Magnetic-Field-Induced Orientation of Photosynthetic Reaction Centers, As Revealed by Time-Resolved D-Band Electron Paramagnetic Resonance of Spin-Correlated Radical Pairs. II. Field Dependence of the Alignment†

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Received: July 30, 2003; In Final Form: February 2, 2004

The magnetic-field-induced orientation of photosynthetic reaction centers has been studied by time-resolved D-band (130 GHz) electron paramagnetic resonance (EPR) of the secondary radical pair, $P_{700}^{+}A_1^{-}$, in plant photosystem I (PSI). Experiments have been performed for fresh and lyophilized whole cells of the deuterated cyanobacterium *S. lividus*. A computer fit of the angle-dependent D-band spectra, measured for two different sample orientations, provides the order parameter $S_{Z'Z'}$ of the symmetry axis, $\boldsymbol{Z'}$, of the susceptibility tensor, relative to the magnetic field. The positive sign of this order parameter indicates that membrane proteins are the major source for the anisotropy of the diamagnetic susceptibility, $\Delta\chi V$. A value for $\Delta\chi V$ has been extracted from the magnetic-field dependence of $S_{Z'Z'}$. The value of $\Delta\chi V = 5.7 \times 10^{-27}$ m^3 is in good agreement with an estimate for the susceptibility anisotropy of a cyanobacterial cell. This demonstrates that whole cells are aligned in the magneto-orientation process. The combination of high-field EPR of a magnetically oriented sample with the analysis of quantum beat oscillations allows determination of the three-dimensional structure of $P_{700}^{+}A_1^{-}$ in the photosynthetic membrane.

1. Introduction

The primary energy conversion steps of photosynthesis proceed via spin-correlated radical pairs[1−4] as short-lived intermediates. In green plant photosystem I (PSI),[5,6] this electron-transfer process involves the primary chlorophyll donor (P_{700}) and a series of membrane bound acceptors (A_0, A_1, and FeS, where A_0 denotes an intervening chlorophyll acceptor,[6] A_1 is a phylloquinone molecule,[7] and FeS are iron−sulfur centers[5,6]). The radical pair $P_{700}^{+}A_1^{-}$ is the first intermediate that has been detectable by time-resolved electron paramagnetic resonance (EPR). At room temperature, $P_{700}^{+}A_1^{-}$ decays in ∼200 ns by forward electron transfer to the FeS centers.[8−11] At low temperature, the lifetime of $P_{700}^{+}A_1^{-}$ is ∼150 μs, and it decays primarily by charge recombination.[12]

Recently, we have determined the geometry of the charge-separated state $P_{700}^{+}A_1^{-}$, using high-time-resolution transient EPR performed at three different microwave frequencies.[13] Structural information was extracted from quantum beat oscillations and high-field EPR spectra of a magnetically aligned sample.[14] In this way, we have evaluated the three-dimensional structure of the radical pair intermediate $P_{700}^{+}A_1^{-}$ in its native membrane. Thus, we also obtained the co-factor arrangement of $P_{700}^{+}A_1^{-}$, with respect to the membrane.

An important milestone in the development of the new structural technique was the observation that the PSI reaction centers are aligned in the field of a W-band (94 GHz) EPR spectrometer.[14] Analysis of the experimental EPR spectra of $P_{700}^{+}A_1^{-}$ provided the orientation of the membrane normal in a magnetic reference system. Knowledge of this orientation made it possible to determine the three-dimensional structure of $P_{700}^{+}A_1^{-}$ in the photosynthetic membrane.[13,14] Until now, however, the details of the magneto-orientation process have not been known. Are membrane proteins the major source for the anisotropy of the diamagnetic susceptibility as assumed in the previous study?[14] How much do lipid membranes contribute to this anisotropy? Are whole bacterial cells oriented by the magnetic field and, if so, in which way? Can we rationalize the extracted geometric and order parameters in terms of a molecular model?

The objective of the present study is to evaluate the mechanism of the magneto-orientation process and, thus, answer the aforementioned questions. To achieve this goal, we examine time-resolved D-band (130 GHz) EPR spectra of the radical pair $P_{700}^{+}A_1^{-}$ in whole cells of the fully deuterated cyanobacterium *S. lividus*. A computer fit of these spectra provides the order parameter of the principal axis of the susceptibility tensor, relative to the magnetic field. The positive sign of this order parameter indicates that membrane proteins are the major source

† Part of the special issue "Jack H. Freed Festschrift".

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10.1021/jp036232c CCC: $27.50 © 2004 American Chemical Society
Published on Web 04/02/2004

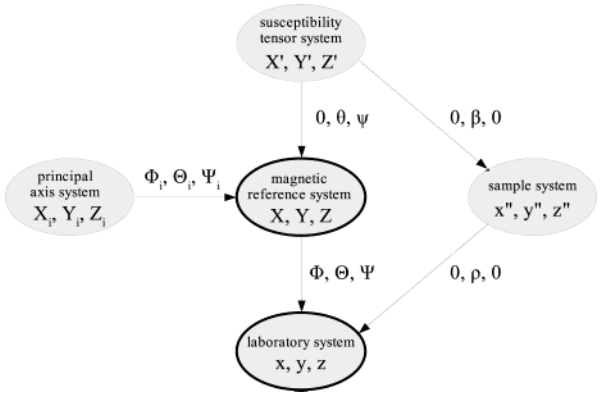

Figure 1. Notation for coordinate systems and Euler transformations used in the EPR model for the spin-correlated radical pairs in photosynthetic reaction centers. ***X***,***Y***,***Z*** represents the molecular reference frame in which one of the two g-tensors of the radical pair is diagonal; ***x***,***y***,***z*** is the laboratory frame with the *z*-axis parallel to the magnetic field; ***X′***,***Y′***,***Z′*** denotes the principal axis system of the susceptibilty tensor axially symmetric along axis ***Z′***; and ***x″***,***y″***,***z″*** represents the sample system, where ***z″*** specifies the macroscopic axis of symmetry of the angular distribution.

for the anisotropy of the diamagnetic susceptibility. An experimental value for this anisotropy can be extracted from the magnetic-field dependence of the degree of orientation. The value is in good agreement with an estimate for the susceptibility anisotropy of a cyanobacterial cell. This demonstrates that whole cells are aligned in the magneto-orientation process. It is shown that high-field EPR of a magnetically aligned sample, in combination with the study of quantum beat oscillations, represents a powerful structural tool for the short-lived intermediates of photosynthesis.

2. Theory

We briefly summarize a stationary model for the spin-polarized EPR spectra of correlated radical pairs in photosynthetic reaction centers.[15] Particular emphasis is given to an anisotropic distribution of the radical pair, with respect to the laboratory frame. The distribution function accounts for magneto-orientation of photosynthetic reaction centers by the magnetic field of the EPR spectrometer.[14]

Spin-Polarized EPR Spectra. Neglecting hyperfine interactions, the spin Hamiltonian, $H(\Omega)$, of a radical pair can be written as

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

where μ_B is the Bohr magneton, B_0 the static magnetic field, $g_i^{zz}(\Omega)$ the *zz*-component of the g-tensor $\mathbf{g}_i$, S_i^z the *z*-component of the electron spin operator $\mathbf{S}_i$, $D^{zz}(\Omega)$ the *zz*-component of the electron dipolar coupling tensor $\mathbf{D}$, J_{ex} the isotropic electron exchange interaction,[16] and S_i^- (S_i^+) the lowering (raising) operator of electron *i*. The orientation dependence of the magnetic tensor elements $g_i^{zz}(\Omega)$ and $D^{zz}(\Omega)$ can be evaluated by a 2-fold transformation from the corresponding principal axis system, $\boldsymbol{X}_i,\boldsymbol{Y}_i,\boldsymbol{Z}_i$. In the first step, we transform to a molecular reference system, ***X***,***Y***,***Z***, in which one of the two g-tensors of the radical pair is diagonal, using the Euler angles[17] $\Omega_i = (\Phi_i, \Theta_i, \Psi_i)$. In the second step, we rotate by the Euler angles $\Omega = (\Phi, \Theta, \Psi)$ in the laboratory frame, ***x***,***y***,***z*** (see Figure 1).

In native photosynthetic reaction centers, even the secondary radical pair is created in a virtually pure singlet state, determined by the spin multiplicity of the excited primary donor. Generally, such a singlet radical pair is formed with spin-correlated population of only one-half of the eigenstates.[1−4] This results in high optical spin polarization, which has been exploited in structural studies of $P_{700}^+A_1^-$, using time-resolved EPR at different microwave frequencies.[15,18−21] In addition, there are coherences between the eigenstates of the radical pair, which can manifest themselves as quantum beats in an EPR experiment with adequate time resolution.[13,22−28]

The basic EPR spectrum of a spin-correlated radical pair with a given orientation Ω consists of four peaks, arranged as two doublets with equal splittings determined by the spin−spin coupling of the radical pair. Both doublets have one emissive component and the other component is absorptive, reflecting the nonequilibrium population of the four electron spin states.[1−4,15] Integration over all possible orientations Ω yields the observable "powder" line shape.

Orientational Distribution Function. 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.[14] The physical origin of this magneto-orientation is an anisotropy in the diamagnetic susceptibility of the photosynthetic system. If we assume that the susceptibility tensor is axially symmetric in its principal axis system, ***X′***,***Y′***,***Z′*** (see Figure 1), the orientational distribution function of the symmetry axis ***Z′*** can be written as[29]

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

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

Here, N is a normalization constant, $\Delta\chi$ the anisotropy in the volume diamagnetic susceptibility, V the effective volume, μ_0 the vacuum permeability, k the Boltzmann constant, and T the absolute temperature of the Boltzmann distribution. The angle β denotes the angle between the principal axis of the susceptibility tensor and the sample axis, ***z″***, determined by the magnetic field direction during sample orientation. Thus, ***z″*** specifies the macroscopic axis of symmetry of the angular distribution. The coefficient A in eq 2 characterizes the magnetic-field-induced alignment of the susceptibility tensor axes, relative to the sample axis ***z″***, whereas the angle ρ specifies the orientation of *z″* in the laboratory frame (see Figure 1). The orientational order parameter, $S_{Z'Z'}$, is related to the coefficient A by a mean value integral:

$$S_{Z'Z'} = \frac{1}{2}\int_0^\pi (3\cos^2\beta - 1) f(\beta) \sin\beta \, d\beta \quad (4)$$

The susceptibility tensor has a fixed orientation, (ϑ,ψ), with respect to the molecular reference system; therefore, $\cos\beta$ can be expressed as a function of the Euler angles Φ, Θ, Ψ (see Figure 1):[14]

$$\cos\beta = -\sin\rho\sin\vartheta\cos\Theta\cos\Psi\cos(\psi+\Phi) + \sin\rho\sin\vartheta\sin\Psi\sin(\psi+\Phi) - \sin\rho\cos\vartheta\sin\Theta\cos\Psi - \cos\rho\sin\vartheta\sin\Theta\cos(\psi+\Phi) + \cos\rho\cos\vartheta\cos\Theta \quad (5)$$

Equations 2 and 5 can be used to evaluate the statistical weight for the EPR spectrum of a given molecular orientation specified by the Euler angles $\Omega = (\Phi, \Theta, \Psi)$. Integration over all possible orientations Ω yields the anisotropic "powder" line shape. The

free adjustable parameters are $S_{Z'Z'}$, ϑ, and ψ, which can be determined by a simultaneous fit of two angle-dependent line shapes, measured with the sample axis either parallel ($\rho = 0°$) or perpendicular ($\rho = 90°$) to the magnetic field.

3. Materials and Methods

Sample Preparation. Deuterated (99.7%) *S. lividus* cells were grown at a temperature of 43 °C, as described previously.[30,31] Three different sample preparations were used in the experiments: fresh whole cells suspended in Tris buffer with an addition of 50% glycerol, lyophilized whole cells rehydrated in Tris buffer with an addition of 50% glycerol, and isolated PSI proteins with 20% glycerol. The samples were filled in quartz capillaries (0.55 mm outer diameter and 0.45 mm inner diameter) and kept in darkness.

Magnetic-field-induced alignment of the samples was achieved at room temperature with the variable magnetic field of the EPR spectrometer. Generally, the samples were kept in the alignment field for 20 min and then cooled to 75 K at a cooling rate of 10 K/min. The EPR spectra were recorded either with the original sample orientation ($\rho = 0°$) or after rotation of the sample by 90° about an axis perpendicular to the magnetic field ($\rho = 90°$). In control experiments, the sample was cooled in the absence of the magnetic field. In this case, two identical line shapes were observed for the $\rho = 0°$ and $\rho = 90°$ orientations.

EPR Mesurements. All EPR experiments were conducted on a home-built D-band (130 GHz/4.5 T) continuous wave/pulsed EPR spectrometer. The microwave bridge was constructed by Dr. V. N. Krymov (HF EPR Instruments, Inc.). The bridge consisted of two independent channels that were driven by a frequency-fixed free-running oscillator. Each channel contained a linear combination of IMPATT amplifiers. The microwave power from each channel was combined by a directional coupler and directed through the circulator to a single-mode TE_{011} cavity. The maximum output power of the circulator in the pulsed mode was 125 mW (34 ns $\pi/2$ pulse).

The cavity has several slits to allow for optical excitation and field modulation. Samples were illuminated in the cavity of the EPR spectrometer. Light excitation was achieved with an optical parametric oscillator (Opotek) pumped by a Nd:YAG laser (Quantel). The output of the laser was coupled to a fiber optic, to deliver light to the cavity (1 mJ per pulse). The excitation wavelength was 550 nm. The sample temperature was regulated by an Oxford temperature controller (model ITC 503) coupled to an Oxford continuous-flow cryostat (model CF 1200).

The D-band EPR spectra of the secondary radical pair in PSI were measured using the time-resolved electron spin−echo technique. Two microwave pulses followed a short 10 ns laser pulse after a fixed delay time. The lengths of the microwave pulses were in the range of 40−70 ns. The spin-polarized EPR spectra of the radical pair were recorded by monitoring the electron spin−echo intensity, as a function of the magnetic field. The delay after laser flash (DAF) time was set to 1 μs for all experiments.

Computations. A Fortran program based on the theoretical approach outlined in the previous section was used to analyze the D-band EPR experiments. The program calculates EPR line shapes of spin-correlated radical pairs in photosynthetic reaction centers with a spatially fixed geometry. Particular emphasis is given to an anisotropic distribution of the radical pair, with respect to the laboratory frame. The free adjustable parameters of the orientational distribution function were evaluated using a nonlinear least-squares fit procedure, based on the Levenberg−Marquardt algorithm.[32]

4. Experimental Results and Analysis

Whole cells and isolated PSI proteins of the deuterated cyanobacterium *S. lividus* were irradiated with a short laser pulse, and the spin-polarized D-band EPR spectra of the radical pair $P_{700}^{+}A_1^{-}$ were measured using the time-resolved electron spin−echo technique. In all experiments, the sample was first oriented in a given alignment field at room temperature and then cooled to 75 K for the EPR measurements. No magneto-orientation effect was observed in the case of isolated PSI proteins. Fresh whole cells and lyophilized whole cells showed reproducible results only if 50% glycerol was added to the sample. Glass formation seems to be an important condition for the observation of magneto-orientation at low temperatures. Yet, the degree of orientation achieved still differed from sample to sample, corresponding to an uncertainty of approximately ±15%.

Angle-Dependent EPR Spectra. Typical D-band EPR spectra of $P_{700}^{+}A_1^{-}$ in fresh whole cells of the cyanobacterium *S. lividus* are shown in Figure 2. Magnetic orientation was achieved at room temperature with an alignment field of 7.0 T. After cooling to 75 K, the line shapes were taken at two different orientations of the frozen sample. The upper EPR spectrum (solid line, Figure 2a) refers to the original sample orientation immediately after cooling ($\rho = 0°$). For the lower spectrum (solid line, Figure 2b) the frozen sample was rotated by 90° about an axis perpendicular to the magnetic field ($\rho = 90°$). Pronounced spectral differences are observed in various regions of the spectra. If, however, the sample was cooled in the absence of the magnetic field, two identical line shapes were detected (results not shown). Evidently, the PSI reaction centers of *S. lividus* cyanobacteria have been aligned in the magnetic field of the D-band EPR spectrometer. The alignment corresponds to previous observations for other photosynthetic systems by optical techniques.[29,33]

The degree of magneto-orientation for a given alignment field can be extracted from the spin-polarized EPR spectra of $P_{700}^{+}A_1^{-}$, using the above outlined EPR model. Table 1 (columns 1−6) summarizes the fixed magnetic[19,34−36] and structural parameters[13] used in the calculations. The free adjustable parameters of the orientational distribution function were determined by simultaneously fitting the two angle-dependent D-band spectra, measured with the sample axis either parallel ($\rho = 0°$) or perpendicular ($\rho = 90°$) to the magnetic field. The dashed lines in Figure 2 represent best simulations based on the parameter values (see Table 1, column 7)

$$S_{Z'Z'} = 0.10 \pm 0.02$$

$$\vartheta = 83^{o} \pm 4^{o}$$

$$\psi = 120^{o} \pm 6^{o}$$

Generally, the agreement achieved is good, although some deviations still exist. The cited errors are linear confidence limits that correspond to a 95% confidence level.

The result of the fit was verified by repeating the fit procedure for a large number of different starting values, covering the whole parameter space. In the majority of all test runs, the same global minimum values were obtained, including the small positive value for the order parameter $S_{Z'Z'}$. The positive sign of $S_{Z'Z'}$ implies that the susceptibility tensor axes $\boldsymbol{Z'}$ orient preferentially parallel to the magnetic field. This is clear evidence for membrane proteins being the major source of the anisotropy in the diamagnetic susceptibility,[37,38] and a similar result has been obtained for membranes studied by a neutron

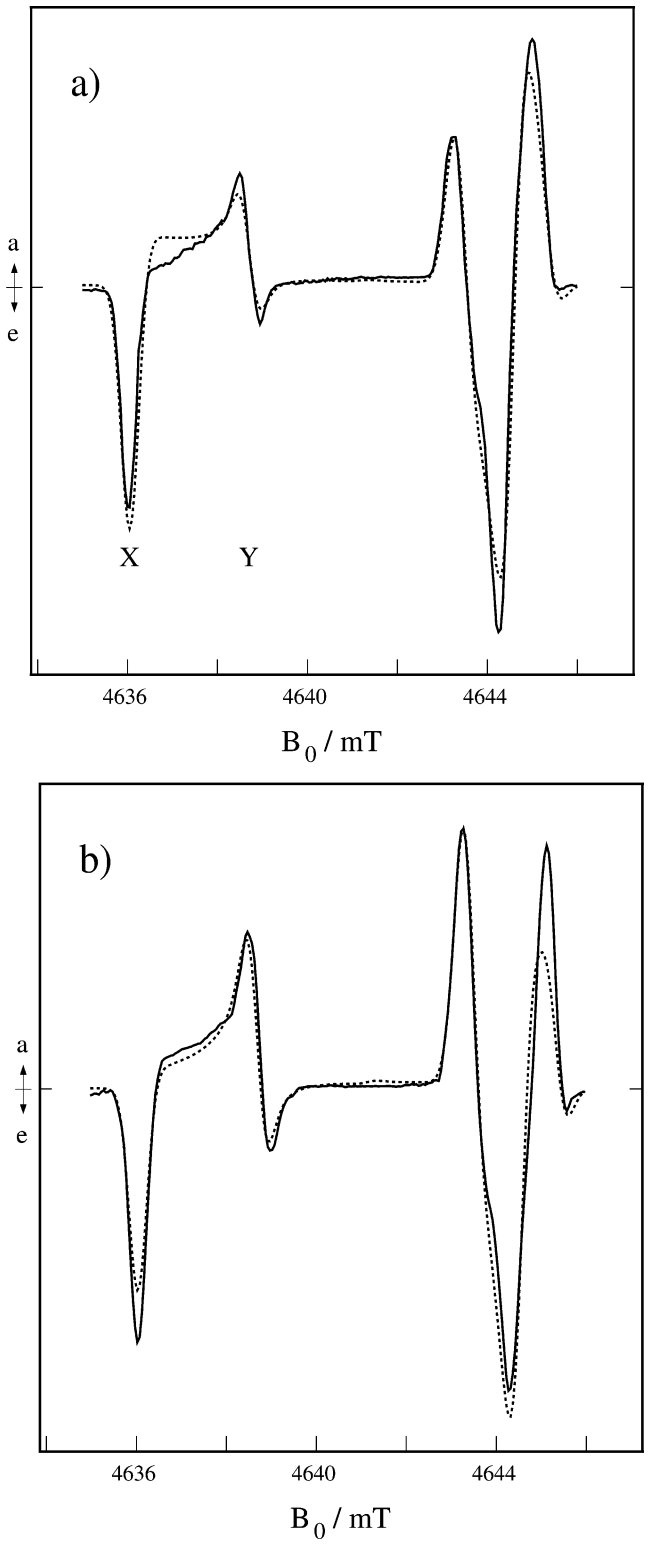

Figure 2. Spin-polarized D-band EPR spectra of the light-induced radical pair, $P_{700}^{+}A_1^{-}$, in plant photosystem I at two different orientational distributions, with respect to the laboratory frame ((a) sample axis parallel to the magnetic field, $\rho = 0°$, and (b) sample axis perpendicular to the magnetic field, $\rho = 90°$). Positive and negative signals indicate absorptive and emissive polarizations, respectively. Full lines represent experimental spectra from the deuterated cyanobacterium *S. lividus*, whereas dashed lines represent calculated spectra using the parameters given in Table 1. Magneto-orientation of the sample was achieved at room temperature with an alignment field of $B_0 = 7$ T. The EPR spectra were recorded using the time-resolved electron spin−echo technique. $T = 75$ K.

scattering technique.[39] If lipid membranes were the dominant source for the anisotropy in the diamagnetic susceptibility, one would expect the susceptibility tensor axes $\boldsymbol{Z}'$ to be oriented mainly perpendicular to the magnetic field; i.e., the order parameter $S_{Z'Z'}$ is negative.[40]

In the cyanobacteria, the photosynthetic proteins are embedded within the thylakoid membrane, which consists of mostly parallel glycolipid bilayers. Theoretical studies indicate that α-helices are a source of anisotropic diamagnetic susceptibility responsible for the alignment of the helix axis parallel to the magnetic field.[37,38] In cyanobacterial PSI, as well as in many other membrane proteins, a total of ∼30 transmembrane α-helices are aligned to within a small angle of the membrane normal,[6] which thus represents a natural choice for the symmetry axis of the susceptibility tensor, $\boldsymbol{Z}'$. Furthermore, any anisotropy in the plane of the membrane will be averaged out for such an arrangement. Therefore, it is reasonable to identify the symmetry axis of the susceptibility tensor with the membrane normal.

The evaluated order parameter of $S_{Z'Z'} = 0.10$ is somewhat smaller than that reported previously (see Table 1, column 8).[14] A value of 0.10 indicates only partial alignment of the membrane normals along the magnetic field. However, we shall see that this small value can be rationalized in terms of a simple molecular model for the magneto-orientation process (see the Discussion section). The computer fit of the experimental D-band spectra also provides the orientation, ϑ and ψ, of the membrane normal in the g-tensor system of P_{700}^{+}. Within the error limits, the values of $\vartheta = 83°$ and $\psi = 120°$ correspond to those determined previously by W-band EPR (see Table 1, column 8).[14] Knowledge of this orientation makes it possible to evaluate the three-dimensional structure of $P_{700}^{+}A_1^{-}$ in the photosynthetic membrane.[13]

Field Dependence of the Alignment. Spin-polarized D-band EPR spectra of the radical pair $P_{700}^{+}A_1^{-}$ were studied for four different alignment fields varying within the range of 0−7 T. In principle, the order parameter $S_{Z'Z'}$ for each field can be obtained from a simultaneous fit of two angle-dependent line shapes, measured with the sample axis either parallel or perpendicular to the magnetic field. However, the method is time- and labor-intensive. Moreover, as the order parameter decreases, the procedure becomes less and less accurate. Therefore, an alternative method has been used to obtain the desired information. In this method, the intensity ratio X/Y of the two low-field peaks in the $\rho = 0°$ EPR spectrum (see Figure 2a) is used as a measure of the order parameter $S_{Z'Z'}$. Model calculations indicate an approximately linear relation between X/Y and $S_{Z'Z'}$ in the range $0 \leq S_{Z'Z'} \leq 0.15$. The order parameter for the upper end of the straight calibration line was determined using a global fit of the $\rho = 0°$ and $\rho = 90°$ line shapes, as described in the previous section. For $S_{Z'Z'} = 0$, the intensity ratio X/Y was extracted from the "isotropic" EPR spectrum of a sample cooled in the absence of the magnetic field.

In Figure 3, the order parameter $S_{Z'Z'}$ is plotted as a function of the alignment field. The experimental values (solid circles) refer to rehydrated lyophilized whole cells of the cyanobacterium *S. lividus*. One sees that the value of $S_{Z'Z'}$ first increases gradually, then increases steeply and finally levels off to a constant limiting value. Thus, the magnetic-field dependence of $S_{Z'Z'}$ can be described by a saturation curve, as defined in eqs 2−4. The free adjustable parameters of this curve, $\Delta\chi V$ and $(S_{Z'Z'})_{\text{limit}}$, were determined by fitting the calculated order parameters $S_{Z'Z'}$ to the experimental ones. The solid line in Figure 3 represents the best-fit simulation based on the parameter values (see Table 2):

TABLE 1: Parameters Used in the Simulation of Spin-Polarized D-Band EPR Spectra of the Radical Pair $P_{700}^{+}A_1^{-}$ Formed by Photoexcitation of Whole Cells of the Fully Deuterated Cyanobacteria *S. Lividus*

g-tensor components[a]		spin−spin	inhomogeneous	g_2-tensor	dipolar tensor	orientational distribution[e]	
P_{700}^{+}	A_1^{-}	coupling[b]	line widths[c]	orientation[d]	orientation[d]	this work	previous work[f]
g_1^X, 2.00311	g_2^X, 2.00624	D, −0.17 mT	ΔB_0 (P_{700}^{+}), 0.53 mT	Φ_2, 116°	Φ_D, arbitrary	$S_{Z'Z'}$, 0.10° ± 0.02°	$S_{Z'Z'}$, 0.13° ± 0.02°
g_1^Y, 2.00256	g_2^Y, 2.00510	E, 0 mT	ΔB_0 (A_1^{-}), 0.45 mT	Θ_2, 50°	Θ_D, 51°	ϑ, 83° ± 4°	ϑ, 81° ± 4°
g_1^Z, 2.00230	g_2^Z, 2.00220	J_{ex}, 0 mT		Ψ_2, 4°	Ψ_D, 114°	ψ, 120° ± 6°	ψ, 133° ± 6°

[a] Data from high-field EPR studies.[19,34,35] [b] Parameter values from an ESEEM study of $P_{700}^{+}A_1^{-}$.[36] [c] Residual hyperfine interactions in $P_{700}^{+}A_1^{-}$ are considered by inhomogeneous Gaussian line widths. [d] Evaluated from the B_0 dependence of Q-band quantum beat oscillations.[13] The Euler angles[17] relate the principal axis system of the respective tensor and the molecular reference system (g-tensor of P_{700}^{+}). [e] Parameter values that characterize the anisotropic orientational distribution of $P_{700}^{+}A_1^{-}$. The listed Euler angles[17] denote the orientation of the symmetry axis of the susceptibility tensor (membrane normal) in the molecular reference system. The cited errors are linear confidence limits (95% confidence level). [f] Parameter values from a previous W-band EPR study.[14]

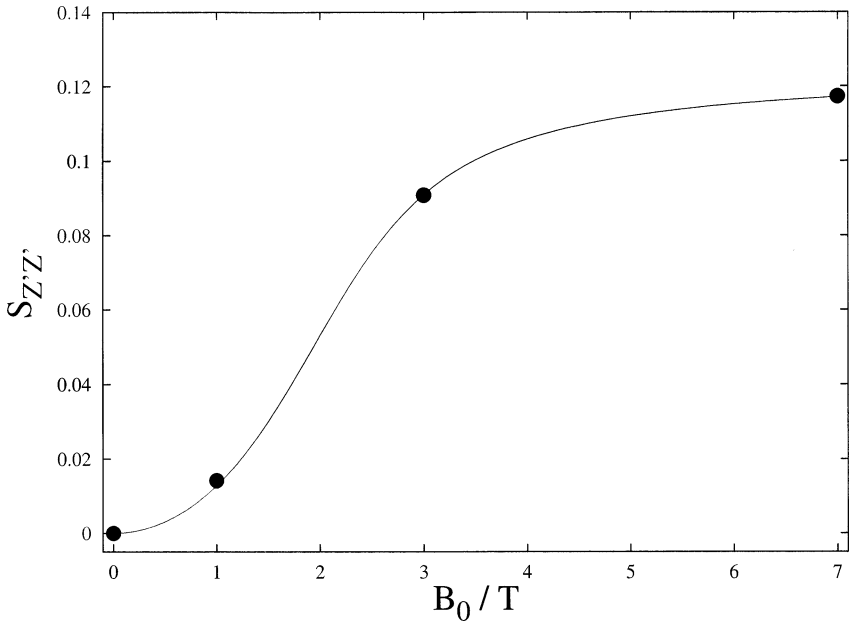

Figure 3. Magnetic-field dependence of the order parameter, $S_{Z'Z'}$, that characterizes the alignment of the symmetry axis of the susceptibility tensor along the magnetic field. The experimental values (denoted by solid circles, ●) were extracted from spin-polarized D-band EPR spectra of the light-induced radical pair, $P_{700}^{+}A_1^{-}$, in plant photosystem I. The solid line represents a best-fit simulation using a simple model for magneto-orientation.[29] The extracted value for the anisotropy of the diamagnetic susceptibility is $\Delta\chi V = 5.7 \times 10^{-27}$ m^3.

$$\Delta\chi V = (5.7 \pm 1.0) \times 10^{-27}\ \mathrm{m}^3$$
$$(S_{Z'Z'})_{\mathrm{limit}} = 0.12 \pm 0.02$$

The agreement achieved is very good. The cited errors are linear confidence limits that correspond to a 95% confidence level.

The large value for the anisotropy of the diamagnetic susceptibility extracted from the saturation curve ($\Delta\chi V = 5.7 \times 10^{-27}$ m^3) suggests that the observed alignment is the result of orientation of the whole cells by the magnetic field. To check this possibility, model calculations have been performed for $\Delta\chi V$, using independent experimental and theoretical information (see Table 2). According to recent electron microscopy studies, a *S. lividus* cell contains thylakoids with a total membrane area of ∼9.8×10^{-11} m^2.[41] Of this, ∼50% are covered by membrane proteins, i.e., 4.9×10^{-11} m^2.[41]

We now estimate the susceptibility anisotropy of a single membrane protein such as PSI. In cyanobacterial PSI, there are 30 parallel transmembrane α-helices,[6] which define the symmetry axis of the susceptibility tensor. Assuming that the alignment is parallel to the magnetic field, we estimate a susceptibility anisotropy of 5.0×10^{-32} m^3. The estimate is based on quantum chemical calculations of the magnetic polarizability of peptide bonds[38] arranged in α-helices.[37] Dividing this value by the area covered by PSI, i.e., 1.0×10^{-16} m^2,[6] we obtain an estimate

TABLE 2: Anisotropy of the Diamagnetic Susceptibility of Whole Cells of the Cyanobacteria *S. Lividus* Determined by D-Band EPR

parameter	value
experimental result	
anisotropy of the diamagnetic susceptibility,[a] $\Delta\chi V$	$(5.7 \pm 1.0) \times 10^{-27}$ m^3
theoretical estimation	
total thylakoid membrane area[b]	9.8×10^{-11} m^2
membrane area covered by proteins[b]	4.9×10^{-11} m^2
susceptibility anisotropy per unit area of membrane proteins[c]	5.0×10^{-16} m
susceptibility anisotropy of a whole bacterial cell,[d] $(\Delta\chi V)_{\mathrm{calc}}$	2.9×10^{-27} m^3

[a] Evaluated from the magnetic field dependence of the order parameter $S_{Z'Z'}$, characterizing the alignment of the symmetry axis of the susceptibility tensor, relative to the magnetic field. The value for $\Delta\chi V$ refers to $T = 220$ K, where the angular distribution is frozen in. The cited error is a linear confidence limit (95% confidence level). [b] Determined by electron microscopy.[41] [c] Calculated on the basis of previous theoretical work.[37,38] [d] In the estimation of $(\Delta\chi V)_{\mathrm{calc}}$, the experimentally determined order parameter of $S_{Z'Z'} = 0.12$ has been considered.

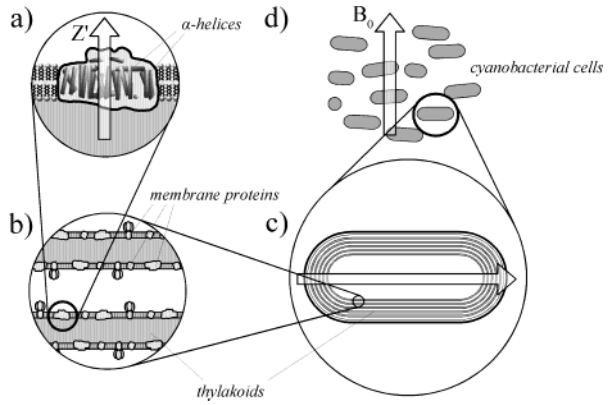

Figure 4. Schematics of the magneto-orientation process proposed for the photosynthetic reaction centers in cyanobacteria: (a) arrangement of the reaction center protein in the photosynthetic membrane (***Z′*** is the membrane normal), (b) structure of the thylakoid membranes, (c) structure of a cyanobacterial cell, and (d) alignment of the cyanobacterial cells in a strong magnetic field $\boldsymbol{B}_0$.

for the susceptibility anisotropy per unit area of 5.0×10^{-16} m, which is typical of membrane proteins.

To calculate the susceptibility anisotropy of a whole cyanobacterial cell, we must consider that the membrane proteins are not all aligned parallel to the magnetic field but are distributed according to an order parameter of $S_{Z'Z'} = 0.12$. This leads to an estimated total susceptibility anisotropy of $(\Delta\chi V)_{\text{calc}} = (4.9 \times 10^{-11}\ \text{m}^2) \times (5.0 \times 10^{-16}\ \text{m}) \times 0.12 = 2.9 \times 10^{-27}\ \text{m}^3$. Given the approximations used in the derivation, the estimated value is in surprisingly good agreement with the observed value of $\Delta\chi V = 5.7 \times 10^{-27}\ \text{m}^3$. Therefore, we conclude that whole cells are aligned in the magneto-orientation process.

A prerequisite for this alignment is an anisotropic shape of the bacterial cell and the thylakoid system. Recent electron microscopy studies indicate that the thylakoid membranes in *S. lividus* cyanobacteria form cylinders ~2.8 μm long with hemispherical caps on both ends.[41] If the long axes of the cells were oriented parallel to the magnetic field, all bilayer normals in the cylinder part would be orthogonal to the field, implying a negative order parameter $S_{Z'Z'}$. This is not observed in the D-band EPR experiments. Therefore, we conclude that the cyanobacterial cells align perpendicular to the magnetic field.

5. Discussion

Magneto-orientation effects were described in several studies on photosynthetic systems more than 25 years ago.[29,33] Yet, for a long time, the mechanism of this process has remained unclear. In this study, we have demonstrated that membrane proteins are the dominant source for the anisotropy of the diamagnetic susceptibility. However, only whole bacterial cells with a large number of ordered membrane proteins are aligned in a magnetic field of a few tesla. This explains why no magneto-orientation effect is observed in the case of isolated reaction center proteins under similar conditions.

Figure 4 depicts a schematic of the magneto-orientation process, as revealed by time-resolved D-band EPR of the radical pair $P_{700}^{+}A_1^{-}$. Spectral analysis indicates that membrane proteins are mainly responsible for the anisotropy of the diamagnetic susceptibility. In cyanobacterial PSI, as well as in many other membrane proteins, ~30 transmembrane α-helices are aligned to within a small angle of the membrane normal,[6] which thus defines the symmetry axis ***Z′*** of the susceptibility tensor (see Figure 4a). The membrane proteins are embedded within the thylakoid membranes that consist of mostly parallel glycolipid bilayers (see Figure 4b). The whole thylakoid system has a regular three-dimensional structure that closely follows the shape of the cyanobacterial cell (see Figure 4c).[41] If a magnetic field of sufficient strength is applied, the cyanobacterial cells align with their long axes perpendicular to the field (see Figure 4d).

Order Parameter of the Membrane Normals. Analysis of the angle-dependent D-band EPR spectra of $P_{700}^{+}A_1^{-}$ provides the order parameter $S_{Z'Z'}$ of the membrane normals, relative to the magnetic field. In the following, we rationalize the extracted value for $S_{Z'Z'}$, in terms of a simple molecular model for the magneto-orientation process. Let us assume that the distribution of the bilayer normals in the laboratory frame can be described by a convolution of two axially symmetric distribution functions f_1 and f_2, as given in eq 2. Specifically, we assume that the function f_1 characterizes the distribution of the bilayer normals, with respect to the symmetry axis of the cyanobacterial cell, and the function f_2 describes the distribution of these axes, relative to the magnetic field. Under these conditions, the order parameter S of the bilayer normals, relative to the magnetic field, can be written as a product of the two order parameters S_1 and S_2, each defined according to eq 4.

Inspection of Figure 4 reveals that the order parameter S_1 is completely determined by the shape of the cyanobacterial cell. Using structural information from a recent electron microscopy study of *S. lividus* cells, we have estimated a value of $S_1 \approx -0.34$.[41] For the order parameter S_2, a strong dependence on the alignment field is expected. Assuming complete alignment for $B_0 = 7$ T, the order parameter S_2 can be assessed to $S_2 \approx -0.5$. This gives a total order parameter of $S = S_1S_2 \approx 0.17$, which compares favorably with the limiting order parameter of $S_{Z'Z'} = 0.12$ that has been observed in this study. Therefore, we conclude that the evaluated mechanism for the magneto-orientation of photosynthetic reaction centers is basically correct.

Orientation of the Membrane Normal. The computer fit of the experimental D-band spectra of $P_{700}^{+}A_1^{-}$ also provides the orientation, ϑ and ψ, of the membrane normal in a magnetic reference system. This stuctural information is unique, because it is not easily available by other EPR techniques. Knowledge of this orientation makes it possible to determine the three-dimensional structure of $P_{700}^{+}A_1^{-}$ in the photosynthetic membrane.[13,14] The structure describes the orientation of the g-tensor of the primary donor, P_{700}^{+}, as well as the position and orientation of the reduced acceptor, A_1^{-}. The new structural information is based on the analysis of quantum beat oscillations, in combination with high-field EPR of a magnetically oriented sample.[13]

With these techniques, it is possible to evaluate the three-dimensional structure of short-lived radical-pair intermediates, following light excitation of photosynthetic proteins in their native membranes. Thus, one can also obtain the co-factor arrangement of the radical pair, with respect to the membrane. The complementary information obtained from quantum beats and a magnetically aligned sample is a powerful structural tool. We expect that this is of general interest, particularly to researchers in the broad fields related to solar energy conversion and storage, because the detailed structure of radical-pair intermediates can be determined on a nanosecond time scale.[13]

6. Conclusions

Magnetic-field-induced orientation of photosynthetic reaction centers has been studied by time-resolved D-band electron paramagnetic resonance (EPR) of the secondary radical pair in plant photosystem I. Experiments have been performed for fresh

and lyophilized whole cells of the deuterated cyanobacterium *S. lividus*. Analysis of the spin-polarized EPR spectra reveals that membrane proteins are the major source for the anisotropy of the diamagnetic susceptibility. From the magnetic-field dependence of the degree of orientation, a value for the anisotropy of the diamagnetic susceptibility has been exctracted. The value is in good agreement with an estimate for the susceptibility anisotropy of a cyanobacterial cell. This demonstrates that whole cells are aligned in the magneto-orientation process. The spin-polarized EPR spectra can be simulated quite well with the correlated radical-pair model, if magneto-orientation is properly taken into account. Combining this technique with the analysis of quantum beat oscillations, it is possible to evaluate the three-dimensional structure of short-lived radical-pair intermediates in their native membranes.

Acknowledgment. We thank Professor P. Gräber (University of Freiburg) for helpful discussions and advice. Financial support by the DFG priority program "High-Field EPR in Biology, Chemistry and Physics" (under No. SPP 1051) is gratefully acknowledged. Work at Argonne National Laboratory was supported by the U.S. Department of Energy, Office of Basic Energy Sciences, Division of Chemical Sciences (Contract No. W-31-109-ENG-38). This work was partially supported by a United States−Israel BSF grant (H.L. and M.C.T.), the Israel Science Foundation, and the Ministry of Science (H.L.). The Farkas Center is supported by the Bundesministerium für Forschung und Technologie and the Minerva Gesellschaft für Forschung GmbH, Germany. This work is in partial fulfillment of the requirements for a Ph.D. degree (E.S.) at the Hebrew University of Jerusalem.

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