Biochimica et Biophysica Acta, 726 (1983) 265–292
Elsevier

BBA 86104

MODEL REACTIONS IN PHOTOSYNTHESIS

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(Received January 20th, 1983)

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IA. Introduction

Photosynthesis involves a very large number of photochemical and chemical transformations. This review focuses on the extraordinarily rapid transport of excitation energy in the antenna pigment-protein complexes and efficient charge separation in the reaction center. These subjects have attracted the attention of investigators from nearly all disciplines. This attention has received added urgency in recent years as the connection between nearly any chemical solar energy conversion scheme and natural photosynthesis becomes more apparent.

This review constitutes an overview of this field from the particular perspective of attempts to model photosynthesis in artificial systems. Such model systems need not be biomimetic, but they

Abbreviations: PS I, II, Photosystem I, II; BChl, bacteriochlorophyll; BPheo, bacteriopheophytin; Chl(s), chlorophyll(s); PChl, pyrochlorophyllides; apoMb, apo-myoglobin; DMA, dimethylaniline; CIDN(E)P, chemically induced dynamic nuclear (electron) polarization; RYDMR, reaction yield detected magnetic resonance; LDAO, lauryldimethylamine *N*-oxide; ENDOR, electron nuclear double resonance.

must share some common features with natural photosynthesis. Because the primary photophysics and photochemistry of photosynthesis involve excitation, energy transport, energy trapping, charge separation, charge transport and charge recombination, it is evident that nearly every study of the interaction of light with matter has some bearing on understanding photosynthesis. As will be seen below, it is not a simple matter to establish precisely which set of observables one is attempting to model. Because the primary events in photosynthesis offer a number of unusual observables, it is natural to focus on these. In addition, models in photosynthesis research play a prominent role in testing theoretical concepts in the general areas of energy and electron transfer.

The particular combination of spectral properties and rates which nature presents in photosynthetic systems has led to the development of new theoretical and experimental methods. Much of the inspiration for developments in the theories of energy and electron transfer has come from photosynthetic systems. On the experimental side, photosynthesis has been a prime area of interest for time-resolved spectroscopic methods, first on the microsecond time scale, and presently on the subpicosecond time scale. Magnetic field effects have been extensively studied in photosynthetic systems and their relevance to model studies justifies the rather detailed discussion in section I*C*.

IB. Reaction-center components

Reaction centers are operationally defined as the units of lowest molecular weight which duplicate the primary charge separation events in photosynthesis. Reaction centers have been isolated from many organisms (see Ref. 1 for a review). The best-characterized reaction centers are obtained from photosynthetic bacteria, in particular the carotenoidless R-26 mutant of the purple bacterium, *Rhodopseudomonas spheroides*. Reaction centers from this organism can be obtained in large quantities and high purity; its properties provide most targets for model studies. There is increasing evidence that several of the unique and most interesting mechanistic aspects of the primary photochemistry of green plant photosynthesis in both Photosystems I and II are shared with bacterial reaction centers, justifying the intense level of study of the latter.

Reaction centers from *Rps. spheroides* R-26 are composed of three hydrophobic polypeptides (apparent M_r 21 000, 24 000 and 28 000), four bacteriochlorophyll *a* molecules (BChl *a*), two bacteriopheophytin *a* molecules (BPheo *a*), two ubiquinones, and one non-heme Fe(II) [1]. The absorption spectrum is shown in Fig. 1 and is compared with the absorption of the pigments extracted into an organic solvent. The reaction center complex is an integral membrane protein and is solubilized either by detergents or lipids. There is no evidence for covalent linkage of any of the components to another. Partial N-terminal sequences are available for all polypeptides [2], and complete sequences are expected in the near future from the DNA sequence [3]. Recently, Michel has reported the successful crystalization of reaction centers from *Rhodopseudomonas viridis* [4], along with diffraction data to 2.5 Å resolution. Similar methods can be expected to produce reaction center crystals from other species. This work promises to revolutionize our knowledge of photosynthesis. As far as is known, none of the reactive components is capable of translation or rotation independent of the entire complex; thus, the complex should be viewed as a solid-state photochemical reactor. It is this solid-state feature which is most difficult to duplicate in models composed of discrete molecular components.

The primary photochemistry in reaction centers

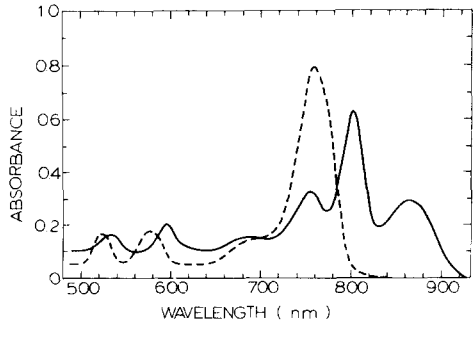

Fig. 1. Electronic absorption spectra of intact reaction centers (———) in 0.01 M Tris-HCl (pH 7.5), containing 0.1% LDAO from *Rps. spheroides* and a neutral organic extract of the pigments (— — —) at the same concentration in dry petroleum ether (taken from Ref. 218).

is commonly described by reference to a kinetic scheme such as that shown in Fig. 2 [5]. The primary electron donor, often called P870 due to its red absorption maximum, is excited either by energy transfer from the antenna pigments or by direct excitation. Many properties of P870 are difficult to explain consistently and offer a challenge to the model builder. (1) No pigment contained in the reaction center absorbs at 870 nm after extraction from the reaction center into organic solvents [6]. (2) The excited state lifetime of P870 is not known, but a transient species with a 350 ps lifetime is detected when electron acceptors are chemically reduced (see below) [7]. By comparison, the singlet lifetimes of BChl *a* and BPheo *a* monomers in organic solvents are about an order of magnitude longer than this [8]. (3) The linewidth of the optical transition at 870 nm is about twice as broad as that for BChl *a* or BPheo *a* monomers in organic solvents or absorption features in the reaction center itself at 760 and 800 nm, generally associated with BPheo *a* and BChl *a*, respectively (see Fig. 1; there is no proof for these spectral assignments at the present time). The fluorescence polarization is constant across the 870 nm band [9]. (4) The absorption feature at 870 nm shows a much larger Stark effect than the absorption features at 760 and 800 nm [10]. (5) The circular dichroism (CD) in the 870 nm region is very different from that associated with the red-most absorption bands of BChl *a* or BPheo *a* monomers in organic solvents [11]. The CD around 870 nm is broad and positive with an overall intensity approximately an order of magnitude greater than that of the monomeric chromophores in organic solvents. (6) The zero-field splitting parameters of the triplet state of P870 ($|D| = 0.01878$ cm^{-1}, $|E| = 0.00322$ cm^{-1}) are smaller than those of either BChl *a* ($D = +0.0224$ cm^{-1}, $E = -0.0053$ cm^{-1}) or BPheo *a* ($D = +0.0259$ cm^{-1}, $E = +0.0046$ cm^{-1}) monomers in organic glasses [12]. Excitation using polarized light at 870 nm produces a triplet EPR spectrum which shows magnetophotoselection along the *Y*-magnetic axis of the triplet state [13–17]. Similar experiments for BChl *a* and BPheo *a* in organic glasses show a much lesser degree of magnetophotoselection [18]. (7) The EPR spectrum of P870$^+$, the hole which remains after several electron transfer steps from P870 to ubiquinone (Q_A in Fig. 2), is narrower than the comparable spectrum of BChl a^+ or BPheo a^+ in organic solvents [19]. Detailed ENDOR analysis reveals that the line-narrowing corresponds to a reduction of approximately a factor of 2 in the nuclear hyperfine coupling constants for a number of protons around a BChl-like macrocycle [20,21]. (8) The absorption spectrum of P870$^+$ has a maximum at 1240 nm, to the red of absorption features associated with BChl a^+ or BPheo a^+ [22,23]. (9) The mid-point potential for the couple P870/P870$^+$ is +450 mV (pH 7) [24], in contrast to that for either BChl *a* (+640 mV) or BPheo *a* (+960 mV) [22,23].

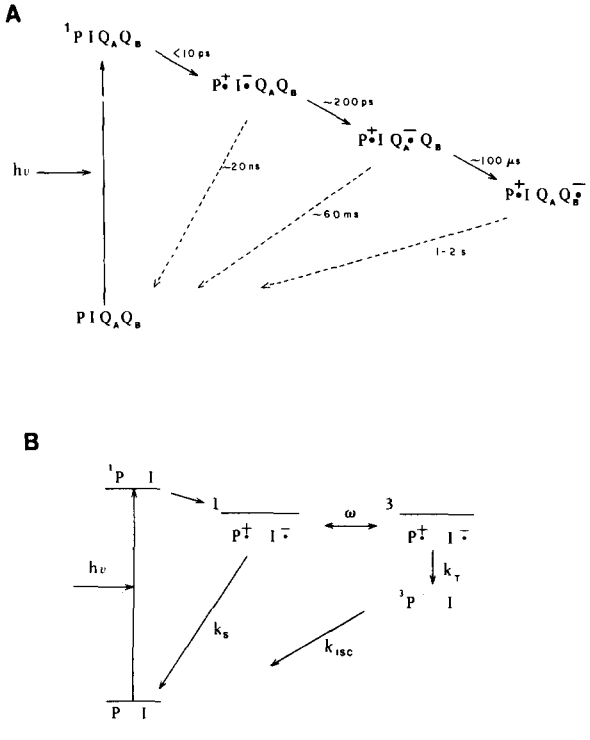

Fig. 2. Scheme illustrating the primary photochemistry in bacterial reaction centers: (A) containing ubiquinone; (B) depleted of ubiquinone. P is the primary electron donor, I is the primary electron acceptor, and Q_A and Q_B are tightly and loosely bound ubiquinones, respectively. The horizontal bars over P^+I^- in (B) denote spin-correlated radical pairs.

From this brief summary of observables, it is evident that BChl *a* or BPheo *a* monomers in organic solvents are poor models for most properties of P870. Based on the EPR data, it has been proposed that P870$^+$ is a dimer [19], considered to

be composed of two BChl *a* macrocycles, although it could conceivably consist of two BPheo *a* molecules or a mixed pair. It should be noted that this does not necessarily mean that P870 is a dimer, or that the excited states of P870 are dimer states. Nonetheless, the EPR and ENDOR data are convincing evidence in favor of a dimer cation radical for $P870^+$. A considerable amount of effort has been expended attempting to build model dimers which duplicate some of the properties listed above for P870; these will be discussed in detail below.

In the discussion above and the literature in this field, BChl *a* monomers in organic solvents are used as the basis for comparison. This is somewhat inappropriate, as the chromophores, irrespective of the details of interchromophore interactions, are unquestionably interacting with the reaction center proteins which surround them. Understanding the degree to which the protein may be responsible for some or all of the differences between BChl *a* monomers in organic solvents and the species in reaction centers is impeded by the complete absence of information on the nature of the BChl *a* and BPheo *a* interactions with the proteins in the reaction center. Model studies have been quite useful in this area, and the properties of synthetic chlorophyll-protein complexes, synthetic chlorophyll-amino acid complexes, and the effects of charged groups on the properties of chlorophylls will be discussed below.

Considerably less is known about the other chromophores in the reaction center, some of which are believed to serve as electron acceptors from the excited singlet state of P870. The absorption spectrum of the intermediate present 10 ps after photoexcitation, the radical pair denoted P^+I^- or P^F, exhibits large changes in absorbance at 540, 760, 800 and 870 nm [25,26]. Because the features at 540 and 760 nm are usually identified with BPheo *a*, it has been proposed that I^- is BPheo a^-. A species believed to be I^- can be produced in reaction centers under reducing conditions at room temperature [1,27,28]. The absorption change at 800 nm complicates this picture, as this is usually identified with two BChls. Thus, it has also been postulated that the electron acceptor part of the initially formed radical pair consists of some mixture of the anion radicals of BChl *a* and BPheo *a*. Shuvalov and Parson [29] have introduced the labels B for the BChl *a* and H for the BPheo *a* molecules which are postulated to comprise I. The reader is cautioned that the assignments of optical bands to particular molecular species is based on analogy with relative band positions and intensities in organic solvents; none of the assignments is certain. Furthermore, because all six reaction center chromophores are associated with two of the polypeptides [1], they are likely to be quite close to each other, and one must be cautious when speaking of the properties of discrete molecular species. From the viewpoint of modeling the reaction center, we do not know with any certainty which of these interactions is important for the primary photochemistry. Thus, most model systems to date have settled for the simplest combinations of molecules.

IC. Reaction-center photochemistry

The scheme shown in Fig. 2 summarizes a great deal of kinetic work, but omits a number of important observations which cannot yet be easily fit into a scheme. Given the extraordinarily fast rate of the forward electron-transfer reaction, one must be very careful to define the exact experiment used to probe the photochemistry, as radiationless relaxation occurs on a comparable time-scale, and there may be multiphoton effects due to intense excitation pulses. Holten and co-workers [30] have studied the forward reaction using subpicosecond excitation pulses at 630 nm; this primarily excites the second excited states of P870 and the BChl molecules. Transient absorption changes are first observed at 600 nm, believed to correspond to the second excited state of one of the BChl molecules. This confirms the earlier measurement of Shuvalov and co-workers [31], and suggests that the initial photochemical reaction is electron transfer from P870 to a BChl molecule. Within a few picoseconds this intermediate changes to another characterized by transient absorption changes at 540 nm, corresponding to a loss of absorption by one of the BPheo molecules. The scheme in Fig. 2 omits this series of events. This is done for simplicity and because it is not clear that these are discrete molecular species (excited state absorption may be responsible for part of what is observed). Another possibility is that the initial changes ob-

served by Holten and co-workers correspond to electron transfer within P870 [30]. This requires that there be some reason for directional electron transfer in the dimer; this could be accomplished by asymmetry in the environment. The large Stark effect observed for P870 might be consistent with this picture [10]. Another variant on this scheme is that P870 and one of the bacteriochlorophylls interact strongly, and the excited state of P870 is a charge transfer state, i.e.:

$$P870 \cdots BChl \xrightarrow{h\nu} [{}^1P870 \cdots BChl \leftrightarrow P870^+BChl^-]$$

A somewhat far-fetched explanation of the linewidth of P870 might be that it is homogeneously broadened by an extremely short lifetime due to electron transfer (this would require a lifetime on the order of 10 fs, if the linewidth were entirely due to this effect).

The triplet state of P in Fig. 2 deserves special attention, because its mechanism of formation provides some of the most stringent requirements for a model of photosynthesis. In functioning reaction centers, triplet states are formed in negligible yield due to the efficiency of the forward electron transfer reactions. Dutton and co-workers [32] showed that a highly spin-polarized triplet EPR signal could be detected with Q_A prereduced or removed (blocked reaction centers). The lifetime of this triplet species is approx. 100 μs below 100 K [33]. Under these conditions, the optical absorption changes which occur within 10 ps of photoexcitation and which correspond to the radical-ion pair P^+I^-, decay within about 20 ns to a new set of changes in which P870 remains bleached for about 100 μs (the state 'P^R') [34]. The correspondence of these lfetimes identifies the optically detected state P^R with ^3P. Schaafsma and co-workers [35] pointed out that the spin-polarization pattern is incompatible with an intersystem crossing mechanism, as the external magnetic field axes (rather than the molecular axes) determine the polarization pattern; the polarization is due to a predominant population in the T_0 high-field state. Thurnauer and co-workers [36] offered the important hypothesis that the ^3P originates from a radical pair precursor. Because the spin multiplicity of the initially formed radical pair is singlet, a singlet-triplet (S-T) mixing mechanism is required before this triplet can be formed. Thus, although triplets do not appear to be formed along the reaction coordinate in normal photosynthesis, they can be formed by blocking photosynthesis, and they offer a relatively long-lived species whose properties provide information on the primary ion pair.

The notion that the spin dynamics in radical pairs control the course of the reaction has been extensively discussed in the literature of Chemically Induced Dynamic Nuclear (Electron) Polarization [CIDN(E)P] [37,38]. As originally developed by Closs, Kaptein and Oosterhoff [39,40], S-T mixing in a radical pair can occur by two mechanisms: nuclear hyperfine coupling in the radicals and the difference in their g-factors. Provided that the singlet and triplet radical pairs can produce chemically or spectroscopically differentiable products, the effects of these mechanisms can be detected.

Aside from the availability of a mechanism to mix singlet and triplet radical pair states, it is essential that these states be nearly degenerate. This is expected for radicals which are free to diffuse in solution, where the S-T splitting (the electron-electron exchange interaction, J) effectively vanishes as the radicals separate, but not for the spatially fixed reaction center components if they are near each other. The consequences of a finite S-T splitting are readily appreciated by reference to Fig. 3, where the states are shown to be split by an isotropic electron exchange interaction. The magnetic field at which a measurement is made is very important: in Fig. 3B, where J is very large, there exists a field where S and T_- are degenerate, and S-T_- mixing would be expected to dominate. An elegant example of this effect has been given by Doubleday and Closs [41], who studied the field dependence of the nuclear polarization intensity for a series of small biradicals of the type $\dot{C}O(CH_2)_n\dot{C}H_2$ ($n = 4$–8). Because the triplet EPR signal detected in reaction centers is predominantly T_0 polarized, the S-T splitting must be very small (e.g., as in Fig. 3A).

It should be noted that spin-orbit interactions can also mix singlet and triplet states. Although it is generally found that this mechanism is not a significant source of S-T mixing for nearly degenerate radical pairs, it may be very important when

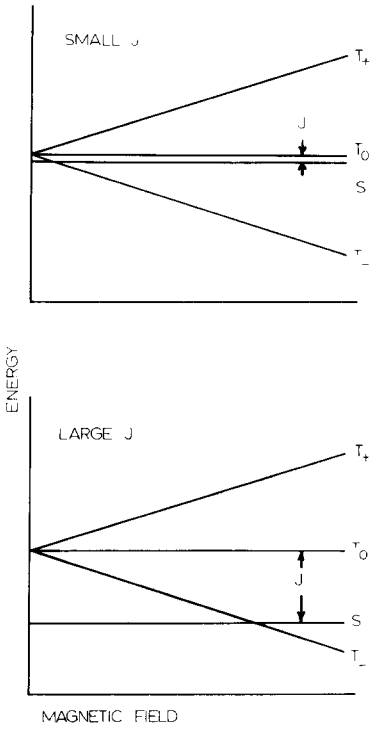

Fig. 3. Simplified radical-pair energy-level diagram, negleting anisotropic dipolar interactions (see text) as a function of the magnitude of the isotropic exchange interaction, J (a singlet ground-state radical pair has been arbitrarily assumed), and the strength of the applied magnetic field. (A) Small J: S-T_0 mixing is likely. (B) Large J: S-T_0 mixing is very unlikely. S-T mixing can only occur for a field strength at which S and T_- levels are approximately degenerate, and falls off both at lower and higher fields.

the S-T splitting is larger [41]. Spin-orbit induced S-T mixing does not depend on nuclear hyperfine interactions or g-factor differences. Spin-orbit interactions may prove to be very important in determining the fate of some strongly coupled radical-ion pairs in reaction centers (Chidsey, C.E.D., Kirmeier, C., Holten, D. and Boxer, S.G., unpublished data).

A more subtle distinction between the reaction center system and any system studied in solution is that one expects an *anisotropic* dipole-dipole interaction to be present, in addition to the isotropic exchange interaction illustrated in Fig. 3. Anisotropic dipolar interactions have been exhaustively studied for stable radical pairs in molecular crystals [43]. Thus, the energy level diagrams in Fig. 3 are inappropriate and should be replaced with diagrams in which the triplet levels are split at zero field and which depend on the orientation of the radical pair zero-field tensor principal axis system and the applied field direction. It may be possible that the dipolar coupling exceeds the exchange interaction for distant radical pairs. This is because the exchange interaction falls off roughly exponentially with distance, whereas the dipolar interaction falls off more slowly with the inverse sixth power of distance.

The observation of an S-T_0 spin-polarized triplet EPR spectrum is an important goal in modeling photosynthesis, and it has not yet been accomplished. A note on experimental methods is in order. Triplet EPR signals are not detectable in fluid solution, as motional modulation of the zero-field splittings leads to rapid electron spin-lattice relaxation, broadening the transitions beyond detection [44,45]. There is no doubt that triplets formed by ion-pair recombination in many organic systems in solution (e.g., pyrene/dimethyl-aniline, to be discussed below) [47,48] would have the T_0 sublevel in a magnetic field populated exclusively initially; however, their triplet EPR spectra cannot be detected. Thus, the goal of observing a T_0-polarized triplet EPR signal requires a solid-state system (note: solid state does not require low temperature).

The simplified energy level diagram shown in Fig. 3A suggests that the quantum yield of triplets (Φ_T) should depend on magnetic field strength. For a very small S-T splitting, the near degeneracy of S with all three triplet sublevels at zero field is lifted as the field strength increases. Thus, as the applied field is increased, the availability of states to mix with S decreases, the rate of S-T mixing diminishes, and the triplet yield should decrease. Magnetic field effects of this type have been observed in a very wide range of radical pair reactions in solution [47–50]. Parson [51] and Hoff [52] and their co-workers observed the predicted decrease in Φ_T with field in blocked reaction centers. With few exceptions (see subsection IIB-2) this is unique for a solid-state reaction. Schulten [53] and Haberkorn [54] and their co-workers developed approximate quantitative treatments of the effect. An important conclusion of their work was that the exchange interaction is very small, on the order of or less than the nuclear hyperfine energy of P^+I^-, or about 10^{-3} cm^{-1}. A disturbing observa-

tion was the absence of any effect of deuteration on the magnetic-field effect [55]. This is surprising, because hyperfine interactions (changed by deuteration) are considered responsible for S-T mixing at low field.

The very small magnitude of the exchange interaction led Haberkorn and co-workers [56,57] to consider more deeply how it could be possible to achieve such a rapid forward electron transfer, $^1PI \rightarrow P^+I^-$, producing such a weakly coupled radical-pair product. A possible escape from this dilemma was to postulate an intermediate state between ^1PI and P^+I^-, where the exchange interaction is very large. Using the notation introduced by Shuvalov and Parson [29]:

$$^1PBH \rightarrow \overset{1}{\overline{P^+B^-H}} \rightleftarrows \overset{1}{\overline{P^+BH^-}}$$

J large $\quad$ J small

That is, rapid hopping between two radical pairs is postulated, and the large exchange interaction in the sate P^+B^-H leads to a very different evolution of the spin multiplicity of the singlet radical pair (by an unspecified mechanism), than in P^+BH^- (dominated by hyperfine and Δg mechanisms). It is not yet clear how one should go about modeling this situation.

As a further test of the mechanism, Boxer and co-workers studied the effects of much larger magnetic fields on Φ_T [58,59]. At high field the g-factor difference between P^+ and I^- provides a mechanism for *increasing* $S\text{-}T_0$ mixing, thereby increasing the triplet yield; this effect was observed. An analytical treatment of the effect gave the g-factor difference and estimates for the recombination rate constants. A most desirable feature of the g-factor difference mechanism is that the $S\text{-}T_0$ mixing rate can be controlled experimentally and be made arbitrarily large simply by increasing the field. Thus, reactions which produce a very low yield of triplets at zero field, due to a short radical pair lifetime for example, can be forced to produce a much larger yield at high field. This provides a useful experimental tool for demonstrating the intermediacy of radical pairs in model systems [60]. Calculated field-dependence curves illustrating this effect are shown in Fig. 4.

Because it proved difficult to fit the magnetic-field dependence of Φ_T at low field with a set of

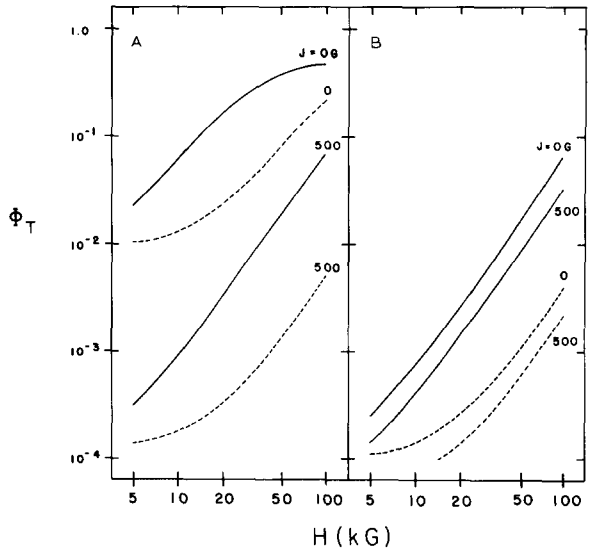

Fig. 4. Theoretical plots of Φ_T as a function of applied magnetic field strength for different values of the magnetic and kinetic parameters, assuming a reaction scheme, comparable to that in blocked reaction centers (Fig. 2B). (A) $k_S = k_T = 10^9$ s^{-1}; (B) $k_S = k_T = 10^{10}$ s^{-1}. Solid lines (———) are calculated for $\Delta g = 4 \cdot 10^{-3}$, dashed lines (- - -) are for $\Delta g = 1 \cdot 10^{-3}$. These plots demonstrate the utility of studying Φ_T in model systems for electron transfer at high magnetic field strength, even for cases in which Φ_T is very small at zero field.

parameters consistent with the data at high field, Boxer and co-workers [61] included a sizable dipolar interaction between P^+ and I^- in the theory. The magnitude of the interaction which fits the data corresponds to a distance of separation between P^+ and I^- of approx. 7–8 Å. The magnitude of the dipolar interaction deduced from the field dependence is comparable to that obtained by Bowman and co-workers [62,63], who measured the magnetic resonance spectrum of P^+I^- by monitoring the effects of intense resonant microwaves on the triplet concentration (Reaction Yield Detected Magnetic Resonance, RYDMR). The reader should be cautioned that distance parameters are not directly measured by any of these methods, but require considerable calculation and approximation (both to deduce the magnitude of the dipolar interaction and to interpret its value in terms of a distance), and ultimately the validity depends upon the appropriateness of the scheme in Fig. 2.

If dipolar interactions in P^+I^- are substantial Φ_T should depend on orientation in a magnetic

field [58,64,65]. If the S-T splitting depends on orientation, those radical pairs whose S-T splittings are smallest lead to the highest Φ_T, whereas those with the largest S-T splittings tend to return to the ground state. Extending this notion, it is evident that the interactions which drive S-T mixing, the nuclear hyperfine interactions and the *g*-factor difference, are both tensor quantities, and their effective magnitudes also depend on orientation in a field. The role played by each in determining Φ_T should be field-dependent, as the *g*-tensor difference is only important at high field. A very substantial orientation and field dependence was observed for Φ_T, and a formalism has been developed to extract structural insight, given a number of as yet unmeasured properties of P^+ and I^- [65]. This field-dependent quantum yield anisotropy is exquisitely sensitive to the magnitudes and orientations of each magnetic parameter in P^+I^-. Effects of this type could provide insight into the structure of radical pair intermediates in models as well.

In establishing a set of criteria to be met by a photosynthetic model, it is useful to distinguish systems which duplicate an operational or mechanistic element from those which seek to duplicate a structural element of the natural system and thereby possibly duplicate the mechanism. The word biomimetic is often used to denote the latter; however, it is possible to use naturally occurring components, such as the chlorophylls, as part of a model to test a general mechanistic concept, so this is a somewhat confusing usage. It is sometimes argued that models can explain photosynthesis; however, it is much more likely that interesting limited aspects of the photochemistry will be duplicated, while many of the observables associated with actual reaction-center structures will not be duplicated. The underlying questions are whether photosynthetic reaction centers are unique and whether the entire set of chemical components, including the protein, is essential to produce the interesting combination of rates which lead to efficient charge separation.

Summarizing the criteria for a photosynthetic model, it is likely to require four molecules: a dimeric electron donor, an electron acceptor which is strongly coupled to the excited state of the dimer, and a second acceptor which is more weakly coupled. All components should be immobilized so that diffusion plays no role in the photochemistry. There should be essentially no fluorescence from the electron donor and the radical ion pair should be stable for many nanoseconds. In order to assist in proving the mechanism, the radical pair produced by electron transfer should have an energy in excess of the triplet energy of the electron donor, so that triplets can be formed by recombination. The yield of triplets so formed should have a strong dependence on applied magnetic field strength, and the triplet EPR spectrum should be T_0 polarized.

ID. Antenna chlorophyll

The majority of Chl in the photosynthetic apparatus is not associated with the reaction center, but transports incident excitation energy to the reaction center. It appears that the vast majority of this antenna Chl is associated with integral membrane proteins in most species, and a number of these proteins have been isolated. These proteins typically contain more than one Chl (there are no reports of pheophytins); however, the excitation energy undoubtedly samples more than one protein, so the act of isolating the proteins disrupts their collective function.

Several complexes have been characterized. The water-soluble complex from the green bacterium *Prosthecochloris aestuarrii* [66] is the only Chl/protein complex to date which has been studied in molecular detail by X-ray crystallography; the structure has been reviewed in detail elsewhere [67]. There is no obvious translational or rotational relationship among the BChls. All are within 12 Å of each other. There have been several attempts to analyze the absorption and CD spectra of this protein with limited success [68–70].

Sauer and Austin [71] have reported the isolation and characterization of an antenna Chl/protein complex from *Rps. spheriodes.* The data suggested that the protein is a dimer of two peptides, each about 8.5 kDa, and each containing a BChl. The red absorption maximum of the BChl is red-shifted to 855 nm and the CD of this band is intense and conservatively split, strongly suggesting exciton coupling between the chromophores in the complex. Dissociation of the aggregate to

monomers eliminates this feature and the red absorption maximum shifts to the blue (ascribed to BPheo formation). The band can be resolved into its two components by linear dichroism [72]. In a related experiment, partial photodestruction of the chromophores in chromatophore preparations [73] likewise shows the loss of the exciton CD feature; however, the red-shifted absorption maximum does not revert to the position associated with BChl in organic solvents. This suggests that the red shift is not primarily a consequence of exciton interactions, but has another origin. This experiment is complicated by a number of assumptions about the homogeneity of dimers in the chromatophore. If, for example, the oscillator strengths of different components in the chromatophore were different, the analysis would be much less certain. Furthermore, it is unclear what the photodestruction produces. A comparable experiment with the homogeneous, isolated dimer would be much more definitive, especially since dissociation of the isolated dimer eliminates the red shift. In spite of these shortcomings, this experiment suggests that a monomer BChl interacting with a protein can have an enormously red-shifted absorption maximum.

The only other well-characterized natural Chl-protein complex is the light harvesting Chl *a/b* protein in plants [74–76]. This protein is found in all Chl *b* containing organisms and contains on the order of 50% of the total Chl in higher plants and green algal chloroplasts. At this time the structural characterization of the *a/b* protein has not progressed to a level where it can be considered an object for modeling studies.

Although it appears that there are no universal structural elements among antenna Chl/protein complexes or even a single Chl-protein within a given organism, the mechanism of energy transport is a common feature. Energy transfer by the Forster dipole-dipole mechanism [77] has been discussed at length in the literature [78]. Because the antenna and reaction-center complexes discussed above position the chromophores within 15 Å of each other, the weak coupling limit, appropriate when applying the Forster mechanism, is inappropriate. At these distances it is necessary to consider the excitonic character of the excited states, especially for degenerate chromophores. There is an enormous literature on singlet and triplet excitons, which often references, but rarely deals explicitly with, photosynthetic systems. Much of this literature is reviewed by Knox [78].

II. Model systems

IIA. Models for reaction-center components

The great majority of model work has focused on the primary electron donor. Models can be divided into three general types: molecular dimers, chemically modified monomeric Chls and monomeric Chls perturbed by the protein environment. It should be noted at the outset that the Chls are much easier to work with synthetically than the BChls. For this reason, most of the model work is based on Chl *a* type chromophores, even if the property being mimicked is best established in bacterial reaction centers (see Fig. 5 for structures).

IIA-1. Dimers

The first relevant model for a dimer was put forward by Katz and co-workers [79], who originally proposed the 'special pair' concept to explain in vivo EPR line narrowing of the donor radical cation [19]. In this model, two Chls are bridged by a water molecule which simultaneously coordinates to the Mg of one macrocycle through oxygen, while both of its hydrogens are hydrogen bonded to carbonyl groups on the other macrocycle (the C-9 keto carbonyl and carbomethoxy carbonyl at position 10). This dimer model is excised from a proposed polymeric structure for Chl *a* in wet

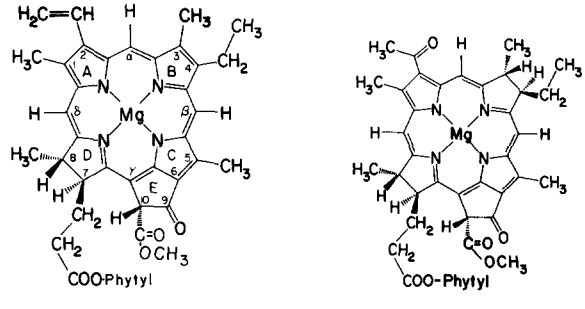

Fig. 5. The chemical structures and numbering systems of Chl *a* (left) and BChl *a*. The chlorophyllides are missing the phytyl ester at carbon 7d; the pheophytins have 2H in place of Mg; the pyrochlorophylls have H in place of carbomethoxy at position 10.

octane and is based on infrared data [80]. A second model was proposed by Fong and Koester [81], in which the two Chls are bridged by two water molecules which interact with the Mg and carbomethoxy carbonyl, not the C-9 keto carbonyl group. Solutions of Chl in wet cyclohexane/pentane at low temperatures were reported to contain this species [82]. Considerable controversy has surrounded the Katz and Fong proposals. As neither is based on a well-defined chemical entity, the debate is based on very indirect evidence and is not very productive. It should be stressed that there is absolutely no evidence at the present time that water is associated with reaction center Chl.

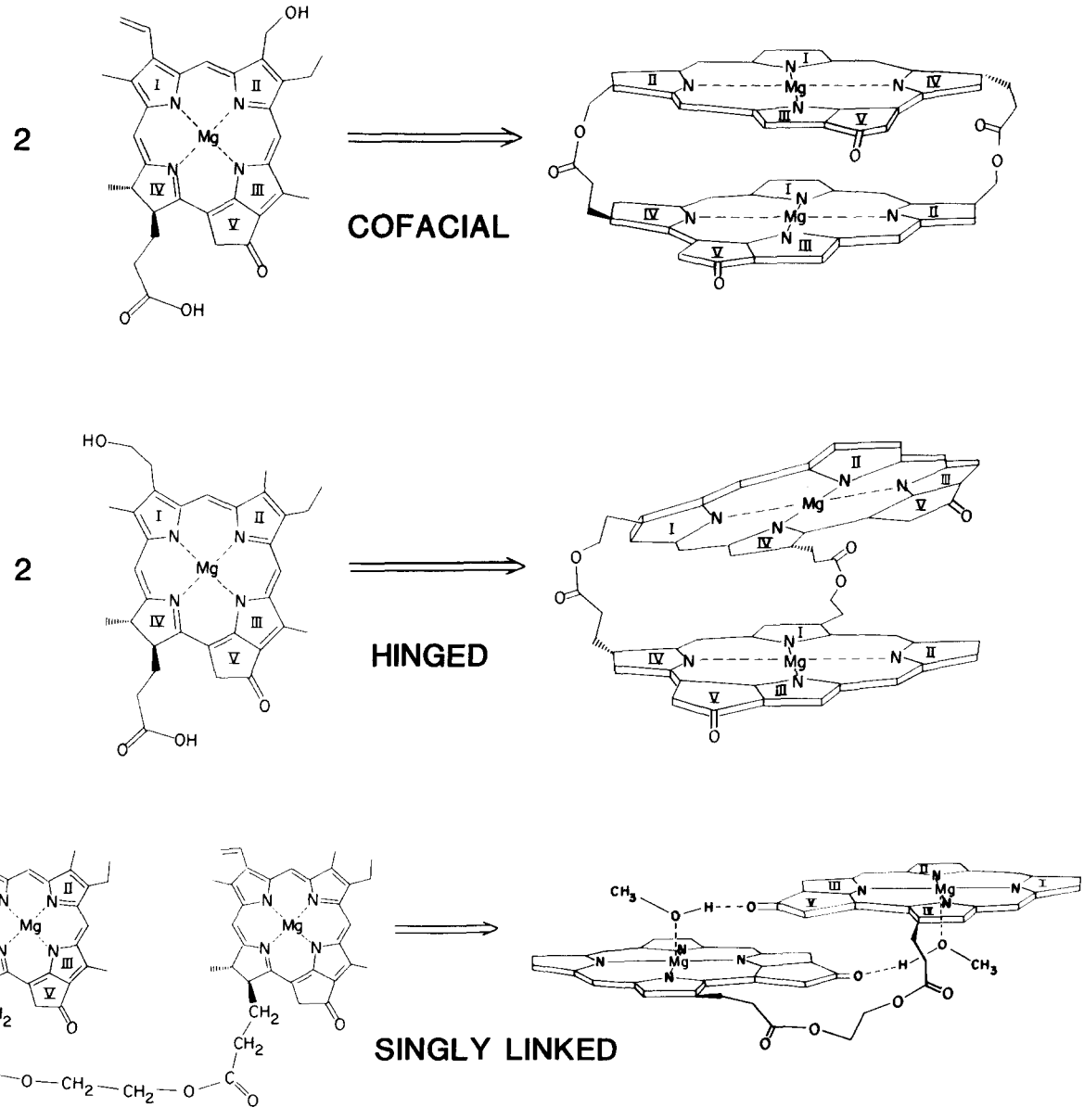

Fig. 6. Chemical structures and schematic molecular conformations for three types of Chl-based dimers: cofacial [94], hinged [93] and singly linked and folded [83].

The first chemically defined Chl dimer was prepared by Boxer and Closs [83], who covalently connected two pyrochlorophyllides by replacing the phytyl chain with ethylene glycol and preparing the diester (Fig. 6C). PChl *a*, which lacks the carbomethoxy group at position 10 (Fig. 5), is spectroscopically and chemically nearly indistinguishable from Chl *a*, but has the very important advantage that only one stereoisomer is present. This is in contrast to native Chl *a* and its derivatives, which epimerize at position 10 to form Chl *a'* (about 15% is *a'* at equilibrium). This epimer complicates NMR spectra, as two sets of peaks are present, and leads to formation of *a*-*a*, *a*-*a'* and *a'*-*a'* dimers, which have different aggregation properties due to the steric bulk of the carbomethoxy group. In the presence of water or alcohols, the Q_y absorption maximum of the synthetic PChl *a* dimer shifts to 700 nm (Fig. 7C), and there are very large changes in the chemical shifts of certain protons in the NMR spectrum. Extensive analysis of this spectrum suggests the 'folded' structure illustrated in Fig. 6C. A molecular model of this structure has the following properties: the macrocycles are about 3.5 Å apart; the centers are offset by about 8.5 Å, and the *Y* molecular axes are approximately antiparallel. Shipman and coworkers [84] have proposed that solutions of Chl *a* in ethanol at low temperature may form structures similar to that proposed by Boxer and Closs [83]. Support for this proposal comes from infrared data; however, there is no proof that the species formed is homogeneous or dimeric.

The absorption and CD properties of the synthetic PChl *a* dimer can be partly explained using the strongly interacting degenerate dipole-dipole treatment introduced for molecular dimers by Kasha [85], and the chiral exciton model introduced by Tinoco [86]. For geometries such as that suggested in Fig. 6C, where the Q_y transition moments of the monomers are assumed to lie along the *Y*-molecular axes, one predicts a very large dimer splitting, but the higher energy band should have negligible oscillator strength: this partly explains the red shift. Note that the oscillator strength of the red-most transition is enhanced in the 'folded' form by about 1.4 ± 0.1 [87], a feature not predicted by the simple model. This is likely due to intensity borrowing from non-degenerate bands in the dimer, and can be calculated using the generalized formalism for the dipole strength introduced by Tinoco [86]. Parson and Scherz, considering only the Q_y, Q_x, B_y and B_x transitions, predict that the Q_y oscillator strength in the 'folded' structure (Fig. 6C) should be enhanced by an amount comparable to that observed experimentally (Parson and Scherz, personal communication). These results suggest that one should be cautious in simply relating the magnitudes of oscillator strengths to the number of interacting chromophores responsible for the band, especially when spectral information is limited (e.g., most bands associated with particular chromophores overlap in the absorption spectrum of reaction centers).

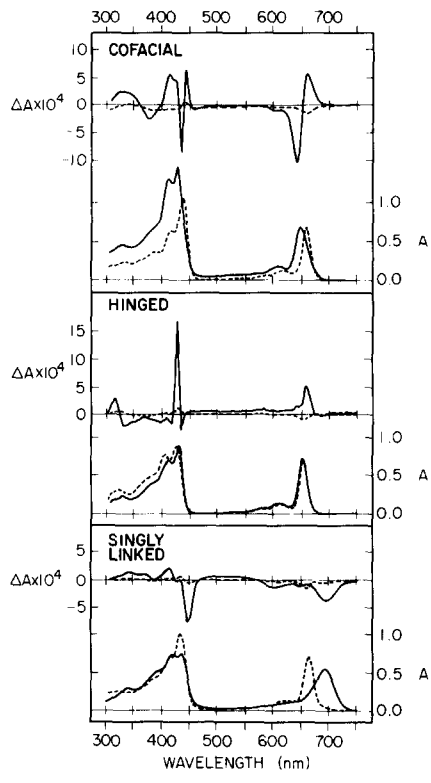

Fig. 7. Comparisons of the absorption and CD spectra for the monomers (— — —) and dimers (———) shown in Fig. 6 (1 cm pathlength). The spectra of the cofacial and hinged dimers and their respective monomers were obtained in CH_2Cl_2 with 0.5 M ethanol (the optical densities were identical for λ_{max} in the red Q_y transition). The spectra of the 'folded', singly linked dimer was obtained in toluene with 0.5 M ethanol, and the 'open' form is the same sample after pyridine was added (10% by volume). The scales for all compounds are identical.

The CD data in Fig. 7C provide further evidence on this point. For the simple model of interacting degenerate transitions, one predicts strong, conservatively split CD bands. The structure proposed in Fig. 6C, however, is a limiting case in which the CD is predicted to vanish; clearly it does not. The observed CD is caused by non-conservative terms in the expression for the rotational strength of the dimer arising from coupled oscillator interactions with nondegenerate excited states of the other monomer comprising the dimer. Similar effects are observed when *monomeric* Chl interacts with proteins (see below). Again the model compounds serve to caution the reader against overinterpreting optical data from natural Chl-protein complexes, where both Chl-Chl and Chl-protein chromophore interactions may be important.

Wasielewski and co-workers have prepared the chlorophyllide *a* [88] and bacteriochlorophyllide *a* [89] analogues of the original synthetic PChl *a* dimer. Both compounds exhibit a red shift for the red absorption maximum in the presence of water or alcohols, and many of the NMR chemical shift changes for the BChl *a* dimer parallel those of the PChl *a* dimer. Oddly, the ring current shifts for comparable protons are greater in the BChl *a* dimer than in the PChl *a* dimer, contrary to expectation, as the ring current for BChl *a* is smaller than that of PChl *a* [90]; no chemical shift data have been reported for the Chl *a* dimer. As expected, a fraction of the Chl *a* dimers does not aggregate due to *a*-*a'* and *a'*-*a'* dimers; for some reason the BChl *a* dimer does not show this effect. No CD data have been reported. The spectral shift for the BChl *a* dimer is from 771 to 803 nm (517 cm^{-1}), compared to the shift from 666 to 696 nm (647 cm^{-1}) for the PChl *a* or Chl *a* dimers. There is an internal inconsistency in these data which is worth noting. *If* the solution structure of the BChl *a* dimer is identical to those of the PChl *a* and Chl *a* dimers in the presence of hydroxylic ligands as proposed [89], and *if* the Q_y transition moments are similarly oriented, then the dimer splitting for the BChl *a* dimer should be about 1.9 times *greater* than that of the PChl *a* or Chl *a* dimer, as this is the ratio of their dipole strengths [91]. This is contrary to what is observed. As pointed out by Shipman and co-workers [92], the spectral shift for a Chl *a* dimer to 700 nm cannot be entirely accounted for by the exciton splitting, and the additional shift is an 'environmental shift', adding to the exciton splitting. It is possible that the environmental shift for the BChl *a* dimer is smaller than that of the Chl *a* dimer or that the environmental shift is causing a shift to higher energy in the BChl *a* dimer.

Two doubly covalently linked Chl-type dimers have been prepared; they are illustrated in Fig. 6A and B, and their absorption and CD properties are shown in Fig. 7A and B. The difference between these compounds is that the dimer in B, prepared first by Wasielewski and co-workers [93], is linked at adjacent pyrrole rings ('hinged'), while that in A, prepared by Bucks and Boxer [94], is connected at apposite pyrrole rings ('cofacial'). These compounds offer an important advantage over the singly linked compounds: the solution structure is much less dependent on solvent. The comparison of optical properties in Fig. 7 affords some insight into the subtle consequences of geometric variations. The CD of the cofacial dimer shows an intense split band; the positive and negative features are comparable, but not equal in intensity. The former is a characteristic of chiral exciton interactions. Both the sign of the CD and the blue shift in absorption are compatible with the structure in Fig. 6A. The CD of the hinged dimer shows no splitting, but is changed in sign relative to the monomer. The CD is predicted to be small in this compound, as the transition moments are roughly perpendicular. The change in sign is again a manifestation of coupling to non-degenerate excited states. Because the 'hinge' in this molecule permits considerable flexibility, the intensities of absorption and CD features are expected to be somewhat solvent-dependent, as has been observed (Scherz, A., personal communication). The spectra in Fig. 7 demonstrate the wide range of properties which can be associated with dimers comprised of the same Chl monomer.

The triplet-state zero-field splitting (ZFS) parameters of all dimers in Fig. 6 are indistinguishable from their appropriate monomers [12,94–96]. The triplet sublevel population and depopulation kinetics for the folded singly linked dimer with Mg as the central metal are not very different from those of the dimer in its open form

[95]. The triplet EPR spectrum of the cofacial dimer is strongly spin polarized, but the monomer and dimer spin polarizations are identical over a wide range of light-modulation frequencies, suggesting that sublevel kinetics are very similar [94]. The zero-field splittings of metal-free monomers (dimers) are greater than those of metal-containing monomers (dimers) by typically 20% [12]. Interestingly, a mixed metal-containing metal-free cofacial dimer has the zero-field splittings and spin polarization of the metal-free half [97]. This suggests that the triplet energy of metal-free Chl is lower than that of metal-containing Chl, contrary to estimates by phosphorescence [98,99] and NMR [87]. In none of the dimers is there a reduction in zero-field splitting comparable to that observed in bacterial reaction centers. Furthermore, a wide range of all compounds (metal-free, Mg or Zn di-metal, or mixed metal/no metal) shows no difference between monomer and dimer, even though these compounds might have a considerable variety of degrees of charge transfer character in the excited triplet state. Dominant population of the T_0 sublevels is *not* observed in any case. Note that the zero-field splitting for triplets in PS I and PS II particles are nearly identical to that of monomer Chl [100–102]. In light of the data on model compounds, it is a mistake to assert that this proves that the primary electron donor in PS I or PS II is a dimer *or* is not.

IIA-2. Chemically modified chlorophylls

The possibility that specialized derivatives of Chl are present in the reaction center has often been suggested, but can generally be ruled out by chemical analysis. This is a simple matter for highly purified reaction centers from the purple bacteria, but is much less reliable for PS I or PS II particles, where the reaction center Chl is only a small fraction of the total Chl. Thus, P870 is definitely not a BChl derivative (it could be BChl *a'* * [103]), but P700 or P680 could be chemically distinct species.

Another possibility is that the electron donor is the Chl enol or that the cation radical is the enol. The latter has been suggested by Fong and Koester [81,82], and has been pursued by Wasielewski and co-workers who have prepared two models: a trapped enol as the silyl enol ether [104] and 9-desoxo-9,10-dehydro-Chl *a* [105]. A very interesting result is that the cation radical of the latter has an EPR linewidth which is very similar to that of $P700^+$. Furthermore, second moment analysis of fully ^{13}C enriched 9-desoxo-9,10-dehydro-Chl a^+ agrees closely with that of fully ^{13}C enriched $P700^+$, but very poorly with that predicted for a delocalized dimer of $(\text{Chl } a)_2^+$ [106]. Most of the properties observed for the enol model itself do not correspond to P700, i.e., the absorption maximum, extinction coefficient or CD spectrum. However, the enol is much easier to oxidize than Chl *a*, as is P700. Thus, the notion that P700 is a dimer remains viable, with the important addition that its radical cation may be localized as the monomeric enol cation. Because the Chl-enol is an unstable species, simple extraction cannot readily prove the role, if any, of the enol in P700 particles.

IIA-3. Chlorophyll-protein interactions

Because Chl or BChl is a prosthetic group in a protein, it is not surprising that its properties should be affected by the protein environment. The nature and magnitude of these effects are difficult to ascertain because all natural Chl-protein complexes isolated to date contain more than one Chl. To some extent, the protein is simply a solvent. The spectral properties of monomeric Chls have been determined in a wide range of solvents, and relatively small changes in absorption features are well documented [107]. Obviously, the 'solvent' is much more ordered in the protein, and particular amino-acid side-chains at particular locations could lead to larger changes in properties.

Two groups independently prepared complexes of Chl-type chromophores and of the protein apomyoglobin (apoMb). Because Chl (minus the phytol side-chain) is structurally related to heme, it is not surprising that it can substitute for heme in the heme pocket. Davis and Pearlstein combined 'chlorophyllin' (supposed to be Mg-chlorin-e_6) and apoMb [108]. These authors observed that the absorption maximum of the chromophore in the complex changed substantially to the red-following irradiation. We have shown that if this chro-

* It is also possible that the esterifying alcohol may vary, as for *Rhodospirillum rubrum* [103].

mophore is dissolved in methanol, the same change in absorption maximum occurs following irradiation, as Davis an Pearlstein [108] observed in the protein [Boxer, unpublished results]. Thus, the protein has nothing to do with the red shift; the nature of the photochemical transformation has not been determined and no further work has appeared on this complex.

At the same time Boxer and Wright [109] reported inserting the natural chlorophyllide *a* chromophore into apoMb; complexes of pyrochlorophyllide *a*, pyrochlorophyllide *b*, and bacteriochlorophyllide *a* have also been prepared [8]. In each case a 1 : 1 complex was formed, and absorption, CD, MCD, EPR and NMR properties of the complexes have been reported (the complexes are photochemically stable). The optical properties are illustrated in Fig. 8 for the pyrochlorophyllide *a* complex. The absorption and MCD spectra change very little when the chromophore is inserted in the heme pocket. On the other hand, the CD spectrum changes substantially. The origin of this effect is identical to that discussed earlier for the dimer models, and involves coupled oscillator interactions with excited states of chromophores in the protein. Of course, the CD result shown in Fig. 8 applies only to this particular protein environment; a different protein, with different side-chains at different orientations and distances, could have completely different effects. Thus, this model study again serves as a warning against overinterpreting the CD spectra of natural Chl-protein complexes. The pyrochlorophyllide *a*-apoMb complex has been crystallized, and the single crystal absorption spectrum has been analyzed [110]. This has permitted the determination of the absolute orientation of the Q_y transition dipole moment with respect to the myoglobin crystallographic axes. ZnPChl *a* has also been inserted into all four heme pockets of apo-hemoglobin, and complementary hybrids containing heme in either the α- or β-chains and ZnPChl *a* in the other have been prepared [111]. The spectroscopy of these complexes is nearly identical to that of the apo-Mb complex; however, degenerate singlet energy transfer is observed. These molecules are models for energy transfer in a completely defined three-dimensional array of chromophores. In the hybrid complexes energy transfer from ZnPChl *a* to deoxy heme (α-chains to β-chains or vice versa) is observed, and shortens the ZnPChl *a* fluorescence lifetime by about 50%, even though ZnPChl *a* is nearly 25 Å from heme [219]. It is evident from this model that the excited state lifetime for *monomeric* Chl in organized biological assemblies must be interpreted with great caution.

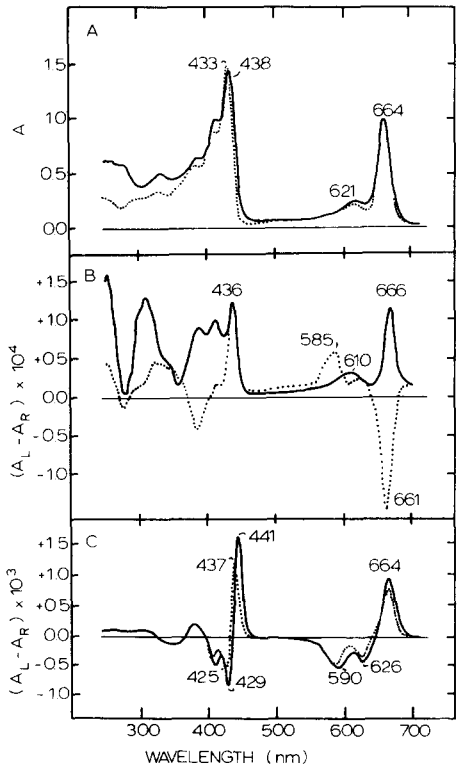

Fig. 8. Comparison of (A) electronic absorption, (B) CD and (C) MCD, 1 cm pathlength at 20°C, spectra for the MgPChl *a*-apomyoglobin complex (———), 10^{-5} M in H_2O, and MgPChl *a* with imidizole as a ligand (·····), 10^{-5} M in CH_2Cl_2.

The effects of nearby charges on the absorption properties of a number of biological chromophores (electrochromic shifts) have been known for many years, but the possible effects on Chl absorption properties have only recently been considered in detail. These effects have been referred to in general terms in studies of reaction center intermediates; for example, the combined spectrum of independently prepared P^+ and I^- is expected to be different from that of P^+I^-. Honig and co-

workers [112] have presented a very detailed analysis of point charge effects on the spectral properties of the visual pigments, and this analysis has been extended recently to the chlorophylls [113]. Substantial spectral shifts are predicted for specific positions of a charged group. In fact, the calculations predict that the whole range of spectral shifts observed in reaction center complexes from many organisms can be explained, in principal, by this effect alone. Note that this analysis requires that an amino acid be ionized within the lipid bilayer to achieve the maximum effects. Davis and co-workers [114] have prepared a model for these effects by converting the formyl group in Chl *b* at position 3 to a titratable primary amine. Very small (4 nm) spectral shifts were observed when the pH was varied through the expected pK_a of the amine, and the sign of the change (blue shift) was as predicted by Eccles and Honig [113]. Unfortunately, this particular position, though synthetically accessible, is predicted to be one of the least sensitive to nearby charges, though it is a first step. Much more work is needed in this area.

A number of amino acids and molecules of related functionality have been covalently connected to PChl *a* and BPChl *a* to determine their impact on the properties of the chromophore [90]. PChl *a* and BPChl *a* esterified with side-chains containing the thio-ether functionality (methyl-6-hydroxyhexylsulfide, a Met analogue) were studied in detail by NMR. Intramolecular coordination at the central metal was observed for the Zn-PChl *a* and Zn-BPChl *a* thio-ether derivatives, but not for the Mg-PChl *a* derivative. The NMR data for the Zn-PChl *a* derivative were interpreted as providing evidence for two distinguishable five-coordinate complexes, with the ligand on either side of the macrocycle. In contrast, the Zn-BPChl *a* derivative appears to be coordinated exclusively on one side. It was proposed that stereoselective coordination may provide a simple mechanism for altering the spectroscopic properties of BPChl *a* when it binds to a protein. PChl *a* was covalently attached to the methyl esters of L-tryptamine, L-tyrosine, L-phenylalanine and L-valine. Very large, similar chemical shift changes in the NMR spectra in the absence of a coordinating ligand indicated that all of these compounds form a novel dimer, in which the carbonyl group of the carbomethoxy group on the amino acid attached to one macrocycle coordinates to the central metal on the other macrocycle, bridging between the macrocycles. An average structure was proposed for this dimer, in which the macrocycle planes are parallel, overlap in ring IV and have their *X*-axes antiparallel.

IIB. Models for reaction-center photochemistry

IIB-1. Solution phase photochemistry

Although the reaction center complex is a solid-state photochemical reactor, the substantial literature on solution phase electron transfer will be very breifly reviewed. A prototypic example is the classic work by Weller's group on the quenching of the singlet excited states of aromatic hydrocarbons (e.g., pyrene, Py) by amines (e.g., dimethylaniline, DMA) [47] shown in Fig. 9. The scheme is *very* reminiscent of that in photosynthesis (compare Fig. 2B). At high DMA concentration, essentially all triplets of Py are formed by ion-pair recombination, none by direct intersystem crossing. Magnetic-field effects are observed on the triplet yield, as are magnetic-isotope effects of the correct magnitude [49,115]. Buchachenko [116] and Turro and co-workers [50] have turned this to advantage by using radical-pair reactions to enrich magnetic isotopes. Pines and co-workers [117] have described quantitatively the consequences of increasing the viscosity on the isotope fractionation. The overall similarity of the phenomenology in reaction centers and in these reactions is apparent; they are sharply distinguished by the absence of

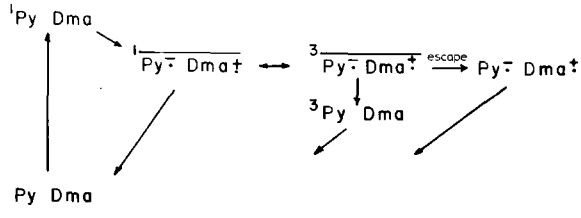

Fig. 9. Scheme showing the photochemistry of pyrene (Py) in the presence of dimethylanaline (Dma) in a polar solvent [47,48]. Note the similarity between this scheme and that in Fig. 2B. The difference is that the radicals are free to diffuse in solution, leading to the escape pathway shown on the right, but not in the reaction center.

magnetic isotope effects in reaction centers [55], and the absence of exchange interactions and anisotropic quantum yields in solution where the radicals are free to diffuse [58].

There have been many biomimetic studies of the quenching of Chl or porphyrin excided states by electron donors and acceptors. Linschitz and Sarkanen [118] originally showed that the Chl triplet state is quenched by quinones to form radical ions, and this reaction has been examined further by others [119–121]. Seely pursued this with a wide range of nitro-aromatic quenchers [122], and further showed that singlet states were also quenched [123]; the quenching efficiencies depended directly on redox potentials, suggesting electron transfer. Recently, Natarajan and Blankenship [124] reached similar conclusions for singlet quenching by quinones. All attempts to observe separated ion pairs from the singlet-quenching pathway have failed [125,126], except for quenching by methyl viologen, which produces a radical ion pair in which both members are positively charged. Detailed CIDNP studies of Chl excited state quenching by quinones [127] or hydroquinones [87,128] showed that only triplet precursors give rise to the degree of solvent separation needed to generate CIDNP. The triplet radical pairs become separated because recombination to the ground state is spin-forbidden; the singlet recombination is allowed. Of course, this begs the question of what is so different in reaction centers (a singlet-state reaction), where singlet recombination is quite slow. This is often ascribed to the large exothermicity of the back reaction, but the same argument applies in solution [129]. The Py/DMA system produces free radical ions from the Py singlet state, whereas Chl/quinone does not. The difference is likely the energetics of the radicals and structural changes which accompany radical formation [129]. Of course, in the reaction center the components have specific spatial arrangements, with a defined 'solvent' environment. If only Chl-type macrocycles are involved (i.e., no motion of protons or participation by the protein), one expects very little change of the macrocycle structure on charge separation.

IIB-2. Non-biomimetic solid-state electron transfer

There are very few relevant solid-state examples of electron-transfer reactions. There is an extensive literature on charge separation in solid semi-conductors and at the semiconductor/liquid interface (see Ref. 130 for a detailed review). One case worth noting is that of amorphous silicon. Many of the phenomena observed in reaction centers have also been observed in amorphous Si, e.g., delayed luminescence from recombination, magnetic-field effects, and RYDMR [131–133]. The connection between this literature and radical-pair spin dynamics in solution or in reaction centers is rarely noted, and the radical-pair mechanism has only recently been introduced to explain some of these effects [134]. The electron and hole migrate in the solid, leading to weak exchange and dipolar interactions, as in solution. Of course, the amorphous nature of the material results in distributions of distances and rates, in contrast to the reaction center. Another example is electron injection from dye molecules (e.g., rhodamine B) adsorbed on anthracene single crystals [135]. Magnetic-field effects were observed, and, due to hopping of the electron in the crystal, magnetic isotope effects were only observed on deuteration of the dye, not of the anthracene. The possible relevance of this system to reaction centers has been stressed by Haberkorn et al. [56,135]. Furthermore, the luminescence yield showed an orientation dependence in the magnetic field. Although this was not analyzed, it may be an example of an anisotropic quantum yield due to factors similar to those in reaction centers [64,65].

An elegant and highly relevant series of papers by Miller and co-workers [136–39] describes one of the few detailed studies of electron transfer involving organic molecules in the solid state. The experimental approach is to produce trapped electrons by pulse radiolysis of glasses and to monitor the rates of electron tunneling to organic electron acceptors. A complication is that the acceptors are randomly distributed in the glass, yielding a distribution of transfer rates. Nonetheless, under these conditions diffusion plays no role and the dependence of the rate on distance and reaction exergonicity can be determined, offering a quantitative test of theories of electron transfer [140–150]. Electron tunneling over distances as large as 40 Å has been observed, albeit at rather slow rates. The rates of highly exothermic reactions were found to

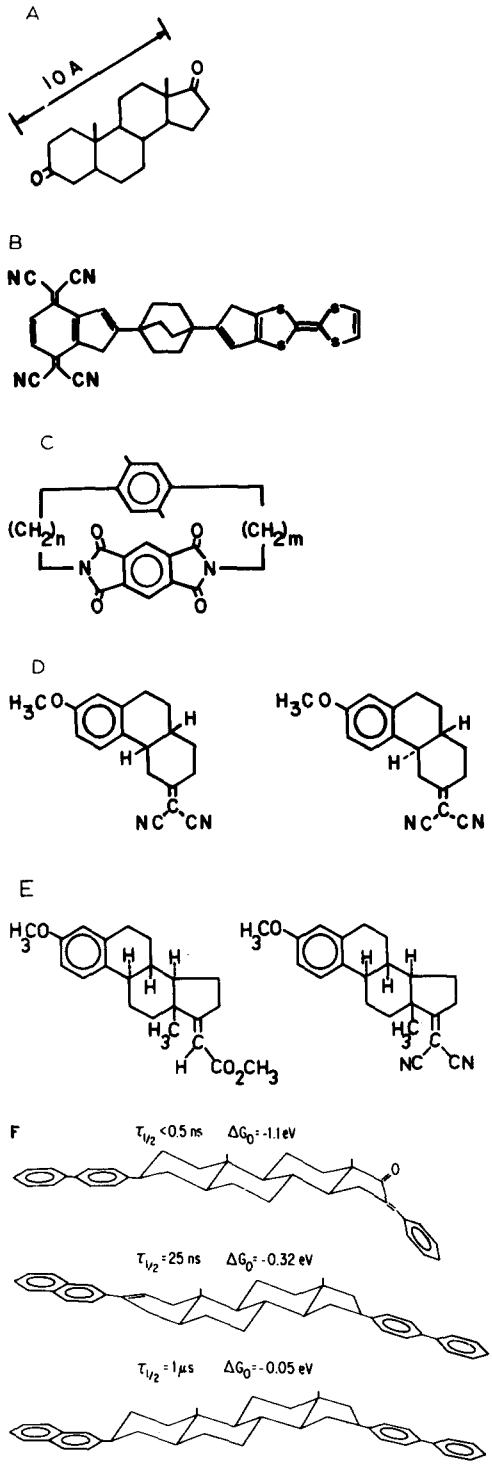

Fig. 10. The structures of several non-biomimetic compounds used to investigate the distance and orientation dependence of electron transfer between organic molecules. (A) Steroid with ketones used as electron scavengers by Huddleston and Miller [138]; (B) molecule proposed (but never prepared) by Aviram and Ratner [158]; (C) donor/acceptor cyclophane studied by Borkent et al. [160]; (D) donor/acceptor pair held rigidly by a decalin bridge [161]; (E) donor/acceptor pair held rigidly by a steroid bridge [162]; (F) structures of diended steroids with half-lives and free-energy changes for intramolecular electron transfer in the radical anions [164].

be much slower than those for moderate exothermicities, consistent with long-standing predictions of theories of electron transfer [148,150]. In order to surmount the uncertainties associated with a distribution of distances, Miller and co-workers studied trapping and transfer in the solid state between two sites held rigidly apart, by virtue of being part of a steroid (Fig. 10A) [138]. The electron-accepting groups in this molecule are approx. 10 Å apart. A detailed tunneling theory was presented to deal with this case and qualitative agreement was found between the experimental results and theory. Although the movement of an electron from one site to another is not identical to the situation encountered in photoinduced electron transfer, all underlying principles are the same, and these studies provide a firm experimental basis for comparison with electron transfer theories. Miller and co-workers have presented an example of photoinduced electron transfer over long distances in rigid glasses by studying the phosphorescence quenching of several organic molecules by electron transfer to or from their excited triplet states [139]. Due to the long lifetime of these states (several seconds), reactions were observed over distances as large as 25 Å.

In order to include the majority of model systems, it is useful to broaden the definition to include models in which the two or more components are constrained to maintain some spatial order relative to each other, although the complex may be in fluid solution. Some of the best-defined intramolecular electron-transfer models were prepared by Taube and his co-workers [151], who connected two transition metals are connected by a bifunctional ligand. The beauty of these complexes is that the intervening ligand can be varied considerably to measure the consequences of its electronic structure on the rate of electron transfer. When mixed-valence compounds are prepared, one

obtains a mixed-valence transition whose position, intensity and lineshape are very sensitive to the degree of coupling between the metal centers [152–154]. Many examples of excited-state electron transfer have also been documented in related compounds (see Refs. 155, 156 for recent examples and references therein). This model work has inspired a great deal of theoretical and experimental work, including an as yet unsuccessful search for very weak infrared bands in reaction-center complexes corresponding to the direct transfer of an electron from one site to another.

Early attempts to study electron transfer in rigidly connected donors and acceptors was motivated by the pioneering work of McConnell and co-workers [157] on charge transfer crystals. The quest for organic conductors and molecular electronic devices (e.g., rectifiers) has inspired proposals of synthetic targets such as that shown in Fig. 10B [158], which has apparently never actually been prepared. Verhoeven and co-workers [159–163] have prepared a series of donor/acceptor molecules covalently connected by flexible chains, and a series of novel, rigidly connected molecules, some of which are illustrated in Fig. 10C–E. These investigators focused on the intensity of charge-transfer emission as a measure of donor/acceptor interactions. The emphasis of the analysis is on the influence of through-bond interactions (three to five sigma bonds) in facilitating the formation of a charge transfer state. These compounds demonstrate unambiguously that through-bond interactions are readily detected when there is no possibility of direct contact between the donor and acceptor groups, a very relevant consideration in any covalently connected donor/acceptor system. These compounds and also the Taube compounds discussed above are examples of relatively strong coupling between electron donors and acceptors.

This type of work has recently been extended by Miller and co-workers [164] who prepared the series of rigidly spaced molecules shown in Fig. 10F. This figure also gives the free-energy change for intramolecular electron transfer in the radical anion (generated by pulse radiolysis) and the observed half-life for intramolecular electron transfer in fluid solution (2-methyl-tetrahydrofuran, 296 K). This experiment provides direct evidence for very fast electron transfer over distances of about 15 Å for a reaction with an exogernicity of about 1 V. The rate of electron transfer in these covalently connected molecules is faster than for equivalent molecules at a comparable separation in glasses. Thus, it appears that even this rather extended steroid 'spacer' permits considerable through-bond interaction (Miller, J., personal communication).

A number of much more flexible, singly covalently connected donor/acceptor systems [D-$(CH_2)_n$-A] have been examined by time-resolved spectroscopy [165–178]. Focusing on one example, anthracene covalently linked to aniline by three methylene groups has been studied by Eisenthal [175–177], Mataga [172–174] and Zewail [178] and their co-workers. In polar solvents the anthracene fluorescence is quenched to a non-fluorescent species, presumably the connected radical ion pair. The ion-pair lifetime is on the order of about 1 ns, with exclusive decay to the ground state. This is in sharp contrast to the unlinked analogue discussed above (Fig. 9), where triplets are formed in high yield on recombination. It is likely that the singlet-triplet splitting in the covalently connected ion pair is too large to permit singlet-triplet mixing at low-magnetic field strengths (see Fig. 3B). No measurements of the effects of large magnetic fields have been reported.

There is a substantial body of work involving electron transfer in organized media, such as micelles [179], semiconductor solid-liquid interfaces (see Refs. 130 and 180 for recent reviews) and in monolayer assemblies (see Refs. 181, 182 and references therein). Change migration over distances as large as 40 Å has been reported in the latter systems [183]. Another very interesting approach is to use the well-defined tertiary and quaternary structure of proteins to hold redox centers at prescribed distances. Gray and co-workers [184] have covalently connected pentaamine ruthenium to His-33 of ferricytochrome *c* (PFeIII) and studied the reaction:

$$\text{PFe(III)-Ru(II)} \xrightarrow{k_{ET}} \text{PFe(II)-Ru(III)}$$

The intramolecular rate constant was found to be $(2.0 \pm 0.5) \times 10^1\ s^{-1}$ and did not depend on temperature between 273 and 310 K. The distance between redox centers is estimated to be about 15

Å and the reaction is weakly exergonic (about 0.1 V). This result was confirmed by Isied and co-workers [185] who obtained $k_{ET} = 82 \pm 20\ s^{-1}$ at 299 K with the same system. Hoffman and co-workers [186] have reported evidence for electron transfer from the triplet state of Zn-protoporphyrin IX substituted in the α-chains of hemoglobin to the Fe(III)-protoporphyrin IX centers in the β-chains. The center-to-center distance is about 25 Å and a rate of less than $100\ s^{-1}$ was reported. It can be expected that a large number of similar systems will be developed in the coming years to test the distance dependence of electron transfer under conditions where the redox centers are not directly connected by sigma bonds. The relevance of these studies to electron transfer in reaction centers is immediately apparent.

IIB-3. Biomimetic models for electron transfer

As discussed in the first section, natural photosynthetic systems exhibit highly efficient electron transfer from Chls or Chl dimers to electron acceptors, including other Chls and quinones. Several groups have prepared prophyrins or Chls covalently connected by a single chain to quinones (Fig. 11). Loach [187–189], Tabushi [190], Ganesh [191–193], Nishitani [194,195] and their co-workers have reported the preparation of tetraphenylporphyrins linked to benzoquinones. Tabushi [190] reported that the porphyrin fluorescence is quenched to a much greater extent than would be the case if an equivalent amount of quinone were added in solution. Greater quenching was observed in more polar solvents. This argues in favor of an electron transfer mechanism, enhanced in yield by the covalent linkage which minimizes diffusion, but otherwise indistinguishable from solution photochemistry. Ho and co-workers [196] reported the observation of radical signals by EPR following photolysis of the compound prepared by Kong and Loach [187,188]. The quantum yield for formation of the EPR signal was very low. By charge balance, radicals in systems of this type are likely the result of irreversible destruction of one half of the photochemically produced ion pair, leaving the other half to be detected (other radical-generating reactions having nother to do with charge separation may also be occurring). Kong and co-workers [189] demonstrated that the fluorescence lifetime decreased with the quinone covalently connected; they also reported long-lived radicals detected by EPR and generated by an unspecified mechanism (assumed to be electron transfer, but likely due to a more complex mechanism as stable ion pairs at such a short separation are very unlikely). Chidsey and Boxer [197,198] have prepared PChl *a* covalently connected to a menaquinone analog (Fig. 11E). The quinone was carefully chosen to be difficult to reduce, so that triplet quenching was energetically prohibited, and ion-pair recombination would be sufficiently exothermic to allow triplet formation. Efficient quenching of the fluorescence, with a parallel reduction in fluorescence lifetime, was observed, which increased with increasing solvent polarity. In the solid state, there was no evidence for the formation of a T_0-polarized triplet state by EPR. Note that in all of these molecules a solid solution contains a very large distribution of linking chain conformations, limiting the usefulness of these molecules as tests of the distance dependence of electron transfer. Also, data obtained in fluid solution (generally fluorescence data) cannot necessarily be used to support EPR data on the same sample in frozen solution and vice versa.

This shortcoming has been partly overcome by Dalton and co-workers, who covalently attached benzoquinone directly to the *meso* position in the place of one or all four of the phenyl rings in tetraphenylporphyrin (Fig. 11F) [199]. Picosecond transient absorption studies of these molecules, in collaboration with Netzel [166], indicated very efficient quenching of the singlet excited state (singlet lifetime less than 6 ps). Rapid repopulation of the ground state was observed, along with a substantial triplet yield. The triplet was reported to be extremely short-lived (about 30 ps), generating a triplet ion pair, which decayed largely to the ground state. The kinetics are summarized as follows (ϕ_3QP = tetraphenylporphyrin with one phenyl group (ϕ) substituted by benzoquinone, Fig. 11F):

$$\phi_3QP \xrightarrow{h\nu} [\phi_3QP]^{S_1} \xrightarrow{0.4} [\phi_2QP]^{T_1} \xrightarrow{0.1} [\phi_3Q^-P^+]$$
$$\uparrow \quad \uparrow 0.6\ (\tau \lesssim 6\ ps) \qquad (\tau = 35\ ps) \qquad (\tau = 10\ ns)$$
$$\uparrow \quad \_\_\_\_\_ 0.9 \_\_\_\_\_ \uparrow$$

There are several very unusual aspects of this proposal; although the scheme is kinetically competent, there is little direct evidence for the iden-

tity of any of the proposed intermediates. The extremely large rates of intersystem crossing, first into the molecular triplet and then from the triplet ion pair to the singlet ground state, are especially difficult to rationalize. It is possible that a very large exchange interaction is present in a singlet ion pair intermediate (not shown), and that rapid, spin-orbit induced triplet-singlet conversion occurs in the ion pair. This requires the questionable assertion that a vibrationally excited triplet ion pair precedes formation of molecular triplet, which

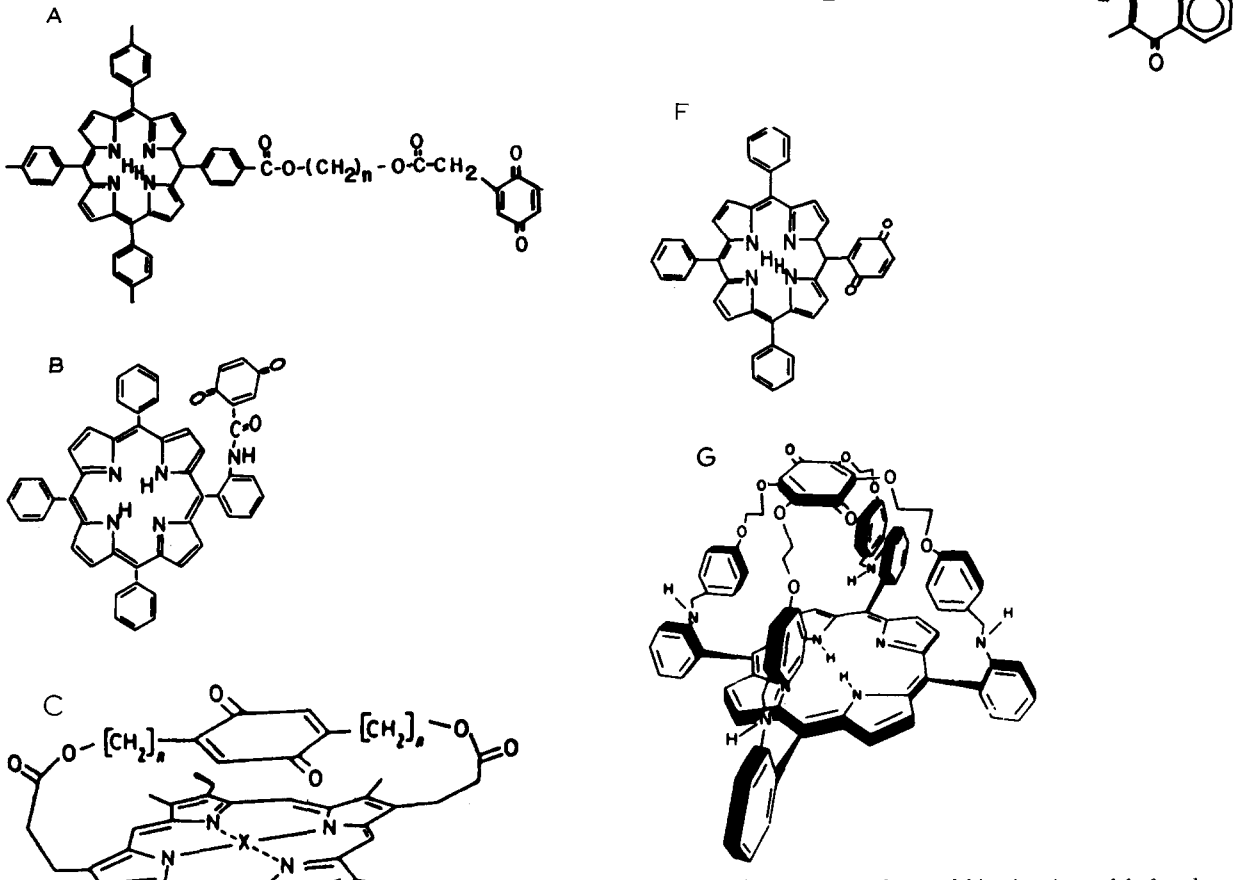

Fig. 11. The structures of several biomimetic models for electron transfer in photosynthesis involving the covalent attachment of quinones to porphyrins or Chls. (A) Kong and Loach [187–189]; (B) Tabushi et al. [190]; (C) Ganesh et al. [191–193]; (D) Nishitani et al. [194,195]; (E) Chidsey and Boxer [197,198]; (F) Dalton et al. [199,200]; (G) Lindsay and Mauzerall [201].

in turn generates the same ion pair, vibrationally relaxed. Although this system needs much greater analysis, it may serve as a model for the strongly interacting, short-lived primary photoproduct,

P^+B^-, in bacterial reaction centers (Chidsey, C.E.D., Kirmeier, C., Holten, D. and Boxer, S.G., unpublished data). Recently, Lindsay and Mauzerall [201] have prepared the multiply covalently connected cofacial porphyrin-quinone shown in Fig. 11G. They report that the fluorescence of the porphyrin is quenched by 60%. Perhaps the most interesting model has been briefly reported by Gust and co-workers [202]. This compound is a synthetic triad consisting of a porphyrin linked to *both* a carotenoid and a quinone. The fully charge-separated species Car^+-Por-Q^- is apparently formed on photoexcitation.

Bucks and co-workers [203] have studied models in which Chl-type chromophores are covalently connected by single chains. These are a very direct attempt to mimic photosynthesis with molecules which may be involved in the primary photochemistry. The open PChl *a* dimer in Fig. 6C can serve as an electron transfer model if one ring contains no central Mg. Further differences in redox potential between the macrocycles can be achieved by adding Cl^- as a ligand, or by using the natural chromophore containing the carbomethoxy group on one half, or by varying solvent polarity. It was found that the excited state lifetime measured by picosecond absorption and fluorescence lifetimes and quantum yields was shortened as the solvent polarity was increased. Although this appears to suggest an electron transfer mechanism, the same trend was observed in the homo-dimers (identical rings), which serve as controls because electron transfer is expected to be energetically forbidden. This suggests that an alternate mechanism of internal conversion is operative, perhaps a tendency for increased intramolecular association as the solvent becomes more hostile to the non-polar chromophores, leading to enhanced fluctuations in the interchromophore coupling and radiationless deactivation. It is worth noting that the combination of picosecond absorption and emission kinetics is very helpful in elucidating excited state deactivation mechanisms.

A further variation on this theme is the trimer shown in Fig. 12B [204]. The Mg-containing dimer portion (rings A and B) folds as in Fig. 6C and the metal-free macrocycle was found to stack on top, as illustrated. Because the singlet lifetime of the folded dimer (electron donor) is only 100 ps

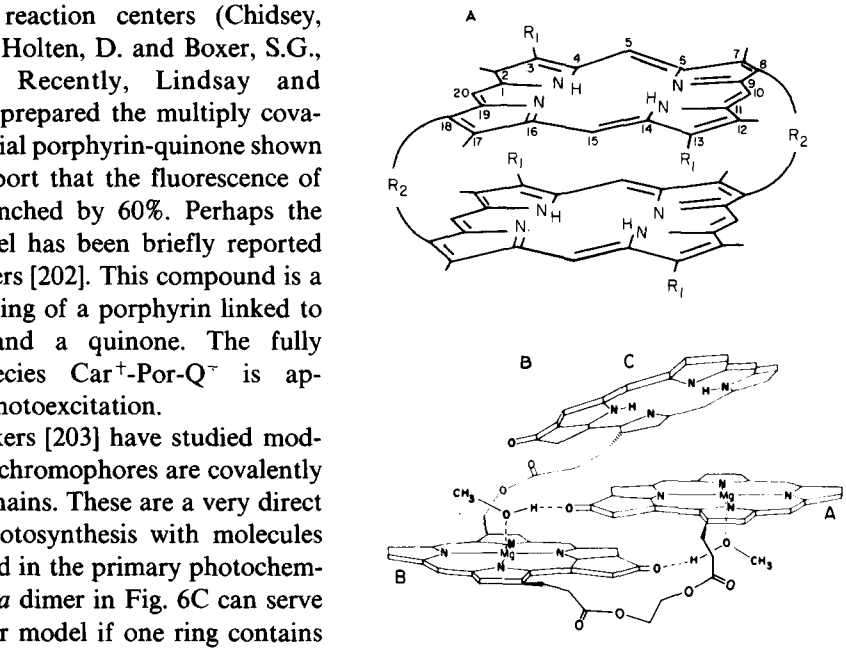

Fig. 12. The structure of several biomimetic models for electron transfer in photosynthesis which employ porphyrin or Chl macrocycles only. (A) Cofacial prophyrin, where R_1 = *n*-octyl and $R_2 = -CH_2C(C{=}O)N(n\text{-Bu})CH_2CH_2-$. Mg-$H_2$, H_2-H_2 and Mg-Mg compounds have been studied [208,212–214]; (B) Chl-based trimer comprised of a dimeric electron donor unit (rings A and B, cf. Fig. 6C) and an electron acceptor (ring C, pyropheophorbide *a* or pheophorbide *a*). The structure shown is the average in solution deduced from NMR measurements [203,204]. See also the molecules in Fig. 6.

[205,206], only very fast electron transfer can be studied. Neither picosecond absorption nor emission showed evidence of electron transfer. When the compound containing ring C with a carbomethoxy group at position 10 (90 meV easier to reduce) was examined, it showed a much more rapid return of the ground state absorbance, suggesting that the triplet yield was low (or the triplet lifetime was orders of magnitude shorter than normal). This hints that electron transfer may be occurring in this trimer, followed by direct recombination to the ground state, and that a rather close association between donor and acceptor and large driving force are needed. Although the possibility of observing electron transfer in all of these compounds was assessed by calculating the free energy change from the redox properties of the partners, and many of the cases studied were predicted to be exergonic by as much as 400 meV,

it is possible that the estimates are systematically in error due to complex solvation effects or subtle ligand effects.

There has been a report in the literature of electron transfer in a molecular aggregate which is quite similar to that in Fig. 12B. Pellin and co-workers [207] non-covalently connected two metal-free PChls to the PChl *a* dimer by using two molecules of the ethylene glycol monoester of pyropheophorbide *a* as the alcohol to 'fold' the dimer, as in Fig. 6C. It was reported that electron transfer occurred within 6 ps with a quantum yield approaching unity, and that the ion pair lived longer than 20 ns. This dramatic result has never been repeated in another laboratory, nor has any further work been reported from the original authors. It has been shown that the experiments were plagued by artifacts due to the extremely high concentrations of all components [97].

The structurally best-defined biomimetic systems at this time are the cofacial porphyrins and Chls, studied by Netzel and co-workers in collaboration with Chang and Boxer and their co-workers [94,208]. Cofacial porphyrins have also been prepared by several other groups [209–211]. It has proved to be very difficult to obtain unambiguous half-wave potentials for the doubly linked cofacial chromophores. In nearly all cases, electrochemical oxidation or reduction is nearly irreversible. The mechanism which destroys the macrocycles is unknown, and may involve disproportionation (Bucks and Boxer, unpublished results). This is unfortunate because it has not been possible to produce sufficiently large quantities of the mono-radicals to examine their absorption CD, and EPR properties in detail for comparison with the in vivo radical P^+. The 'hinged' Chl dimer (Fig. 6B) is reported to undergo reversible one-electron oxidation at a potential about 70 meV more negative than the monomer [106]. This provides an estimate of the effects of dimerization on the redox properties of Chl, and is much less than the change in oxidation potential of the primary electron donors in photosynthesis relative to monomeric Chls in organic solvents [106].

Symmetric cofacial porphyrins are not expected to show photoinduced electron transfer (see Fig. 12A for structure); neither is the mixed metal containing/metal-free ($Mg\text{-}H_2$) diporphyrin in non-polar solvents. However, in more polar solvents, electron transfer in the latter is predicted to be energetically feasible, and the $Mg\text{-}H_2$ diporphyrin does show quite different transient absorbance features within 6 ps under these conditions, in a spectral region expected to contain radical ion absorption [212,213]. These features decay with a half-life dependent on solvent (0.20 ± 0.01 ns in CH_2Cl_2, 0.85 ± 0.05 ns in *N*-methylacetamide and 1.3 ± 0.1 ns in dimethylformamide). Thus, it appears that very efficient singlet quenching is occurring to produce a new state; proving that this is, in fact, an ion-pair state remains a goal. Some evidence favoring this hypothesis has been obtained. Quinone added to a solution of the $Mg\text{-}H_2$ cofacial porphyrin quenched the primary photoproduct, suggesting that it is an ion pair which further reduces the quinone [214]. The analogous dimeric porphyrin with a four-atom linking chain was also examined and the absorption changes and decay kinetics were quite similar to those of the five-atom linked cofacial porphyrin. Although this may appear surprising, it is possible that the five-atom linked cofacial porphyrin slips to give Van der Waals contact between the macrocycles; consequently, the distance between the rings in these two molecules may be quite similar. At this time, there have been no transient absorption studies in the solid state. The triplet EPR spectra are extremely difficult to detect (commensurate with efficient singlet quenching). A T_0-polarized triplet was not detected. This does not rule out the formation of radical ions, as it is likely that the singlet-triplet splitting is substantial. Thus, the important characteristic of in vivo reaction centers, that the ion-pair exchange interaction be small enough to allow S-T mixing, has not yet been achieved using two chromophores.

A brief critique of these efforts may be useful. It is imperative that the search for electron transfer products employ ps absorption, emission and fluorescence quantum yield measurements on the same sample under the same conditions. Each method plays an important role, but none is conclusive by itself. Ps transient absorption measurements are complicated by the dilemma of distinguishing excited singlet and triplet or ion-pair absorption. These are often very similar. The combination of these approaches provides a much

more complete bookkeeping of the intermediates. Ordinary EPR following photoexcitation is useful only if both radicals in the pair can be detected and identified. The observtion by EPR of a single radical or a very long-lived superposition of radicals is likely due to selective destruction of the molecule. A radical pair in the solid state will give a very different EPR spectrum due to spin-spin coupling; however, it is very unlikely that such a species would be sufficiently stable to be detected by ordinary EPR (lifetime longer than 30 μs). Reaction centers provide a good model in this case: the EPR spectrum of P^+I^- has not been directly detected (exchange interaction about 10^{-3} cm^{-1}), but the radical pair P^+Q^- can be detected (exchange interaction immeasurably small). Thus, it is unlikely that EPR spectra showing splitting ascribable to spin-spin interactions will be detected in models. Magnetic-field effects or time-resolved EPR/RYDMR offer a definitive approach to proving the intermediacy of species which possess spin angular momentum. No results of this type of measurement have yet been reported for any of the biomimetic models discussed above, thus the goal of duplicating the primary photochemistry of photosynthesis remains unmet.

Most of the impact of model studies to date has been to provide examples of the complexities of Chl spectroscopy which serve as warnings against oversimplified interpretations of in vivo results. The electronic absorption, CD spectrum and fluorescence lifetime of monomeric Chl are found to be highly dependent on the environment, especially on the presence of charged groups, asymmetric chromophores and chromophores with low-lying excited states. Most of the possible spectral shifts and CD patterns predicted for dimers of asymmetric chromophores have been illustrated with synthetic Chl dimers, along with the complexities inherent in treating only interactions among degenerate states. The fluorescence lifetimes of dimers of defined structure are found to vary from more than an order of magnitude shorter to longer than the monomer lifetime. The widely held notion that dimer lifetimes are necessarily shorter than monomer lifetimes is not correct, though this is often true for a flexible dimer, where the sampling of many structures during the excited state lifetime finds conformations where the lifetime is much shorter, quenching the excited state.

It is somewhat ironic that the models in which the spatial relationship between donor and acceptor is enforced by covalent connection may lose some of their generality by virtue of the substantial through-bond interactions which are present. Even in the case of the protein-based electron-transfer systems [184,185], donor-acceptor interactions through the peptide-bond backbone may be very important, even when the distance over which this interaction operates is greater than the shortest through-space distance. There is no evidence at the present time for or against the notion that the Chls in reaction centers experience strong through-bond interactions via their coordination to amino-acid side-chains. This suggests important model experiments to measure the magnitudes of such effects for Chls; this will be especially important when crystallographic data reveal the specific protein contacts. It may even turn out that the making and breaking of such through-bond interaction pathways plays an important role in steering the kinetics of the primary events in the desired direction.

IIC. Future directions

It is evident from this review of efforts to duplicate photosynthesis that progress has been slow. The most promising systems to date are the doubly linked porphyrins and Chls. As was the case with reaction centers, picosecond absorption measurements indicate electron transfer and establish the kinetics. However, methods which are sensitive to the existence and nature of the radical-ion pair intermediate (magnetic-field effects, RYDMR) have not yet been applied to these systems. It is probable that strong electron exchange coupling in the ion pair and short ion-pair lifetimes will make these experiments very difficult.

Another potentially useful direction is the application of recently developed optical coherence methods to reaction center components. For example, molecular dimers in single crystals have been studied by four-wave mixing methods (e.g., coherent Stokes-Raman scattering [215]) and photon echo methods [216,217]. These methods offer an approach to measuring intermolecular interactions

in pairs of molecules (or higher aggregates), as well as studying excited state dynamics. Although transient absorption spectroscopy provided picosecond kinetic data in reaction centers and models, it is not possible to detect directly paramagnetic intermediates whose lifetimes are less than 1–10 ns by time-resolved EPR or RYDMR methods. Time-resolved Raman spectroscopy, particularly coherent Raman scattering, may fill in the very short time-scale with a method which is more sensitive to the nature of the intermediates.

It is unlikely that the primary donors and acceptors in photosynthesis which produce moderately stable radical pairs (20 ns) are as close as the macrocycles in any of the cofacial compounds. It is a much greater synthetic challenge to position donors and acceptors *rigidly* at greater distances. Irrespective of the success or failure to duplicate photosynthesis, these efforts are essential to provide an experimental test for both the distance and orientation dependence predicted by electron-transfer theories for weakly interacting systems. Proteins offer an interesting alternative to chemical synthesis. To date the only example of a synthetic multi-Chl containing protein is the work from the author's laboratory on Chl substituted into apo-hemoglobin. In this molecule the inter-chromophore separation is around 25 Å, too large for an appreciable yield of electron transfer products on the time-scale of the singlet lifetime. However, other protein-based systems may position the chromophores much closer to each other [184–186].

Finally, it is evident that the primary photochemistry of photosynthesis takes place in the unique environment of the reaction center protein. It is tempting to speculate that some or all of the unique properties of the primary photochemistry are a consequence of this environment and that photosynthesis cannot be duplicated without duplicating this environment. A simple question which remains to be answered, for example, is how the radical cation P^+ and anion I^- can be so stable when formed chemically in the reaction center complex, yet these radicals are highly unstable when formed electrochemically, unless great care is taken to eliminate many of the reactive groups common to most proteins. As charged intermediates are formed in photosynthesis, it is interesting to speculate further that very selective protonation or deprotonation by amino acid side-chains in the protein may play a key role in stabilizing intermediates. A new concept is that the natural system itself can be considered a model. This is because the protein can be modified, in principle, using the recombinant DNA technique of site-directed mutagenesis. The availability of this technology offers an entirely new approach to studying the primary photochemistry and spectroscopic properties of photosynthetic systems and may ultimately prove to be the most useful approach.

Acknowledgements

The author wishes to thank Professors Closs, Dervan, Hush, Parson and Verhoeven and Drs. Miller, Netzel and Scherz for sharing results prior to publication. Special thanks go to Drs. Bucks, Chidsey, Kuki, Moog and Roelofs from the author's laboratory. The work from the Stanford group was supported by NSF, USDA and DOE grants PCM 7926677/8303776, 78-59-2066-0-1-147-1 and DE-FG02-80CS84006, respectively. S.G.B. is an Alfred P. Sloan and Camille and Henry Dreyfus Teacher-Scholar Fellow.

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