

## Chemically Induced Magnetic Polarization in Photosynthetic Systems

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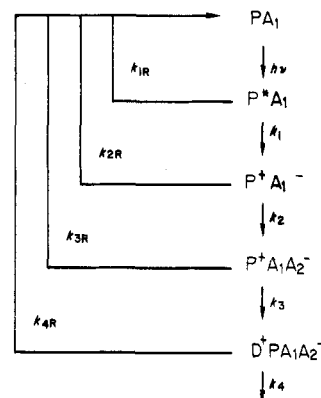
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Research on photosynthetic organisms has long provided a meeting ground for scientists from a variety of disciplines. The photosynthetic system has been able to challenge and interest solid-state physicists, chemists, and biologists by presenting them with a multitude of physical and biological processes ranging from exciton transfer in pigment arrays to the growth of forests. In this diverse field it is therefore not surprising that a remarkable range of physical techniques has been employed to elucidate various mechanistic aspects of photosynthetic energy storage. Experiments exploiting the phenomenon of chemically induced magnetic polarization (CIMP) have been extraordinarily useful in photosynthetic research in the past few years.

Photosynthesis<sup>1</sup> begins when a photon is absorbed by a pigment molecule embedded in a biological membrane (the chloroplast membrane of green plants or the cytoplasmic membrane of photosynthetic bacteria), promoting it from the ground state to an excited state. The energy is rapidly transferred to a chlorophyll (or bacteriochlorophyll in the case of photosynthetic bacteria) in a specialized chlorophyll-protein complex called the reaction center. In this excited state the pigment molecule is an extremely strong reductant, and an electron is lost to an acceptor molecule strategically placed nearby. The oxidized chlorophyll and reduced acceptor then rapidly react with secondary electron donors and acceptors to separate the charges and stabilize the system against recombination losses. Remarkably, these recombination losses, in which a reduced acceptor transfers an electron back to an oxidized donor with loss of the photon energy as heat, appear not to occur to any appreciable degree in the functioning system. Essentially every photon that initiates photochemistry eventually produces stable products.<sup>2</sup>

Thermodynamically the charge recombination process returning the system to the ground state is favored over any other fate of the system. That it does not occur to any appreciable degree in the functioning system is clearly the result of kinetic factors. A sim-

plified scheme of photosynthetic electron transfer illustrates this principle of "kinetic steering." P signifies a photoactive pigment molecule, while D's and A's signify electron donor and acceptor species, and  $k$ 's are rate constants of the various reactions.



For the system to arrive at state  $D^+PA_1A_2^-$  in nearly 100% yield, the rates of the recombination reactions ( $k_{1R}$ ,  $k_{2R}$ , etc.) must be negligible compared to the rate of the forward reaction at the same point ( $k_1$ ,  $k_2$ , etc.). Indeed, the rates of many of the recombination reactions have been measured by artificially blocking the forward reaction; the recombination rate constants are invariably a factor of 100–1000 slower than the rate constant of the forward reaction.<sup>3</sup>

The photosynthetic system is relatively free from recombination losses because the components are positioned such that the rates of favorable reactions are maximized and the rates of unfavorable reactions are minimized. Although most membranes have been found to be quite fluid, with considerable lateral mobility of the lipid and protein components, this is almost certainly not the case within the reaction center complex. One almost invariably observes first-order kinetics

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(2) Wright, C. A.; Clayton, R. K. *Biochim. Biophys. Acta* **1973**, *333*, 246–260.

(3) Blankenship, R. E.; Parson, W. W. In "Photosynthesis in Relation to Model Systems"; Barber, J., Ed.; Elsevier: Amsterdam, 1979; pp 71–114.

Robert Blankenship was born in Auburn, NE, in 1948 and received his undergraduate education at Nebraska Wesleyan University and a Ph.D. in Chemistry under Kenneth Sauer at the University of California, Berkeley. He was then a postdoctoral fellow in Biochemistry with William Parson at the University of Washington in Seattle and since 1979 Assistant Professor of Chemistry at Amherst.

for reactions between two reaction center components which are present in equal concentrations, suggesting that diffusion within the membrane is unimportant. Also, many of the earliest reactions which follow the photochemical step still occur at 4 K, where membrane fluidity is negligible.<sup>4</sup> The picture of the reaction center that emerges is of a complex with a relatively rigid geometry: the various components have fixed distances and orientations with respect to each other. This is not to say that motion does not occur within the complex; indeed small movements may be important, but almost certainly reacting groups have a relatively narrow range of orientations and distances over which electron transfer occurs.

Two of the most relevant questions about the photosynthetic system are (1) what is the nature of the electronic excited state of the system which precedes the initial electron transfer and (2) how are the reaction center components arranged so that electron transfer is highly efficient? CIMP techniques can answer the first question outright and can make a contribution toward the answering of the second.

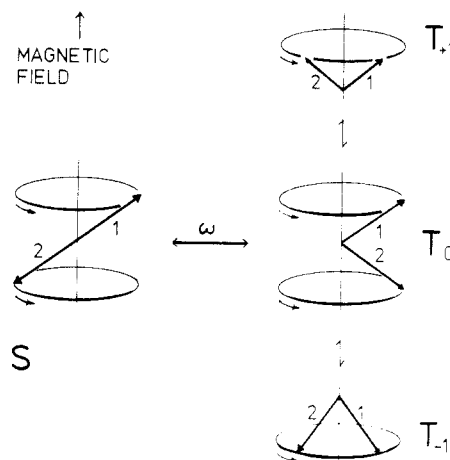
Spin-polarized, or non-Boltzmann, electron spin resonance (ESR) spectra result if a chemical reaction has a preference for one of the spin states of the products of the reaction. Two mechanisms for the generation of the nonequilibrium spin distribution have been identified in chemical systems,<sup>5</sup> the radical pair and triplet mechanisms. A radical pair is simply two radicals whose electron spins are correlated with respect to each other; i.e., the relative orientation of the two electron magnetic moments is not random. This correlation can exist in a single molecule before radical formation or can be produced by spin-selective reactions between independently generated radicals. A single molecule with two unpaired electrons that interact strongly is usually called a triplet rather than a radical pair.

The first step in the spin polarization process is radical pair formation. In the case of photosynthetic systems, the radicals are oxidized and reduced species produced by the photochemical electron-transfer reaction. The two electron spins were highly correlated just before the reaction, and the chemical reaction preserves this correlation. Thus an excited singlet produces a singlet radical pair and an excited triplet produces a triplet radical pair. The basis set that describes the spin system consists of a singlet and three triplet radical pair states. If the radicals are far enough apart, the individual spin vectors are free to precess about the field direction at a frequency determined by the electron  $g$  factor and nuclear hyperfine state of the radical. In the presence of a magnetic field and the absence of any exchange coupling between the two radicals, only the singlet (S) and middle triplet ( $T_0$ ) levels are mixed. The spin Hamiltonian can be divided into two parts, one of which gives the frequency of  $S \rightarrow T_0$  mixing.<sup>6</sup>

$$\omega_{ab} = \hbar^{-1}(\frac{1}{2}\beta H_0(g_1 - g_2) + \frac{1}{2}\sum_n A_{1n}M_{1n} - \frac{1}{2}\sum_m A_{2m}M_{2m}) \quad (1)$$

(4) Feher, G.; Okamura, M. Y. In "The Photosynthetic Bacteria"; Clayton, R. K.; Sistrom, W. R., Eds.; Plenum: New York, 1979; pp 349-386.

(5) "Chemically Induced Magnetic Polarization"; Muus, L. T.; Atkins, P. W.; McLaughlin, K. A.; Pedersen, J. B., Eds.; D. Reidel: Dordrecht-Holland, 1977.



**Figure 1.** Vector diagram illustrating singlet-triplet interconversion of a radical pair at high magnetic field and negligible exchange coupling between the two spins, from ref 8. Arrows labeled 1 and 2 represent spin angular momentum vectors for the unpaired electrons on radicals 1 and 2. Mixing of S and  $T_0$  states occurs if the precession frequencies of spins 1 and 2 are not equal; cf eq 1.

In eq 1  $\omega_{ab}$  represents the difference in angular precession frequency of the two electrons,  $\hbar$  is Planck's constant divided by  $2\pi$ ,  $\beta$  is the Bohr magneton,  $H_0$  is the applied magnetic field,  $g_1$  and  $g_2$  are the electronic  $g$  factors of the two radicals, and  $A_{1n}$  (or  $A_{2m}$ ) are the isotropic hyperfine coupling constants of nucleus  $n$  (or  $m$ ) on radical 1 (or 2) with magnetic quantum number  $M_{1n}$  (or  $M_{2m}$ ). Anisotropic effects can be incorporated into eq 1 by substituting the appropriate tensor quantities for  $g$  and  $A$ .<sup>7</sup> The process of  $S \rightarrow T_0$  mixing can be easily visualized through the use of a simple vector diagram for the motion of the electron spins (Figure 1).<sup>5,6,8</sup> The S and  $T_0$  radical pair states are nearly degenerate in energy due to the large distance and therefore weak exchange coupling between the two radicals. These quasi-degenerate levels are rapidly mixed, but the populations of the energetically remote  $T_{+1}$  and  $T_{-1}$  levels do not change in the absence of spin-lattice relaxation, usually expected to be slow relative to the nanosecond time scale of  $S \rightarrow T_0$  mixing. The simple  $S \rightarrow T_0$  mixing process described above can account for some of the CIMP observations in photosynthetic systems. However, for understanding of the spin-polarized ESR signals which arise from isolated radicals a somewhat more complicated picture will be discussed later.

### The Nature of the Precursor Excited State

Is the excited state of the reaction center which directly precedes electron transfer a singlet or a triplet? This question has been hotly debated for over 25 years.<sup>9</sup> An unambiguous answer to this question is essential for

(6) (a) Adrian, F. J. *Chem. Phys.* 1971, 54, 3912-3917. (b) Closs, G. L. *J. Am. Chem. Soc.* 1969, 91, 4552-4554. (c) Kaptein, R. *Ibid.* 1972, 94, 6251-6262.

(7) (a) Friesner, R.; Dismukes, G. C.; Sauer, K. *Biophys. J.* 1979, 25, 277-294. (b) Friesner, R., Ph.D. Thesis, University of California, Berkeley, 1979.

(8) Blankenship, R. E.; Schaafsma, T. J.; Parson, W. W. *Biochim. Biophys. Acta* 1977, 461, 297-305.

(9) (a) Robinson, G. W. *Proc. Natl. Acad. Sci. U.S.A.* 1963, 49, 521-529. (b) Franck, J.; Rosenberg, J. L. *J. Theor. Biol.* 1964, 7, 276-301. (c) Fong, F. K. *Ibid.* 1974, 46, 407-420. (d) Clayton, R. K. "Molecular Physics in Photosynthesis"; Blaisdell: New York, 1965; Chapter 13.

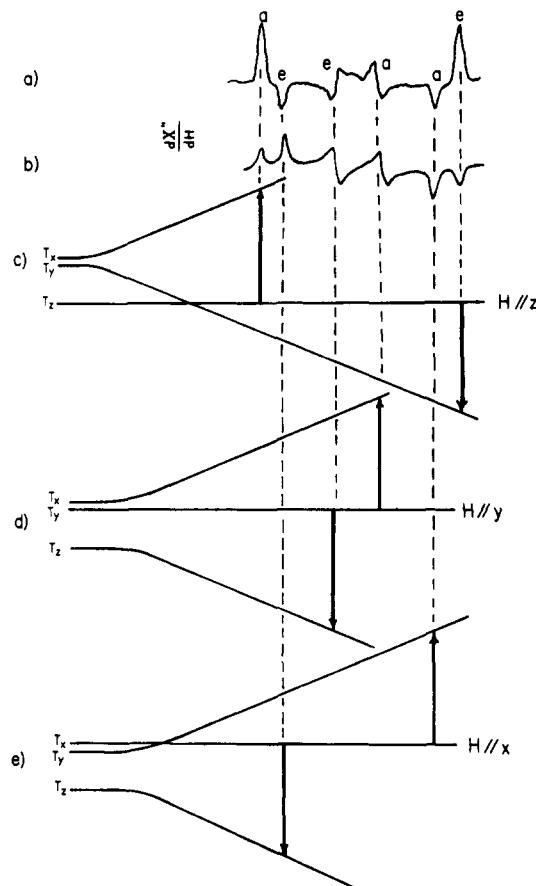
an understanding of the photochemistry of photosynthesis.

The development of picosecond spectroscopy in the last several years has permitted a detailed study of the kinetics of formation of the product radicals in isolated reaction centers of photosynthetic bacteria.<sup>10</sup> Results from a number of laboratories agree; the lifetime of the precursor excited state is <10 ps. The extremely short excited-state lifetime of the reaction center has led most workers to favor the excited singlet as the state which precedes electron transfer. The assumption has been that intersystem crossing to form a triplet state of the complex prior to electron transfer could not proceed at such a high rate. However, this conclusion is severely weakened by the observation of ultrafast intersystem crossing in a number of organic molecules.<sup>11</sup> The presence of an energetically nearby excited triplet state of the proper symmetry can allow very rapid intersystem crossing.<sup>12</sup> Since the bacterial reaction center contains six interacting pigment molecules and the details of its electronic structure are not yet available, such a state is possible. Rapid intersystem crossing to a triplet state of a reaction center pigment followed by electron transfer from the excited triplet state is consistent with all picosecond studies reported so far. The nature of the precursor excited state is therefore not unambiguously determined by kinetic studies of the excited-state lifetime. CIMP studies have, in conjunction with optical studies, given a definite answer to the question of precursor multiplicity.

### The $T_0$ Spin Polarized Triplet

In 1972 Dutton et al.<sup>13</sup> observed an unusual ESR spectrum in membranes of the photosynthetic bacterium *Chromatium D*. The spectrum showed the line shape which is characteristic of triplet states of organic molecules,<sup>14</sup> with zero-field splitting parameters,  $|D|$  and  $|E|$ , of 0.018 and 0.003  $\text{cm}^{-1}$ . Figure 2a shows a similar spectrum for *Rhodospseudomonas sphaeroides*, R-26 mutant, along with a simulated spectrum of a thermally equilibrated triplet state with similar zero-field splitting parameters (Figure 2b). The most dramatic difference between the two spectra is that three of the six lines of the experimental spectrum are inverted, indicating ESR emission rather than absorption.

The ESR spectrum of Figure 2a is only observed if the normal course of photochemistry is blocked, either by chemical reduction<sup>15-17</sup> or by extraction<sup>18</sup> of the endogenous electron acceptor, ubiquinone. This triplet state could either be a normally occurring state of the



**Figure 2.** (a) First derivative triplet ESR signal from *Rhodospseudomonas sphaeroides* R-26 reaction centers, taken from ref 15. Emissive lines are labeled e and absorptive lines are labeled a. (b) Simulated first derivative ESR spectrum of a randomly oriented thermally equilibrated triplet. (c-e) Energy level diagrams of molecules in which the magnetic field is parallel to the z, y, and x zero-field magnetic axes. Observed transitions are indicated with heavy black arrows.

reaction center which is stabilized when its preferred decay pathway is inhibited or it could be a state of the system that does not form to any significant degree when photochemistry is not blocked. A closer examination of the spin-polarized triplet ESR spectrum, together with other experiments, indicates almost certainly that this triplet state is not formed during unblocked electron flow. Interestingly, a careful analysis of this "side reaction" permits the unambiguous conclusion that photochemistry proceeds from the excited singlet state, a conclusion that could not be reached otherwise.

Three of the peaks in the first derivative ESR spectrum represent transitions between the  $T_{-1}$  and  $T_0$  triplet levels and three represent transitions between the  $T_0$  and  $T_{+1}$  triplet levels. Figure 2b-f diagrams the energy levels and resulting spectrum.<sup>19-21</sup> Note that the three lines corresponding to the  $T_0 \rightarrow T_{+1}$  transitions are absorptive in Figure 2a and the three corresponding to the  $T_{-1} \rightarrow T_0$  transitions are emissive, indicating that the population of the  $T_0$  level is much larger than the  $T_{\pm 1}$  levels. The radical-pair  $S \rightarrow T_0$  mixing process described earlier and diagrammed in Figure 3a is a candidate for the source of the excess

(10) Holten, D.; Windsor, M. W. *Annu. Rev. Biophys. Bioeng.* 1978, 7, 189-227.

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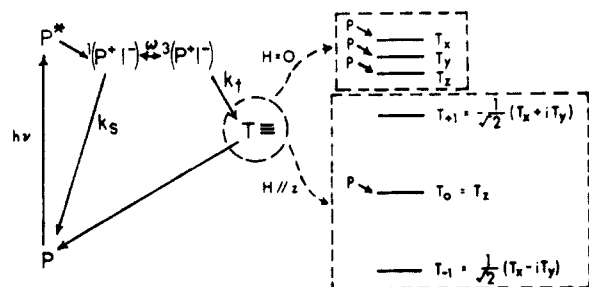
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(19) Levanon, H.; Norris, J. R. *Chem. Rev.* 1978, 78, 185-198.

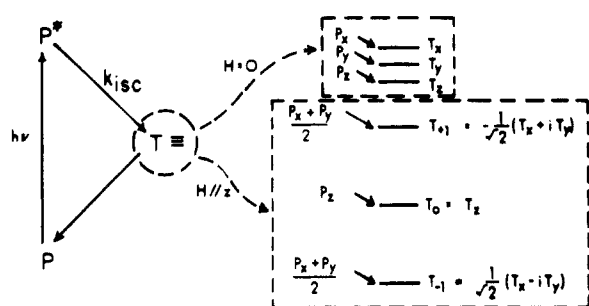
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a) via Radical Pair Intermediate



b) via Intramolecular Intersystem Crossing



**Figure 3.** Mechanisms of triplet formation (a) via radical pair intermediate or (b) via intramolecular intersystem crossing.  $k_S$  and  $k_T$  refer to recombination rate constants from singlet and triplet radical pairs, while  $k_{isc}$  is the rate constant for intersystem crossing. The dashed boxes are details of the circled triplet states in zero and high magnetic field (parallel to the molecular  $z$  axis).  $P$ 's in the left of each box represent populating probabilities, while the high-field triplet wave functions are listed on the right.

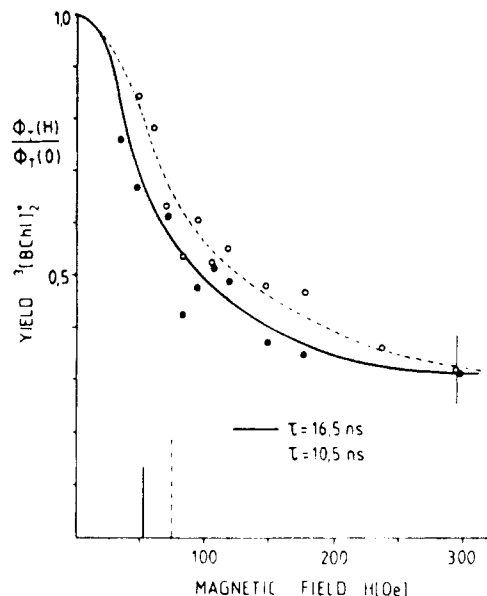
population of the  $T_0$  level, but other possibilities must first be considered before a final assignment can be made.

Can the  $T_0$  spin-polarized triplet species arise by any other mechanism? In particular, can the normal intramolecular intersystem crossing mechanism generate a triplet with this polarization pattern? Certainly, spin-polarized triplet ESR spectra are observed in systems where no radical intermediates occur.<sup>22</sup> To answer this question we need to look closely at Figure 3b, especially noting the differences in spin states between zero and high external field. The high-field spin eigenfunctions  $T_{+1}$ ,  $T_0$ , and  $T_{-1}$  can also be expressed as appropriate linear combinations of the zero-field spin eigenfunctions  $T_x$ ,  $T_y$ , and  $T_z$ . If the molecule's  $x$  axis is parallel to the external magnetic field, the  $T_0$  high-field spin function is identical to the  $T_x$  zero-field spin function, while  $T_{+1}$  and  $T_{-1}$  are linear combinations of the  $T_y$  and  $T_z$  zero-field functions. Analogous behavior occurs for molecules whose  $y$  and  $z$  magnetic axes are parallel to the external magnetic field (see Figure 2).<sup>19-21</sup>

Intramolecular intersystem crossing is described by using the spin-orbit coupling operator. The important point for the present discussion is that the three zero-field triplet states are coupled to both the excited singlet (populating) and ground singlet (depopulating) states by this operator. At high field the populating probabilities ( $P$ ) and depopulating rates are mixed in a manner which reflects the mixture of zero-field eigenstates<sup>23</sup> (Figure 3b). The aeeaae pattern observed

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**Figure 4.** Magnetic field effect on yield of triplet in *Rps. sphaeroides* R-26 reaction centers detected optically at room temperature 10.5 and 16.5 ns after a laser flash [reprinted with permission from ref 34. Copyright 1979, Elsevier/North-Holland.]

in vivo (Figure 2a) cannot be predicted by the intramolecular intersystem crossing mechanism.<sup>17,24,25</sup> The  $T_0$  state is always populated to excess, regardless of the reaction center's orientation with respect to the external field. Such behavior is simply inconsistent with the intramolecular intersystem crossing mechanism. However, the radical-pair mechanism of triplet formation diagrammed in Figure 3a very naturally predicts the observed spin polarization pattern.

Optical experiments identify the triplet species as P870, the primary electron donor of the bacterial photosystem, and also strongly suggest a radical precursor to the triplet state.<sup>27</sup> The triplet ESR spin polarization pattern coupled with the optical data unambiguously implicate a radical pair mechanism for formation of the triplet state. The initial radical pair has singlet character, indicating that electron transfer indeed occurs from the excited singlet state.<sup>28,29</sup> The above argument is self-consistent and relies on a minimum number of assumptions; however, independent corroborating evidence is desirable. Fortunately, such evidence is available from studies of the magnetic field dependence of the amount of triplet formed when normal photochemistry is blocked.

(24) Schaafsma, T. J.; Kleibeuker, J. F.; Platenkamp, R. J.; Geerse, P. *Mol. Spectros. Dense Phases* 1975, 491-494.

(25) It has been shown [Ponte Goncalves, A. M.; Spindel, W. U. *Chem. Phys. Lett.* 1978, 54, 611-615] that the aeeaae pattern could result from very different rates of intersystem crossing of the three triplet levels. The measured values of these rates (20, 21, and 26) rule out this possibility.

(26) Hoff, A. J.; de Vries, H. G. *Biochim. Biophys. Acta* 1978, 503, 94-106.

(27) Parson, W. W.; Clayton, R. K.; Cogdell, R. J. *Biochim. Biophys. Acta* 1975, 387, 265-278.

(28) This conclusion depends on the fact that the zero-field splitting parameter  $D$  is positive. If  $D$  were negative, the polarization pattern would indicate an excess population of  $T_{+1}$  and  $T_{-1}$  over  $T_0$ . This could easily arise via a triplet radical pair precursor in which  $T_0 \rightarrow S$  mixing had occurred, selectively depleting the population of  $T_0$ . However, the sign of  $D$  has been measured by a relaxation method and found to be positive.<sup>21</sup>

(29) Another suggested mechanism<sup>17</sup> is singlet fission, in which a singlet excited state splits into two triplets. This mechanism is ruled out by measurements of the energies of the two states.<sup>30</sup>

### Magnetic Field Dependence of Triplet Yield

The same state that exhibits the  $T_0$  polarized ESR spectrum can be monitored by optical spectroscopy by observing triplet-triplet absorption.<sup>31</sup> The probability of triplet formation is only 10–20% at room temperature; at low temperature it is much higher, perhaps as high as 80–100%.<sup>27,32</sup> At room temperature the application of a weak magnetic field causes the triplet yield to decrease by as much as a factor of three<sup>8,33,34</sup> (Figure 4). No such effect is observed on triplet formation in vitro or on normal photochemistry in unblocked systems.<sup>8</sup> These observations are easily explained by reference to the mechanisms of triplet formation diagrammed in Figure 3. Note especially the difference in behavior at zero magnetic field vs. high magnetic field. The earlier discussion of selective  $S \rightarrow T_0$  mixing in radical pairs is only applicable at high magnetic field. At zero field, the four radical pair states are very nearly degenerate in energy. The exchange and dipolar interactions between the two spins of the radical pair are undoubtedly much weaker than in the molecular triplet due to the greater average distance between the unpaired electrons. Mixing of the singlet and all three triplet radical pair states is now possible.<sup>35</sup> Consequently, the amount of triplet character the radical pair develops will be greater at zero field than at high field, and the decay to the molecular triplet will preserve this difference. The exact magnitude of the magnetic field modulation of the triplet yield depends on a number of factors, including the frequency of  $S \rightarrow T$  mixing, the intrinsic rate constants for decay of the singlet and triplet phased radical pairs to the molecular singlets and triplets, and the magnitude of the exchange parameter  $J$ . The modulation factor  $\Phi_H/\Phi_0$ , where  $\Phi_H$  is the triplet yield at high field and  $\Phi_0$  is the yield at zero field, can range from 1 (no effect) to  $1/3$ .

Considerable experimental and theoretical effort has resulted in the conclusions that  $k_t$ , the rate of triplet radical pair reaction, is greater than  $k_s$ , the rate of singlet radical pair reaction, and that exchange coupling between the two electrons is very small ( $\leq 10$  G).<sup>20,36</sup> These calculations are hampered by the fact that the experimental values for the field-induced triplet yield modulation and the triplet yield in zero field are variable from one preparation to another. The variability presumably reflects small structural differences in the reaction center samples and points out the extraordinary sensitivity of this measurement to the details of the magnetic and electronic environment of the reaction center.

(30) Shuvalov, V. A.; Parson, W. W. *Proc. Natl. Acad. Sci. U.S.A.*, 1981, 78, 957–961.

(31) Early uncertainty about whether the optically and ESR observed triplet states correspond to the same species has been resolved. See ref 19–21 for discussion.

(32) Schenck, C.; Blankenship, R. E.; Parson, W. W. *Biochim. Biophys. Acta* (submitted).

(33) Hoff, A. J.; Rademaker, H.; van Grondelle, R.; Duysens, L. M. N. *Biochim. Biophys. Acta* 1977, 460, 547–554.

(34) Michel-Beyerle, M. E.; Scheer, H.; Seidlitz, H.; Tempus, D.; Haberkorn, R. *FEBS Lett.* 1979, 100, 9–12.

(35) One might naively expect the three zero-field populating probabilities to be equal. The measured values (26) deviate slightly from this expectation. Perhaps this reflects some small dipolar splitting in the radical pair.

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One particularly difficult problem in the analysis of field dependence data concerns the source of the driving force for the  $S \rightarrow T$  mixing. Equation 1 indicates that either the hyperfine or electron Zeeman effects are the likely candidates. The Zeeman effect is ruled out as the major contributor by the fact that under some conditions the triplet yield can be quite high even at zero applied field. The shape of the field dependence curve is quite suggestive of the hyperfine effect, and most analyses have assumed this was the sole source of  $S \rightarrow T$  mixing in the radical pair.<sup>36</sup> This assumption is weakened by the observation that deuterated reaction centers, in which all proton magnetic moments are replaced by the 6.5-fold smaller deuterium magnetic moment, have virtually the same triplet yield as normal protonated reaction centers.<sup>32,37,38</sup> However, in very high magnetic fields the triplet yield begins to rise again due to the Zeeman effect.<sup>39</sup> This result argues that the rate of  $S \rightarrow T$  mixing at zero field is slow relative to the very high field rate. Clearly more work is needed to settle this question.

Regardless of the precise mechanism of  $S \rightarrow T$  mixing in the radical pair, the direction of the effect of a modest magnetic field on the triplet yield clearly implicates a formation pathway from an initially *singlet* radical pair. A triplet radical pair origin predicts a field-induced *increase* while the intramolecular mechanism of triplet formation predicts no effect of a field on the total yield of triplet (Figure 3b). Recent experiments on a field-induced fluorescence yield increase observed in blocked reaction centers and whole cells further strengthen this conclusion.<sup>40</sup>

The  $S \rightarrow T_0$  mixing process is very sensitive to the magnitude, but not the sign, of the exchange parameter  $J$ .<sup>5</sup> The most effective mixing occurs when  $J$  is zero. Analysis of the field dependence of the triplet yield can in principle provide a value for  $J$ , and preliminary evidence indicates that it is quite small. There are, however, other constraints on the magnitude of  $J$ , indicating that it is significantly greater than zero. The rates of various electron-transfer reactions within the reaction center complex are related to  $J$ .

### CIDEP in Bacterial Systems

Spin-polarized doublet ESR spectra have also been observed by Hoff et al.<sup>20,41</sup> in some preparations from photosynthetic bacteria. These CIDEP (chemically induced dynamic electron polarization) signals are observed only when photochemistry is blocked and the nonheme Fe atom of the reaction center is removed. The spectra are entirely compatible with the radical pair mechanism, in agreement with the triplet ESR, magnetic field effect, and fluorescence data. In addition they afford an estimate of both the magnitude (1–5  $G^{41}$ ) and sign (positive<sup>42</sup>) of  $J$ , the exchange coupling between the two halves of the radical pair.

(37) Blankenship, R. E.; Parson, W. W. *Biochim. Biophys. J.* 1979, 25, 205a.

(38) Rademaker, H.; Hoff, A. J.; van Grondelle, R.; Duysens, L. N. M. *Biochim. Biophys. Acta* 1980, 592, 240–257.

(39) Chidsey, C. E. D.; Roeloffs, M. G.; Boxer, S. G. *Chem. Phys. Lett.* 1980, 74, 113–118.

(40) (a) Voznyak, V. M.; Elfimov, E. I.; Sukovatitzina, V. K. *Biochim. Biophys. Acta* 1980, 592, 235–239. (b) Rademaker, H.; Hoff, A. J.; Duysens, L. N. M. *Biochim. Biophys. Acta* 1979, 546, 248–255.

(41) (a) Hoff, A. J.; Gast, P.; Romijn, J. C. *FEBS Lett.* 1977, 73, 185–190. (b) Hoff, A. J.; Gast, P. *J. Phys. Chem.* 1979, 83, 3355–3358.

(42) An analysis of the data of Hoff and Gast using the suggestion of Pedersen [Pedersen, J. P. *FEBS Lett.* 1979, 97, 305–310] permits the conclusion that  $J$  is positive.

The magnetic requirements for production of polarized doublets are different from the requirements for production of the  $T_0$  spin-polarized triplets and the field effect on triplet and fluorescence yields. The latter effects require only  $S \rightarrow T_0$  mixing, which alone is insufficient to cause doublet polarization, as examination of Figure 1 will reveal. Doublet polarization requires both  $S \rightarrow T_0$  mixing and an exchange interaction between the two radicals. The process is more difficult to visualize than  $S \rightarrow T_0$  mixing alone, although another vector diagram aids in this regard.<sup>43</sup>

The  $S \rightarrow T_0$  mixing and exchange can either occur simultaneously on a single radical encounter or sequentially on two encounters. The doublet polarization  $\rho$  (excess of upper spin state) which an initially singlet radical pair can generate in a single encounter is<sup>5,6a,7</sup>

$$\rho = \frac{2\omega_{ab}J}{\omega^2} \sin^2(\omega t) \quad (2)$$

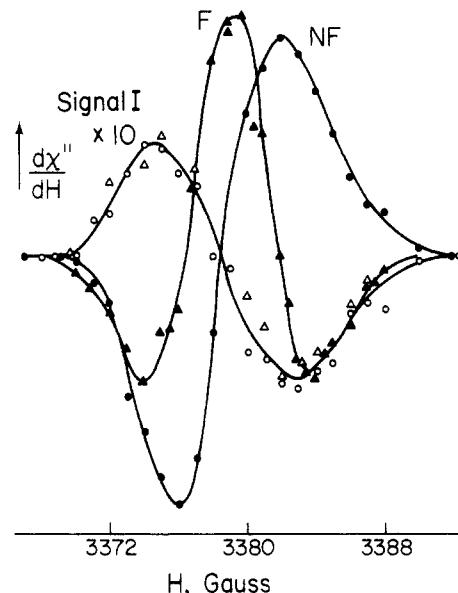
where  $2J = E_s - E_t$  is the singlet-triplet splitting and  $\omega = (\omega_{ab}^2 + J^2)^{1/2}$ . This term is only significant when  $\omega_{ab} \approx J$ ; in diffusing systems due to the rapid Brownian motion of the molecules ( $\approx 10^{-12}$  s) this condition is not satisfied for a long enough time for significant polarization to develop in this manner. Multiple encounters between freely diffusing radicals can also generate doublet polarization.<sup>5,6a</sup> In most systems this mechanism accounts for all the observed polarization, and the (incorrect) feeling has arisen that radical-pair CIDEP can only arise in a diffusing system. The fixed geometry of the photosynthetic reaction center effectively precludes free diffusion and multiple encounters of the radicals but is ideal for development of single-encounter polarization via eq 2, if the radical separation is such that  $\omega_{ab} \approx J$ .

Another CIDEP mechanism is operative in some systems, especially photolytic ones, in which intramolecular intersystem crossing to a triplet-state precedes radical formation. This "triplet" mechanism has no diffusion requirement, relying instead on differential rates of intersystem crossing.<sup>5</sup> The two mechanisms make very different predictions of the polarizations of the product radicals. The radical pair mechanism predicts no net polarization; most commonly both radicals show mixed emission-absorption. If the  $g$  factors of the two radicals are very different, one will be observed in emission and the other in enhanced absorption. The triplet mechanism predicts that both radicals will have the same sign of polarization; most commonly both are emissive. If spectra of both radicals are not observed, it may be difficult to decide which mechanism is operative.

### CIMP in Plant Systems

Oxygen-evolving photosynthetic organisms also exhibit a variety of CIMP effects. Interestingly, the initial manifestations of CIMP in plant systems were very different than in bacterial systems and chiefly consisted of spin-polarized doublet radicals observed under physiological conditions rather than under conditions of blocked photochemistry. Oxygen-evolving organisms have two photochemical systems operating in series, called photosystem I and photosystem II, rather than the single cyclic bacterial photosystem. The electron

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**Figure 5.** CIDEP spectra of flowing ( $\Delta$ ,  $\blacktriangle$ ) and nonflowing ( $\circ$ ,  $\bullet$ ) chloroplasts. Filled symbols signify the amplitude of the 2- $\mu$ s transient ESR signal, while open symbols represent the amplitude of the relaxed P700<sup>+</sup> ESR signal. (Reprinted with permission from ref 45. Copyright 1978, The Rockefeller University Press.)

donor of photosystem I is called P700, after the wavelength of maximum photobleaching.<sup>1</sup>

Early CIDEP observations in green plants were interpreted in terms of the triplet mechanism, partly based on the mistaken assumption that diffusion is required for the operation of the radical pair mechanism.<sup>44</sup> More recent data are interpreted more easily by using the radical pair mechanism, so the triplet mechanism has been abandoned. The most important recent finding is that the spin-polarized ESR spectra are dependent on the orientation of the chloroplasts in the magnetic field.<sup>45</sup> Nonrandom orientation is achieved by flowing the ellipsoidal chloroplasts through the thin ESR flat cell, where they are partially aligned by shear forces. Point-by-point ESR spectra obtained under both flowing and nonflowing conditions are shown in Figure 5. CIDEP spectra observed in plant systems to date all appear to be largely originating in photosystem I.<sup>44-46</sup>

To understand the origin of the flow dependence of the CIDEP spectra, recall that in the radical pair theory the line shape of the polarized ESR spectrum depends on the interplay of the  $\Delta g$  and the hyperfine terms in the spin Hamiltonian. If  $\Delta g$  is small, the system shows mixed emission-absorption behavior, whereas if  $\Delta g$  is large, pure emission or absorption is observed.<sup>5</sup> These two extremes are just the behavior observed in the flow and no-flow spectra of Figure 5. It is possible for the  $g$  factor of one component of the radical pair to depend on membrane orientation, if the species has both an appreciable  $g$  factor anisotropy and is preferentially oriented in the membrane. A number of acceptor species which have been proposed as candidates for early electron acceptors in photosystem I have consid-

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erable  $g$  anisotropy.<sup>1</sup> However, only one, called  $X^-$ , also has the proper membrane orientation such that the alignment produced by flow is sufficient to account for the spectral features observed in Figure 5.<sup>47,48</sup> In the nonflowing sample all possible membrane orientations are equally likely, and the average  $g$  factor is 1.92. The flowing sample has an average  $g$  factor of about 2.0, very close to that of  $P700^+$ , so mixed emission-absorption is observed. The nonflowing sample has a large average  $\Delta g$ , and a purely emissive ESR signal is observed.

While this simple mechanism accounts for the gross features of the CIDEP spectra of photosystem I, a detailed calculation reveals that it is unable to simulate the observed spectra quantitatively. Friesner et al.<sup>7</sup> extended the model of Dismukes et al.<sup>45</sup> by proposing that  $X^-$  is preceded by an earlier transient acceptor with an isotropic  $g$  factor near  $g = 2.00$ . Independent optical<sup>49</sup> and ESR<sup>50</sup> experiments have confirmed the existence of this early acceptor and tentatively identified it as a chlorophyll anion radical.

The CIDEP studies discussed above do not unambiguously determine the multiplicity of the initial radical pair. Application of the sign rules for CIDEP<sup>51</sup> indicates that either the initial radical pair is a singlet and  $J$  is positive or the initial radical pair is a triplet and  $J$  is negative. Recently  $T_0$  spin-polarized triplets<sup>52</sup> and a magnetic field induced increase in the fluorescence yield of photosystem I<sup>53</sup> have been observed in photosystem I samples in which photochemistry has been blocked. This ensures that the initial radical pair is a singlet and that photochemistry occurs from the excited singlet state, similar to the situation in photosynthetic bacteria. The sign of  $J$  must then be positive. Since triplet formation via the radical pair mechanism is observed at very low temperatures, the energy of the radical pair states must lie above that of the triplet. In this situation Adrian<sup>51</sup> predicts that  $J$  will be negative; this discrepancy has not yet been resolved. The magnitude of  $J$  for the  $P700^+ A_1^-$  radical pair has been estimated to be 75 G<sup>7</sup> and 30–50 G.<sup>53</sup>

Green plant photosystem II is somewhat less studied. However, the extraordinary similarity between the electron acceptor side of photosystem II and the single bacterial photosystem suggests that it too will proceed from the excited singlet. Magnetic field dependence of fluorescence from photosystem II<sup>40b,54</sup> and  $T_0$  spin-polarized triplets<sup>52b</sup> also supports this conclusion.

### Rates of Electron Transfer in Photosynthetic Systems

Earlier, it was asserted that the kinetic steering that occurs in the electron-transfer reactions of the reaction

center complex is a consequence of the details of the reaction center structure. We will now briefly explore this subject, emphasizing the contribution CIMP studies can make to an understanding of this process.

The rate of an electron-transfer reaction depends on the values of a number of parameters, including the extent of orbital interaction of donor and acceptor species, the free energy change of the reaction, and the vibrational energy level structure of the products, reactants, and surroundings.<sup>3,55</sup> Electron-transfer processes, similar to the absorption and emission of electromagnetic energy, are described by Fermi's golden rule. When the Born-Oppenheimer approximation for the separability of electronic and vibrational wave functions is employed, the observed rate constant is<sup>55,56</sup>

$$k = \frac{2\pi}{\hbar} |T_{ab}|^2 \sum_{i,f} P_i |\langle \chi_i | \chi_f \rangle|^2 \delta(E_i - E_f) \quad (3)$$

In eq 3, the  $|T_{ab}|^2$  matrix element represents the electronic interaction of the initial and final states of the system,  $P_i$  is the probability of being in initial state  $i$ ,  $\chi_i$  and  $\chi_f$  are nuclear wave functions, and the Dirac  $\delta$  function ensures conservation of energy.  $T_{ab}$  depends in a sensitive way on the relative distance and orientation of the reaction partners. CIMP studies can give us some indication of the magnitude of this term, as discussed below. The Franck-Condon factor,  $|\langle \chi_i | \chi_f \rangle|^2$ , represents the overlap of nuclear wave functions of the initial and final states, and it is this term that gives one information about the relative vibrational energy level structure of the system. This term does not depend on the orientation or distance of species. A large number of parameters are needed to describe the system fully, and not all of them are easily measurable. Consequently uncertainty exists as to the values of the other parameters, which are often obtained by curve-fitting of the temperature dependence of the rate constant. Clearly, an experimentally measurable quantity which allows independent determination of any of the heretofore freely adjustable parameters used in the theoretical description of the electron-transport process will be of considerable utility. The exchange parameter  $J$  measured in a CIMP experiment is related to  $T_{ab}$ , the orbital overlap parameter of eq 3.<sup>56-57</sup> The exchange parameter is found to vary exponentially with distance, as  $|T_{ab}|^2$  is expected to do.<sup>58</sup> It thus seems that  $J$  is directly proportional to  $|T_{ab}|^2$ , although the constant of proportionality is still not well defined.  $J$  is not expected to be dependent on the Franck-Condon factor of eq 3, so measurement of  $J$  affords an estimate of  $|T_{ab}|^2$  apart from the complexities of the vibrational energy level structure of the system. More theoretical

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work is needed to clarify the relation between  $|T_{ab}|^2$  and  $J$ .

Haberkorn et al.<sup>56</sup> and Hoff<sup>20,41</sup> have used the very low value of  $J$  ( $\leq 10$  G) which results from an analysis of the magnetic field dependence of the triplet yield in bacterial systems to suggest the existence of an earlier ion pair state which precedes the radical pair. An earlier state has also been suggested based on picosecond optical studies,<sup>59</sup> so this seems a likely possibility. The recombination reaction may also be slowed by an unfavorable Franck-Condon factor, although it is unclear whether the rates of very exothermic reactions are slowed by this process.<sup>60</sup>

### Conclusions and a Look to the Future

A major conclusion that has emerged from CIMP studies of photosynthesis is that the initial electron transfer unquestionably occurs from an excited singlet state. In addition, new states of the system have been predicted and subsequently found, and a great deal of optical data have been more easily interpreted. The next stage of research using CIMP in photosynthetic systems will be more quantitative. A complete de-

scription of the structure of the reaction center, including the distances and orientations of various components and the reasons why that structure gives rise to the observed properties of the system, is still some years away. CIMP along with a variety of other experimental and theoretical approaches will contribute in this regard.

Clearly CIMP can tell us a great deal about the mechanism of energy storage in photosynthesis. But it also seems that photosynthetic systems can tell us something about CIMP. The requirement for diffusion in the radical-pair mechanism is clearly not an absolute one, if the molecules are properly positioned relative to one another. Anisotropic CIMP effects have so far only been observed in photosynthetic systems. The photosynthetic system also is an ideal choice for study of electron-transfer reactions. Considerable application of CIMP studies in this regard is to be expected in the future.

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