Electron spin polarization in photosynthetic reaction centres: Strategies for extracting structural and functional information

Yuri E. Kandrashkin*1, *2 and Art van der Est*1

*1 Department of Chemistry, Brock University, Canada
*2 Kazan Physical Technical Institute, Russian Academy of Sciences, Russia

A simple and elegant treatment of sequential radical pairs is presented using assumptions based on the properties of the radical pairs in photosynthetic reaction centres. Analytical expressions for the spin polarization patterns of the radical pairs are derived and it is shown that when the exchange coupling is negligible, a unique solution for the geometry is only obtainable if the value of the arbitrary scaling factor relating the experimental and calculated spectra is known. It is also shown that the influence from spin dynamics in the precursor radical pair can be separated into several contributions and calculated easily. Strategies for dealing with the restrictions on the geometric information and for obtaining information about the kinetic phases in the electron transfer in Photosystem I are discussed.

Introduction

The spin polarization generated by the light-induced electron transfer in photosynthetic reaction centres (RC) has been widely used to study their structure and function.1, 2 The electron transport generates a sequence of radical pairs (RP), according to the general scheme:

\[ P \rightarrow P^* \rightarrow RP_0 \rightarrow RP_1 \rightarrow RP_2 \rightarrow \cdots \]

where \( P \) is the primary donor and \( RP_0, \) \( RP_1 \) and \( RP_2 \) are the radical pairs generated by the electron transfer. The first radical pair, \( RP_0, \) is generated very rapidly so that initially, it inherits the singlet spin orientation from \( P^*. \) Usually, \( RP_0 \) is too short-lived to be observed by transient electron paramagnetic resonance (TREPR) but its spin dynamics may influence the polarization pattern of the subsequent pair, which nonetheless retains a high degree of singlet character. Further electron transport is much slower, and it is usually possible to separate its influence on the polarization pattern from the influence of the spin dynamics. Here, we present a straight forward analytical treatment of the polarization patterns of such pairs and apply it first to singlet-born RPs, and then to the influence of spin evolution in the precursor states.

The most important feature of this treatment is that we make assumptions based on properties specific to RCs. Although this limits the generality, it provides greater insight into the origins of the polarization. The properties used are: (1) a fixed geometry of the RC in the surrounding protein matrix, (2) a random distribution of RC orientations relative to the laboratory frame, (3) a large distance between the radicals in the observable RPs (ca. 25-30 Å) so that the spin-spin coupling between the electrons is small compared with other spin interactions, i.e. Zeeman and hyperfine interactions. Only these few, very reasonable assumptions allow us to develop a simple and elegant method for the study of the functional and structural properties of RCs.

Stick spectrum

We begin by constructing the stick spectrum of a singlet-born weakly coupled RP3, 4 comprised of a donor radical, \( P, \) and an acceptor radical, \( A. \) The spin dynamics of the RP are described by the Hamiltonian:

\[
\hat{H} = \hbar \left[ \Delta \omega_P \hat{S}_P + \Delta \omega_A \hat{S}_A - 2a \hat{S}_P \hat{S}_A \right. \\
\left. - b \left( \hat{S}_P \hat{P}_A + \hat{S}_P \hat{S}_A \right) \right],
\]

which includes the precession frequencies of the two radicals in the rotating frame, \( \Delta \omega_P \) and \( \Delta \omega_A \), and two secular spin-spin coupling parameters, \( a \) and \( b, \) which describe the longitudinal two-spin order and the frequency of the flip-flop transitions, respectively. The parameters \( a \) and \( b \) are determined by the exchange coupling \( J \) and the dipole-dipole interaction parameter \( d. \)

\[ a = J - d, \quad b = 2J + d. \]

The latter is a function of the zero-field splitting parameter, \( D \) and the angle \( \Psi \) between external magnetic field direction and the vector connecting two spins:

\[ d = D(\cos^2 \Psi - 1/3). \]

The zero-quantum transitions, with frequency, \( \Omega = \sqrt{q^2 + b^2}, \) are an important feature of singlet-born RPs. In the limit of weak coupling, \( \Omega \) reduces to the difference of the precession frequencies \( q. \)

\[ q = \Delta \omega_P - \Delta \omega_A. \]

The EPR transitions of the singlet-born RP can be described by delta functions in the linear response regime and after the decay of the initial zero-quantum coherence. With our assumption of weak spin-spin interactions, the EPR signal can be expressed as the sum of four delta-functions:

\[ I \sim I_P^S + I_A^S, \]

\[ I_{M-P,A}^S = \frac{1}{2} \left( \delta(\Delta \omega_M - a) - \delta(\Delta \omega_M + a) \right). \]

* Note, that this assumes transient phenomena like quantum beats, transient nutations, etc. can be ignored.
The resulting stick-spectrum consists of two antiphase doublets with a splitting, \( a \), centred at the resonance positions of the two radicals. The polarization pattern depends on the sign of \( a \), the longitudinal magnetic order part of the spin-spin coupling. With a positive it is \( E/A/E/A \) and \( A/E/A/E \) with a negative. Because the initial state is a singlet, the intensities of all four transitions are equal and are independent of the structural parameters. At first sight this would appear to make the spectra insensitive to the geometry of the RP. However, we must also take into account the inhomogeneous broadening due to local fields.

**Influence of the local magnetic fields**

Unresolved hyperfine couplings to magnetic nuclei lead to broadening of the EPR lines. Typically, the inhomogeneous linewidths \( 2D_{M=P,A} \) are 0.7–1.0 mT for protonated samples and 0.3–0.4 mT for deuterated samples, while the value of \( a \) is of the order of 0.1 mT. This means the linewidth is larger than the separation between the absorptive and emissive parts of the antiphase doublet and thus, there is a large degree of cancellation. An important consequence of the cancellation is that the signal of the doublet associated with each radical can be expanded in a Taylor series in \( a \) about the resonance magnetic field of the radical. Because of the asymmetric shape of the doublet, there are only odd terms in this series. Moreover, the small value of \( a \), allows the series to be truncated after the linear term. The relative error associated with the truncation is of the order \( (a/D_{M})^2 \), which is rather small for protonated samples. Thus, to a good approximation, we can consider the EPR signal of the singlet-born RP as a linear function of the spin-spin coupling parameter \( a \). Numerical simulations show that in practice, the effective error is even smaller and the truncation is also acceptable for deuterated samples. However, in this case it is important to explicitly test the validity of the truncation. Note, that the well-known fact that the spin-polarization patterns are insensitive to the strength of spin-spin coupling is a reflection of this linearity.

Next, we derive a lineshape function for the two antiphase doublets by integrating over a Gaussian distribution of local fields. The integrated lineshapes are indicated by overlined symbols:

\[ I \sim \bar{I}_P + \bar{I}_A, \quad \bar{I}_{M=P,A} = \frac{a \Delta \omega_M}{\sqrt{2\pi}D_M^2} \exp\left\{-\frac{\Delta \omega_M^2}{2D_M^2}\right\}. \]  

Equation (7) clearly shows the linear dependence of the intensity on the splitting of the doublets, \( a \). As we will see, this property is crucial to simplifying the extraction of structural information from the polarization patterns.

**Averaging over the random distribution of RCs**

In addition to the inhomogeneous broadening, we also take into account the random distribution of RCs in the sample by integrating the spectra over the surface of a sphere. We use angled brackets to indicate this integration, e.g. the integral of the function \( f \) over the spherical variables \( \theta \) and \( \phi \) is \( \langle f \rangle \).

Using the symmetry properties of the sine and cosine functions and the fact that the dipole interaction is traceless, we are able to express the experimental spectrum of each radical as the sum of three functions:

\[ I_{\text{exp}} = \sum_{M=P,A} \kappa_{M,J} \langle I_{M,J} \rangle + \kappa_{M,\Delta} \langle I_{M,\Delta} \rangle + \kappa_{M,s} \langle I_{M,s} \rangle. \]  

The important feature of Eq. (8) is that the powder averages \( \langle I_{M,J} \rangle, \langle I_{M,\Delta} \rangle \) and \( \langle I_{M,s} \rangle \) do not depend on the mutual orientation of two radicals:

\[ \langle I_{M,J} \rangle = \frac{1}{\sqrt{2\pi}D_M^2} \left( \Delta \omega_M \exp\left\{-\frac{\Delta \omega_M^2}{2D_M^2}\right\} \right), \]
\[ \langle I_{M,\Delta} \rangle = -\frac{1}{\sqrt{2\pi}D_M^2} \left( \Delta \omega_M \exp\left\{-\frac{\Delta \omega_M^2}{2D_M^2}\right\} \sin^2 \theta \cos 2\phi \right), \]
\[ \langle I_{M,s} \rangle = -\frac{1}{\sqrt{2\pi}D_M^2} \left( \Delta \omega_M \exp\left\{-\frac{\Delta \omega_M^2}{2D_M^2}\right\} \left( \cos^2 \theta - 1/3 \right) \right). \]  

The structural information is contained in the weighting factors \( \kappa_{M,J}, \kappa_{M,\Delta}, \) and \( \kappa_{M,s} \).

\[ \kappa_{M,J} = \varepsilon J, \quad \kappa_{M,\Delta} = -\frac{1}{2} \varepsilon D \sin^2 \theta \cos 2\phi_M, \]
\[ \kappa_{M,s} = -\frac{1}{2} \varepsilon D (3 \cos^2 \theta_M - 1). \]  

The angles \( \theta_M \) and \( \phi_M \) describe the orientation of the dipole axis in the molecular frame of radical \( M = P, A \). The parameter \( \varepsilon \) is a scaling factor relating the amplitudes of the calculated and experimental spectra.

The advantage of Eqs. (8)–(10) is that the powder averages are calculated only once and the structural information is determined by fitting the experimental spectrum with a linear combination of the powder spectra. More importantly, these equations also demonstrate the restrictions on information obtainable from the data. For RCs, the exchange interaction, \( J \), is negligibly small in the observable RPs and for TREPR data the value \( \varepsilon \) is generally unknown. Equations. (8)–(10) show that under these conditions, it is only possible to determine three independent \( \kappa \)-values for the four independent angles. In other words, the set of angles is a function of the scaling factor \( \varepsilon \). We have demonstrated this recently\(^5\) for zinc-containing bacterial RCs by fitting the W-band polarization pattern.\(^6\) The fit does not give a single set of angles but rather two curves, one for the donor \( P^+ \) and one for the acceptor \( Q^- \) as functions of \( \varepsilon \). Each point on the curves corresponds to a pair of angles, consistent with the data, for a particular value of \( \varepsilon \). In general, the value of \( \varepsilon \) can be determined using independent information to fix one angle and the curve for either the donor or acceptor. The curve for the other radical partner can then be used to obtain structural information. The crucial point is that the angles cannot be determined from the polarization patterns alone because at least one angle or the value of \( \varepsilon \) must be known.

**The influence of the precursor**

In many cases, the model outlined above is inadequate because spin evolution during the lifetime of the precursor RP causes an admixture of triplet character to the initial singlet state and thus influences the polarization pattern.\(^7\)–\(^9\) It is convenient\(^8\)–\(^10\) to describe the overall polarization as the sum of a contribution corresponding to a singlet precursor and an additional polarization, \( p \), due to spin evolution in the precursor state(s). When the eigenstates of the observable pair are close to the direct product spin states of non-interacting radicals (i.e. when the coupling is weak), the additional polarization depends only on the parameters of the
p = q₀b₀/(q₀² + b₀² + k²) \qquad (11)

The parameters \( q₀ \) and \( b₀ \) have the same meaning as in the Eqs. (3), (5), and \( k \) is the inverse mean life-time of the precursor pair. Here, we use the subscript “0”, to indicate the precursor pair which may be either \( R₁ \) or \( P₁ \) depending on the particular case under consideration. Even though the value of \( p \) is small when \( k \) is large, it can have a significant effect on the observed polarization pattern because, the contribution to both lines of the doublet has the same sign. Thus, unlike the singlet contribution line broadening does not lead to cancellation. From Eq. (11) it is obvious, that for very short-lived precursors (\( k² >> b₀² + q₀² \)), the polarization \( p \) is linearly proportional to \( q₀ \), i.e. to the difference of the precession frequencies of the precursor radicals. This difference can be divided into contributions from the Zeeman and hyperfine interactions and the corresponding contributions to the polarization are known in the CIDEP literature as net and multiplet polarization, respectively.

In RCs, \( R₁ \) is usually short-lived and its spin-spin coupling, \( b₀ \) is usually dominated by the exchange interaction, \( J \). From Eq. (11) it can be seen that under these conditions the extra polarization, \( p \), observed in \( R₁ \) can be used to determine the sign of the exchange integral in \( R₁ \). Eq. (11) also shows that \( p \) is maximal around \( q₀² \sim b₀² + k² \) and since \( q₀ \) is field dependent, EPR measurements at different bands allow the lifetime of precursor to be estimated if the value of its spin-spin coupling is known and vice versa. It is also important to note, that since \( p \) has a maximum, measuring the polarization at sufficiently high field reduces the amplitude of the extra polarization. We recently demonstrated this property for the precursor pair.

Here, we investigate the influence of biphasic kinetics on the polarization patterns in PS1. Optical data\(^{11}\) show that in many samples the electron transfer from \( A₁ \) to \( F₅ \) is governed by lifetimes of \( \sim 20 \) ns and \( \sim 200 \) ns at 300 K. However, the relative amplitude of the two phases appears to be species and preparation dependent, which has lead to considerable discussion about whether the two phases represent bi-directional electron transfer or heterogeneity.\(^{12-14}\) Decay associated spin polarization patterns of the states \( P₇⁰⁰⁻\) and \( P⁷ₐ⁻ \) can be extracted from the time/field TREPR data sets,\(^{15}\) but because the lifetime of the fast kinetic phase is below the \( \sim 50 \) ns response time of the spectrometer it is difficult to determine its amplitude accurately by fitting the data. In Fig. 1, spectra associated with the slow kinetic phase are shown for PS1 from two different organisms for different assumed ratios of the fast and slow components. The solid spectra are the amplitudes associated with the slow kinetic phase extracted from the experimental data and the dashed spectra are simulations based entirely on independent information.\(^{9,10}\) As can be seen, the agreement between the simulations and experiment for the cyanobacterium \textit{Synechocystis} 6803 (Fig. 1 top left) is very good when a single 200 ns kinetic component is assumed. In particular, the relative amplitudes of the two spectra, which are not scaled in any way, are reproduced satisfactorily. In contrast, for the eukaryotic green-alga \textit{Chlamydomonas reinhardtii}, the spectra extracted using the same assumption (Fig. 1 top right) do not agree with the simulation. However, when we assume a 2:1 ratio of the two kinetic phases the \textit{Chlamydomonas} spectra (Fig. 1 bottom right) agree with the simulation and the \textit{Synechocystis} spec-

---

\(^{10}\) Chemical induced dynamic electron polarization.
tra (Fig. 1 bottom left) do not. Care must be taken in making quantitative conclusions from this result because of the uncertainty associated with some of the parameters used in the simulation and assumptions made in the kinetic model used to extract the spectra. For instance, the function used to extract the spectra assumes the same spectral form for the two kinetic phases, taking into account the lifetime dependence of the extra polarization, $p$. Simulations show that although the deviations are small, this assumption is not strictly correct and probably accounts for the discrepancies in the high field emission between the simulated and experimental spectra of *Chlamydomonas*. We have also assumed the same geometry and magnetic parameters for both species and although, this is very reasonable we cannot exclude the possible that differences could exist. Nonetheless, it is clear that the proportions of the two kinetic phases are very different in the two organisms with a much larger fraction of the fast phase in the *Chlamydomonas* sample. An important aspect of this analysis is that we have overcome the limitation of the response time of the spectrometer in separating the two kinetic phases. Moreover by including the magnetic field/microwave frequency dependence and independent structural information, the analysis of the polarization patterns yields details about the species involved in the electron transfer and their lifetimes.

Conclusions and Outlook

The procedures outlined here provide a straightforward procedure for analyzing the spin polarization patterns of RCs and give better insight into their physical origins and the limitations on the information obtainable. This is of considerable importance for the next generation of TREPR experiments particularly in PSI, in which we are studying details regarding the directionality of the light-induced electron transfer using site-directed mutagenesis as well as structure/function relationships by quinone replacement.

Acknowledgements

This work was supported by a NATO science fellowship to Yu. K. and grants from NSERC and CFI to AvdE

References