the free-electron *g*-value suggest that many interactions occur between the unpaired spin and carbon and hydrogen nuclei for both the *in vivo* and *in vitro* chlorophyll free radicals. This supposition is buttressed by a comparison between free radicals produced in organisms of ordinary isotopic composition and those in photosynthetic organisms of unnatural isotopic composition containing ^2H in place of ^1H. In fully deuteriated algae or bacteria, the e.p.r. line-width is reduced by about 60%, reflecting the considerably weaker electron–nuclear hyperfine interaction of ^2H. The ^2H effect in simple aromatic molecules reduces the line-width by a maximum of about 4 G. The ^2H effect in deuteriated organisms of only about 2.4 G can be accounted for by additional interactions in these systems with the nitrogen atoms present in the chlorophyll macrocycle. That the ^2H effect on the e.p.r. line-width is the same for both *in vivo* and *in vitro* chlorophyll systems is itself good proof that a chlorophyll species is the origin of the *in vivo* signal.

TABLE 1

Comparison of e.p.r. properties of $P700^{+\cdot}$ and $P865^{+\cdot}$ in selected photosynthetic organisms

System	ΔH_{pp}[a]	R[b]
[^{1}H]Chl $a^{+\cdot}$	9.3 ± 0.3	2.4
[^{2}H]Chl $a^{+\cdot}$	3.8 ± 0.2	
[^{1}H]Bchl $a^{+\cdot}$	12.8 ± 0.5	2.4
[^{2}H]Bchl $a^{+\cdot}$	5.4 ± 0.2	
[^{1}H]*Syneccochocus lividus*	7.1 ± 0.2	2.4
[^{2}H]*Syneccochocus lividus*	2.95 ± 0.5	
[^{1}H]*Rhodospirillum rubrum*	9.5 ± 0.5	2.3
[^{2}H]*Rhodospirillum rubrum*	4.2 ± 0.3	

[a]All lines are Gaussian and have $g = 2.0025 \pm 0.0002$.
[b]Ratio of the line-width of the ^{1}H-system to the ^{2}H-system: $[^1H]\Delta H_{pp}/[^2H]\Delta H_{pp}$.

Thus, the features of the e.p.r. Signal I in green plants and the corresponding signal from photosynthetic bacteria and bacterial reaction centre are consistent with the formation of Chl $a \cdot L_1^{+\cdot}$ or Bchl $a \cdot L_1^{+\cdot}$ (L_1 is a ligand nucleophile). The exception is the line-width. The *in vivo* signals are about 40% narrower than the e.p.r. signals from monomeric Chl $a \cdot L_1^{+\cdot}$ or Bchl $a \cdot L_1^{+\cdot}$. (For convenience in comparison, the relevant e.p.r. data on *in vitro* and *in vivo* signals are collected in Table 1.) The discrepancy in line-width makes it impossible to equate $P700^{+\cdot}$ or $P865^{+\cdot}$ with monomeric chlorophyll free radicals. In a similar fashion, the optical properties of P700 and P865 are not satisfactorily accounted for in terms of monomeric chlorophylls. To account for the discrepancies, it is necessary to invoke the participation of more than one chlorophyll molecule in the photooxidation of P700 or P865, and this leads to new views about the structure and function of the photoreaction centre.

THE CHLOROPHYLL SPECIAL PAIR

The e.p.r. data discussed above make it plausible that chlorophyll is the primary electron donor in the photoreaction centre (Katz & Norris 1973). Discrepancies between the line-width of the e.p.r. signal and the visible absorption of P700 and P865 and those of monomeric Chl *a* and Bchl *a* make it impossible to identify the *in vivo* primary electron donor with monomeric chlorophyll. Attribution of the discrepancy to the consequences of biological environment of an unspecified nature is no longer satisfactory. The unusual photo-e.p.r. signal that can be elicited from *in vitro* P740, however, points a ywa to the resolution of the dilemma.

E.p.r. and the $\sqrt{2}$ effect

The chlorophyll–water adduct absorbing maximally at 740 nm (P740) has an extraordinarily narrow e.p.r. signal with a line-width of about 1 G, far narrower than the signals from either $P700^{+\cdot}$ or Chl $a \cdot L_1^{+\cdot}$. The unusual line-width of the $P740^{+\cdot}$ species can be rationalized by delocalization of the unpaired spin over the entire assembly of chlorophyll molecules in the aggregate. The delocalization can be viewed as a rapid process of spin migration between equivalent sites. Given a sufficiently high rate of spin migration, the e.p.r. signal from the effectively delocalized electron collapses to a narrow line. When a 'free' electron is delocalized over an aggregate of N molecules, it can readily be proved (Norris *et al.* 1971) that ΔH_N, the line-width when the unpaired spin is delocalized over N equivalent chlorophyll molecules, is given by equation (1),

$$\Delta H_N = \Delta H_M / N^{1/2} \quad (1)$$

where ΔH_M is the line-width of the monomeric chlorophyll free radical. A value of $N = 2$ accounts with considerable precision for the 40% narrowing of the $P700^{+\cdot}$ and $P865^{+\cdot}$ signals relative to Chl $a \cdot L_1^{+\cdot}$ and Bchl $a \cdot L_1^{+\cdot}$ (Table 2). The $\sqrt{2}$ narrowing in the *in vivo* $P700^{+\cdot}$ and $P865^{+\cdot}$ signals is analogous to that observed in organic dimeric cationic free radicals where the unpaired spin is shared by molecules (for a review, see Bard *et al.* 1976). The $\sqrt{2}$ narrowing in line-width holds reasonably well for all photoreaction centres containing Chl *a* or Bchl *a*. The relationship applies from ambient temperatures down to

TABLE 2

The $\sqrt{2}$ e.p.r. line-width effect in plants and photosynthetic bacteria

Organism	ΔH_{pp} (G)[a]	*Calculated special-pair* ΔH_{pp} (G)[b]	R[c]
[^{1}H]*S. lividus*	7.1 ± 0.2	6.6 ± 0.3	1.08 ± 0.06
[^{2}H]*S. lividus*	2.95 ± 0.1	2.7 ± 0.1	1.10 ± 0.05
[^{1}H]*C. vulgaris*	7.0 ± 0.2	6.6 ± 0.3	1.06 ± 0.05
[^{2}H]*C. vulgaris*	2.7 ± 0.1	2.7 ± 0.1	1.00 ± 0.05
[^{1}H]*S. obliquus*	7.1 ± 0.2	6.6 ± 0.8	1.08 ± 0.06
[^{2}H]*S. obliquus*	2.7 ± 0.1	2.7 ± 0.1	1.00 ± 0.05
[^{1}H]HP700	7.0 ± 0.2	6.66 ± 0.3	1.06 ± 0.05
[^{1}H]R. *rubrum*	9.5 ± 0.5	9.1 ± 0.4	1.05 ± 0.07
[^{2}H]*R. rubrum*	4.2 ± 0.3	3.8 ± 0.1	1.10 ± 0.09

[a]From Norris *et al.* (1971).
[b]Calculated from equation (1) with $N = 2$.
[c]$R = \Delta H_{\text{in vitro}} / \Delta H_{\text{in vivo}}$.

1.8 K and is followed equally well by the intact living organism and by isolated reaction-centre preparations. It is equally valid for green plants and for photosynthetic bacteria containing Bchl *a*. The almost universal occurrence of the 1/2 effect in photosynthetic reaction centres strongly implies that the primary donor in the photoreaction centre is a special pair of chlorophyll molecules. We hesitate to call the pair of chlorophyll molecules acting as the primary donor a 'dimer'. The term dimer has been preempted to describe a true chlorophyll dimer formed by a keto C=O---Mg coordination interaction between two chlorophyll molecules. The electronic transition spectra, redox properties, and the geometry of the true dimer differ in major respects from those of the two chlorophylls that act as donor in the reaction centre. In addition, there is evidence to suggest that the geometry of the two chlorophylls results from the intervention of a bifunctional nucleophile. To avoid confusion, we therefore refer to the primary donor in the reaction centre as a chlorophyll special pair, Chl_{sp} or $Bchl_{sp}$.

Endor and the one-half effect

Electron-nuclear double resonance (Endor) spectroscopy, a high-resolution variant of e.p.r. (Feher 1956), has made a valuable contribution to establishing the special-pair nature of P865 (Norris *et al.* 1973, 1974, 1975; Feher *et al.* 1973, 1975). In an aggregate of size N over which an unpaired electron is shared equally (effectively delocalized), the electron–proton hyperfine coupling constants are related to those in the monomer by the equation (2),

$$a_{Ni} = a_{Mi}/N \tag{2}$$

where a_{Mi} is the electron–nuclear hyperfine coupling constant for the ith nucleus in the monomer, and a_{Ni} is the coupling constant for that site in an aggregate of size N. For a Chl_{sp} where $N = 2$, the hyperfine coupling constant will be halved relative to those in the monomer, i.e. equation (3).

$$a_{2i} = a_{1i}/2 \tag{3}$$

One such equation applies to each different nuclear site in the molecule which makes up the aggregate. Consequently, a comparison of proton–electron hyperfine coupling constants in $Chl_{sp}^{+\cdot}$ or $Bchl_{sp}^{+\cdot}$ and Chl $a\cdot L_1^{+\cdot}$ or Bchl $a\cdot L_1^{+\cdot}$ is a much more rigorous and demanding test of the special-pair hypothesis than is line-shape analysis. Assignment of the coupling constants accounting for $> 80\%$ of the line-width of Chl $a^{+\cdot}$ has been done by endor spectroscopy on a suite of isotopically substituted derivatives of Chl *a* (Scheer *et al.* 1977). Table 3 lists the aggregation numbers deduced from endor experiments

TABLE 3

Endor evidence for special-pair chlorophyll[a]

Protons	*Hyperfine coupling constants (G)*		*Aggregation number*	
	Bchl$^{+\cdot}$	*Chl a*$^{+\cdot}$	*R. rubrum*	*S. lividus*
(α, β, δ, 10)	0.49	0.24	1.7	
1a	1.88		2.4	
(1a, 3a, 4a)		1.13		1.9
		1.31		2.2
5a	3.46	2.63	2.1	2.0
7, 8	4.95	4.17	2.0	2.2
		Average	2.0	2.1

[a]Data taken from Norris *et al.* (1974).

on P700$^{+\cdot}$ and P865$^{+\cdot}$ *in vivo*. In both cases the *in vivo* coupling constants are (approximately) halved relative to the monomer free radical, thus providing convincing support for the Chl_{sp} model. For photosynthetic bacteria the assignment of the *in vivo* endor spectra is straightforward and is compatible with the Chl_{sp} model. For green plants, the endor spectra are more complicated and the interpretation is not as direct. Nevertheless, here also the simplest interpretation of the endor data requires a pair of chlorophyll molecules.

Evidence from electron spin-echo spectroscopy

Electron spin-echo spectroscopy is a pulsed form of e.p.r. spectroscopy in which resonance is detected by 'spin-echo' from the free radical excited by a suitable sequence of high-intensity radio-frequency pulses (Mims 1972). This relatively new technique can be applied to good advantage to the special-pair problem. Electron spin-echo spectroscopy has been used to study nitrogen hyperfine interactions that cannot be observed by endor. Fig. 2 shows two sets of spin-echo envelopes in which P700$^{+\cdot}$ is compared with Chl $a\cdot L_1^{+\cdot}$ and P865$^{+\cdot}$ with Bchl $a\cdot L_1^{+\cdot}$. The pulse spin-echo envelopes have superimposed on them a modulation pattern caused by interactions of the nitrogen atom with the unpaired electron. It can be deduced immediately from these patterns that the simple monomer chlorophyll cation cannot be responsible for the *in vivo* signals. There are such large differences in the spin-echo envelope modulations between the *in vivo* and *in vitro* nitrogen environments experienced by the unpaired spins in P700$^{+\cdot}$ and Chl $a\cdot L_1^{+\cdot}$ as to eliminate the possibility that

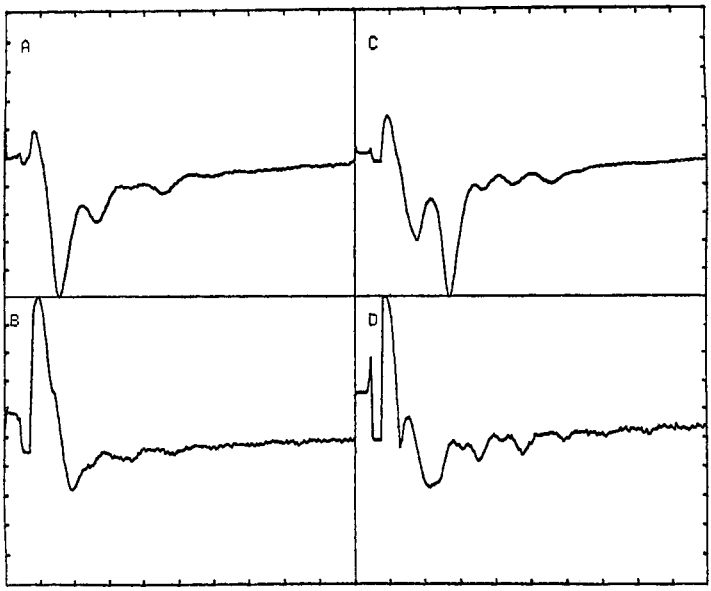

FIG. 2. Comparison of *in vivo* and *in vitro* spin-echo envelope modulations: spin-echo intensity (ordinate in arbitrary units) is plotted against time (abscissa, one division corresponds to 40 ns). A, *in vitro* [^{2}H]Bchl $a \cdot L_1^{+\cdot}$; B, *in vivo* $P865^{+\cdot}$ in [^{2}H]*R. rubrum*; C, *in vitro* [^{2}H]Chl $a \cdot L_1^{+\cdot}$; D, *in vivo* $P700^{+\cdot}$ in [^{2}H]*C. vulgaris*.

Chl $a \cdot L_1^{+\cdot}$ is the origin of the *in vivo* signal (J. R. Norris & M. K. Bowman, unpublished work).

A possible alternative to the Chl_{sp} explanation for the endor data is a 'special environment' effect. For example, the differences in the endor of $P700^{+\cdot}$ and Chl $a \cdot L_1^{+\cdot}$ could conceivably arise from distortions in the geometry of a monomer cation produced by some aspect of the *in vivo* environment, or rotation of methyl groups in the chlorophyll could be hindered. Either of these two possible *in vivo* perturbations could be adequate to explain the differences between the *in vivo* and *in vitro* endor data. The nitrogen atoms, on the other hand, are embedded in the conjugated system and thus are not nearly so sensitive to geometry. Consequently the nitrogen hyperfine interactions with the unpaired spin provide, in many respects, an even better test for the validity of the Chl_{sp} model than does endor. The best evidence yet against a monomeric chlorophyll primary donor in green plant reaction centres comes from the electron spin-echo experiments.

Evidence from the bacterial triplet state

The discovery by Dutton *et al.* (1972, 1973) that Bchl *a* triplet states could be detected by e.p.r. or optically detected magnetic resonance in intact photosynthetic bacteria or bacterial reaction centres when the normal course of forward photosynthesis is blocked has provided a new approach to the study of the structure and function of the bacterial reaction centre. The extensive literature on chlorophyll triplets and their significance for photosynthesis has recently been thoroughly reviewed (Levanon & Norris 1978). Here we shall only say that comparison of the properties of *in vitro* monomeric ^{3}Bchl $a \cdot L_1$ with ^{3}P865 in the reaction centre again rules out monomeric ^{3}Bchl *a* as the origin of the e.p.r. triplet signal. The zero-field splitting parameters and the unusual spin polarization in the triplet spectra from the *in vivo* reaction centre exclude monomer ^{3}Bchl *a* but, since the triplet-state parameters are sensitive to geometry, the triplet results do not distinguish unambiguously between a triplet state confined to only two Bchl *a* molecules and a triplet state involving several Bchl *a* molecules. The triplet results, however, do furnish valuable information about some of the important details of special pair function; these are briefly described later (see pp. 28–31).

Evidence from optical properties for Chl_{sp}

It has been a perplexing question why P700 and P865 are red-shifted relative to the red absorption maxima of Chl $a \cdot L_1$ and Bchl $a \cdot L_1$. Earlier investigators were impressed by the red shifts that can be readily observed in solid chlorophyll films, concentrated solutions in non-polar solvents, and in colloidal dispersions. Consequently, chlorophyll aggregation has been advanced as an explanation alternative to a 'biological environment' or a simple 'protein–chlorophyll interaction' for the *in vivo* red shifts. A red shift can be expected on theoretical grounds whenever chlorophyll molecules are forced into close proximity with their transition moments aligned. The red shift in chlorophyll aggregates arises in part because of the electronic perturbations induced by chlorophyll acting as a donor in coordination or hydrogen-bonding interactions, and in part by transition dipole–transition dipole interactions between closely positioned chlorophyll molecules. The optical consequences of chlorophyll aggregation are perhaps most vividly illustrated by the Chl *a*–water aggregate that absorbs maximally at 740 nm. The large optical shift relative to Chl $a \cdot L_1$ ($\lambda_{max} = 660$ nm) in this aggregate can readily be rationalized (Shipman *et al.* 1976; Shipman & Katz 1977) by a combination of environmental and transition-dipole interactions using intermolecular distances derived

from the Strouse X-ray crystal structure of ethyl chlorophyllide *a*.$2H_2O$ and the formalism developed by Shipman *et al.* (1976). It can be shown that a stack of two (or three) Chl *a* molecules arranged with one water molecule between them and having the same geometrical arrangement as in the Strouse (1974) linear stack is expected to have an absorption maximum near 700 nm. Thus, to a first approximation, a pair of Chl *a* molecules arranged as in the ethyl chlorophyllide $2H_2O$ crystal structure would reasonably be expected to have the optical properties of P700. As we shall show (pp. 13–16) there are several ways of arranging two Chl *a* molecules so that they have an absorption maximum at about 700 nm. For a $Bchl_{sp}$ the situation is more complex. On the basis of the experimental evidence now available, orienting two Bchl *a* molecules in the same geometry as in ethyl chlorophyllide *a*.$2H_2O$ does not produce the required optical red shift to 865 nm. However, purified reaction-centre preparations from photosynthetic bacteria contain at least four Bchl *a* and two Bpheo *a* molecules, which makes it plausible that it is the further interaction of the additional Bchl *a* and Bpheo *a* with a $Bchl_{sp}$ that is responsible for the red shift to 865 nm. In any event, monomeric Chl *a*·L_1 and Bchl *a*·L_1 have optical properties inconsistent with P700 and P865, but on both experimental and theoretical grounds Chl_{sp} and $Bchl_{sp}$ have optical properties entirely consistent with those required for a valid model for P700 or P865.

We have summarized a sizable body of optical and magnetic resonance data that casts serious doubt on a role for monomeric Chl *a* or Bchl *a* as the primary electron donor in P700 or P865, but does support the view that a special pair of chlorophyll molecules functions as the primary donor in light-energy conversion. We can now consider specific models for Chl_{sp} and $Bchl_{sp}$.

MODELS FOR Chl_{sp} AND $Bchl_{sp}$

Various models for Chl_{sp} and $Bchl_{sp}$ have been advanced, which have much in common but which also have important differences. All the models use two chlorophyll molecules with the macrocycle planes arranged in a parallel orientation, but they differ in such details as symmetry, the relative orientations of the two chlorophyll molecules, the distance between the macrocycle planes, and the functional groups and nucleophiles used to cross-link the two chlorophylls to form the special pair.

There are several requirements that must be met by a valid special pair model. The lowest energy $S_0 \rightarrow S_1$ electronic transition (called Q_y in the literature) must be red-shifted relative to the Q_y transition in the antenna to assure effective trapping of the singlet excitation energy in the reaction centre. The necessary shift in the Q_y transition band by the special pair requires a parallel alignment

and the shortest practicable distance between the two Q_y transition moments. An appropriate Chl_{sp} model should also provide the necessary redox properties: that is, it must be more easily and rapidly oxidizable than either monomeric or antenna chlorophyll. Such will be the case when the highest occupied molecular orbitals (HOMOs) of the two chlorophylls in the special pair mix to form two 'supermolecular' HOMOs (see p. 18), from the higher of which it will be easier to remove an electron than from the HOMOs of the monomer chlorophylls. And finally, the arrangement of the two chlorophylls in the special pair model must provide for overlap of the π-systems of the macrocycles and for equality of corresponding sites in the two chlorophyll molecules to make possible the equal sharing of the unpaired spin in $Chl_{sp}^{+\cdot}$.

Model of Shipman et al. (1976)

The most satisfactory model so far proposed, or at least the one that has the best basis in experiment, is that of Shipman *et al.* (1976) (see Fig. 3). A similar but less-detailed model has been proposed by Boxer & Closs (1976). In the former model, the two Chl *a* molecules are held together by two molecules of

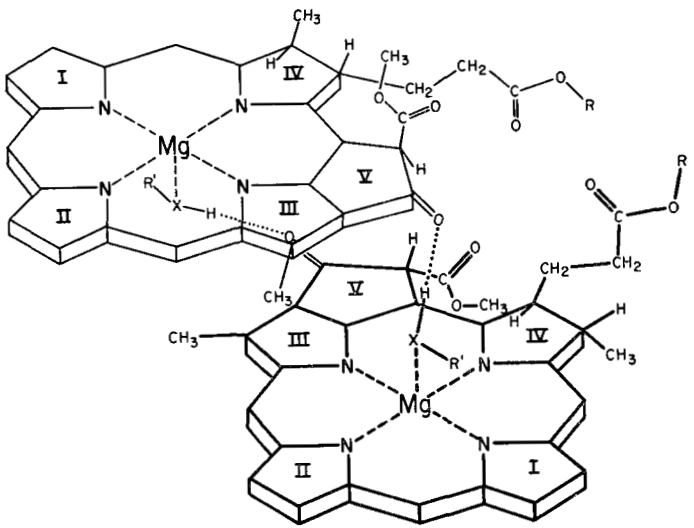

FIG. 3. Model of Chl_{sp} proposed by Shipman *et al.* (1976): note the hydrogen-bonding to the keto C=O functions. Many hydrogen-bonding nucleophiles can act as cross-linking agents.

a bifunctional ligand. The bifunctional ligand, in addition to an electron lone pair available for coordination to Mg, must have hydrogen-bonding properties. Ligands of the general type R′XH, where R′ = H or alkyl group and X = O, NH or S are suitable. Typical nucleophiles of this general class are water, HOH, or ethanol, $CH_3 \cdot CH_2 \cdot OH$. The electron lone pair on the oxygen atom of these ligands is coordinated to the Mg atom of one of the Chl *a* molecules and hydrogen-bonded to the keto carbonyl group of the other Chl *a* molecule in the pair. The arrangement in Fig. 3 sets the macrocycles at a π–π stacking distance of 0.36 nm, a distance that just brings the π-systems into contact and provides optimum π-overlap. The extent of π-overlap in this special-pair configuration assures spin delocalization in the special-pair cationic free radical.

The optical properties expected for this model are also consistent with the requirement for a 700 nm absorption maximum. In the orientation of Fig. 3, the Q_y transitions are parallel and, from exciton theory, the red-shifted Q_y exciton transition will have all the oscillator strength. If each of the monomer Chl *a* molecules is considered to be environmentally shifted to 686 nm by the hydrogen-bond interaction at its keto carbonyl group and if π–π stacking is taken into account, then the model of Shipman *et al.* (1976) is calculated to have its Q_y transition at 700 nm. An environmental shift to 686 nm is required in a Chl *a* molecule strongly hydrogen-bonded at its keto carbonyl function to account for the experimentally observed red shift in the 740 nm-absorbing Chl *a*–water adduct. Thus this model appears to have the necessary red shift in its Q_y transition.

One of the interesting features of this model is that a considerable variety of nucleophiles can be used to form it. The geometrical arrangement in Fig. 3 is such that the cross-linking nucleophile is not restricted to a small ligand such as water. Nucleophilic groups present in the generic class R′XH are characteristically also present in protein side-chains. We can contemplate the use of the OH groups of serine or threonine, the NH_2 group of lysine, the SH group of cysteine etc. to form the Shipman *et al.* (1976) special pair. The proposed structure is open enough to allow the entry of large nucleophilic groups without difficulty. The possibility that special pairs could be formed by the intervention or participation of protein is of considerable interest. The composition of the bacterial reaction-centre preparations (polypeptides and chlorophyll) and the current activity in the isolation of chlorophyll–protein complexes from photosynthetic membranes or organelles make such a possibility more immediate. A chlorophyll–protein interaction in the sense described here to form a Chl_{sp} does not involve formation of covalent bonds. Extraction of the chlorophyll with organic solvents from a chlorophyll–protein complex would be accompanied by a change in protein conformation, thereby providing a rationale for

the difficulty encountered up to now in reconstituting a disassembled photosynthetic membrane. The kind of interaction suggested here would provide a reasonable explanation for a red shift in a chlorophyll–protein complex in terms of chlorophyll–chlorophyll interactions mediated but not directly caused by interaction with the protein. Protein participation in special-pair formation also raises the possibility that there could be many reaction centres *in vivo* formed with different nucleophiles or combinations of nucleophiles which would have essentially the same optical properties and the same ability to share an unpaired spin, but which might have significantly different redox properties. A Chl_{sp} linked by two water molecules might have excited-state properties different from those of one formed from, say, one water molecule and one seryl OH group, or from a special pair formed from a lysyl NH_2 group and a cysteinyl SH group. Other ways in which the nucleophilic groups in protein side-chains could be used to form Chl_{sp} should also be considered. Given two nucleophilic groups (selected from OH, NH_2, SH etc.) in a polypeptide it is conceivable that coordination of a nucleophile to either of the fifth coordination sites of the Mg atoms of two chlorophyll molecules could position the two chlorophylls with a geometry appropriate to a special pair. In this mode of organization by protein, coordination would be from the rear, and no nucleophiles would be present between the chlorophylls. The question here would be whether a peptide structure could be made sufficiently rigid to maintain the geometry of the special pair formed in this way. Hydrophobic interactions involving the phytyl group of the chlorophylls and the hydrophobic regions of the protein or polypeptide could also be considered as a possible mode of interaction in the formation of organized chlorophyll species. All these possibilities suggest new experimental initiatives to the general question of chlorophyll–protein interactions and the possible role of protein in the formation of Chl_{sp} (and antenna chlorophyll too for that matter).

Symmetry considerations in Chl_{sp} models

The models of Shipman *et al.* (1976) and Boxer & Closs (1976) have C_2 symmetry which makes the two chlorophyll molecules identical. Fong (1974*a*) originally raised the issue of symmetry in the special pair and proposed the first model with C_2 symmetry. The symmetry requirement for Fong's special-pair model was introduced to satisfy the presumed requirements of a singlet–triplet (up-conversion) scheme for photosynthesis (Fong 1974*a*, *b*). Whatever the merits of the up-conversion scheme (see, for example, Menzel 1976; Warden 1976; Govindjee & Warden 1977), Fong's Chl_{sp} model has some serious problems. Cross-linking is exclusively by hydrogen-bonding to the ring V

methoxycarbonyl groups of the chlorophylls, and the keto carbonyl functions are not used in any way. From molecular models the spacing between the macrocycle planes in the Fong structure is 0.57 nm, a substantially greater distance than the van der Waals' contact distance of 0.34–0.36 nm that is optimum for porphyrin and chlorophyll π-system overlap. Molecular overlap between the macrocycles in the Fong structure is expected to be small because orbital overlap falls off exponentially with distance. Further, when the methoxycarbonyl C=O groups are used, the transition moments in the two macrocycles are at a 60° angle, not parallel as is required for strong exciton coupling. Exciton considerations indicate that the optical red shifts in this structure are too small to be compatible with a 700 nm absorption requirement for the Chl_{sp} and, moreover, predict a blue shift. Models formed by hydrogen-bonding to the keto carbonyl function, however, avoid the intractable problems in the Fong structure (see previous section).

Symmetry in the special pair, as we see it, may be important not because of its possible contribution to a long-lived triplet state for $Chl_{sp}^{+\cdot}$ but because it makes for better geometry and promotes the near-equivalency of corresponding sites in the two chlorophyll molecules of the Chl_{sp}. A more complete discussion of the possible participation of the triplet state in photosynthesis can be found in a review by Katz *et al.* (1978*a*).

Lower symmetry models for Chl_{sp}

Although the C_2 models of Shipman *et al.* (1976) and Boxer & Closs (1976) have an undeniable attraction, other configurations for the Chl_{sp} structure cannot be excluded on the basis of the experimental evidence now available. The original model for the chlorophyll special pair proposed by the Argonne group (Ballschmiter & Katz 1968; Katz & Norris 1973) was based on the structure inferred at that time for the P740 Chl *a*–water adduct. In this structure two Chl *a* molecules are held together by a single water molecule coordinated to the Mg of one Chl *a* molecule and simultaneously hydrogen-bonded to the keto and methoxycarbonyl C=O groups of the second Chl *a* in the Chl_{sp}. The principal problems with this model are the distance between the two macrocycles (because of the presence of the hydrogen-bonded methoxycarbonyl group) and the unfavourable angles for hydrogen-bonding. The two Chl *a* molecules are not identical as one functions as a donor, the other as acceptor with respect to hydrogen bonding. There is, therefore, a question whether the two Chl *a* molecules are sufficiently equivalent to assure delocalization of an unpaired electron.

A modification of the asymmetric model that avoids its worrisome aspects