

# Magnetic-Field-Induced Orientation of Photosynthetic Reaction Centers As Revealed by Time-Resolved W-Band EPR of Spin-Correlated Radical Pairs

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Received: June 17, 1999; In Final Form: September 8, 1999

The spin-polarized W-band EPR spectra of the secondary radical pair in plant photosystem I indicate a magnetic-field-induced orientation of the photosynthetic reaction centers in the field of the EPR spectrometer. This orientation arises due to the anisotropy of the diamagnetic susceptibility of the reaction center protein. Analysis of the angular-dependent EPR spectra on the basis of the spin-correlated radical pair concept provides new information on the cofactor arrangement in plant photosystem I.

## 1. Introduction

Spin-correlated radical pairs<sup>1–3</sup> are the short-lived intermediates of the primary energy conversion steps of photosynthesis. Numerous publications have now appeared, reporting on these radical pairs in purple photosynthetic bacterial reaction center proteins and in green plant photosystem I (PSI) (for recent reviews see refs 4–6). Such interest is justified because the analysis of the spin-polarized EPR spectra provides information on the structural organization of the cofactors of the reaction center proteins (RCs) in a functional state other than the ground state. Similar is true for the analysis of quantum beat oscillations, detectable at early times after laser pulse excitation.<sup>7–9</sup>

To increase the spectral resolution, high-field EPR studies have been performed on various noncrystalline RC preparations.<sup>10–12</sup> So far, it has generally been assumed that the radical pairs are randomly distributed with respect to the magnetic field. In the present study, however, we show that magnetic-field-induced orientation of photosynthetic RCs occurs in the field of a W-band EPR spectrometer. This orientation arises due to the anisotropy of the diamagnetic susceptibility of the RC protein. When taking the orientation effect into account, the correlated radical pair model<sup>2,3</sup> satisfactorily describes the observed EPR spectra, providing new structural information.

## 2. Theory

In this section we briefly summarize a model for time-resolved EPR of spin-correlated radical pairs in photosynthetic RCs.<sup>7</sup> Specifically, we consider a sudden light-induced generation of the radical pair in a singlet state. Particular emphasis is given to an anisotropic distribution of the radical pair with respect to the laboratory frame. Neglecting hyperfine interactions, the spin Hamiltonian,  $H(\Omega)$ , can be written as

$$H(\Omega) = \beta B_0(g_1^{zz}(\Omega)S_1^z + g_2^{zz}(\Omega)S_2^z) + 2(D^{zz}(\Omega) - J_{ex})S_1^zS_2^z - \left(\frac{1}{2}D^{zz}(\Omega) + J_{ex}\right)(S_1^-S_2^+ + S_1^+S_2^-), \quad (1)$$

where  $\beta$ ,  $B_0$ ,  $g_i^{zz}$ ,  $S_i^z$ ,  $D^{zz}(\Omega)$ ,  $J_{ex}$ , and  $S_i^\pm$  ( $S_i^+$ ) are the Bohr magneton, the static magnetic field, the  $zz$  component of the  $g$ -tensor  $\mathbf{g}_i$ , the  $z$  component of the electron spin operator  $\mathbf{S}_i$ , the  $zz$  component of the electron dipolar coupling tensor  $\mathbf{D}$ , the isotropic electron exchange interaction, and the lowering (raising) operator of electron  $i$ , respectively. The orientation dependence of the magnetic tensor elements  $g_i^{zz}(\Omega)$  and  $D^{zz}(\Omega)$  can be evaluated by a two-fold transformation from the corresponding principal axis system,  $\mathbf{X}_i$ ,  $\mathbf{Y}_i$ ,  $\mathbf{Z}_i$ , in which the tensor is diagonal (Figure 1).<sup>7</sup> In the first step we transform to a magnetic reference system,  $\mathbf{X}$ ,  $\mathbf{Y}$ ,  $\mathbf{Z}$ , using the Euler angles,  $\Omega_i = (\Phi_i, \Theta_i, \Psi_i)$  (Figure 1). In the second step we rotate by the Euler angles  $\Omega = (\Phi, \Theta, \Psi)$  in the laboratory frame,  $\mathbf{x}$ ,  $\mathbf{y}$ ,  $\mathbf{z}$ , (Figure 1).

The basic EPR spectrum of a singlet radical pair with a given orientation,  $\Omega$ , consists of four peaks arranged as two doublets with equal splittings determined by the spin–spin coupling between the radicals. Both doublets have one component emissive and the other absorptive, reflecting the non-equilibrium population of the four electron spin states.<sup>2,3</sup> Integration over all possible orientations yields the observable “powder” line-shape.

High-field EPR spectra from samples, frozen in the presence of the magnetic field, indicate an *anisotropic distribution* of the radical pair with respect to the laboratory frame. The most probable physical origin of this effect is an anisotropy in the diamagnetic susceptibility of the RC protein. If we make the simplifying assumption that the susceptibility tensor is axially symmetric in its principal axis system,  $\mathbf{X}'$ ,  $\mathbf{Y}'$ ,  $\mathbf{Z}'$  (Figure 1), the *orientational distribution function* of the RCs can be written as

$$f(\beta) = N \exp(A \cos^2 \beta) \quad (2)$$

$$A = \frac{1}{2} \Delta\chi V B_0^2 / (\mu_0 kT) \quad (3)$$

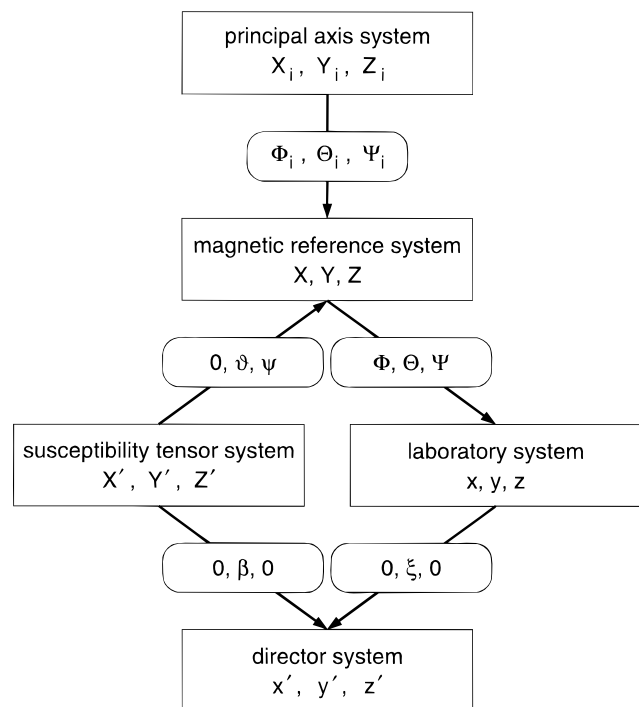
Here,  $N$  is a normalization constant,  $\beta$  is the angle between the

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**Figure 1.** Notation for coordinate systems and Euler transformations, used in the EPR model.  $z$  = magnetic field direction,  $z'$  = director axis (net ordering axis).

symmetry axis of the susceptibility tensor and the director axis (net ordering axis),  $\Delta\chi$  is the anisotropy in the volume diamagnetic susceptibility,  $V$  is the effective volume,  $\mu_0$  is the vacuum permeability,  $k$  is the Boltzmann constant, and  $T$  is the absolute temperature. The coefficient  $A$  in eq 2 characterizes the magnetic-field-induced alignment of the RCs along the director axis,  $z'$ , while the angle  $\xi$  specifies the orientation of  $z'$  in the laboratory frame (Figure 1). The orientational order parameter,  $S_{ZZ'}$ , is related to the coefficient  $A$  by a mean value integral:

$$S_{ZZ'} = \frac{1}{2} N \int_0^\pi (3 \cos^2 \beta - 1) \exp(A \cos^2 \beta) \sin \beta d\beta \quad (4)$$

If we assume that the principal axis of the susceptibility tensor,  $Z'$ , has a fixed orientation,  $\vartheta, \psi$ , with respect to the magnetic reference system (Figure 1), it is possible to express  $\cos \beta$  as a function of  $\Phi, \Theta, \Psi$ :

$$\begin{aligned} \cos \beta = & [(\sin \Phi \sin \Psi - \cos \Phi \cos \Theta \cos \Psi) \sin \xi - \\ & \cos \Phi \sin \Theta \cos \xi] \sin \vartheta \cos \psi + [(\cos \Phi \sin \Psi + \\ & \sin \Phi \cos \Theta \cos \Psi) \sin \xi + \\ & \sin \Phi \sin \Theta \cos \xi] \sin \vartheta \cos \psi + (\cos \Theta \cos \xi - \\ & \sin \Theta \cos \Psi \sin \xi) \cos \vartheta \quad (5) \end{aligned}$$

Combining eqs 5 and 2 yields the *orientational distribution function* of the radical pair with respect to the laboratory frame, necessary for a proper analysis of high-field EPR experiments on *magnetically aligned* RCs.

### 3. Materials and Methods

Lyophilized, whole cells of deuterated (99.7%),  $^{15}\text{N}$ -substituted (95%) *S. lividus* cyanobacteria were rehydrated in deuterated Tris buffer (uncorrected pH = 7.5), containing 50% glycerol as cryo-protection. About 0.6  $\mu\text{L}$  of the sample was used to fill a quartz capillary (0.9 mm o.d., 0.5 mm i.d.), located

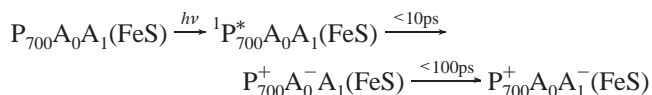
in the symmetry axis of the microwave resonator. Magnetic-field-induced alignment of the RCs was achieved at room temperature with a field of 3.35 T. The samples were then cooled to 90 K and the EPR spectra were measured as a function of the angle  $\xi$  between director axis and magnetic field.

All EPR experiments were carried out on a Bruker ELEXSYS E680 W-band EPR spectrometer, equipped with a TE<sub>011</sub> cylindrical cavity and high bandwidth mixer detection. The field of the superconducting magnet was calibrated against LiF:Li (Institute of Crystallography, Moscow), which is a good standard for low-temperature measurements.<sup>13</sup> Optical excitation of the sample was performed using 2.5 ns pulses of a Q-switched, frequency-doubled Nd:YAG laser (Spectra Physics Quanta Ray GCR 130–15). The laser output was attenuated to approximately 1 mJ/pulse. Excitation of the sample in the resonator was achieved using a fiber optic light path through the sample rod (silica optical fiber, 400  $\mu\text{m}$  core diameter, Fiberguide Industries), as described previously.<sup>14</sup>

The time-resolved EPR signal (CW mode) was recorded and averaged with a LeCroy 9354A digital oscilloscope. Typically, 400 transients were accumulated at off-resonance conditions and subtracted from those on-resonance to get rid of the laser background signal. A weak chlorophyll triplet signal, superimposed on the radical pair signal, was subtracted using a linear interpolation procedure. Generally, a complete data set consisted of transient EPR signals taken at equidistant field points covering the total spectral width. Transient spectra were extracted from the two-dimensional plot at any given time after the laser pulse as slices along the magnetic field axis.

### 4. Results and Discussion

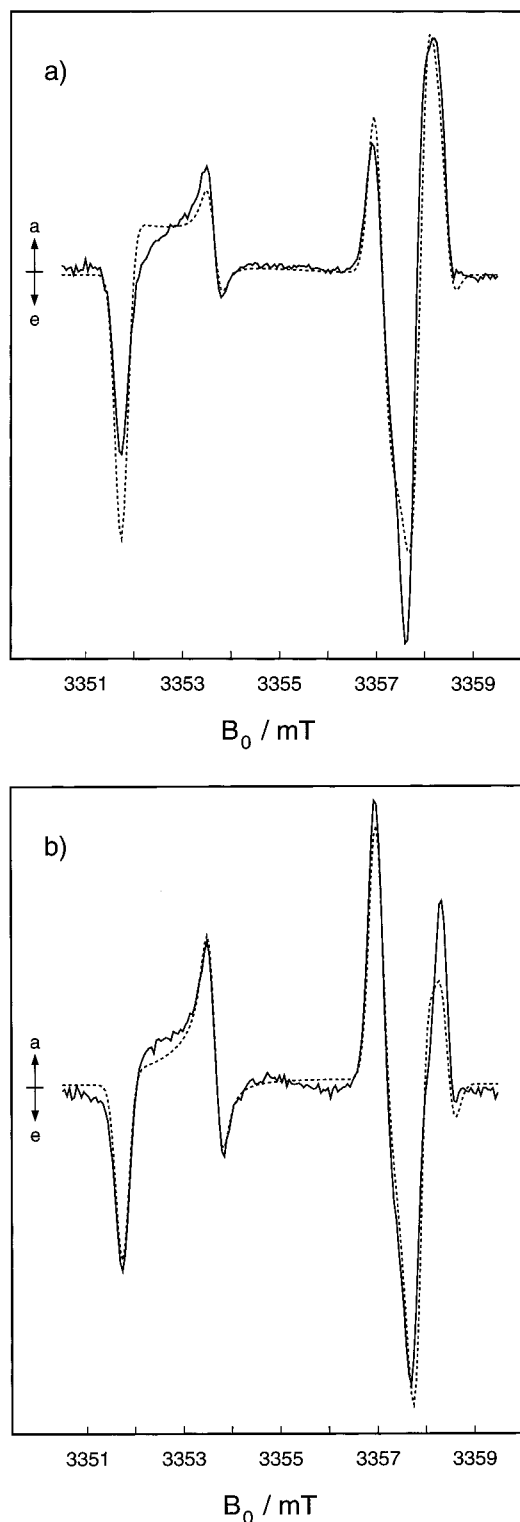
The initial photochemical conversion steps in PSI are described as follows:<sup>15</sup>



Here  $\text{P}_{700}$  is the primary chlorophyll donor,  $\text{A}_0$  is an intervening chlorophyll acceptor,  $\text{A}_1$  is a phylloquinone molecule, and FeS are iron–sulfur centers. After photoexcitation of  $\text{P}_{700}$ , charge separation takes place in a few picoseconds, forming the primary radical pair,  $\text{P}_{700}^+\text{A}_0^-$ . The electron is then transferred to the secondary acceptor  $\text{A}_1$  in less than 100 ps. At low temperatures,  $\text{P}_{700}^+\text{A}_1^-$  has a lifetime of about 150  $\mu\text{s}$ , and it decays primarily by charge recombination.

Transient W-band EPR spectra of  $\text{P}_{700}^+\text{A}_1^-$  were measured for suspensions of deuterated,  $^{15}\text{N}$ -substituted *S. lividus* cyanobacteria, cooled to 90 K in the presence of the static magnetic field of 3.35 T. To establish any field-induced alignment of the RCs, the line shapes were taken at two different orientations of the frozen sample. Typical results are shown in Figure 2. The upper EPR spectrum (solid lines, Figure 2a) refers to the original sample orientation immediately after cooling ( $\xi = 0^\circ$ ). For the lower spectrum (solid line, Figure 2b) the frozen sample was rotated by  $90^\circ$  about an axis perpendicular to the magnetic field ( $\xi = 90^\circ$ ). Pronounced spectral differences are observed particularly in the high field region. If, however, the sample was cooled in the absence of the magnetic field, two identical line shapes were detected (results not shown).

Apparently, the PSI RCs of *S. lividus* cyanobacteria have been aligned in the static magnetic field of the W-band spectrometer, as expected on theoretical grounds.<sup>16</sup> The alignment corresponds to previous observations for other photosynthetic systems. Using



**Figure 2.** Transient W-band EPR spectra of the light-induced radical pair,  $P_{700}^+A_1^-$ , in plant photosystem I at two different orientational distributions with respect to the laboratory frame. Positive and negative signals indicate absorptive and emissive polarizations, respectively. (a) Director parallel to the magnetic field,  $\xi = 0^\circ$ . (b) Director perpendicular to the magnetic field,  $\xi = 90^\circ$ . Full lines: Experimental spectra from deuterated,  $^{15}\text{N}$ -substituted cyanobacteria *S. lividus*. The signal intensity was averaged in the time window 0.4–2.4  $\mu\text{s}$ . Microwave field,  $B_1 = 0.01$  mT. Microwave frequency,  $\omega/2\pi = 94.1163$  GHz,  $T = 90$  K. Dashed lines: Calculated spectra using the parameters given in Table 1.

fluorescence polarization and linear dichroism measurements, magneto-orientation could be established for whole cells of

various green algae and photosynthetic bacteria.<sup>17,18</sup> Interestingly, no such effects were reported in recent high-field EPR studies of photosynthetic RCs.<sup>10–12</sup> It appears that the degree of magneto-orientation crucially depends on the sample preparation as well as on the field strength employed.<sup>17,18</sup>

Analysis of the transient W-band EPR spectra of  $P_{700}^+A_1^-$  was performed using the model outlined in the Theory section. Table 1 summarizes the structural and magnetic parameters used in the calculations. The g-tensor components of  $P_{700}^+$  and  $A_1^-$  were adapted from recent high-field EPR studies of various PSI preparations.<sup>10,19,20</sup> The listed spin–spin coupling parameters are based on electron spin–echo envelope modulation (ESEEM) studies of  $P_{700}^+A_1^-$ .<sup>21</sup> Residual hyperfine interactions in the fully deuterated and  $^{15}\text{N}$ -substituted sample were considered by inhomogeneous Gaussian line widths. The orientation of the magnetic tensors (geometry of  $P_{700}^+A_1^-$ ) was determined from the  $B_0$ -dependence of Q-band EPR quantum beats.<sup>22</sup> The listed Euler angles<sup>23</sup> relate the principal axis system of the respective magnetic tensor and the magnetic reference system (g-tensor of  $P_{700}^+$ ).

The parameters of the orientational distribution function (see eq 5) were determined by simultaneously fitting the two angular-dependent W-band EPR spectra, measured with the director axis either parallel ( $\xi = 0^\circ$ ) or perpendicular ( $\xi = 90^\circ$ ) to the magnetic field. The dashed lines in Figure 2a,b represent best simulations based on the parameter values  $S_{ZZ} = 0.13$ ,  $\vartheta = 81^\circ$ , and  $\psi = 133^\circ$ . Generally, the agreement achieved is good. In particular, the differential intensities in the high field part of the spectrum are faithfully reproduced by the calculations. This provides unambiguous confirmation of the magnetic-field-induced alignment of photosynthetic RCs.

Generally, the PSI complexes are embedded within the thylakoid membrane, consisting of mostly parallel phospholipid bilayers. Theoretical studies indicate that  $\alpha$ -helices might be a source of anisotropic diamagnetic susceptibility.<sup>16</sup> In cyanobacterial PSI, a total of 34 transmembrane  $\alpha$ -helices are aligned to within a small angle of the membrane normal,<sup>24</sup> which thus represents a natural choice for the principal axis of the susceptibility tensor,  $Z'$ . Furthermore, any anisotropy in the plane of the membrane will be averaged out for such an arrangement. It is therefore reasonable to identify the symmetry axis of the susceptibility tensor with the membrane normal.

An order parameter of  $S_{ZZ} = 0.13$  indicates only partial magnetic alignment of the RC proteins. This is not surprising in view of the complex biological material, which might orient nonuniformly, e.g., with a small fraction of well-aligned PSI and a large fraction of disordered PSI. Simulations of the experimental W-band spectra employing such an approach yielded similarly good fits as obtained on the basis of the unimodal distribution function (eqs 2 and 5). Notably, the obtained values of  $\vartheta$  and  $\psi$  did not vary significantly from those given in Table 1. We are therefore confident that the extracted geometry is basically correct.

Evidently, the present results do not allow for a more detailed characterization of the orientational distribution of the RC proteins. However, analysis of the angular-dependent W-band spectra as a function of the alignment field could provide the desired information. Studies along these lines are currently in progress.

Knowledge of the orientation of the membrane normal in the magnetic reference system, makes it possible to depict the geometry of  $P_{700}^+A_1^-$  in the photosynthetic membrane, as shown in Figure 3. The structural model for the quinone acceptor,  $A_1^-$ , is in substantial agreement with recent EPR and crystallographic



