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ELECTRON SPIN POLARIZATION
FROM THE PHOTOSYNTHETIC LIGHT
REACTION: PHOTOSYSTEM I*

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I. Introduction

This review summarizes our experimental and theoretical studies of electron spin polarization associated with the Photosystem I light reaction of green plants and algae. Populations of the electron spin sublevels of the oxidized reaction-center electron donor complex, which are not in thermal equilibrium with their surroundings, can be observed promptly after formation by photooxidation. This polarization serves as a rich source of information about the magnitude of the coupling between neighboring electron transport cofactors, their organization within the photosynthetic membranes, and the kinetics of electron transfer.

Following a brief account of the current knowledge of the sequence of electron transfer reactions in Photosystem I, the unique contributions provided by studies of electron spin polarization are presented in detail.

A. Electron Acceptors of Photosystem I

In the reaction centers of photosynthetic membranes the excitation produced by light absorption leads to a rapid transfer of electrons in an oxidation-reduction reaction. The electron donor and acceptor molecules are incorporated into a well-defined complex fixed in the membranes. For Photosystem I of higher plants and algae the electron transfer results in optical absorption changes associated with the species P700, the electron donor, and P430, an early electron acceptor.¹ Characteristic EPR signals associated with the unpaired electrons present in the donor and acceptor species are also observed.²

The photoinduced changes in P700, easily monitored by optical absorption or EPR at room temperature under physiological conditions, have been interpreted in terms of the one-electron oxidation of a pair of chlorophyll-a molecules located in the reaction center.³ The reduced acceptors are less readily seen, and their identity and sequence are the subject of active investigation. The optical changes designated P430 are reported only for detergent-treated membrane fragments,¹ and these correlate with a low temperature EPR signal characteristic of an iron-sulfur center⁴ that has been designated center A.⁵ Upon illumination of membrane fragments enriched in Photosystem I⁶ or intact chloroplasts⁷ which have center A reduced, another low temperature EPR signal is observed characteristic of an iron-sulfur center that has been designated center B. Iron sulfur centers A and B (also known as ferredoxins A and B) appear to be the 4Fe4S centers⁸ of a single 8Fe8S protein⁷ which we designate as Fd(A, B).

The proposal that centers A or B act as the primary electron acceptor has been challenged in recent studies. McIntosh and Bolton,⁹

Evans et al.^{10,11}, and Ke et al.^{12,13} report evidence of an unidentified species, designated X, observed in photosystem I membrane fragments which exhibits properties characteristic of the primary acceptor when Fd(A, B) has been reduced chemically. A light-induced EPR signal observed for X⁻ forms reversibly in parallel with P700⁺. Dismukes and Sauer⁷ have observed X⁻ by EPR in intact chloroplasts and without prior chemical reduction, thus supporting its involvement in the native membranes. These authors suggested that X is probably another iron-sulfur center. This is supported by recent analysis of the stoichiometry of membrane fragments enriched in photosystem I, which indicates 12 Fe atoms and 12 S atoms per reaction center.¹⁴ This agrees with an assignment in which X is a 4Fe4S center.

Sauer et al.¹⁵ recently observed the kinetics of re-reduction of P700⁺ following flash excitation in reduced Photosystem I membrane fragments using transient absorption spectrophotometry. This serves to monitor the state of the electron acceptors and determine something about their sequence. They found evidence for two acceptors preceding P430, and that two electrons may be transferred at the redox level of P430. The following sequence was proposed.

The species A₁ and A₂ were not seen directly by optical methods. Presumably X must be either A₁ or A₂. Golbeck et al.¹⁶ have performed similar experiments in which the iron-sulfur centers are denatured but P700 is still active and find evidence that A₂ is an iron-sulfur center. These results are in agreement with time-resolved EPR studies reported by Dismukes et al.¹⁷ and Friesner et al.¹⁸ which provide information on the identities of A₁ and A₂ from their magnetic properties.

B. Historical Background

Time resolved EPR spectroscopy has been the only technique used to date to investigate the early Photosystem I electron acceptors in unfractionated native membranes in which transfer is not altered by chemical reduction. As such, these experiments provide the clearest observation of the in vivo events. Blankenship et al.¹⁹ were the first to observe non-Boltzman occupation of the electron spin sublevels of a transient radical intermediate formed by flash illumination. Dismukes et al.¹⁷ showed that this electron spin polarization was due to $P700^+$, and that it displayed a significant dependence on the orientation of the radicals relative to the direction of the EPR magnetic field. Friesner et al. interpreted these results within the framework of the radical pair mechanism, which was extended to include systems of membrane-bound radicals where g anisotropy is important.¹⁸ These results indicate that A_1^- possesses isotropic magnetic properties and that A_2 is very likely the acceptor X. A likely candidate for A_1 is a chlorophyll-a molecule, based on magnetic properties and what is known about the chemical composition of Photosystem I membrane fragments.

II. Experimental Techniques

The technique of time-resolved EPR spectroscopy for the measurement of spin polarization is well-established.²⁰ Signal detection is the same as in conventional steady-state methods except that the response time is faster.

A standard practice for signal detection is to employ modulation of the static magnetic field and synchronous detection at the modulation frequency. This has the advantage that it rejects field independent noise such as flash artifacts from light sources. The instrumental response time is restricted by the modulation frequency (ω_m) which, in turn, restricts the maximum resolution because of the appearance of modulation sidebands on the signal. We have employed 1 Mhz field modulation in the work presented here.²¹ The observed response time (0-90% of maximum) is 2 μ s, and resolution is restricted to linewidths greater than $\omega_m/\gamma = 0.35$ G.

A. Modulation Effects

We will be concerned with the lineshape of the absorption signal in the discussion of the spin polarization of $P700^+$. It is therefore necessary to ascertain the experimental features which contribute to lineshape.

The theory of the effects of modulation amplitude and frequency on steady-state EPR lineshapes is well known²² and no derivation will be presented. The modulating magnetic field H_m at frequency ω_m does not appreciably influence the observed resonance lines when²³

$$\omega_m/\gamma \ll 1/\gamma T_1 \leq \Delta H$$

$$\omega_m \ll \gamma H_1$$

$$H_m \ll \Delta H$$

where ΔH is the linewidth of the absorption derivative signal and T_1 is the longitudinal relaxation time. We measured the magnitude of the H_1 field in our TE_{102} cavity and found $H_1 = (0.74 \text{ G watt}^{1/2})\sqrt{P}$ where P is the incident microwave power (in watts) when the cavity impedance is matched to the bridge. Typically, $P = .025 \text{ W}$ in our experiments so that

$H_1 = 0.12 \text{ G}$ is in the region of linear dependence of signal amplitude on $\sqrt{P}$ at room temperature. The modulation amplitude H_m was typically 3.8 G or 1.9 G which is below the

linewidth of $P700^+$ $\Delta H \sim 7.5 \text{ G}$. T_1 is less than $2 \mu\text{s}$ based on the observation that the decay of spin polarization occurs faster than can be resolved by our detection system. The above conditions are fulfilled so that no appreciable modulation effects are expected. In the fast modulation region $\omega_m T_1 > 1$ at power levels above saturation the signal lineshape is expected to depend on ω_m , H_m and the lock-in detector phase.

We have not observed this behavior in samples examined at room temperature. However, at low temperatures $\leq 100 \text{ K}$ where relaxation times

are considerably longer and power saturation is difficult to avoid, lineshape distortion is clearly evident. These conditions lead to rapid passage effects, resulting in the inability to separate absorption and dispersion lineshapes. Rapid passage effects may have contributed to the unusual signals observed at low temperature by other workers.^{24,25} By using a paramagnetic standard with short relaxation times, such as divalent manganese solutions, it is simple to obtain the correct detector phase for isolation of the absorption signal. With the same detector phase setting, photosynthetic samples at room temperature show a normal absorption lineshape for P700⁺, and no signal is observed when the out of phase component is sought, as expected.²²

B. Generation and Detection of Transient Signals

Light excitation directly into the EPR microwave cavity using short duration, pulsed light sources is the best method for the observation of large transient populations of oxidized P700. We used both a ruby laser with 50 ns pulse width $\lambda = 694$ nm and a xenon flash lamp with 10 μ s pulse width. Light energies of 25 mJ per pulse at 694 nm typically produced radical concentrations near the saturation limit.

At the usual radical concentration of $1-2 \times 10^{-5}$ M, signal averaging is a necessity. Typically, the signals generated by 200-700 laser flashes or lamp flashes were added to obtain the kinetic behavior at a single magnetic field position. The field dependence was then constructed by stepping the field and repeating the measurements.

C. Orientation of Photosynthetic Membranes

The chemical species which carry out the light reactions in photosynthetic organisms are located in highly developed lipid membranes known as thylakoids. In plants, such as spinach, these are found in chloroplasts, subcellular organelles which can be isolated readily from the plant. By providing soluble ferredoxin and NADP as terminal electron acceptors to compensate for that which is washed away during isolation of the chloroplasts, nearly complete retention of the in vivo electron transport chain can be obtained.

The thylakoid membranes of chloroplasts are highly ordered. They occur as membrane stacks and sheets that are nearly parallel throughout the chloroplast. Spinach chloroplasts are shaped like oblate ellipsoids with typical dimensions of $2.5 \times 5 \times 5$ microns. The orientation of the normal to the thylakoid membranes is predominantly along the short axis of the chloroplast.²⁶ Therefore, alignment of the chloroplast is sufficient to produce alignment of the membranes. To orient chloroplasts we have used flow gradients and strong magnetic fields. Both methods are known to result in orientation, as has been deduced from optical absorption dichroism^{27,28} or fluorescence polarization.²⁹ We induced orientation by running the suspension through a flat quartz sample cell of cross-section $0.025 \text{ cm} \times 1.0 \text{ cm}$. Flow rates as low as 0.6 ml/min produced saturation of orientation. The geometry of the sample cell relative to the spectrometer's magnetic field axis is fixed by the design of the EPR cavity, as indicated in Fig. 1a. This configuration results in the alignment of the short axis of the chloroplast along the velocity gradient, perpendicular to the magnetic field of the spectrometer. This aligns the normal to the

thylakoid membranes predominantly perpendicular to the EPR magnetic field.

Spectra of nonflowing chloroplasts were recorded with a fresh chloroplast suspension for each magnetic field position. In the absence of flow, the chloroplasts align partially (10-15%) in the 3.4 kG magnetic field used in the EPR spectrometer. This is caused by the anisotropic diamagnetic susceptibility of the chloroplast membranes and results in the short axis of the chloroplast aligning parallel to the field. Examination of the chloroplasts with an optical microscope at higher field strengths confirms this orientation. Magnetic field orientation saturates above 10 kG.

The EPR signal observed in chloroplasts in the dark and designated Signal II can be used as a marker of the extent of orientation, because it displays partially resolved anisotropic hyperfine splitting and probably g tensor anisotropy that are sensitive to the orientation with respect to the EPR magnetic field. Signal II has been attributed to a membrane-bound semiquinone radical, most probably plastosemiquinone.^{30,31} We find that orientation of chloroplasts by a velocity gradient yields Signal II spectra with features identical to those of chloroplasts oriented and frozen in intense magnetic fields. The spectra are shown in Fig. 2, and the corresponding orientations of the thylakoid membranes induced by flow or magnetic fields are shown in Figs. 1b and 1c.

III. Measurement of Spin Polarization

In a typical experiment we monitor the first derivative of the EPR absorption at a fixed field during a laser pulse. This is shown in the upper trace of Fig. 3 for broken spinach chloroplasts at a field position on the high-field side of the $P700^+$ spectrum. A large transient change in intensity occurs during the laser pulse, followed by a slower decaying signal. Both signals are field-dependent, indicating that they are not due to flash artifacts. Flow orientation of the photosynthetic membranes causes the brief pulse to invert for some field positions, as shown in the lower trace of Fig. 3. The 5- μ s width of the short intense signal is limited by the spectrometer response time. The slow, smaller amplitude change is independent of sample flow at all field positions. The decay kinetics of the slow change is the same at all field positions and is the same as found for $P700^+$ with both optical and EPR detection.

The dependence of both signals on magnetic field strength for flow-oriented and for nonoriented samples is plotted in Fig. 4. The amplitude of the rapid signal is taken as the difference between the peak amplitude and the amplitude after the pulse (see Fig. 3). The phases of the derivative signals indicate that the rapid component appears in emission, while the slower component is in absorption. The g value (2.0025), linewidth (7.5 G, ΔH_{pp}), and isotropic character of the absorption signal are identical to those of spin-relaxed $P700^+$. Preferential excitation of Photosystem I occurs at the 694 nm laser wavelength. The lineshape of the emission signal is a sensitive function of the orientation of the chloroplast with respect to the magnetic field. Without flow orientation we observe

a symmetric emission signal displaying a g value identical to $P700^+$, but having a narrower line width of 5.5 G. For flow-oriented chloroplasts, an enhanced absorption peak appears on the high-field side and the polarization pattern is that typical of a hyperfine multiplet ensemble. The unresolved linewidth of $P700^+$ is due predominantly to isotropic proton hyperfine structure. The integral areas of the polarized signals for the oriented and unoriented samples are approximately equal and are 13 times greater than the integral areas under the spectrum of relaxed $P700^+$ prior to decay. This is a lower limit because the amplitude of the transient signals are clearly limited by our instrumental response time.

The polarized signal and the relaxed $P700^+$ signal are proportional to $\sqrt{P}$ below 25 mW, indicating no power saturation. The polarized spectrum is independent of modulation amplitude up to the maximum attainable value of 3.8 G. 90° rotation of the phase detector abolished all signals. This test confirms the non-dispersive nature of the signals.

Several tests have shown that the polarized signal and $P700^+$ share a common origin. Blankenship et al.¹⁹ showed that treatment of chloroplasts with DCMU, an inhibitor of electron transport, abolished the polarized signal and the relaxed $P700^+$ signal. Also, when the sample was exposed to two light flashes separated by a delay, the reappearance of the relaxed and polarized signals at the second flash required several tens of milliseconds. Likewise, the chemical oxidation of P700 by ferricyanide lead to the loss of the light-induced $P700^+$ signal and the polarized signal both by an apparent one-electron process with an identical midpoint potential of $E_m = 500-525$ mV (ref. 17).

The signals observed in Figs. 3 and 4 using laser excitation at 694 nm are also observed when excitation with a xenon flash lamp is used. Because the pulse duration of the xenon lamp is 10 μ s, the polarized signals are decreased further because of relaxation.

To determine unequivocally if the polarized signal arose from photosystem I reactions, experiments were performed on membrane fragments isolated with the detergent digitonin. This enriches the Photosystem I activity considerably. Furthermore, since these membrane fragments are far too small to orient by either flow gradients or magnetic fields, it was also possible to test for the absence of orientation effects. The results, which are given in Fig. 5, show that the polarized signal and the relaxed P700⁺ signal are both observed, and that no effects occurs upon flowing the suspension.

A further confirmation of the origin of the flow effect as an orientation phenomenon is demonstrated in Fig. 6, which shows the results obtained from the unicellular alga Chlorella pyrenoidosa. The green algae, such as Chlorella, possess a photosynthetic apparatus essentially identical to ^{that of} green plants except for the morphology of the membranes. Chlorella is a nearly spherical alga which, of course, cannot orient in a weak velocity gradient. The results of Fig. 6 confirm these expectations since no significant flow effect is found. The small effect on the polarized signal that is observed is probably due to the disruption of magnetically aligned cells which are partially oriented by the 3.4 kG EPR field. Partial orientation of Chlorella at this field strength has been observed by fluorescence polarization methods.²⁹

The integrated areas and linewidths of the polarized signals observed in the preceding experiments are summarized in Table I. A linewidth of 5.6-6 G is typically observed for the polarized signal, in comparison with 7.5-8 G for P700⁺ at thermal equilibrium. The amplitude of polarization is largest for membranes which have not been extensively disrupted during isolation.

A dependence of the lineshape on orientation of the membranes can also be observed for the polarized signal in chloroplasts which are aligned by intense magnetic fields and frozen in this ordered state prior to observation below 100 K. The kinetics of decay are then resolvable and show a clear dependence on the microwave power, indicating power saturation of the signal. These samples are prepared with iron-sulfur centers A and B reduced chemically to insure reversible photo-oxidation of P700. In samples prepared with the X center also reduced, a five-fold reduction in the amplitude of the polarized signal was measured under identical instrumental conditions. However, the lineshape is complex and does not integrate to zero as is necessary for a physically reasonable absorption signal. The lineshape depends on modulation amplitude and the signal is not lost when phase detection is shifted 90°. These observations indicate that rapid passage effects contribute to the complex lineshape at low temperatures.

IV. Origin of Spin Polarization

The results of the preceding section show clearly that the polarized signal is observed only upon photo-oxidation of P700. These observations can be explained if the polarized signal arises from either $P700^+$ or a counter radical formed by electron transfer. If it does arise from a counter radical then it cannot be associated with the iron-sulfur centers, for they are not observable by EPR at room temperature due to efficient spin relaxation.

We believe that the polarized signal is due to $P700^+$, and that the lineshape dependence on orientation of the membranes arises from a radical pair interaction with an early electron acceptor which possesses g anisotropy and is oriented in the membranes. The polarized signal changes from a total emission spectrum for non-flowing chloroplasts to one with a near symmetric pattern of emission and enhanced absorption across the $P700^+$ hyperfine linewidth for flow-oriented chloroplasts. This may occur if the g tensor of the paramagnetic acceptor is oriented such that its average value changes upon disorientation of the chloroplasts from a value close to the $P700^+$ value of 2.0025 (i.e., within the 8 G linewidth) to a g -value that is significantly smaller. Net emissive polarization occurs when g value differences predominate, while a multiplet effect occurs when the hyperfine field is the predominant polarization term. The change in sign of the hyperfine field in the center of an EPR spectrum leads to a

symmetric emission/enhanced absorption pattern of polarization. This qualitative picture has its limitations because it does not consider the magnitude of the polarization. As we shall see in Section VI, ——— to account for the similar integral intensities of the polarized signals we are led to the conclusion that $P700^+$ participates in radical pair formation/successively with two reduced acceptor molecules, designated A_1^- and A_2^- , which form by consecutive electron transfer steps

We obtain information on the identities of A_1 and A_2 from the magnetic properties deduced from quantitative simulation of the polarized spectra.

The triplet mechanism of spin polarization may be discarded as a plausible alternative to the mechanism proposed here because it does not predict a hyperfine dependent polarization pattern.

V. Orientation of Iron-Sulfur Centers

To test the hypothesis that the orientation dependence of the polarized $P700^+$ signal arises from an exchange interaction with a paramagnetic partner possessing g anisotropy and oriented in the membranes, we examined the EPR spectra at low temperatures of the iron-sulfur centers Fd(A, B) and the X center in magnetically ordered chloroplasts.⁷ The results are presented in Fig. 7 for Fd(A, B). Reduced center A, which has g values of $g_x = 1.87$, $g_y = 1.95$, and $g_z = 2.05$, is shown in the middle trace. This center shows no dependence on alignment of the chloroplasts. The spectrum of fully reduced Fd(A, B)⁻ is shown in the bottom trace. The features ascribed to center B are marked. It is found that center B is oriented with its g_x peak (1.89) preferentially in the membrane plane and its g_y peak (1.93) normal to the membrane plane. The average g value difference between $P700^+$ and center B would therefore increase in going from a randomly oriented distribution of chloroplasts to a flow-oriented distribution, which places the membrane plane parallel to the EPR field. Accordingly, center B cannot be the paramagnetic partner which causes the orientation dependence of polarized $P700^+$.

The EPR spectrum of X^- in oriented chloroplasts is given in Fig. 8. The g_x peak (1.78) is aligned preferentially perpendicular to the membrane plane, while the g_y peak (1.90) and the g_z peak (2.09) lie close to the membrane plane. Flow orientation would then lead to an average increase in g value for X^- , because the smallest principal g tensor component is normal to the plane of the membrane. This causes a decrease in the g value difference from $P700^+$, which is consistent with the orientation effect predicted for the paramagnetic partner from the spin polarization results.

VI. Radical Pair Mechanism for Membrane Bound Radicals

The radical pair mechanism offers a method for the quantitative calculation of spin polarization. We have extended this theory to include the effects of g tensor anisotropy and to incorporate modifications appropriate for a system of membrane-bound radicals. The theory is then used to simulate the lineshapes of the polarized signals and from this to determine something about the organization of the species in the membrane, the magnitude of the spin exchange interaction between radicals, and the radical pair lifetimes.¹⁸

For the case of a radical pair created by electron transfer from an excited donor molecule (D) to an unexcited acceptor (A), we can write an approximate spin Hamiltonian as

$$\begin{aligned} \mathcal{H}_{RP} = & \beta \vec{H}_0 \cdot [\hat{g}_D \cdot \vec{S}_D + \hat{g}_A \cdot \vec{S}_A] - 2J(\vec{S}_D \cdot \vec{S}_A + 1/2) \\ & + \sum_{\ell} \underline{A}_{\ell}^{(D)} \vec{I}_{\ell}^{(D)} \cdot \vec{S}_D + \sum_j \underline{A}_j^{(A)} \vec{I}_j^{(A)} \cdot \vec{S}_A \end{aligned} \quad (1)$$

where β is the Bohr magneton, $\vec{H}_0$ is the applied magnetic field, $\hat{g}_A$ and $\hat{g}_D$ are the g tensors of the acceptor and donor species, respectively, $\vec{S}_A$ and $\vec{S}_D$ are spin operators for the unpaired electrons on the donor and acceptor radicals, J is the magnitude of the isotropic exchange interaction between A^- and D^+ , $\vec{I}_{\ell}^{(m)}$ is the spin operator for the ℓ th nucleus on molecule m ($m = A, D$), and $\underline{A}_{\ell}^{(m)}$ is the isotropic hyperfine coupling constant for the ℓ th nucleus on molecule m.

The eigenstates of the Hamiltonian (1) have been determined by Adrian³² for the case where $\hat{g}_A$ and $\hat{g}_D$ are isotropic (i.e. scalars). We obtain solutions

analogous to those of Adrian, except that the spin states are quantized in the direction of the effective field

$$\vec{h}' = \vec{H}_0 \cdot (\hat{g}_A + \hat{g}_D) \quad (2)$$

The eigenstates are then linear combinations of the spin functions $|S\rangle$, $|T_{-1}\rangle$, $|T_{+1}\rangle$, and $|T_0\rangle$. We shall assume from this point onward that the radical pair is created initially from a singlet state. As shown elsewhere,¹⁸ the mixing of $|S\rangle$ with $|T_{\pm 1}\rangle$ has a negligible effect on the net polarization when the g tensor anisotropy is sufficiently small. Therefore, we adopt an S- T_0 basis set.

The solution to the time-dependent Schrödinger equation for the Hamiltonian in (1) is a linear combination of singlet and triplet states

$$\psi(t) = C_S(t) |S\rangle + C_T(t) |T_0\rangle$$

with

$$C_S(t) = C_S(0)(\cos \omega t - i J/\omega \sin \omega t) - i \frac{H_{AD}}{\omega} C_T(0) \sin \omega t \quad (3)$$

$$C_T(t) = C_T(0)(\cos \omega t + i J/\omega \sin \omega t) - i \frac{H_{AD}}{\omega} C_S(0) \sin \omega t$$

where $\omega = (H_{AD}^2 + J^2)^{1/2}$ and H_{AD} is given by

$$H_{AD} = \langle S | \mathcal{H}_{RP} | T_0 \rangle \quad (4)$$

$$= \frac{1}{2} \beta \vec{H}_0 \cdot (\hat{g}_D - \hat{g}_A) \cdot \hat{z} + \frac{1}{2} \left(\sum_{\ell} A_{\ell}^{(D)} m_{\ell z}^{(D)} - \sum_j A_j^{(A)} m_{jz}^{(A)} \right)$$

$\hat{z}$ is a unit vector in the direction of $\vec{h}'$, $m_{\ell z}^{(m)}$ is the z component of nuclear spin of the ℓ th nucleus on molecule m, and $\pm \omega$ are the energies of the stationary states of the radical pair.

The polarization of the donor radical, ρ , is obtained by following the time evolution of the spin wave function. For a time interval t during which U is constant, $\rho(t)$ is given by

$$\begin{aligned}\rho(t) &= 2 \langle \psi(t) | S_{Dz} | \psi(t) \rangle \\ &= [C_T(0)C_S^*(0) + C_T^*(0)C_S(0)] \\ &\quad \times \{ \cos^2 \omega t + [(H_{AD}^2 - J^2)/\omega^2] \sin^2 \omega t \} \\ &\quad + (iJ/\omega)[C_T(0)C_S^*(0) - C_T^*(0)C_S(0)] \sin(2\omega t) \\ &\quad + 2(JH_{AD}/\omega^2) \sin^2 \omega t [|C_S(0)|^2 - |C_T(0)|^2]\end{aligned}\tag{5}$$

where $C_T(0)$, $C_S(0)$ are the coefficients of $|T_0\rangle$ and $|S\rangle$ for the spin wave function at the beginning of the time interval of constant J ($t=0$).

To simplify the ensuing calculations, we set $|C_S(0)|^2 = 1$, $|C_T(0)|^2 = 0$, corresponding to the assumption of creation of the radical pair from an initial singlet state. The results which follow could easily be generalized by retaining the terms dependent upon $C_S(0)$ and $C_T(0)$.

The simplified expression for the polarization during a time interval t of constant J is, from eq. (5),

$$\rho(t) = (2H_{AD}J/\omega^2) \cdot \sin^2 \omega t\tag{6}$$

This expression will be larger than the thermal population difference ($\sim 10^{-3}$ at room temperature) only if H_{AD} and J are of comparable magnitude for a time interval $\sim \omega^{-1}$.

We consider here a system in which the radical species are bound to a membrane at fixed sites. A radical pair is produced by transfer of an electron from a donor molecule (D) to an initial acceptor (A_1). The

electron is then transferred to successive acceptors in a fixed sequence.

We shall assume that all of the electron transfers are irreversible. This assumption is not necessary, but it simplifies the calculations considerably. Then, transfer away of an electron is analogous to diffusion. However, there can be no "return" of the radical pair, and the development of polarization has an origin distinct from that of diffusive systems.

The development of spin polarization on D^+ is a consequence of the time evolution of the coupled spin wave functions of the electrons on D^+ and A_n^- . This process will change the polarization with time as long as there is a sufficiently large exchange coupling, J_n , between D^+ and A_n^- . Therefore, we must consider the interaction of all radical pairs formed by successive electron transfer in which J_n is appreciable.

We will assume that J_n is zero for $n \geq 3$, because $A_3 \dots A_n$ are ~~too distant~~ too distant from D^+ to have a significant exchange coupling. Then there are two reasonable models for the development of polarization. The one-pair model assumes that J_2 is also negligible, and that only the interaction within $D^+ A_1^-$ need be considered. The two-pair model assumes that both J_1 and J_2 are significant, and that the interaction between D^+ and A_2^- must be included in a calculation of the spin polarization.

A. One Pair Model

An acceptor radical A_n^- is characterized by a lifetime, τ_n , which determines the duration of the existence of the radical pair $D^+A_n^-$. (This is in fact the case in Photosystem I, where $P700^+$ has a life greater than 30 μs which is much longer than the lifetimes of either A_1^- or A_2^- .) The probability that the radical pair will exist for time t is given by $\left(\frac{dt}{\tau_n}\right) e^{-t/\tau_n}$. The time-averaged polarization for the one site model is then

$$\rho(\tau_1) = 4H_1J_1\tau_1^2/(1 + 4\omega_1^2\tau_1^2) \quad (7)$$

where H_1 is the off-diagonal matrix element H_{AD} for the radical pair $D^+A_1^-$, and $\omega_1 = (H_1^2 + J_1^2)^{1/2}$.

B. Two Pair Model

For this model we need to calculate the net polarization on D^+ after the electron leaves A_2 . The polarization after the radical pair $D^+A_2^-$ has existed for time t_2 can be found by using the coefficients $C_S(t_1)$, $C_T(t_1)$ from eq. (3) as the initial conditions in eq. (5)

Following time averaging over t_1 and t_2 , we obtain

$$\begin{aligned} \rho(t_1, t_2) = & \frac{4H_1 J_1 \tau_1^2}{1 + 4\omega_1^2 \tau_1^2} \cdot \left(1 - \frac{4J_2^2 \tau_2^2}{1 + 4\omega_2^2 \tau_2^2} \right) \\ & + \frac{4J_2 H_1 \tau_1 \tau_2}{1 + 4\omega_1^2 \tau_1^2} \cdot \left(\frac{1}{1 + 4\omega_2^2 \tau_2^2} \right) \\ & + \frac{4H_2 J_2 \tau_2^2}{1 + 4\omega_2^2 \tau_2^2} \cdot \left(1 - \frac{4H_1^2 \tau_1^2}{1 + 4\omega_1^2 \tau_1^2} \right) \end{aligned} \quad (8)$$

C. Orientation Effects Due to g Tensor Anisotropy

Because of the dependence of the matrix elements H_n on the orientation of the radicals in the applied magnetic field $\vec{H}_0$ the polarization is orientation dependent. We shall restrict ourselves to a situation where only one radical involved in the development of spin polarization on D^+ possesses g anisotropy; the coordinate system defining the orientation is then chosen to be the principal axis system of the anisotropic species. $\vec{H}_0$ is specified by a magnitude, $|\vec{H}|$, and the spherical polar angles θ and ϕ .

The polarization of a hyperfine line i of D^+ is an ensemble average over all possible orientations of the membrane-bound radical system with respect to $\vec{H}_0$;

$$\overline{\rho_i} = \frac{2}{\pi} \int_0^{\pi/2} \int_0^{\pi/2} \rho_i(\theta, \phi) P(\theta, \phi) d\theta d\phi \quad (9)$$

where $P(\theta, \phi)$ is the probability that the radicals possess orientation (θ, ϕ) relative to $\vec{H}_0$, and $\rho_i(\theta, \phi)$ is the spin density developed on D^+ in hyperfine state i from either eq. (7) or eq. (8), with (see ref. 18, appendix).

$$H_n(\theta, \phi) = (\alpha_i + \beta |\vec{H}|) \Delta g_n / 2 \quad (10)$$

and the definitions,

$$\alpha_i = \sum_j A_j^{(D)} m_j^{(D)}$$

and is the hyperfine field of D^+ in the overall hyperfine state i;

$$\Delta g_n = [g_D - (g_n^x \sin^2 \theta \cos^2 \phi + g_n^y \sin^2 \theta \sin^2 \phi + g_n^z \cos^2 \theta)]$$

g_n^x , g_n^y and g_n^z are principal g tensor components of acceptor A_n^- , and g_D is the isotropic g value of D^+ . For the one site model, we assume that A_1 has an anisotropic g tensor; then, substitution of eqs. (7) and (10) into (9) yields, with suitable rearrangement,

$$\bar{\rho}_i(\text{one site}) = 4\tau_1^2 J_1 (U_1 + \frac{\alpha_1}{2} V_1) \quad (11)$$

where we have defined

$$U_n = \frac{2}{\pi} \beta |\vec{H}| \int_0^{\pi/2} \int_0^{\pi/2} \frac{\Delta g_n(\theta, \phi) P(\theta, \phi) d\theta d\phi}{I_n(\theta, \phi)} \quad (12)$$

$$V_n = \frac{2}{\pi} \int_0^{\pi/2} \int_0^{\pi/2} \frac{d\theta d\phi P(\theta, \phi)}{I_n(\theta, \phi)} \quad (13)$$

$$I_n = 1 + 4\omega_n^2(\theta, \phi)\tau_n^2 \quad (14)$$

For the two site model, we anticipate the next section and assume that the g tensor of only A_2^- is anisotropic, and that $g_1 = g_D$ are both scalars. Then, noting that $H_1 = \alpha_1/2$ (since $\Delta g_1 = 0$) and that both H_1 and ω_1 are orientation-independent, we obtain

$$\begin{aligned} \bar{\rho}_i(\text{two sites}) = \frac{\alpha_1}{2} \left[\frac{4J_1\tau_1^2}{1 + 4\omega_1^2\tau_1^2} \cdot (1 - 4J_2^2\tau_2^2V_2) \right. \\ \left. + \frac{4J_2\tau_1\tau_2}{1 + 4\omega_1^2\tau_1^2} \cdot V_2 + 4\tau_2^2J_2V_2 \cdot \left(1 - \frac{4H_1^2\tau_1^2}{1 + 4\omega_1^2\tau_1^2}\right) \right] \\ + 4U_2\tau_2^2J_2 \cdot \left(1 - \frac{4H_1^2\tau_1^2}{1 + 4\omega_1^2\tau_1^2}\right) \end{aligned} \quad (15)$$

The experimental EPR intensity I_D of D^+ as a function of field position H is given by

$$I_D(H) = \sum_{\text{all hyperfine configurations of } D^+(i)} (-\bar{\rho}_i) e^{(H-H_i^0)^2/\delta^2} \quad (16)$$

where H_i^0 is the field position of the center of hyperfine line i , and δ is the half-width of the individual hyperfine lines. Note that because ρ is defined as $N_\alpha - N_\beta$, an excess population of the state higher in energy (α) leads to $\bar{\rho}_i > 0$ and a net emission of radiation.

VII. Calculated Polarization of P700⁺

The possible assignments of electron acceptors in photosystem I and the results of the previous section suggest two alternate schemes for the development of spin polarization: (1) Acceptor A₁ is the species X, and polarization develops as in the one-site model; (2) Acceptor A₁ is a small organic molecule, possibly Chl, and A₂ is X; polarization develops as in the two-site model.

We have rejected two other conceivable schemes. A one-site model with Chl as A₁ would be inappropriate because it would not account for the orientation dependence of the polarized signal.

The effect of orientation upon the development of polarization can now be determined for both the one and two-site models. The only orientation-dependent terms in eqs. (11) and (15) are the integrals U_n and V_n. We first note that U₁(one site) = U₂ (two site), and V₁(one site) = V₂ (two site), since all of these integrals involve the g tensor components, lifetime, and J value of the same anisotropic species, X⁻. We therefore drop the subscripts, and refer to these integrals as U and V, respectively.

For a random orientation (no flow, NF), P(θ, φ) = sinθ for all θ, φ, and

$$U^{NF} = \frac{2}{\pi} \int_0^{\pi/2} \int_0^{\pi/2} \frac{A_{gX}^{NF} \sin\theta d\theta d\phi}{1 + 4\tau_X^2 [J_X^2 + H_X^2(\theta, \phi)]} \quad (17)$$

where τ_X is the lifetime of X^- , J_X is the exchange interaction between $P700^+$ and X^- , $H_X = (\alpha_1/2) + \Delta g_X^{NF}$, and $\Delta g_X^{NF}(\theta, \phi) = 2.0026 - (1.78 \sin^2\theta \cos^2\phi + 1.90 \sin^2\theta \sin^2\phi + 2.09 \cos^2\theta)$.

$$V^{NF} = \frac{2}{\pi} \int_0^{\pi/2} \int_0^{\pi/2} \frac{\sin\theta \, d\theta \, d\phi}{1 + 4\tau_X^2 (J_X^2 + H_X^2)} \quad (18)$$

For the flow oriented system, (F), we set $\phi = \pi/2$ [i.e. $P(\theta, \phi) = \delta(\phi - \frac{\pi}{2})$]. Then, U and V are given by

$$U^F = \frac{2}{\pi} \int_0^{\pi/2} \frac{\Delta g_X^F(\theta) \, d\theta}{1 + 4\tau_X^2 [J_X^2 + H_X^2(\theta)]} \quad (19)$$

$$V^F = \frac{2}{\pi} \int_0^{\pi/2} \frac{d\theta}{1 + 4\tau_X^2 [J_X^2 + H_X^2(\theta)]} \quad (20)$$

where $\Delta g_X^F(\theta) = 2.0026 - (1.90 \sin^2\theta + 2.09 \cos^2\theta)$, and $H_X(\theta) = (\Delta g_X^F(\theta) + \alpha_1)/2$.

We can now evaluate the predictions for the polarized $P700^+$ lineshape in the context of the two models described above. There are three important experimental observations which a successful model must explain:

- (1) The orientation dependence of the lineshape.
- (2) The ratio of integrated signal intensities between the polarized signal and the relaxed $P700^+$ signal is approximately 13:1. Spin relaxation is not formally included in our calculations, although the data of Fig. 3 show that it does reduce the amplitude of the observed signal. Therefore, the calculated area ratios should be in excess of 13:1.
- (3) For the polarized signal, the ratio of integrated signal intensities between the unoriented and oriented samples is between 1:1 and 2:1 (this number is at present experimentally uncertain).

A. One Pair Model

We make the simplifying approximation that $|\Delta g_1| + |J_1| > |\alpha_1/2|$, since $\alpha_1/2$ for P700⁺ is $\sim 4G$. Then, $\omega_1^2 \sim [(\Delta g_1)^2 + J_1^2]$, and we can write eq. (11) as

$$\bar{\rho}_1(\text{one site}) = k_1[\alpha_1 + \Delta g_1] \quad (21)$$

where

$$k_1 = 2V\tau_1^2 J_1, \text{ and } \Delta g_1 = 2U/V$$

The Δg_1 term is mathematically isomorphic to the g value difference term in Adrian's original formulation. Both k_1 and Δg_1 are independent of α_1 .

The orientation dependence of eq. (21) is easily described. The integral U decreases by a factor of 10 to 100 upon orientation, i.e. $10 < U^{NF}/U^F < 100$. The integral V is relatively insensitive to orientation, $V^{NF}/V^F \sim 1$ for a wide range of τ_1 and J_1 . Thus, $k_1^{NF}/k_1^F \sim 1$, and $10 < \Delta g_1^{NF}/\Delta g_1^F < 100$. The absolute amplitudes of Δg_1^{NF} and k_1 are dependent upon the specific values of τ_1 and J_1 .

The one-site model correctly predicts the signal in unoriented samples to be in total emission. Δg_1^{NF} is large and positive and the net polarization of the signal is sufficiently greater than the thermal population difference to account for the 13:1 area ratio of the polarized to unpolarized signal.

However, the one-site model fails completely for the oriented signal. The integral V is always small; therefore, k_1 is always small, less than .0025. Since Δg_1 is inversely proportional to k_1 , the hyperfine term α_1 is dominated by Δg_1 even for the oriented system. Furthermore, the total polarization for the oriented system is insufficient to account for the observed area ratios. Even for the most favorable values of

τ_1 and J_1 , the one-site model predicts that the oriented signal is much smaller than the unoriented signal (a factor of 10 or more) and in total emission.

These arguments can be summarized somewhat more lucidly by reference to Fig. 9 which is a plot of eq. (7) vs. H_{AD} for several values of J . The magnetic field range spanned by the g tensor of X^- is indicated by the horizontal lines for oriented and unoriented samples. The net polarization for a particular hyperfine state is the integral of ρ over these regions with a weighting factor determined by the orientational distribution. It is then clear that $\rho > 0$ for the random orientation and emission results. α_i is very small on the scale of Fig. 9, so that net emission is predicted for all hyperfine states. The oriented sample also results in a large Δg factor; however, a more symmetric distribution of positive and negative Δg 's occurs. The net effect is a substantial reduction in the observable polarization since these produce populations of radicals with opposite polarization. We therefore conclude that the one-site model is incapable of explaining our results.

B. Two Pair Model

The polarization equation for the two-site model can be written as

$$\bar{\rho}_i(\text{two site}) = k_2(\alpha_i + \Delta g_2) \quad (22)$$

where

$$k_2 = \frac{2J_1\tau_1^2}{1 + 4\omega_1^2\tau_1^2} + V \cdot [2\tau_1^2J_2 + \frac{2\tau_1J_2\tau_2}{1 + 4\omega_1^2\tau_1^2}(1 - J_1J_2\tau_1\tau_2)]$$

$$\Delta g_2 = 2U\tau_2^2J_2/k_2$$

We have assumed that $|\alpha_i| \ll |J_1|$ and $|\alpha_i| \ll \Delta g_2$, so that ω_1, ω_2 are independent of α_i , and the term $[4H_1^2\tau_1^2/(1 + 4\omega_1^2\tau_1^2)] \ll 1$ in eq. (15) and thus has been neglected. Both k_2 and Δg_2 are then independent of α_i .

The major difference between the one and two site models is the amplitude of k . k_1 is directly proportional to the integral V , which is small for all values of τ_X and J_X . k_2 is a sum of two terms, one proportional to V and one independent of V . It is the latter term, $2J_1\tau_1^2/(1 + 4\omega_1^2\tau_1^2)$, which can have a relatively large amplitude for appropriate values of τ_1 and J_1 . This term arises from the interaction between $P700^+$ and A_1^- , and is large when Δg_1 is zero, so that $H_1 \ll J_1 \sim \omega_1$. Effectively, the interaction of $P700^+$ with A_1^- produces a substantial polarization term proportional to the hyperfine field of $P700^+$.

The experimental signals can be generated from eq. (22) when k_2 is sufficiently large (so that the polarized signals have enough amplitude relative to the relaxed signal) and when the average value of

α_i (2-3 gauss) falls between Δg_2^F and Δg_2^{NF} . Then, for the oriented system the term linear in α_i dominates, the sign of $\bar{\rho}_i$ is governed by the sign of α_i , and a mixed emissive-enhanced absorptive signal results. For the unoriented system, the sum $(\alpha_2 + \Delta g_2)$ is positive for all values of α_i , and the polarized signal is seen in total emission.

Fig. 10 displays the dependence of the calculated EPR lineshape on the value of Δg_2 in eq. (22). For $\Delta g_2 < 0.7$ G, a nearly symmetrical mixed emissive-enhanced absorptive pattern results. For $\Delta g_2 > 4$ G, the signal is essentially in total emission.

These simulations are made by using eq. (16) to calculate an EPR spectrum for an isolated, relaxed $P700^+$ radical, assuming that it is an oxidized chlorophyll dimer.³³ The relative magnitudes of the hyperfine coupling constants were obtained from NMR studies,^{34,35} the absolute magnitudes were scaled to the ENDOR result for the largest coupling constant.³⁶ This yields a linewidth of 7.5 G and a gaussian lineshape.

The narrowing of the polarized signal (see Conclusions) was introduced phenomenologically by uniformly decreasing all of the hyperfine coupling constants until the experimental linewidth was reproduced. The reduced coupling constants were used to simulate the signals for both the oriented and unoriented samples.

It is possible to set limits on k_2 and Δg_2 such that the three fitting criteria for the experimental signals described above are satisfied. The general lineshape analysis requires that $0 < \Delta g_2^F < 0.7$ G, while $\Delta g_2^{NF} > 4$ G. Also, $k_2/.001 > 3.7$, so that the net area ratio is greater than 13:1. This restricts the values of the parameters τ_1 , τ_2 , J_1 and J_2 . Table II presents several sets of parameters for which k_2 , Δg_2^F , and Δg_2^{NF} fall within these limits.

It is not possible to deduce the absolute magnitudes of any of the parameters from the data available at present. However, it is necessary that $\tau_1 \geq 350$ psec, and $J_1 < 200$ G, for k_2 to be greater than .0037. Once τ_1 and J_1 are fixed, a limited set of pairs (τ_2 , J_2) will generate acceptable values of Δg_2^F and Δg_2^{NF} .

For a comparison of theory and experiment, we chose a value of τ_1 which is comparable to the lifetime of I^- , the initial acceptor* observed in photosynthetic bacteria.^{37,38} We also chose $J_1 > J_2$, because A_1 is presumably closer to $P700^+$. The resulting values of J_1 and J_2 are reasonable ones for exchange interactions between organic molecules separated by 5 - 25 Å.³⁹ They are also within the neighborhood of exchange interactions observed between electron acceptors in photosynthetic bacteria.⁴⁰

Fig. 11 displays the theoretical and experimental EPR signals for the oriented and unoriented samples. The amplitudes of the theoretical signals, which are larger than the experimental signals, are reduced by equal amounts to account for the effects of relaxation. It is seen that excellent agreement is obtained within the limits of experimental error.

* I^- is the designation given to the "intermediary" electron acceptor, probably a bacteriopheophytin anion.

VIII. Conclusions

The two-site model successfully predicts most of the important features of the polarized signals arising from oriented and unoriented chloroplasts.

We believe that our results provide compelling evidence for the existence of two acceptors in Photosystem I preceding Fd(A, B). The orientation dependence of the polarized signal indicates that one of these is X, while the amplitudes indicate the presence of an acceptor preceding X and possessing an isotropic g value close to that of $P700^+$. The most likely candidate for A_1 at present is chlorophyll, because it is known to be present in sufficient quantity in reaction-center preparations, and Chl^- has the requisite isotropic g value. Also, the midpoint reduction potential of chlorophyll-a is -0.78 V (vs. NHE, in dimethylsulfoxide),⁴¹ which is consistent with its role as an earlier acceptor than X.⁴² In analogy with photosynthetic bacteria, pheophytin might also be considered as a suitable candidate for A_1 . However, Thornber et al have found no pheophytin in enriched photosystem I preparations.⁴³ We have no direct information concerning the chemical identity of A_1 .

The radical pair theory as developed by Adrian appears to be applicable to membrane-bound systems of radicals; the fundamental origin of spin polarization is, as in diffusive systems, S- T_0 mixing. The simple approach taken in this paper provides an adequate explanation for the experimental results to date; however, more sophisticated treatments are possible and may be needed in the future. One could, for example, allow back transfer

of an electron, or postulate more than one site for the electron in X, or investigate the possibility that at room temperature reduced or unreduced X may have appreciable unpaired spin density due to mixing in of low lying excited spin states. Development along these lines may become profitable when more data are available.

For conditions involving an initial singlet state and $\Delta g > 0$ we must have $J = 1/2(E_S - E_T) > 0$ for the polarization to be emissive. This ordering of singlet and triplet states is not the same as that usually found for neutral radicals for which covalent bonding interactions lead to a bonding singlet state and an antibonding triplet state. However, other factors may be important in determining the exchange interaction for weakly coupled ion pairs such as are created in Photosystem I. Quantum mechanical mixing in of the states^{1,3} ($D^* - A$) with the radical pair states^{1,3} ($D^+ - A^-$) could result in a lowering of the triplet radical pair state relative to the singlet.⁴⁴ For this to occur, the energy of the state $^3(D^* - A)$ must be greater than that of the radical pair. This may account for the inability to observe the triplet state of P700 at low temperature, in contrast to photosynthetic bacteria for which reaction center triplets are readily observed.⁴⁵ This is an area for future research.

The narrowing of the polarized signal relative to the relaxed $P700^+$ signal is an interesting phenomenon for which we currently do not have a satisfying explanation. The polarized signal from the unoriented sample has a peak-to-peak linewidth of 5.6 G, as compared to the value of 7.5 G measured for the relaxed $P700^+$ signal. The polarized signal from the oriented sample is the derivative of a mixed emissive-enhanced absorptive

lineshape, and therefore its linewidth cannot be compared directly with those of the other signals. However, good simulation of the oriented signal requires that the starting linewidth be narrowed to the value of 5.6 G found for the unoriented signal.

A reduction in linewidth is not predicted by the simple radical pair mechanism presented here. The polarization is either a constant across the hyperfine field (A_{H} large) or linear in α_i (A_{H} small). Neither of these polarization functions leads to a symmetrical narrowing of ^{the} hyperfine envelope of the P700^+ signal. Furthermore, one would not expect the effect to be identical for the oriented and unoriented systems.

By improving the response time of the spectrometer it should be possible to observe polarization prior to the onset of significant spin relaxation. This may reveal relaxation rates which increase with $|\alpha_i|$ and which could produce the "apparent" narrowing. Linewidth studies have shown that the spin-spin relaxation time T_2 increases with $|\alpha_i|$ in some systems.⁴⁶ The theoretically predicted time dependence of polarized EPR signals has terms which decay exponentially with T_2 (ref. 47). ENDOR studies of reaction-center chlorophyll cations might also yield this linewidth information. Other mechanisms have been offered to account for the narrowing.¹⁷ This is a rich area for future work.

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X. References

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Abbreviations

DCIP, 2,6-dichlorophenol indophenol

DCMJ, 3-(3,4-dichlorophenyl)-1,1-dimethylurea

HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid

P700, Photosystem I reaction center chlorophyll

P430, bound iron-sulfur centers A and B in photosystem I
(also Fd(A, B))

X, early electron acceptor in photosystem I

Table I: Relative Integral Areas and Linewidths of the Polarized EPR Signals

Sample	Conditions*	I_{NF}	I_F	I_{NF}/I_F	ΔH	ΔH_{NF}
Broken chloroplasts	Laser, 2 μs	13	13	1	7.5	5.6
Broken chloroplasts	Flashlamp, 10 μs	5	3	1.7	8	6.5
D-144 particles	Flashlamp, 10 μs	1.7	1.7	1	8	5.8
<u>Chlorella pyrenoidosa</u>	Flashlamp, 10 μs	2.5	2.3	1	8	6

I_{NF} and I_F are normalized integral areas for the polarized signals in non-flowing (NF) and flowing (F) samples.

----- ΔH , peak-to-peak linewidth for the relaxed $P700^+$ signal;

ΔH_{NF} , peak-to-peak linewidth for the polarized signal in nonflowing samples.

*Light source and overall instrument response time.

Table II: Calculated Values of k_2 , Δg_2^F and Δg_2^{NF} for selected values of τ_1 , τ_2 , J_1 and J_2 .

J_1 (G)	J_2 (G)	τ_1 (nsec)	τ_2 (nsec)	k_2	Δg_2^F (G)	Δg_2^{NF} (G)	$\frac{A_{pol.}^F}{A_{sig. I}}$	$\frac{A^{NF}}{A^F}$
10	75	1.0	2.1	.029	.45	5.7	106	1.84
50	10	0.35	0.35	.0046	.29	5.3	16.6	1.74
50	20	1.0	1.0	.0111	.17	5.1	40.0	1.69
100	10	0.35	0.35	.0045	.28	5.0	16.6	1.67
100	10	1.0	2.1	.067	.07	4.5	241	1.58
150	10	0.35	0.35	.0039	.33	6.0	13.1	1.92
150	20	1.0	0.35	.0047	.27	4.9	16.6	1.65
150	10	3.5	35	.0047	.076	6.4	16.9	2.02
100	150	0.35	0.035	.0051	.54	5.4	18.4	1.76
50	50	3.5	35	.0128	.08	4.5	46.1	1.58
75	3.5	0.35	35	.0047	.13	5.0	16.7	1.67

Figure Captions

Figure 1: (a) EPR cell geometry. $\vec{V}$, flow direction; H_0 , EPR spectrometer magnetic field axis; $\nabla \vec{V}$, direction of the velocity gradient and illumination. (b) Orientation of the thylakoid membranes of spinach chloroplasts relative to the EPR field when caused by the flow gradient. (c) Orientation of the thylakoid membranes caused by an intense magnetic field.

Figure 2: The effects of magnetic field orientation (A) and flow orientation (B) on the dark EPR signal in spinach chloroplasts. (A) chloroplasts oriented at 13 kG in 50% ethylene glycol, then frozen; spectrum recorded at 77°K. Scans are shown for both parallel and perpendicular orientations of the aligning field direction in the sample relative to the spectrometer field. Spectrometer conditions, 0.5 mW power, 2.0 G modulation amplitude, 100 kHz modulation frequency, 12.5 G/min scan, 1.0 s time constant, 1.5×10^5 gain. (B) chloroplasts under nonflowing (NF) and flowing (F) condition. Flow rate, 0.6 ml/min; EPR cell, 0.025 cm x 1 cm; temperature, 20°C; spectrometer conditions, 25 mW power, 4 G modulation amplitude, 1 MHz modulation frequency, 25 G/min scan, 1.0 s time constant, 30 dB attenuation, 5.0 gain.

Figure 3: The effect of sample flow on the EPR signal from broken spinach chloroplasts. The average of 500 kinetic traces is shown. Flow rate was 0.5 ml/min through a cell of cross-section 0.025 cm x 1.0 cm; chlorophyll concentration, 8.3 mg/ml; room temperature; spectrometer conditions: microwave power, 25 mW; modulation amplitude, 3.8 G; time constant, 0.5 μ s; magnetic field strength, 3,385 G; pulsed ruby laser excitation: wavelength, 694 nm; duration 50 ns; repetition rate 2s^{-1} .

Figure 4: The first derivative of the EPR absorption is plotted as a function of magnetic field for the slow (Signal I) and fast (polarized) kinetic components of Fig. 3. Signal I: Δ , flowing sample and $\circ$, nonflowing sample. Polarized signal: $\blacktriangle$, flowing sample and $\bullet$, nonflowing sample. The experimental conditions are the same as in Fig. 3, except that 2 G modulation amplitude was employed and 700 flashes were averaged to obtain the flow spectra. The amplitudes are normalized to the same number of flashes and modulation amplitude. The amplitude of Signal I is multiplied by 10 for the purpose of display.

Figure 5: EPR spectra of the emission signal and Signal I in Photosystem I particles (D-144) under flow and no-flow conditions. Each point represents the average of 300 events. Flow rate, 0.6 ml/min; 25 mW microwave power; 2.5 G modulation amplitude; 1 MHz modulation frequency; chlorophyll content, 0.83 mg/ml. The solution contained $2 \times 10^{-3}\text{M}$ NADP, 70 $\mu\text{g/ml}$ ferredoxin, $2 \times 10^{-4}\text{M}$ EDTA,

1.4×10^{-7} M ferredoxin-NADP reductase, 1×10^{-2} M ascorbate, and 4×10^{-5} M DCIP, in 0.05 M HEPES, 0.4 M sucrose, and 0.01 M NaCl at pH 7.5. The scale for Signal I is 2.5 times that for the emission signal.

Figure 6: EPR spectra of the emission signal and Signal I in whole cells of Chlorella pyrenoidosa. Each point represents the average of 400 events. Flow rate, 0.6 ml/min; 25 mW microwave power; 4 G modulation amplitude; 1 MHz modulation frequency; time constant 10 μ s. The scale for Signal I is 2.5 times that for the emission signal.

Figure 7: EPR spectra of oriented chloroplasts frozen in buffer in the dark, D (top trace); following exposure to light at very low temperature, L (middle trace). Bottom traces; oriented chloroplasts reduced with dithionite and dimethyltriquat at pH 10 under anaerobic conditions. Peaks attributable to ferredoxin species are designated A and B. Measurement temperature, 12 K; microwave power, 20 mW (top and middle), 1 mW (bottom); modulation amplitude, 10 G. The concentration of chloroplasts in the two samples is not the same, but is typically 3 mg/ml total chlorophyll. The parallel and perpendicular designations refer to the relative orientation of the membrane normal and the Zeeman field.

Figure 8: EPR spectra of oriented chloroplasts observed in the dark following trapping of X^- by illumination during cooling from room temperature to 200 K. The headings on each spectrum indicate the treatment given during trapping. The parallel and perpendicular designations refer to the relative orientation of the membrane normal and the Zeeman field. Dimethyltriquat is present in the dithionite reduced samples, which were also prepared anaerobically. Temperature, 12 K.; total chlorophyll concentration 5 mg/ml in all samples; microwave power: 200 mW; modulation amplitude, 32 G; illumination intensity, $2 \times 10^{13} \text{ J m}^{-2} \text{ s}^{-1}$ in the visible region. A and B designate features due to ferredoxin centers A and B.

Figure 9: Polarization vs. off-diagonal matrix element H_{AD} for the one-pair model. The horizontal lines indicate the range of the g values of X^- when the EPR field is in the plane of the thylakoid membrane (F) and for a random distribution of membranes (NF).

Figure 10: Simulated EPR spectra for the polarized signal for $A_{\text{g2}} = 0.1 \text{ G}, 0.5 \text{ G}, 1.0 \text{ G}$ and 5.0 G .

Figure 11: Calculated and experimental EPR spectra for the oriented and unoriented polarized signal from spinach chloroplasts. Values of the parameters used in the simulation are $\tau_1 = .35 \text{ ns}$, $\tau_2 = 35 \text{ ns}$, $J_1 = 75 \text{ G}$, $J_2 = 3.5 \text{ G}$. Solid triangles ($\blacktriangle$) are experimental intensities for flow-oriented chloroplasts. Open circles (o) are experimental intensities for unoriented chloroplasts.² Solid lines are theoretical curves.

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