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EVIDENCE FOR AN ANISOTROPIC MAGNETIC INTERACTION BETWEEN THE (BACTERIOPHEOPHYTIN) INTERMEDIARY ACCEPTOR AND THE FIRST QUINONE ACCEPTOR IN BACTERIAL PHOTOSYNTHESIS

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We have investigated electron spin polarization effects occurring in protonated and perdeuterated reaction centers of *Rhodospirillum rubrum* with electron spin resonance at 9 and 35 GHz (X- and Q-band). As for *Rhodopseudomonas sphaeroides* strains 2.4.1 and R-26 (Gast, P. and Hoff, A.J. (1979) Biochim. Biophys. Acta 548, 520–535; Gast, P., Mushlin, R.A. and Hoff, A.J. (1982) J. Phys. Chem. 86, 2886–2891), electron spin polarization effects of the prereduced first quinone acceptor Q_A^- in *R. rubrum* are strongly nonuniform. This nonuniformity is due to an anisotropic magnetic coupling between the intermediary bacteriopheophytin acceptor (I^-) and Q_A^-. It is argued that the anisotropy is too strong to arise solely from an anisotropy in the exchange interaction between I^- and Q_A^- and that dipolar contributions to the magnetic coupling between I^- and Q_A^- are important. The anisotropy in the magnetic coupling for reaction centers of *Rps. sphaeroides* strains 2.4.1 and R-26 is different from that of *R. rubrum* wild type. The combination of the 4-fold higher resolution at Q-band and the line narrowing upon deuteration has enabled us to obtain the principal g values and two hyperfine interaction constants of the reduced first quinone acceptor Q_A^-. The principal g values are $g_x = 2.0067$, $g_y = 2.0056$ and $g_z = 2.0024$; the hyperfine constant of the CH_2 group at position 1 is 1.6 G and that of the CH_3 group at position 2 is 2.1 G. These values are close to those found for ubisemiquinone in vitro (Okamura, M.Y., Debus, R.J., Isaacson, R.A. and Feher, G. (1980) Fed. Proc. 39, 1802; Hales, B.J. (1975) J. Am. Chem. Soc. 97, 5993–5997).

Introduction

The first stable (often called primary) acceptor in photosynthetic purple bacteria has been identified by optical and ESR spectroscopy to be an iron-ubi- (or mena-)quinone complex (Q_A-Fe) [1–3]. In whole cells and in intact, isolated reaction centers the ESR line of the semiquinone-iron complex is composed of a broad resonance at $g \approx 1.8$ with a line width of 200–300 G [1]. Upon dissociation of the quinone-iron complex the broad ESR line is transformed into a narrow, near-Gaussian line at $g = 2.0046$ with width $\Delta H_{pp} = 8.1$ G [4]. This line has been assigned to the reduced semiquinone by an ESR study at 9 and 35 GHz (X- and Q-band) of the in vivo signal in iron-depleted reaction centers and of ubisemiquinone in vitro [4]. By Mossbauer spectroscopy it was demonstrated that the iron in the quinone-iron complex is a high-spin ($S = 2$) Fe^{2+} that does not change its valence upon photoreduction of the complex [5]. Apparently, in intact reaction centers the ESR line of the photoreduced quinone is broadened and shifted as a result of a magnetic coupling between the paramagnetic iron ion and the semiquinone [6]. This is confirmed by magnetic susceptibility measurements as a function of temperature [6]. The Q-band ESR spectrum of the uncoupled Q_A^- signal has a characteristic shape that is reproduced in the in vitro spectrum of ubisemiquinone. Moreover, it could be demonstrated that illumination of dark-frozen iron-depleted reaction centers produced signals due to the oxidized primary donor and the reduced first stable acceptor with equal integrated intensity [4]

The near-Gaussian narrow ESR line of the semiquinone can also be observed in reaction centers in which the iron is not removed, but magnetically decoupled from the quinone by detergent treatment [7–9]. We have shown [10,11] that in such particles illumination at low temperature generates spin-polarized ESR signals that arise from the spin-polarized primary donor, P^+-870 and the first stable acceptor Q_A^-. The polarization is thought to be generated in the radical pair P^+-870I^-, where I^- is the transient bacteriopheophytin acceptor. When Q_A was photochemically prereduced, illumination at low temperature caused the ESR line of Q_A^- to turn emissive [9,12]. This effect was attributed to transfer of polarization from the polarized transient acceptor I^- to prereduced Q_A^- [9]. It was subsequently shown that this transfer of polarization is nonuniform over the ESR line of Q_A^- [13]. This nonuniformity is caused by the polarization transfer itself and not by possible anisotropy in spin-spin or spin-lattice relaxation times [14]. It was concluded that the magnetic coupling between the radical I^- and Q_A^- that is responsible for the transfer of polarization is anisotropic. We have now further investigated this phenomenon with Q-band ESR experiments on protonated (^{1}H) and perdeuterated (^{2}H) reaction centers. The higher g resolution offered by Q-band spectroscopy of ^{2}H-labeled reaction centers especially provides clear evidence that the magnetic coupling is indeed highly anisotropic, and that it is different for the two bacteria *Rhodopseudomonas sphaeroides* and *Rhodospirillum rubrum.* The high degree of anisotropy makes it likely that the magnetic coupling contains a relatively large dipolar contribution

To aid in the interpretation of our spin-polarization results, we have simulated the four spectra of Q_A^- obtained by ESR spectroscopy at 9 and 35 GHz on ^{1}H- and ^{2}H-labeled reaction centers using an anisotropic g matrix [15] and hyperfine splitting constants derived from model quinones and from ENDOR experiments on Q_A^- [16]. The good fit of our simulations to the experimental spectra demonstrates clearly that the ESR spectrum of Q_A^- can be interpreted as a powder spectrum of a radical with rhombic g values which is broadened by unresolved hyperfine interaction

Materials and Methods

The X-band ESR experiments were performed at 120 K as described in Ref. 9. Most of the Q-band ESR experiments were performed at the University of California, San Diego, using a superheterodyne spectrometer of local design equipped with a glass immersion-type helium cryostat. For experiments at 80 K helium gas served as heat conductor between the liquid nitrogen inside the outer mantel of the cryostat and the sample. The cavity temperature was monitored with a calibrated carbon and/or platinum resistor. The ESR spectra were stored and processed in a Nicolet dedicated computer Samples of 2 mm inner diameter could be illuminated through slots in the wall of the cylindrical cavity. g values were determined by measuring the magnetic field with an NMR Gaussmeter and the frequency of the microwave field with a transfer oscillator and frequency counter. Q-band spectra at 120 K were recorded at the University of Leiden using a Varian V4595 spectrometer.

^{1}H-labeled reaction centers (so-called AUT-s particles) of *R. rubrum* and *Rps. sphaeroides* were prepared according to the method of Slooten [2,17] which involves SDS treatment. Iron-depleted LM particles of ^{1}H-labeled *Rps. sphaeroides* R-26 reaction centers [18,19] were kindly prepared by R. Debus. Perdeuterated cells of *R. rubrum* were a gift of Dr. J.J. Katz. Since the available amount of these perdeuterated cells was not enough to isolate proper AUT-s reaction centers, an alternative method was used for this material to dissociate the quinone-iron complex, which is based on SDS treatment of whole cells. The optimal SDS concentration was found using ^{1}H-labeled cells by first sonicating the cells for 10 min followed by incubation with different amounts of SDS at 20°C. During the incubation period aliquots were taken and the ubiquinone acceptor was reduced as for reaction centers The amplitude of the ESR signal around $g = 2.00$ in the dark, measured with X-band ESR at 5 K, was used as an indicator for the uncoupling of the iron from Q_A in the Q_A-Fe complex. The amplitude of the ESR signal of the triplet state of the primary donor, P^T-870, photogenerated in the same sample at 5 K, was used as a marker for the photoactivity of the reaction center in the treated cells at low temperature. With this method the optimum incubation procedure with maximal iron uncoupling and minimal reaction center destruction was found to consist of incubation in 1% SDS at 20°C for $2\frac{1}{2}$ h using cells with an absorbance at 850 nm of 20 cm^{-1}. This method was followed for the incubation of the perdeuterated cells which after treatment were kept permanently under liquid nitrogen No further destruction of the cells or changes in the polarization effects were observed even after 3 years of storage. The fact that the iron had probably not been dissociated from the acceptor complex in all the reaction centers does not present a problem for the investigation of the spectrum of Q_A^-, since reaction centers with an intact acceptor complex do not show ESR intensity in the $g = 2.00$ region when the quinone is prereduced. The presence of the dissociated iron does not affect the shape of the dark or the emissively polarized spectrum of Q_A^- [9]. In contrast to the reduction method used for isolated reaction centers [9], in the SDS-treated cells of *R. rubrum* photoaccumulation of Q_A^- in the presence of sodium ascorbate resulted in the accumulation of some P^+-870, of which the ESR spectrum interfered with that of Q_A^-. Therefore, in the SDS-treated cells Q_A was reduced by adding sodium dithionite under anaerobic conditions For comparison the AUT-s and LM preparations were also reduced with sodium dithionite with results identical to those of the more gentle ascorbate-plus-light reduction method

Computer simulations of the ESR spectra were performed with a program based on the program MAGNSPEC from the Quantum Chemistry Program Exchange, Indiana University, Bloomington, U S.A., that was modified for the handling of more nuclei.

Results

The ESR spectrum of Q_A^-

The X- and Q-band ESR spectra of the reduced first stable acceptor Q_A^- in ^1H- and ^2H-labeled reaction centers and in SDS-treated ^2H-labeled cells of *R rubrum,* in which the Q_A-Fe complex was dissociated are depicted in Fig 1. Fig. 1A shows the familiar, almost Gaussian X-band spectrum centered around $g = 2.0048 \pm 0.0002$ of ^1H-labeled Q_A^- present in AUT-s particles from *R. rubrum* [4,7,9]. At Q-band this spectrum is clearly split due to the higher g resolution (Fig. 1B) The spectrum differs slightly from the spectra in Refs 4 and 13, probably due to the difference in temperature. When perdeuterated SDS-treated cells are used (Fig. 1C and D) the separate lines of the spectrum of Q_A^- narrow due to the reduction of hyperfine interaction by a factor $\gamma_H/\gamma_D = 6.5$ Owing to this narrowing, an extra splitting of the spectrum is now observed in the Q-band spectrum (Fig 1D) The spectrum clearly shows the char-

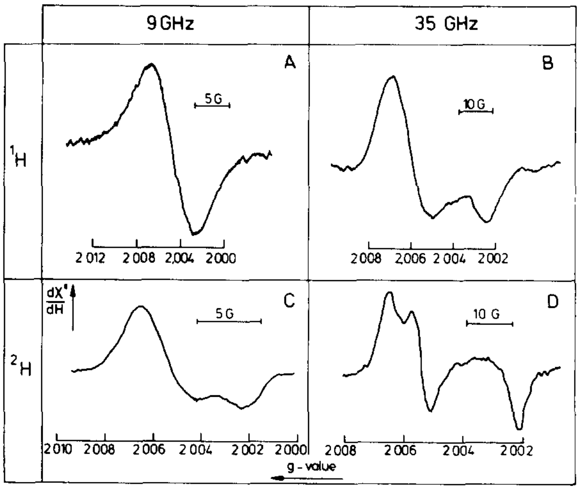
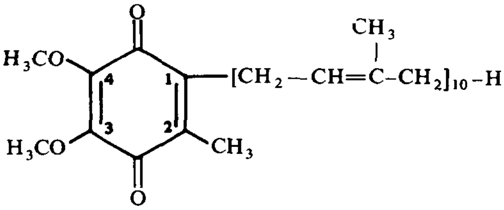


Fig 1 (A) ESR spectrum at 9 GHz (X-band) of Q_A^- in prereduced AUT-s particles of *R rubrum* Conditions Microwave power 20 μW, temperature 120 K (B) Q-band ESR spectrum of Q_A^- in prereduced AUT-s particles of *R rubrum* Conditions microwave power about 50 μW, modulation amplitude 2 G, temperature about 120 K (C) ESR spectrum at 9 GHz (X-band) of Q_A^- in prereduced SDS-treated, perdeuterated cells of *R rubrum* Conditions microwave power 20 μW, modulation amplitude 1 G, temperature 120 K (D) Q-band ESR spectrum of Q_A^- of the same preparation as in C Conditions, microwave power 1 μW, modulation amplitude 3 G, temperature 80 K

acteristics of a powder ESR spectrum with principal g values at 2.0067, 2.0056 and 2.0024 ($\pm$0.0002). The same low-field splitting around $g = 2.0060$ was also observed when these samples were reduced by accumulation of Q_A^- in the light in the presence of sodium ascorbate, although then the high-field part of the spectrum of Q_A^- was obscured by the presence of P^+-870. This result excludes the possibility that under the rather drastic reduction conditions with sodium dithionite a second quinone with a different axial g value is reduced, which could give rise to one of the two peaks at low field.

Computer simulations with the above principal g values of the powder spectrum, measured at 9 and 35 GHz for protonated and perdeuterated samples are depicted in Fig. 2. In the simulations for protonated samples we have incorporated the following hyperfine interactions for the $S = 1/2$, $I = 1/2$ system (Fig. 2A and B): 1.6 G for the CH_2 group at position 1 (as shown below, structure of ubiquinone), and 2.1 G for the CH_3 group at position 2. The small hyperfine interaction with the methoxy groups at positions 3 and 4 (0.3 G) was absorbed in the 1.5 G wide Gaussian spin-packet that was used in the simulation of the spectra. The above values for the hyperfine interactions were derived from the recently published ENDOR data on the reduced acceptor Q_A^- [16]. The spectra of ^{2}H-labeled Q_A^- were simulated using a 6.5-fold reduced hyperfine interaction for the deuterons ($I = 1$). For ^{2}H-labeled Q_A^- the required spin-packet width of 1.5 G is larger than expected on the basis of the measured $T_2 = 2.2$ μs of ^{1}H-labeled Q_A^- [14]. Apparently, the packets are broadened by another mechanism, such as a small contribution of exchange interactions.



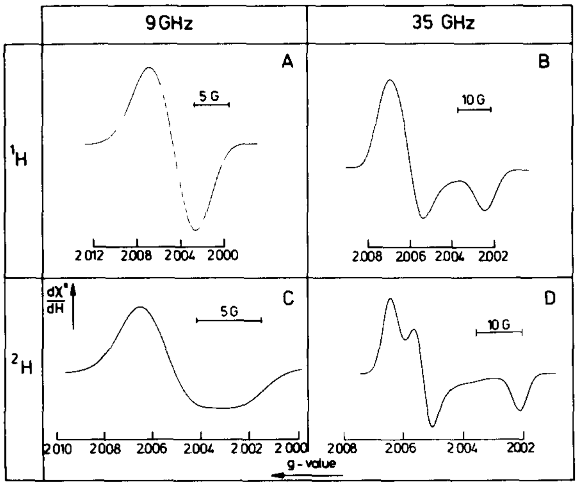


Fig 2 Computer simulation of the spectrum of Q_A^- for deuterated and nondeuterated material at X- and Q-band. The parameters used were $g_x = 2.0067$, $g_y = 2.0056$ and $g_z = 2.0024$, CH_3 hyperfine interaction 2.1 (0.32) G, CH_2 hyperfine interaction 1.6 (0.25) G (the values between brackets are the values used for the simulation of the ^{2}H spectra), Gaussian spin-packet line width 1.5 G

Electron spin polarization of Q_A^-

Illumination of the SDS-treated perdeuterated cells reduced with sodium dithionite generated an unexpected spin-polarization pattern that is shown in Fig. 3. The nonuniformity in the polarization in these particles is clearly different from that found in AUT-s particles of *Rps. sphaeroides* [9] and is

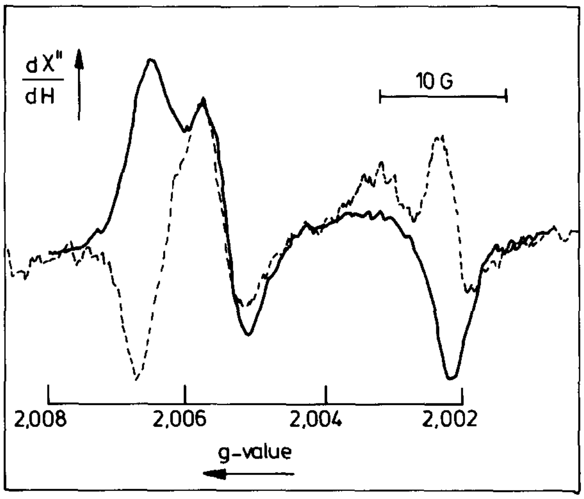


Fig 3 Light (------) and dark (———) ESR spectra of the prereduced acceptor Q_A^- in SDS-treated perdeuterated cells of *R rubrum*. Conditions as in Fig 1D

strongly enhanced. The peak at $g = 2.0056$ (the 'g_y peak') is hardly spin polarized while the remaining spectrum shows large polarization effects. Similar effects were also observed at 2.1 K. The X-band ESR spectra of light and dark spectra of the ^{2}H-labeled *R. rubrum* sample measured at 120 K show the same phenomena albeit less well resolved (data not shown) Using ^{1}H-reaction centers of *R rubrum,* the polarized spectrum shifted to $g = 2.0044$. This shift is a result of the absence of polarization around the g_y peak as resolved at Q band in ^{2}H-labeled samples (see Discussion).

Discussion

Dark ESR spectrum of the reduced primary acceptor Q_A^-

The combination of ESR spectra of the reduced primary acceptor in SDS-treated particles, measured for ^{1}H- and ^{2}H-labeled particles at 9 and 35 GHz enables us to obtain some physical parameters of the acceptor Q_A. Substitution of protons by deuterons, which have a 6.5-times lower hyperfine constant than ^{1}H, causes the high- and low-field parts of the Q-band spectrum to narrow (Fig. 1D). Thus, part of the line width in ^{1}H spectra is caused by unresolved hyperfine interaction. In addition, in the ^{2}H spectra at 35 GHz, g anisotropy is clearly observed (Fig. 1D). The principal g values ($g_x = 2.0067$, $g_y = 2.0056$, $g_z = 2.0024$ (± 0.0002)) are very close to those observed for in vitro benzosemiquinone in methanol ($g_x = 2.0065$, $g_y = 2.0053$, $g_z = 2.0023$) [15] The simulated spectra of Fig 2 agree well with the observed spectra. The negative peak at the g_y position in the simulations is somewhat larger than that in the observed spectrum, perhaps because of a slight anisotropy in hyperfine interactions responsible for the spin-packet width of 1.5 G With the same parameters used for the simulation of the Q-band spectrum, the X-band spectrum of ^{2}H-labeled Q_A^- could also be fitted well (Fig. 2C). In addition, the X- and Q-band spectra of ^{1}H-labeled material were well simulated by enhancing all hyperfine interactions 6.5-fold while retaining the g values of the ^{2}H fits (Fig. 2A and B) and keeping the spin-packet line width 1.5 G. Our successful simulations give a satisfactory explanation of the line shape of the X- and Q-band signals of Q_A^- and support the assignment of the ENDOR lines given in Ref. 16

Electron spin-polarized spectra of Q_A^-

Transfer of polarization is thought to occur via a magnetic spin-exchange interaction J ($I^-Q_A^-$) between the photoreduced polarized intermediary acceptor I^- and the prereduced acceptor Q_A^- [9,12]. Such an interaction randomizes and averages to zero the so-called multiplet polarization that depends on the nuclear hyperfine interactions (the exchange operator does not contain the nuclear spin operator). Thus, only the so-called net polarization, that depends on the difference in g factor (Δg) between the members of the geminate radical pair (here P^+-870 and I^-) in which the polarization initially develops, is transferred to Q_A^- (see, for an introduction to the theory of spin polarization by the radical pair mechanism, e.g., Refs. 11, 20 and 21). This randomizing effect was first noted by Pedersen [22] for the case in which there is actual electron transport between one of the geminate radicals and a neighboring species. Were the analogy between polarization transfer by electron transport and by exchange interaction complete, then in both cases the transferred polarization should be independent of the position within the line. Yet, this is manifestly not so. This is especially clear in perdeuterated cells of *R. rubrum* (Fig. 3), where the g_y peak seems only slightly polarized. Also in X-band ESR of the perdeuterated cells this effect is clearly observed (data not shown). For protonated AUT-s particles of *R rubrum* at X-band the absorptive dark and the emissive light signals have very nearly the same shape (apart from the sign) but here the g values operationally defined as the baseline crossing, are different The apparent g value of the light signal is shifted to a lower value. The baseline crossing of the light-minus-dark spectrum is at 2.0043, consistent with the observation with perdeuterated cells. Since in ^{1}H-labeled AUT-s particles of *R. rubrum* at X-band the baseline crossing of the dark spectrum is close to the average g value $1/3(g_x + g_y + g_z) = 2.0047$, in the light-minus-dark spectrum one would expect it to be approx $\frac{1}{2}(g_x + g_z) = 2.0044$ for weak or no polarization at the g_y position. In *Rps. sphaeroides* the nonuniformity of the polarization [9,14] is different from that observed in *R. rubrum,* inasmuch that the high-field part of the Q-band spectrum corresponding to the g_z region seems to be less polarized [13]. If this effect is translated to X-band one expects the baseline crossing of the light-minus-dark spectrum to be at a lower field than that of the dark spectrum of Q_A^-, as was indeed observed [9].

If $J(I^-Q_A^-)$ is a scalar, then anisotropy in the resulting Q_A^- polarization pattern can occur if the initial net polarization on I^- is anisotropic In principle, such anisotropy can arise because of an anisotropic Δg term. However, for *R rubrum* [23,24] and for *Rps sphaeroides* [25] the anisotropy in the difference of g_{P^+} and g_{I^-} is too small (approx. 1 G at X-band [23–25]) to explain the nonuniform polarization of Q_A^-. Also, *Rps. sphaeroides* has an anisotropic polarization pattern that is clearly different from that developed in *R. rubrum* [13]

The above arguments lead to the conclusion that the nonuniformity of the polarization of Q_A^- arises because the transfer of spin polarization itself is anisotropic This means that the magnetic interaction between I^- and Q_A^- must be anisotropic. The anisotropy relative to the average value of the interaction is large. From a quantitative analysis of the polarization data of ^{1}H-labeled samples at X-band we have previously concluded that the exchange interaction $J(I^-Q_A^-)$ is 3–5 G [9]. From Fig. 3 it is seen that reaction centers oriented with the y-axis of the quinone molecule parallel to the magnetic field exhibit only very little polarization, hence $J_y(I^-Q_A^-) \approx 0$. This means that the anisotropy in J is larger than the average value. This is an uncommon phenomenon, which indicates that dipolar interactions are important. Consider the term in the Hamiltonian describing a general exchange interaction between spins A and B

$$\mathcal{H}_{\mathrm{exch}} = \vec{S}_A \overset{\rightrightarrows}{J} \vec{S}_B \qquad (1)$$

where $\vec{S}_{A,B}$ represents the spin operator of radicals A and B and $\overset{\rightrightarrows}{J}$ is a second-rank tensor. Eqn. 1 is isomorphous with the term in the Hamiltonian describing a dipole-dipole interaction:

$$\mathcal{H}_{\mathrm{dip}} = \vec{S}_A \overset{\rightrightarrows}{D} \vec{S}_B \qquad (2)$$

where $\overset{\rightrightarrows}{D}$ represents the dipolar interaction tensor.

Although their physical origin is quite different, $\mathcal{H}_{\mathrm{exch}}$ and $\mathcal{H}_{\mathrm{dip}}$ have similar mathematical structure. Therefore, without detailed information on the geometry and distances of the two spins it is a priori not possible to conclude from experiments whether $\mathcal{H}_{\mathrm{exch}}$ or $\mathcal{H}_{\mathrm{dip}}$ is predominant The dipolar tensor $\overset{\rightrightarrows}{D}$ has trace equal to zero, hence it is purely anisotropic (it does not contain an isotropic part). The tensor $\overset{\rightrightarrows}{J}$ has trace unequal to zero and can be written

$$\overset{\rightrightarrows}{J} = J\overset{\rightrightarrows}{1} + \overset{\rightrightarrows}{J}_{\mathrm{anis}} \qquad (3)$$

where J represents the isotropic part of the exchange interaction needed to explain our nonuniform polarization of Q_A^- and $\overset{\rightrightarrows}{J}_{\mathrm{anis}}$ the anisotropic part with trace equal to zero. To decide which of the two interactions ($\overset{\rightrightarrows}{J}_{\mathrm{anis}}$ or $\overset{\rightrightarrows}{D}$) is predominantly responsible for the effect, we are guided by experimental evidence that the anisotropy in $\overset{\rightrightarrows}{J}$ (which may be defined as the maximal difference between the diagonal elements in the principal axis system, taken two by two) is usually much smaller than the isotropic value J [26]. This contrasts with our experimental findings that the anisotropy is larger than the average interaction. Hence, we conclude that the magnetic interaction between I^- and Q_A^- has a significant dipolar contribution. This result is a corollary of the recent finding that the magnetic interaction between the oxidized primary donor P^+-870 and the photoreduced intermediary acceptor I^- contains a dipolar contribution that is at least equal in magnitude to that of the isotropic exchange interaction [27,29]. Note that, in the simple picture of transfer of polarization being governed by an isotropic exchange interaction, introduction of a dipolar splitting between the energy levels reduces the magnitude of the transfer rate. Thus, our result that in *R. rubrum* the transfer is minimal along the y-axis of the quinone means that for this species the dipolar interaction along this direction is maximal.

The elements of the dipolar interaction tensor $\overset{\rightrightarrows}{D}(I^-Q_A^-)$ depend on the mutual orientation of I^- and Q_A^- Quantitative determination of the dipolar interaction will permit delineation of the geometric configuration of the bacteriopheophytin and the quinone acceptor. To this end, experiments on the electron spin polarization of Q_A^- in oriented reaction centers will clearly be of much value.

Acknowledgements

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