Title

DEVELOPMENT OF ELECTRON SPIN POLARIZATION IN PHOTOSYNTHETIC ELECTRON TRANSFER BY THE RADICAL PAIR MECHANISM

Author

Friesner, Richard

Publication Date

1978-03-01

Submitted to Biophysical Journal

LBL-7543 c 2
Preprint

DEVELOPMENT OF ELECTRON SPIN POLARIZATION IN PHOTOSYNTHETIC ELECTRON TRANSFER BY THE RADICAL PAIR MECHANISM

Richard Friesner, G. Charles Dismukes, and
Kenneth Sauer

March 1978

Prepared for the U. S. Department of Energy
under Contract W-7405-ENG-48
and National Science Foundation
Grant PCM 76-5074

DEVELOPMENT OF ELECTRON SPIN POLARIZATION IN PHOTOSYNTHETIC ELECTRON TRANSFER BY THE RADICAL PAIR MECHANISM

by

Richard Friesner, G. Charles Dismukes and Kenneth Sauer

Laboratory of Chemical Biodynamics
Lawrence Berkeley Laboratory

and

Department of Chemistry
University of California
Berkeley, CA 94720

ABSTRACT

Development of Electron Spin Polarization in Photosynthetic Electron Transfer by the Radical Pair Mechanism

R. Friesner, G. C. Dismukes, and K. Sauer
Department of Chemistry and the Lawrence Berkeley Laboratory
University of California, Berkeley

We have extended the radical pair theory to treat systems of membrane-bound radicals with g tensor anisotropy. Analysis of the polarized EPR signals of $P700^+$, originating from Photosystem I of higher plants, in terms of the radical pair mechanism provides information about the sequence of early electron acceptors. In order to account for the orientation dependence of the lineshape and integrated area of this polarized signal, we propose the electron transfer sequence to be P700 → A → X → Fd(A,B) where A is a small organic molecule (possibly chlorophyll), X is the acceptor species observed recently in low temperature EPR studies, and Fd(A,B) are the ferredoxin iron-sulfur centers A and B.

Our calculations provide information about the lifetimes of A^-, and X^-, and their exchange interactions with $P700^+$. We also find supporting evidence for the orientation of X^- in the thylakoid membrane reported recently by G.C. Dismukes and K. Sauer (submitted for publication to Biochim. Biophys. Acta). The linewidth narrowing of the polarized signal is proposed to be due to delocalization of the unpaired electron on the oxidized reaction chlorophyll complex over 3 or 4 chlorophyll molecules immediately following photo-oxidation.

INTRODUCTION

In two preceding papers[1,2] (hereafter designated as I and II, respectively) we reported the observation of a polarized EPR signal from spinach chloroplasts arising from Photosystem I. It was proposed in paper II that this signal is produced by a non-Boltzmann distribution of spins of the cation radical of P700, the primary electron donor of Photosystem I.

In this paper, we propose a model for the development of spin polarization in $P700^+$ which quantitatively explains the results reported in paper II. The model is based on the radical pair theory[3,4], which has succeeded in accounting for chemically induced dynamic nuclear polarization (CIDNP) and electron polarization (CIDEP) in systems of freely diffusing radicals.

We extend the radical pair theory to include the effects of g tensor anisotropy, and incorporate modifications appropriate for a system of membrane-bound radicals. The results are qualitatively similar to those obtained for diffusive systems. For immobilized radicals that are also ordered, g tensor anisotropy leads to a marked dependence of the intensity and sign of the polarization on orientation of the sample.

Conclusions about the initial photochemical events arise from application of this model to Photosystem I. Our results indicate that there are two electron acceptors in series between P700 and P430 (ferredoxins A and B) which function under normal photosynthetic conditions. The first acceptor, which we shall call A_1, is probably a small organic molecule, possibly a chlorophyll. The anion radical of this species formed upon one electron reduction has an isotropic g tensor similar in magnitude to that of $P700^+$. The second acceptor has g tensor anisotropy and an orientation in the thylakoid membrane like that of the X^- species, which has been observed previously in chloroplasts and membrane fragments under conditions of chemical reduction and/or intense illumination.[5]

These conclusions are in agreement with the interpretation of recent optical results of Sauer et al.[6] By monitoring the kinetics of reduction of $P700^+$ following flash excitation in reduced photosystem I membrane fragments, evidence was provided for two acceptors preceding ferredoxins A and B.

EARLY EVENTS IN PHOTOSYSTEM I

The early electron transfer events in Photosystem I of higher plants have been investigated primarily by EPR and optical spectroscopy. The initial step following the absorption of a photon is the transfer of an electron by the reaction center chlorophyll complex, designated P700.[7] The optical properties of P700 have been established,[8] and the steady-state EPR spectrum of $P700^+$ can readily be observed upon illumination.[9]

The reduced electron acceptor, which we shall refer to as A_1, forms a radical pair $P700^+$-A_1^- with the oxidized reaction center species. Subsequently, the electron is transferred from A_1 to additional electron acceptors, ultimately reducing $NADP^+$.

Sauer et al[6] have proposed an acceptor scheme based on the kinetics of reduction of $P700^+$ after flash illumination. This scheme is illustrated in Fig. 1. The species A_1 and A_2 are detected by observation of changes in P700 absorption and have not been seen directly by optical methods. The optical properties of P430, the first stable photoreduced species, have been characterized by Ke and co-workers.[10]

Various EPR signals corresponding to reduced Photosystem I acceptors have been reported in the literature. Table I lists the principal signals observed, their g tensor components, and midpoint potentials.

Electron acceptor centers A and B have been associated with bound ferredoxin species,[11] and they also correlate with P430.[14] X^- can be observed upon flash illumination when centers A and B are reduced, or by photochemical trapping.[15] It has been inferred, therefore, that X is closer to P700 than is P430. In the scheme of Fig. 1, it seems likely that X is either A_1 or A_2.

The spin polarized EPR signals reported in paper II are observed at very short (microsecond) times during flash illumination of the sample. The signals are observed from a variety of preparations containing Photosystem I, including broken spinach chloroplasts. A transient signal is present at $g = 2.0026$ (where signal I is normally observed) which is strongly polarized, indicating that the radical from which it arises has a non-Boltzmann population of spin states. This signal undergoes significant lineshape changes when the chloroplasts are oriented in a velocity gradient. The changes in lineshape and amplitude with orientation provide a basis for deducing the mechanism of charge separation in Photosystem I, and the organization of the electron transport cofactors within the membrane.

THE RADICAL PAIR MECHANISM

The radical pair mechanism was originally proposed to explain the anomolous spin polarization which developed in radicals observed in solution following the creation of a radical pair or following a spin selective reaction. An early quantitative formulation was that of Adrian,[16] which predicted a dependence of the polarization on the hyperfine states and g values of the two radicals. In this section we present a brief description of the essential features of the radical pair mechanism following Adrian; in the following sections we adapt and apply these results to the photosynthetic system under consideration.

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

$$\mathcal{H}_{RP} = \beta\vec{H}_o\cdot[\hat{g}_D\cdot\vec{S}_D + \hat{g}_A\cdot\vec{S}_A] + J\vec{S}_D\cdot\vec{S}_A$$

$$+ \sum_i \underline{A}_i^{(D)}\vec{I}_i^{(D)}\cdot\vec{S}_D + \sum_j \underline{A}_j^{(A)}\vec{I}_j^{(A)}\cdot\vec{S}_A \quad .$$

where β is the Bohr magneton, $\vec{H}_o$ 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, $\vec{I}_i^{(m)}$ is the spin operator for the ith nucleus on molecule m (m=A,D), and $\underline{A}_i^{(m)}$ is the isotropic hyperfine coupling constant for the ith nucleus on molecule m.

Eq.(1) neglects the dipolar spin-spin interaction and the anisotropic exchange and hyperfine terms. We assume that, to a first approximation, these terms are small enough so that they have a minimal effect on the fixed energy levels of the radical pair and on the spin polarization.

The eigenstates of Hamiltonian (1) have been determined by Adrian for the case where $\hat{g}_A$ and $\hat{g}_D$ are isotropic (i.e. scalars). In the appendix, it is shown that we obtain solutions analogous to those of Adrian, except that the spin states are quantized in the direction of the effective field

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

The eigenstates are then linear combinations of the spin functions $|S\rangle$, $|T_{-1}\rangle$, $|T_{+1}\rangle$, and $|T_o\rangle$. We shall assume from this point onward that the radical pair is created initially from a singlet state. In the appendix, we demonstrate that the mixing of $|S\rangle$ with $|T_{\pm1}\rangle$ has a negligible effect on the net polarization when the g tensor anisotropy is sufficiently small. Therefore, we adopt an S-T_o basis set.

The solutions to (1) are then

$$\phi_1 = (\omega+J/2\omega)^{1/2}|S\rangle + (\omega-J/2\omega)^{1/2}|T_o\rangle$$

$$\phi_2 = (\omega-J/2\omega)^{1/2}|S\rangle - (J+\omega/2\omega)^{1/2}|T_o\rangle \qquad (3)$$

$$E_1 = +\omega \qquad E_2 = -\omega$$

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

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

$$= \frac{1}{2}\beta\vec{H}_o\cdot(\hat{g}_D - \hat{g}_A)\cdot\hat{z} + \frac{1}{2}(\sum_i \underline{A}_i{}^{(D)}m_{iz}{}^{(D)} - \sum_j \underline{A}_j{}^{(A)}m_{jz}{}^{(A)})$$

$\hat{z}$ is a unit vector in the direction of $\vec{h}'$, $m_{iz}{}^{(m)}$ is the z component of nuclear spin of the ith nucleus on molecule m, and E_1 and E_2 are the energies of ϕ_1 and ϕ_2, respectively.

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 J is constant, $\rho(t)$ is given by

$$\begin{aligned}\rho(t) &= \langle\Psi(t)|S_{iz}|\Psi(t)\rangle \\ &= [C_T(o)\,C_S{}^*(o) + C_T{}^*(o)\,C_S(o)] \\ &\times \{\cos^2\omega t + [(H_{AD}{}^2-J^2)/\omega^2]\sin^2\omega t\} \\ &+ (iJ/\omega)[C_T(o)\,C_S{}^*(o) - C_T{}^*(o)\,C_S(o)]\sin(2\omega t) \\ &+ 2(JH_{AD}/\omega^2)\sin^2\omega t[|C_S(o)|^2-|C_T(o)|^2] \qquad (5)\end{aligned}$$

where $C_T(o)$, $C_S(o)$ are the coefficients of $|T_o\rangle$ and $|S\rangle$ for the spin wave function at the beginning of the time interval of constant J (t=0). In the following sections, we use eq.(5) to calculate the spin polarization predicted by two alternative models of our experimental system.

CIDEP OF MEMBRANE-BOUND RADICALS

The radical pair mechanism described in the previous section has been applied primarily to diffusive systems. In these systems, it is necessary for the two radicals to diffuse apart and then re-encounter one another in order for appreciable polarization to develop.

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 (see the discussion for the justification of this assumption in Photosystem I). 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),

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

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 $\sim \omega^{-1}$. Because ω^{-1} is typically of the order of 10^{-9} sec, and the diffusion correlation time is $\sim 10^{-12}$ sec, this condition is ordinarily not satisfied for freely diffusing radicals in solution, and the net polarization upon initial separation of the radicals is negligible. After a re-encounter, other terms in Eq. (5) become significant, and the polarization develops as described by Adrian's model.

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. (It is a good assumption in Photosystem I, since the electron transfer has a quantum efficiency greater than 90%).[17] 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 unpaired electrons on D^+ and A_n^-. This process will change the polarization with time as long as there is a large enough exchange coupling, J_n, between D^+ and A_n^-. We therefore 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 $n \geq 3$, as $A_3 \ldots A_n$ are presumably too distant from D^+ to have a significant exchange coupling. Then, there are two reasonable models for the development of polarization.

The one-site model assumes that J_2 is also negligible, and that only the interaction $D^+ - A_1^-$ need be considered. The two-site model assumes that both J_1 and J_2 are significant, and that the interaction $D^+ - A_2^-$ must be included in a calculation of the spin polarization.

One Site 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 lifetime of 30 msec 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 e^{-t/τ_n}. The time-averaged polarization for the one site model is then

$$\rho(\tau_1) = \frac{2}{\tau_1} \int_0^\infty e^{-t/\tau_1} (H_1 J_1/\omega_1^2) \sin^2\omega_1 t \, dt \qquad (7)$$

$$= 4H_1 J_1 \tau_1^2/(1 + 4\omega_1^2 \tau_1^2)$$

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

Eq. (7) predicts a large value for ρ for suitable values of J_1 and τ_1. This is possible because, in contrast to the diffusive system, τ_1 may well be of the order of ω^{-1}. Thus, if J_1 is of the order of H_1, eq. (7) may attain values greatly in excess of the thermal population difference.

Two Site Model

For this model we need to calculate the net polarization on D^+ after the electron leaves A_2. The spin wave function at the time of transfer to A_2 (i.e. immediately after the electron has left A_1) is given by

$$\psi_1(t_1) = |S\rangle \cdot [\cos(\omega_1 t_1) - i(J_1/\omega_1)\sin(\omega_1 t_1)] \qquad (8)$$

$$- |T_o\rangle \cdot [(H_1/\omega_1)\sin(\omega_1 t_1)]$$

where t_1 is the duration of existence of $D^+ - A_1^-$.

The polarization after the radical pair $D^+ - A_2^-$ has existed for time t_2 can be found by obtaining the coefficients $C_S(t_1)$, $C_T(t_1)$ from eq. (8) and substituting into eq. (5)

$$\rho(t_1,t_2) = \frac{2H_1J_1}{\omega_1^2}\sin^2\omega_1 t_1(1 - \frac{2J_2^2}{\omega_2^2}\sin^2\omega_2 t_2) \qquad (9)$$

$$+ \frac{J_2H_1}{\omega_1\omega_2}\sin(2\omega_1 t_1)\sin(2\omega_2 t_2)$$

$$+ \frac{2H_2J_2}{\omega_2^2}\sin^2\omega_2 t_2(1 - \frac{2H_1^2}{\omega_1^2}\sin^2\omega_1 t_1)$$

Time averaging over t_1 and t_2, we obtain

$$\rho(\tau_1,\tau_2) = \frac{4H_1J_1\tau_1^2}{1 + 4\omega_1^2\tau_1^2}\cdot(1 - \frac{4J_2\tau_2^2}{1 + 4\omega_2^2\tau_2^2}) \qquad (10)$$

$$+ \frac{4J_2H_1\tau_1\tau_2}{1 + 4\omega_1^2\tau_1^2}\cdot(\frac{1}{1 + 4\omega_2^2\tau_2^2})$$

$$+ \frac{4H_2J_2\tau_2^2}{1 + 4\omega_2^2\tau_2^2}\cdot(1 - \frac{4H_1^2\tau_1^2}{1 + 4\omega_1^2\tau_1^2}).$$

ORIENTATION EFFECTS

We now investigate the effect of g tensor anisotropy on the expressions for the polarization derived in the previous section. The effect arises from the dependence of the matrix elements H_n on the orientation of the radicals in the applied magnetic field $\vec{H}_o$. We shall restrict ourselves to a situation where only one radical involved in the development of spin polarization on D^+ is anisotropic; the coordinate system defining the orientation is then chosen to be the principal axis system of the anisotropic species. The location of $\vec{H}_o$ is specified by a magnitude, $|H|$, 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 $\vec{H}_o$;

$$\rho_i = \frac{2}{\pi} \int_0^{\pi/2} \int_0^{\pi/2} \rho_i(\theta,\phi) P(\theta,\phi) d\theta d\phi \qquad (11)$$

where $P(\theta,\phi)$ is the probability that the radicals possess orientation (θ,ϕ) relative to $\vec{H}_o$, and $\rho_i(\theta,\phi)$ is the spin density developed on D^+ in hyperfine state i from either eq. (7) or eq. (10), with $H_n(\theta,\phi)$ given by eq. (10a) of the appendix.

We anticipate the next section and assume that the g tensor of D^+ is predominantly isotropic. For the one site model, we assume that A_1 is anisotropic; then, substitution of eqs. (7) and (10A) into (11) yields, with suitable rearrangement,

$$\rho_i(\text{one site}) = \frac{8}{\pi}\tau_1{}^2 J_1 \cdot \left[\int_0^{\pi/2}\int_0^{\pi/2} \frac{\Delta g_1(\theta,\phi)P(\theta,\phi)d\theta d\phi}{I_1(\theta,\phi)} + \frac{\alpha_i}{2}\int_0^{\pi/2}\int_0^{\pi/2} \frac{P(\theta,\phi)d\theta d\phi}{I_1(\theta,\phi)}\right] \tag{12}$$

where

$$\Delta g_n = -\tfrac{1}{2}[g_n{}^x \sin^2\theta\cos^2\phi + g_n{}^y \sin^2\theta\sin^2\phi + g_n{}^z\cos^2\theta - g_D] \tag{13}$$

$g_n{}^x$, $g_n{}^y$, and $g_n{}^z$ are the principal g tensor components of $A_n{}^-$, g_D is the isotropic g value of D^+, α_i is the total hyperfine field, $\sum_j A_j{}^{(D)} M_{j_i}{}^{(D)}$, of D^+ in hyperfine state i, and $I_n(\theta,\phi) = 1 + 4\omega_n{}^2(\theta,\phi)\tau_n{}^2$.

Defining

$$U_n = \frac{2}{\pi}\int_0^{\pi/2}\int_0^{\pi/2} \frac{\Delta g_n(\theta,\phi)P(\theta,\phi)d\theta d\phi}{I_n(\theta,\phi)} \tag{14}$$

$$V_n = \frac{2}{\pi}\int_0^{\pi/2}\int_0^{\pi/2} \frac{d\theta d\phi P(\theta,\phi)}{I_n(\theta,\phi)} \tag{15}$$

we have

$$\bar{\rho}_i(\text{one site}) = 4\tau_1{}^2 J_1 (U_1 + \frac{\alpha_i}{2} V_1) \tag{16}$$

For the two site model, we again 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 $H_1 = \alpha_i/2$ (since $\Delta g_1 = 0$) and that both H_1 and ω are orientation-independent, we obtain

$$\bar{\rho}_i(\text{two site}) = \frac{\alpha_i}{2}\left[\frac{4J_1\tau_1^2}{1+4\omega_1^2\tau_1^2}\cdot(1-4J_2^2\tau_2^2V_2) + \frac{4J_2\tau_1\tau_2}{1+4\omega_1^2\tau_1^2}\cdot V_2 + 4\tau_2^2J_2V_2\cdot\left(1-\frac{4H_1^2\tau_1^2}{1+4\omega_1^2\tau_1^2}\right)\right] + 4U_2\tau_2^2J_2\cdot\left(1-\frac{4H_1^2\tau_1^2}{1+4\omega_1^2\tau_1^2}\right) \qquad (17)$$

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

$$I_D(H) = \sum_{\substack{\text{all hyperfine}\\ \text{configurations}\\ \text{of } D^+}} (-\bar{\rho}_i)\, e^{(H-H_i^\circ)^2/\delta^2} \qquad (18)$$

where H_i° is the center of hyperfine line i, and δ is the half-width of the individual hyperfine lines. Note that a positive value of $\bar{\rho}_i$ results in a negative EPR intensity, i.e. $\bar{\rho}_i > 0$ means that hyperfine line i will be found in emission. This is the case because ρ is defined as $N_\alpha - N_\beta$, and an excess population of the state higher in energy (α) leads to a net emission of radiation.

In the next section, we examine the ability of eqs. (16) and (17) to predict the intensity patterns of the signals observed in Photosystem I, and thereby deduce a mechanism for the development of this polarization.

CIDEP IN PHOTOSYSTEM I

Fig. (3), in paper II, displays the CIDEP signals from flow oriented and from randomly oriented broken spinach chloroplasts. The effect of the velocity gradient in the configuration of the EPR spectrometer is to orient the short axis of the thylakoid membranes in the chloroplasts normal to the applied magnetic field.[2,5]

Paper II presents arguments to support the view that the CIDEP signals from both the oriented and the unoriented systems are due to the $P700^+$ cation radical. We shall adopt this as a working hypothesis which is supported by the calculations which follow.

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 A_1 is the species X, polarization develops as in the one-site model; (2) acceptor A_1 is a small organic molecule, possibly Chl, and A_2 is X, polarization develops as in the two-site model.

We have rejected two other conceivable schemes. A one-site model with Chl as A_1 would be inappropriate because it would not account for the orientation dependence of the polarized signal. A two-site model with X as A_1, bound Fd (center A or B) as A_2, would fail to correctly predict the mixed-emissive-enhanced absorptive pattern of the oriented signal for much the same reason as the one-site model (see the analysis of the one-site model for details), i.e. the term proportional to the hyperfine field of $P700^+$ would be too small.

It has been shown[5] that the x component of the g tensor of X^- (1.78) is oriented parallel to the short axis of the thylakoid membranes. Thus, the result of flow orientation is to align the g_x component normal to $\vec{H}_o$.

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. (16) and (17) are the integrals U_i and V_i. We first note that U_1(one site) = U_2(two site), and V_1(one site) = V_2(two site), since all of these integrals involve the g tensor components, lifetime, and J value of the same anisotropic radical, X^-. We therefore drop the subscripts, and refer to these integrals as U and V, respectively.

For a random orientation (no flow), $P(\theta,\phi) = \sin\theta$ for all θ,ϕ, and

$$U^{NF} = \frac{2}{\pi} \int_0^{\pi/2} \int_0^{\pi/2} \frac{\Delta g_x^{NF} \sin\theta \; d\theta d\phi}{1+4\tau_x^2[J_x^2+H_x^2(\theta,\phi)]} \qquad (19)$$

where τ_x is the lifetime of X^-, J_x is the exchange interaction between $P700^+$ and X^-, $H_x = (\alpha_i/2) + \Delta g_x^{NF}$, and $\Delta g_x^{NF}(\theta,\phi) = -(1.78 \sin^2\theta \cos^2\phi + 1.90 \sin^2\theta \sin^2\phi + 2.09 \cos^2\theta) + 2.0026$.

$$V^{NF} = \frac{2}{\pi} \int_0^{\pi/2} \int_0^{\pi/2} \frac{\sin\theta \; d\theta d\phi}{1+4\tau_x^2(J_x^2+H_x^2)} \qquad (20)$$

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

$$U^F = \frac{2}{\pi} \int_0^{\pi/2} \frac{\Delta g_x^{\,F}(\theta)\, d\theta}{1+4\tau_x^{\,2}[J_x^{\,2}+H_x^{\,2}(\theta)]} \qquad (21)$$

$$V^F = \frac{2}{\pi} \int_0^{\pi/2} \frac{d\theta}{1+4\tau_x^{\,2}[J_x^{\,2}+H_x^{\,2}(\theta)]} \qquad (22)$$

where $\Delta g_x(\theta) = 1.90 \sin^2\theta + 2.09 \cos^2\theta - 2.0026$, and $H_x(\theta) = \Delta g_x(\theta) + \alpha_i/2$.

We have set g_D (the isotropic g value of the donor radical) equal to 2.0026, the experimental value for P700$^+$.

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 EPR spectrum from the unoriented sample is in total emission, i.e. the polarization is positive across the entire hyperfine field of P700$^+$. The signal from the oriented system displays a mixed emissive-enhanced absorptive pattern; the polarization changes sign near $\alpha_i = 0$.

(2) The integrated area ratio for either polarized signal to the relaxed P700$^+$ signal is approximately 13:1 (i.e., the population difference, $|N_\alpha - N_\beta|$, is more than 10 times the thermal value at 300°K, 10^{-3}). Because relaxation has already begun when the EPR measurements are made, the calculated area ratios should be in excess of 13:1.

(3) The area ratio of the unoriented signal to the oriented signal is between 1:1 and 2:1 (this number is at present experimentally uncertain).

One Site Model

We make the simplifying approximation that $|\Delta g_1| + |J_1| \gg |\alpha_i/2|$, since α_i for P700$^+$ is typically a few gauss (the peak to peak linewidth of the steady-state P700$^+$ signal is 7.5 G). Then, $\omega_1{}^2 \sim (\Delta g_1)^2 + J_1{}^2$, and we can write eq. (16) as

$$\bar{\rho}_i(\text{one site}) = k_1[\alpha_i + \Delta \underline{g}_1] \qquad (23)$$

where $k_1 = 2V\tau_1{}^2 J_1$, and $\Delta \underline{g}_1 = 2U/V$.

The $\Delta \underline{g}_1$ term is mathematically isomorphic to the Δg_1 value difference term in Adrian's original formulation. Both k_1 and $\Delta \underline{g}_1$ are independent of α_i.

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 $10 < \Delta \underline{g}_1^{NF}/\Delta \underline{g}_1^{NF} < 30$. The absolute amplitudes of $\Delta \underline{g}_1^{NF}$ and k_1 are dependent upon the specific values of τ_1 and J_1.

The one-site model correctly predicts the unoriented signal to be in total emission. $\Delta \underline{g}_1^{NF}$ is large and positive, 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 $\Delta \underline{g}_1$ is inversely proportional to k_1, the hyperfine term α_i is dominated by $\Delta\underline{g}_1$ 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 be much smaller than the unoriented signal (a factor of 10 or more) and in total emission. We therefore conclude that the one-site model is incapable of explaining our results.

Two Site Model

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

$$\bar{\rho}_i(\text{two site}) = k_2(\alpha_i + \Delta\underline{g}_2) \qquad (24)$$

where

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

$$\Delta\underline{g}_2 = 2U\tau_2^2J_2/k_2$$

We have again assumed that $|\alpha_i| \ll |J_1|$ and $|\alpha_i| \ll \Delta\underline{g}_2$, 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. (17), and thus has been neglected. Both k_2 and $\Delta\underline{g}_2$ 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 this second term, $4J_1\tau_1^2/(1+4\omega_1^2\tau_1^2)$, 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 because Δg_1 is zero, so that $H_1 \ll J_1$. Effectively, the interaction of P700$^+$ with A_1^- produces a substantial polarization term proportional to the hyperfine field of P700$^+$. The corresponding term in the one site model is small because the only radical pair interaction available here is P700$^+$ - X^-. For this radical pair, the g value difference is quite large relative to α_i for almost all orientations of X^-.

The experimental signals can be generated from eq. (24) 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 $\underline{g}_2^F$ and $\underline{g}_2^{NF}$. 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 $(\alpha_1 + \Delta\underline{g}_2)$ is positive for all values of α_i, and the polarized signal is seen in total emission.

In the next section, we simulate the polarized signals quantitively by substituting eq. (24) into eq. (18) and summing over all configurations of the P700$^+$ hyperfine system.

RESULTS OF CALCULATIONS WITH THE TWO-SITE MODEL

We first calculated an EPR spectrum for an isolated, relaxed $P700^+$ radical, assuming that it is an oxidized chlorophyll dimer.[18] The relative amplitudes of the hyperfine coupling constants were obtaiend from NMR studies,[19] the magnitudes were scaled to the ENDOR result for the largest coupling constant.[20]

The narrowing of the polarized signal (see Discussion) was introduced phenomenologically by decreasing the hyperfine coupling constants. An identical adjustment was used to simulate the signals for both the oriented and unoriented systems.

Fig. (2) displays the dependence of the EPR lineshape on the value of $\Delta\underline{g}_2$ in eq. (24). For $\Delta\underline{g}_2 < 0.7$ G, a nearly symmetrical mixed emissive-enhanced absorptive pattern results. For $\Delta\underline{g}_2 > 4$ G, the signal is essentially in total emission. For $0.7 \text{ G} < \Delta\underline{g}_2 < 4$ G, a lineshape intermediate between the two previous cases is found.

The integrated area of a polarized signal depends linearly on k_2, and in a complicated fashion upon $\Delta\underline{g}_2$. Table II lists the integrated area of $|dI/dH|$ as a function of $\Delta\underline{g}_2$; the area of the unpolarized signal is set equal to 1.0, and the polarized signals normalized to this. The net integral area relative to the thermal equilibrium value for signal I at 300°K is found by multiplying the value in table II by $k_2/.001$ (.001 is the thermal population difference at 300°K).

From these results we can set limits on k_2 and $\Delta\underline{g}_2$ such that the three fitting criteria for the experimental signals described above are satisfied. The general lineshape analysis requires that $0 < \Delta\underline{g}_2^F < 0.7$ G, while $\Delta\underline{g}_2^{NF} > 4$ G. Since the polarized signals have an area 3.5 - 6 times greater than that of the unpolarized signal when k_2 is set equal to .001 , we require that $k_2/.001 > 3.7$, so that the net area ratio is greater than 13:1. An upper limit of 2:1 on the area ratio of the oriented and unoriented signals can be insured by setting the limit $\Delta\underline{g}_2^{NF} < 6.5$ G.

The values of k_2, $\Delta\underline{g}_2^F$, and $\Delta\underline{g}_2^{NF}$ are determined by the parameters τ_1, τ_2, J_1 and J_2. Table III presents several sets of parameters for which k_2, $\Delta\underline{g}_2^F$, and $\Delta\underline{g}_2^{NF}$ fall within the limits prescribed above. The exact values of the individual exchange energies or lifetimes are not critical; a small change in τ_n or J_n will produce a correspondingly small change in the simulated EPR spectrum.

It is clearly not possible to deduce the absolute magnitudes of any of the parameters from the data available at present. We can, however, set some limits on τ_1 and J_1. It is necessary that $\tau_1 \geqslant 350$ psec, and $J_1 < 200$ G, in order 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 $\Delta\underline{g}_2^F$ and $\Delta\underline{g}_2^{NF}$.

For a comparison of theory and experiment, we chose a value of τ_1 which is comparable to the lifetime of I^- observed in photosynthetic bacteria. We also chose

$J_1 > J_2$, because A_1 is presumably in closer proximity 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.[21] They are also within the neighborhood of exchange interactions observed between electron acceptors in photosynthetic bacteria.[22]

Fig. (2) 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 to account for the effects of relaxation. It is seen that excellent agreement is obtained within the limits of experimental error.

DISCUSSION

The two-site model successfully predicts most of the important features of the polarized signals arising from oriented and unoriented chloroplasts. Many of the values of τ_1, τ_2, J_1 and J_2 which generate the correct lineshapes are consistent with what is known about early photosynthetic events. The model is relatively insensitive to the details of the calculations, i.e. small errors in the polarization function (as are introduced by neglect of $S\text{-}T_{\pm 1}$ mixing) would have a minimal effect on the predicted lineshapes and area ratios.

We believe that our results provide compelling (although indirect) evidence for the existence of an acceptor in Photosystem I preceding X. A radical pair mechanism with X as the initial acceptor is inconsistent with the mixed emissive-absorptive lineshape and relative area of the oriented signal. The presence of an earlier acceptor with an isotropic g value close to that of $P700^+$ provides a simple and satisfying explanation for these features. 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 g tensor properties. Also, the midpoint reduction potential of chlorophyll a is 0.78 V (vs. NHE, in dimethylsulfoxide),[23] which is consistent with its role as an earlier acceptor than X. 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.[24] However, we have no direct information concerning the chemical identity of A_1.

The assignment of X as A_2 is also supported by our results. The alignment of the high field component of the g tensor of A_2 normal to the plane of the thylakoid membrane is required to produce the transformation from a totally emissive spectrum to a mixed emissive-enhanced absorptive spectrum upon orientation. Neither ferredoxin signal (centers A or B) displays the proper orientation in the membrane to generate the observed lineshape changes.[5] The observation that the simulation of the oriented and unoriented signals, assuming that A_2 is X, gives excellent quantitative agreement is convincing evidence that this interpretation is valid.

Paper I proposed a triplet mechanism for the development of spin polarization. This can now be eliminated, because it never predicts a mixed emissive-enhanced absorptive lineshape. The triplet and radical pair mechanisms are the only theories proposed to date to explain chemically induced spin polarization. The model presented here thus appears to be the only reasonable explanation which fits the experimental results.

The radical pair theory as developed by Adrian appears to be applicable to membrane-bound systems of radicals; the fundamental driving mechanism 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.

We have assumed throughout our calculations that the initial radical pair state is a singlet. This can be justified qualitatively without invoking any EPR results. The initial state of P700* is surely a singlet. If the rate of electron transfer from P700* to A_1 is comparable to that observed in bacterial systems ($<$ 20 psec),[25,26] there would be insufficient time for intersystem crossing to a triplet state to occur. Also, the unusual spin polarization of the reaction center triplet state in bacteria can be explained if electron transfer occurs from the excited singlet state.[27] (A spin flip as a consequence of electron transfer is quantum mechanically forbidden). We thus expect the radical pair to initially have the same singlet character as P700*.

The narrowing of the polarized signal relative to the relaxed P700$^+$ signal is an interesting phenomenon for which we currently do not have a completely 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.

The above observations are not predicted by the radical pair mechanism. The polarization is either a constant across the hyperfine field ($\Delta\underline{g}_2$ large) or linear in α_i ($\Delta\underline{g}_2$ small). Neither of these polarization functions leads to a symmetrical narrowing of hyperfine envelope of the P700$^+$ signal. Furthermore, one would not expect the effect to be identical for the oriented and unoriented systems.

One explanation of the narrowing is that, immediately following photo-oxidation, the unpaired electron on P700$^+$ is delocalized over 3 or 4 chlorophyll molecules. The steady-state P700$^+$ complex is believed to be a strongly coupled chlorophyll dimer;[16] delocalization of the unpaired electron over two molecules leads to a narrowing of $\sqrt{2}$ compared to the Chl$^+$ monomer EPR signal. Full delocalization over 3 or 4 molecules would result in a further narrowing of $\sqrt{3/2}$ or $\sqrt{4/2}$, respectively; the experimental narrowing is between these two values. Following electron transfer, the oxidized reaction center complex reaches a new equilibrium structure which favors delocalization over only two chlorophylls.

Paper II discusses other hypotheses concerning the narrowing phenomenon. Verification of these proposals will require further theoretical and experimental work.

There are many interesting areas of future research which are suggested by this paper. Further EPR and optical experiments on photosystem I are needed to evaluate details of the two-site model, determine values for lifetimes and exchange interactions, and determine the identity of A_1. An approach similar to the one described here can also be applied to the CIDEP signals reported from photosynthetic bacteria.[28]

ACKNOWLEDGEMENTS

This work was supported by the Division of Biomedical and Environmental Research of the U.S. Department of Energy, and National Science Foundation Grant PCM 76-5074. We would also like to thank Dr. A. Pines for helpful discussions concerning the signal narrowing.

APPENDIX

The radical pair Hamiltonian given in eq. (1) can be split into two parts

$$\mathcal{H}_{RP} = \mathcal{H}_{D} + \mathcal{H}_{OD} \qquad (1A)$$

where

$$\mathcal{H}_{D} = \frac{1}{2}\beta\vec{H}_{o}\cdot(\hat{g}_{A} + \hat{g}_{D})\cdot(\vec{S}_{A} + \vec{S}_{D}) + J\vec{S}_{A}\cdot\vec{S}_{D}$$

$$+ \frac{1}{2}[\sum_{i} \underline{A}_{i}^{(D)}\vec{I}_{i}^{(D)} + \sum_{j} \underline{A}_{j}^{(A)}I_{j}^{(A)}]\cdot(\vec{S}_{A} + \vec{S}_{D})$$

$$\mathcal{H}_{OD} = \frac{1}{2}\beta\vec{H}_{o}\cdot(\hat{g}_{D} - \hat{g}_{A})\cdot(\vec{S}_{D} - \vec{S}_{A})$$

$$+ \frac{1}{2}\ (\sum_{i} \underline{A}_{i}^{(D)}\vec{I}_{i}^{(D)} - \sum_{j} \underline{A}_{j}^{(A)}\vec{I}_{j}^{(A)})$$

$\mathcal{H}_D$ is diagonal in the basis $\{|S\rangle, |T_o\rangle\ |T_{+1}\rangle\ \ |T_{-1}\rangle\}$, provided that the spin functions $|\alpha\rangle$ and $|\beta\rangle$ are quantized in the direction of the effective field

$$z = (\hat{g}_{A} + \hat{g}_{D})\cdot\vec{H}_{o}/||(\hat{g}_{A} + \hat{g}_{D})\cdot\vec{H}_{o}|| \qquad (2A)$$

The radical pair eigenfunctions and energies depend upon the off-diagonal elements of the above basis set of the operator $\mathcal{H}_{OD}$. We now show that, for small g tensor anisotropy, the mixing of $|S\rangle$ with $|T_{+1}\rangle$ and $|T_{-1}\rangle$ is of negligible importance, and an $|S\rangle - |T_o\rangle$ basis set is sufficient for calculation of the polarization. We also derive an approximate expression for the matrix element $\langle S|H_{OD}|T_o\rangle \equiv H_{AD}$ as a function of orientation of radicals A^- and D^+.

We shall assume that the donor radical is isotropic, with scalar g value g_D. We choose as a coordinate system the principal axis system of the acceptor radical. Then

$$H_o = |\vec{H}|(\sin\theta\cos\phi \quad \sin\theta\sin\phi \quad \cos\theta) \tag{3A}$$

$$\tilde{\hat{g}}_A = \begin{bmatrix} g_A^x & 0 & 0 \\ 0 & g_A^y & 0 \\ 0 & 0 & g_A^z \end{bmatrix}$$

$$g_D = \begin{bmatrix} g_D & 0 & 0 \\ 0 & g_D & 0 \\ 0 & 0 & g_D \end{bmatrix}$$

We define

$$\bar{g}_A = \frac{1}{3}(g_A^x + g_A^y + g_A^z) \tag{4A}$$

$$\Delta x = \bar{g}_A - g_A^x$$

$$\Delta y = \bar{g}_A - g_A^Y$$

$$\Delta z = \bar{g}_A - g_A^Z$$

$$g_+ = \bar{g}_A + g_D$$

$$g_- = g_D - \bar{g}_A$$

We wish to calculate the matrix elements $\langle S|H_{0D}|T_o\rangle$, $\langle S|H_{0D}|T_{+1}\rangle$, and $\langle S|H_{0D}|T_{-1}\rangle$. We first define

$$H_{OD} = H_{HF} + H_{\Delta g} \tag{5A}$$

where

$$H_{HF} = \frac{1}{2}\Big(\sum_i \underline{A}_i^{(D)}\vec{I}_i^{(D)} - \sum_j \underline{A}_j^{(A)}\vec{I}_j^{(A)}\Big)\cdot(\vec{S}_D - \vec{S}_A)$$

$$H_{\Delta g} = \frac{1}{2}\beta\vec{H}_o\cdot(\hat{g}_D - \hat{g}_A)\cdot(\vec{S}_D - \vec{S}_A)$$

Because we are interested in the spin polarization of the donor radical, we set the sum over the acceptor hyperfine field equal to its ensemble average, i.e.

$$\sum_j \underline{A}_j^{(A)} m_j^{(A)} \rightarrow \langle\Sigma\underline{A}_j^{(D)} m_j^{(A)}\rangle = 0 \tag{6A}$$

The nuclear spin operators $I_i^{(D)}$ are quantized in the direction of the effective field, $\hat{z}$. Then

$$\langle S|H_{HF}|T_{\pm 1}\rangle = 0 \tag{7A}$$

$$\langle S|H_{HF}|T_o\rangle = \frac{1}{2}\sum_j \underline{A}_j^{(D)} m_j^{(D)}$$

where $m_j^{(D)}$ is the projection of $I_j^{(D)}$ on $\hat{z}$.

The matrix elements of $H_{\Delta g}$ must now be evaluated. Substitution of (3A) and (4A) into (5A) yields

$$\begin{aligned}\langle S|H_{\Delta g}|T_o\rangle = \frac{1}{2}\beta|H|\{&\cos^2\phi\sin^2\theta(g_+-\Delta x)(g_--\Delta x) \\ &+ \sin^2\theta\sin^2\phi(g_+-\Delta y)(g_--\Delta y) + \cos^2\theta(g_+-\Delta z)(g_--\Delta z)\} \\ &\{\cos^2\theta\sin^2\phi(g_+-\Delta x)^2 + \sin^2\theta\sin^2\phi(g_+-\Delta y)^2 \\ &+ \cos^2\theta(g_+-\Delta z)^2\}^{-1/2}\end{aligned} \tag{8A}$$

$$\langle S|H_{\Delta g}|T_{\pm 1}\rangle = \mp i[\tfrac{\beta}{2}||(\hat{g}_D-\hat{g}_A)\cdot\vec{H}_o||^2-(\langle S|H_{\Delta g}|T_o\rangle)^2]^{1/2}$$

We now make the approximation

$$g_- \sim \Delta x, \Delta y, \Delta z \ll g_+$$

Then, algebraic manipulation of (8A) leads to

$$\langle S|H_{\Delta g}|T_o\rangle \approx \tfrac{1}{2}\beta|H|(g_- - \cos^2\phi\sin^2\theta\Delta x + \sin^2\phi\sin^2\theta\Delta y + \cos^2\theta\Delta z) \qquad (9A)$$

$$\langle S|H_{\Delta g}|T_{\pm 1}\rangle \approx \mp i[(g_- -\Delta x)^2\cos^2\phi\sin^2\theta + (g_- -\Delta y)^2\sin^2\theta\sin^2\phi + (g_- -\Delta z)\cos^2\theta - (\langle S|H_{\Delta g}|T_o\rangle)^2]^{1/2}$$

This gives as a final expression for $\langle S|H_{OD}|T_o\rangle$

$$\langle S|H_{OD}|T_o\rangle \equiv H_{AD} = \tfrac{1}{2}\Sigma\,\underline{A}_j^{(D)}M_j^{(D)} + \tfrac{1}{2}\beta|H|[g_D - (g_A^x\cos^2\phi\sin^2\theta + g_A^y\sin^2\phi\sin^2\theta + g_A^z\cos^2\theta)] \qquad (10A)$$

We estimate the effects of $T_{\pm 1}$ mixing by calculating the ensemble average value

$$<\langle S|H_{\Delta g}|T_{\pm 1}\rangle> = \frac{2}{\pi}\int_0^{\pi/2}\int_0^{\pi/2}\langle S|H_{\Delta g}|T_{\pm 1}\rangle \sin\theta\, d\theta d\phi$$

$$\approx [\tfrac{1}{3}(\Delta x^2 + \Delta y^2 + \Delta z^2)]^{1/2}\cdot\tfrac{1}{2}\beta H \qquad (11A)$$

Substitution of values of Δx, Δy, and Δz for the species X^- yields

$$<\langle S|H_{\Delta g}|T_{\pm 1}\rangle> \approx \mp\frac{i}{2}\beta|H|\cdot(0.13) \qquad (12A)$$

The mixing coefficients, $C_{ST_{\pm 1}}$, are given to first order by

$$C_{ST_{\pm 1}} = \langle S | H_{OD} | T_{\pm 1} \rangle / (E_S - E_{T_{\pm 1}}) \qquad (13A)$$

$$= \mp \frac{i}{2} \beta H(0.13)/g\beta H \approx \mp i(.0325).$$

This 3.25% mixing in of the $T_{\pm 1}$ states leads to an error of less than 3% in the calculated polarization.

REFERENCES

1. Blankenship, R., A. McGuire and K. Sauer. (1975) Chemically induced dynamic electron polarization in chloroplasts at room temperature: Evidence for triplet state participation in photosynthesis. Proc. Nat. Acad. Sci. USA 72:4943-4947.
2. Dismukes, G.C., A. McGuire, R. Blankenship and K. Sauer. (1978) Electron spin polarization in photosynthesis and the mechanism of electron transfer in Photosystem I. Experimental observations. Biophys. J. 21: in press.
3. Wan, J.K.S. and A.J. Elliot. (1977) Chemically induced dynamic magnetic polarization in photochemistry. Acc. Chem. Res. 10:161-166.
4. Freed, J.H. and J.B. Petersen. (1976) The theory of chemically induced dynamic spin polarization. Adv. Mag. Res. 8:1-84.
5. Dismukes, G.C. and K. Sauer. The orientation of membrane bound radicals: an EPR investigation of magnetically ordered spinach chloroplasts. Submitted to Biochim. Biophys. Acta.
6. Sauer, K., P. Mathis, S. Acker and J.A. Van Best. Electron acceptors associated with P700 in triton solubilized Photosystem I particles from spinach chloroplasts. Submitted to Biochim. Biophys. Acta.

7. Sauer, K. (1975) Primary events and the trapping of energy. In "Bioenergetics of Photosynthesis" (Govindjee, ed.) Academic Press, New York. 115-181.

8. Kok. B. (1961). Partial purification and determination of the oxidation reduction potential of the photosynthetic chlorophyll complex absrobing at 700 nm. Biochim. Biophys. Acta 48:527-533.

9. Bearden, A.J. and R. Malkin. (1976) Chloroplast photosynthesis: the reaction center of Photosystem I. In Brookhaven Symposium in Biology No. 28, (J.M. Olson and G. Hind, eds.) Brookhaven National Laboratory, Upton, New York. 247-266.

10. Ke, B. The primary electron acceptor of Photosystem I. Biochim. Biophys. Acta. 301:1-33.

11. Evans, M.C.W., S.G. Reeves and R. Cammack. (1974) Determination of the oxidation-reduction potential of the bound iron-sulfur proteins of the primary electron acceptor complex of Photosystem I in spinach chloroplasts. FEBS Lett. 49:111-114.

12. Ke, B., R.E. Handen and H. Beinert. (1973). Oxidation-reduction potentials of bound iron-sulfur proteins in Photosystem I. Proc. Nat. Acad. Sci. USA 70:2941-2945.

13. Ke, B., E. Dolan, K. Sugahara, R.M. Hawkridge, S. Demeter and E.R. Shaw. (1977) Electrochemical and kinetic evidence for a transient electron acceptor in the photochemical charge separation in Photosystem I.

In "Photosynthetic Organelles", a special issue of Plant Cell Physiol. No. 3, (Mijachi, Katch, Fujita and Shibota, eds.) Jap. Soc. Plant. Physiol., Japan. 187-199.

14. Ke, B. and H. Beinert. (1973) Evidence for the identity of P430 of photosystem I and chloroplast bound iron-sulfur protein. Biochim. Biophys. Acta. 305:689-693.

15. McIntosh, A.R., and J. Bolton. (1976) Electron spin resonance spectrum of species "X" which may function as the primary electron acceptor in Photosystem I of green plant photosynthesis. Biochim. Biophys. Acta 430:555-559.

16. Adrian, F.J. (1971) Theory of anomolous electron spin resonance spectra of free radicals in solution. Role of diffusion-controlled separation and re-encounter of radical pairs. J. Chem. Phys. 54:3918-3923.

17. Sun, A.S.K. and K. Sauer. (1971) Pigment systems and electron transport in chloroplasts I. Quantum requirements for the two light reactions in spinach chloroplasts. Biochim. Biophys. Acta 234:399-414.

18. Katz, J.J. and J.R. Norris, Jr. (1973) Chlorophyll and light energy transduction in photosynthesis. In "current Topics in Bioenergetics", (D.R. Sanadi and L. Packer, eds.) Vol. 5, 41-75.

19. Sanders, J.K.M. and J.C. Waterton. (1976). The chlorophyll-a radical cation: determination of hyperfine coupling constants by NMR. J. Chem. Soc. Chem. Commun. 247-248.

20. Scheer, H., J.J. Katz and J.R. Norris. (1977) Proton-electron hyperfine coupling constants of the chlorophyll a cation radical by ENDOR spectroscopy. J. Amer. Chem. Soc. 99:1372-1381.

21. Metzner, E.K. (1974) Spin exchange in biradicals. Ph.D. Thesis. University of California, Berkeley, CA.

22. Prince, R.C., D.M. Tiede J.P. Thornber and P.L. Dutton. (1977) Spectroscopic properties of the intermediary electron carrier in the reaction center of *Rhodopseudomonas viridis*. Biochim. Biophys. Acta 462:467-490.

23. Dryhurst, G. (1977) In "Electrochemistry of Biological Molecules", Academic Press, New York, NY. p. 412.

24. Thornber, J.P., R.S. Alberts, F.A. Hunter, J.A. Shiazawa and K.S. Kan. (1976). In "Brookhaven Symposium in Biology, No. 28" (J.M. Olson and G. Hind, eds.) Brookhaven National Laboratory, Upton, N.Y. p. 148.

25. Kaufmann, K.J., P.L. Dutton, T.L. Netzel, J.S. Leigh and P.M. Rentzepis. (1975) Picosecond kinetics of events leading to reaction center bacteriochlorophyll oxidation. Science 188:1301-1304.

26. Holton, D., M.W. Windsor, W.W. Parson and J.P. Thornber. (1978) Primary photochemical processes in isolated reaction centers of *Rhodopseudomonas viridis*. Biochim. Biophys. Acta 501:112-126.

27. Thurnauer, M.C., J.J. Katz and J.R. Norris. (1975) The triplet state in bacterial photosynthesis: possible mechanisms of the primary photo-act. Proc. Nat. Acad. Sci. 72:3270-3273.

28. Hoff, A.J., P. Gast and J.C. Romijn. (1977) Time-resolved ESR and chemically induced dynamic electron polarization of the primary reaction in a reaction center particle of *Rhodopseudomonas spheroides* wild-type at low temperature. FEBS Lett. 73:185-190.

FIGURE CAPTIONS

Figure 1: Proposed scheme of electron transfer in Photosystem I.

Figure 2: Simulated EPR spectra for the polarized signal for $\Delta \underline{g}_2$ = 0.1 G, 0.5 G, 1.0 G and 5.0 G.

Figure 3: Calculated and experimental EPR spectra for the oriented and unoriented polarized signal from spinach chloroplasts. Values of the parameters used in the simulation are τ_1 = .35 ns, τ_2 = 35 ns, J_1 = 75 g, J_2 = 3.5 g. Solid triangles (▲) are experimental intensities for flow-oriented chloroplasts. Open circles (○) are experimental intensities for unoriented chloroplasts.[2] Solid lines are theoretical curves.

$$\mathrm{P700} \rightarrow A_1 \rightarrow X \rightarrow \mathrm{P430}$$

Fig. 1

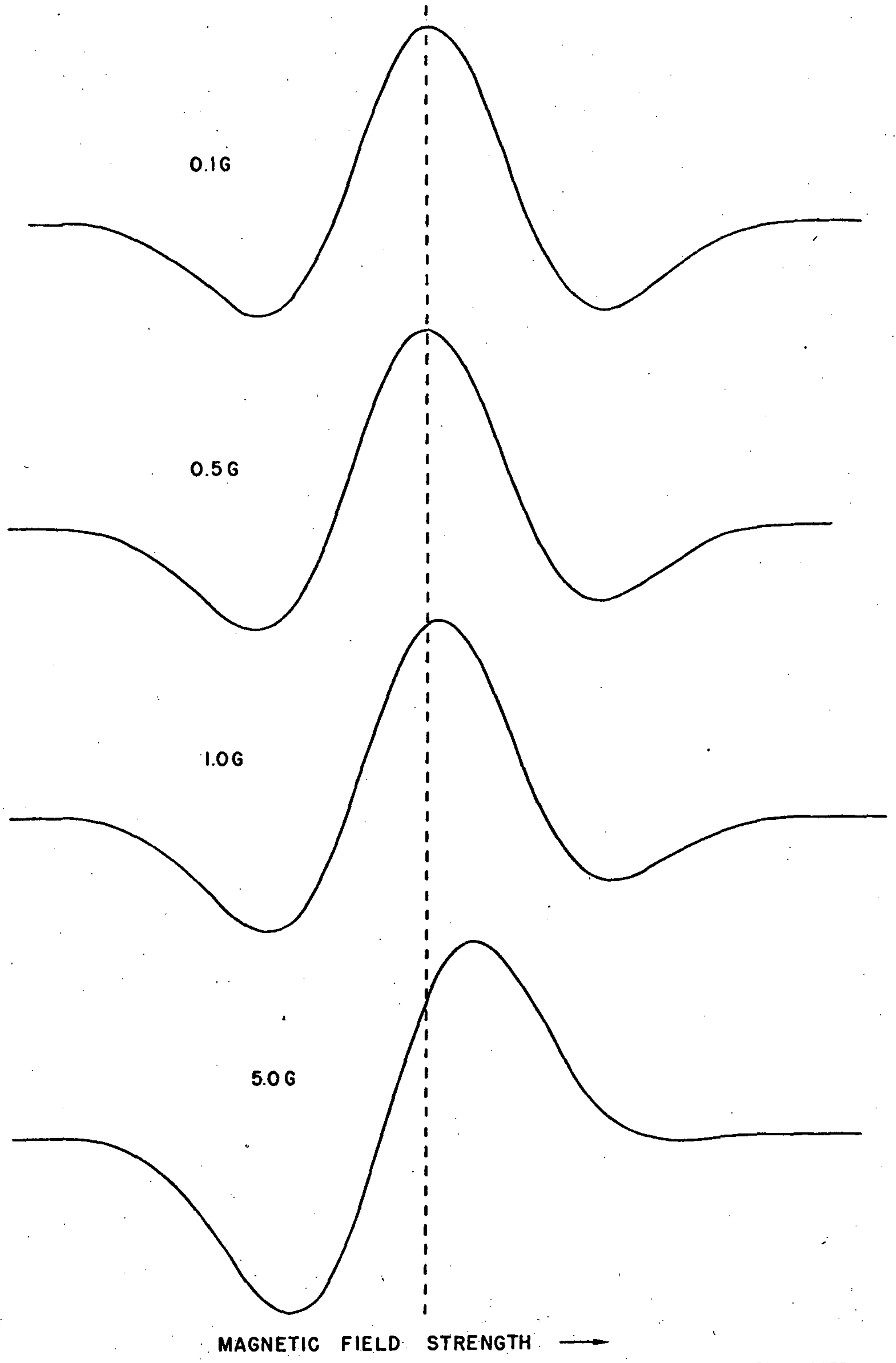
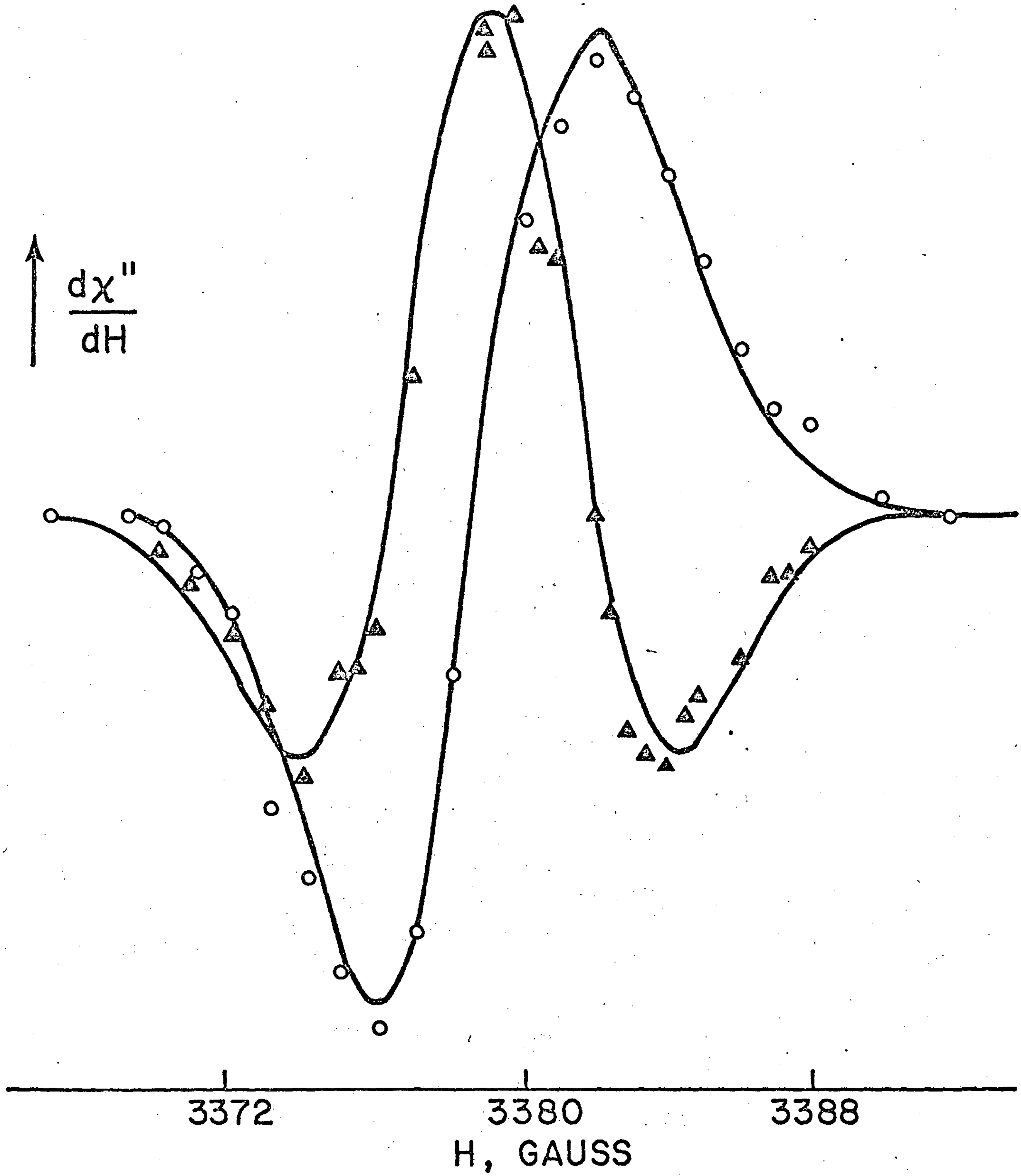


Fig. 2



XBL 775-4394

Fig. 3

TABLE I

g Tensor Values and Midpoint Potentials of Photosystem I Electron Acceptors

Species	g_x	g_y	g_z	Midpoint Potential (mV)	Refs.
X^-	1.78	1.90	2.09	-730	5,13
Center A	1.87	1.95	2.05	-553, -530	11,12
Center B	1.89	1.93	2.05	-594, -580	11,12

TABLE II

Relative Area of the Polarized Signal as a Function of $\Delta \underline{g}_2$

(Signal I = 1.0)

$\Delta \underline{g}_2$	Area
0.0	3.6
0.1	3.6
0.2	3.6
0.5	3.6
1.0	3.7
2.0	4.0
5.0	6.0
10.0	10.5

TABLE III

Calculated Values of k_2, $\Delta \underline{g}_2^F$ and $\Delta \underline{g}_2^{NF}$ for selected values of τ_1, τ_2, J_1 and J_2.
Area ratios are also calculated using Table II

J_1(G)	J_2(G)	τ_1(nsec)	τ_2(nsec)	k_2	$\Delta \underline{g}_2^{NF}$(G)	$\Delta \underline{g}_2^F$(G)	$\frac{A^F_{pol}}{A_{sig.I}}$	$\frac{A^{NF}}{A^F}$
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

TECHNICAL INFORMATION DEPARTMENT
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720