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Magnetic interactions between the triplet state of the primary donor and the prereduced ubiquinone acceptor in reaction centers of the photosynthetic bacterium *Rhodopseudomonas sphaeroides* 2.4.1

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The lineshape of the polarized prereduced primary quinone acceptor of *Rhodopseudomonas sphaeroides* after a laser flash was studied using time-resolved continuous wave EPR and electron spin echo techniques. The EPR as well as the electron spin echo experiments show that the lineshape of the ubiquinone shortly after a laser flash is time-dependent. This is attributed to magnetic interactions between the ubiquinone and the triplet state of the primary donor. Taking into account both dipolar and exchange interactions, a simulation of the EPR lineshape of the ubiquinone at 50 μs after the laser flash is performed, yielding a distance of 1.85 nm between the primary donor and the primary acceptor. From the simulation it is also concluded that the plane of the ubiquinone is approximately parallel to the surface of the membrane.

Introduction

Recently, we have documented in a series of papers [1–4] that the EPR signal of the first quinone acceptor in bacterial photosynthesis can become emissively spin-polarized upon continuous illumination when the quinone is prereduced. This phenomenon has been observed in reaction centers in which the iron that is nomally complexed to the quinone is either removed or magnetically uncoupled from the quinone. The electron spin polarization of the quinone was attributed to transfer of polarization from the short-living, spin-polarized anionic intermediary acceptor bacteriopheophytin through a spin exchange interaction between this acceptor and the prereduced quinone.

In this communication we report a study of the time dependence of the emissive signal (hereafter labeled U^-_{pol}) after laser flash illumination in the range 600 ns to 40 ms by fast continuous wave EPR and electron spin echo techniques. We have found a fast decaying feature in the U^-_{pol} signal with a lifetime similar to that of the triplet state of the primary donor, ^{3}P-860, which is photogenerated simultaneously with U^-_{pol} by recombination of the charges on P-860 and the bacteriopheophytin acceptor. This feature is indicative of magnetic interactions between U^-_{pol} and ^{3}P-860.

The continuous wave EPR lineshape of the U^-_{pol} signal at 50 μs after the flash could be simulated using a combination of dipolar and exchange interactions between U^-_{pol} and ^{3}P-860. The simulation yields a distance of $r = 1.85 \pm 0.2$ nm between these two entities. The g tensor of the ubiquinone appears to be oriented such that the g_z axis is approximately parallel to the distance vector between the quinone and ^{3}P-860, i.e., more or less perpendicuar to the surface of the photosynthetic membrane.

Abbreviation: EPR, electron paramagnetic resonance.

A preliminary account of this work was presented at the 6th International Congress of Photosynthesis, Brussels, Belgium, June 1983 [5].

Materials and Methods

Rhodopseudomonas sphaeroides 2.4.1 was grown as in Ref. 6. Reaction centers (alkali-urea-Triton X-100 particles) were prepared by treatment with alkali-urea-Triton-SDS as described in Refs. 7–9. The SDS treatment used in the preparation reduced the iron content to an averaged value of 1–2 atoms of iron per reaction center [2]. No signal of the iron-quinone complex was seen under the conditions of measurement normally used for its observation [10], indicating that in those reaction centers that still did contain iron, the ubiquinone and the iron are magnetically uncoupled [11]. It has previously been shown that in iron-depleted so-called LM particles of reaction centers of *Rps. sphaeroides* R-26 an emissively spin-polarized signal of the quinone is found that is identical to that of alkali-urea-Triton X-100 particles [2].

The EPR and electron spin echo samples consisted of quartz tubes of 3 mm diameter, containing 150 μl of alkali-urea-Triton X-100 particles with an absorbance (800 nm) of 30 cm^{-1}, 250 μl glycerol solution in water (87%) and a few crystals of solid sodium dithionite, were frozen to 77 K in the dark, immediately after addition of the dithionite and gassing with nitrogen.

The continuous wave X-band (9 GHz) EPR spectrum at 20 K in the dark showed the familiar signal of the reduced primary ubiquinone acceptor at $g = 2.0048 \pm 0.0004$ with a near-Gaussian lineshape of width 8.0 ± 0.4 gauss. This form of the ubiquinone EPR signal will be referred to as U^-_{unpol}. The absorptive $g = 2.0048$ signal became totally emissive under continuous illumination, in agreement with earlier results [1]. Together with U^-_{pol} the polarized triplet signal of P-860 was observed.

Time-resolved continuous wave EPR experiments at X-band were performed on a modified Varian E-9 spectrometer with a response time of 20 μs [1]. The output of the 100 kHz lock-in detector was fed into a PAR model 162 boxcar averager mainframe, equipped with a model 165 gated integrator plug-in unit, while keeping the time between the laser pulse and the boxcar gate fixed. Time-resolved EPR spectra were obtained by scanning the external field. At the low microwave powers used, no transient nutation oscillations were observed [12,13]. To avoid lineshape distortion, field scanning was always slow enough to be compatible with the time constant of the boxcar.

The electron spin echo experiments were carried out with a home-built phase-sensitive electron spin echo spectrometer similar to that described in Ref. 2. Typical operation took place at a microwave power of 20 W, resulting in a 180° pulse of 120 ns. The time-resolved EPR and electron spin echo experiments were performed at a temperature of 20 and 4.2 K, respectively, employing a standard Varian rectangular TE102 transmission cavity equipped with an Oxford Instruments helium gas flow cryostat. For the electron spin echo experiments the normally high Q of 5000 of the cavity was spoiled to 1000 by strong overcoupling to admit the bandwidth of the frequencies present in the short pulses. The echo intensity was measured by setting the gate of the boxcar on the maximum of the echo. The output of the boxcar was fed into a LSI 11/23 microcomputer which was linked to a VAX computer (Digital Equipment Corporation). Data processing and computer simulations were performed on the VAX. The computer simulation of the U^-_{pol} lineshape was performed using a program designed to calculate powder EPR lineshapes as described in Ref. 3 and modified to accomodate dipolar and exchange interactions.

As a light source in our flash experiments we used in the EPR experiments a JK Nd-YAG oscillator plus amplifier yielding 130 mJ/10 ns pulses which pumped a home-built single step broadband dye laser using Rhodamine B as a dye with an output of 30 mJ/pulse, centered around 580 nm. In the electron spin echo experiments we used a coaxial flashlamp-pumped dye laser with Rhodamine 6G as a dye operating at 560 nm. The flash had an energy of 55 mJ/pulse with a pulsewidth of 250 ns.

Results

Time-resolved continuous wave EPR

The dark continuous wave EPR spectrum at 20 K of alkali-urea-Triton X-100 particles of

Rhodopseudomonas sphaeroides, reduced with dithionite and frozen to 77 K in the dark, showed the familiar near-Gaussian EPR lineshape of U^-_{unpol} at $g = 2.0048$ [1].

Upon flashing the sample, time-resolved EPR spectra of the polarized ubiquinone U^-_{pol} were obtained at various times after the laser flash using the boxcar technique as described in the previous section (Fig. 1). The spectrum at 40 ms (Fig. 1D) after the flash shows an 8-G wide absorptive lineshape at $g = 2.0042 \pm 0.0008$, which is attributed to U^-_{unpol}. At a delay of 250 μs between flash and boxcar gate the spectrum consists of a totally emissive signal that closely resembles the totally emissive signal created under continuous illumination [1]. At 10 ms (not shown) the whole line had disappeared. This is interpreted as an intermediate relaxed state between the emissive 250 μs and the absorptive 40 ms spectra. At times shorter than 250 μs after the laser flash the spectrum shows an unexpected splitting at the high field side of the spectrum. This peculiar lineshape is most evident at 50 μs (Fig. 1A) after the flash.

Electron spin echo spectra

In order to study the peculiar high field feature with a higher time resolution, we repeated our time-resolved experiments with the electron spin echo spectrometer. First an extensive study of the dependence of the U^-_{pol} lineshape on the delay time τ_{12} between the first (90°) and the second (180°) microwave pulse was made.

τ_{12} dependence

The τ_{12} dependence of the U^-_{pol} lineshape at 600 ns after the laser flash is shown in Fig. 2. It is clearly seen that at $\tau_{12} = 1150$ ns and especially at 950 ns the lineshape shows a high field shoulder, while at 600 ns and 1500 ns the signal shape is more symmetric. In principle, the observed τ_{12} dependence could be due to incomplete excitation of the EPR line by the microwave pulses, as our pulses are typically in the order of 100 ns, i.e., the excitation band width is smaller than the width of the EPR signal. Then, at different field positions a different set of spin packets contributing to the echo intensity is excited.

In order to check this possibility, we recorded

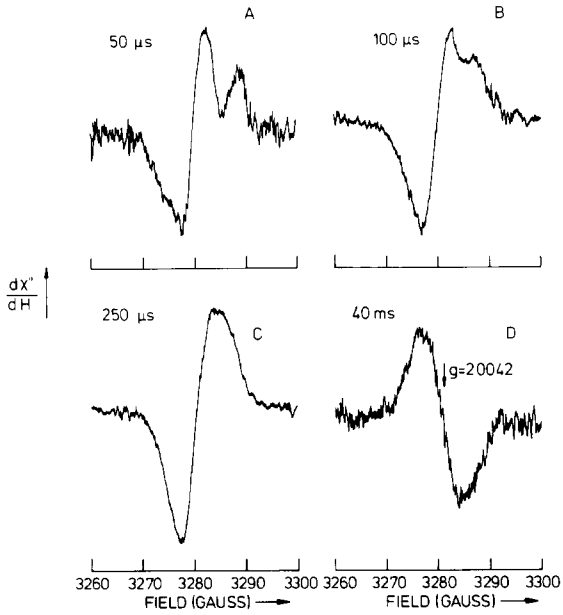

Fig. 1. Continuous wave EPR boxcar spectra of the prereduced ubiquinone acceptor of *Rps. sphaeroides* 2.4.1 at various times (as indicated) after a flash. Temperature, 20 K; modulation amplitude, 2 gauss; microwave power, 20 μW.

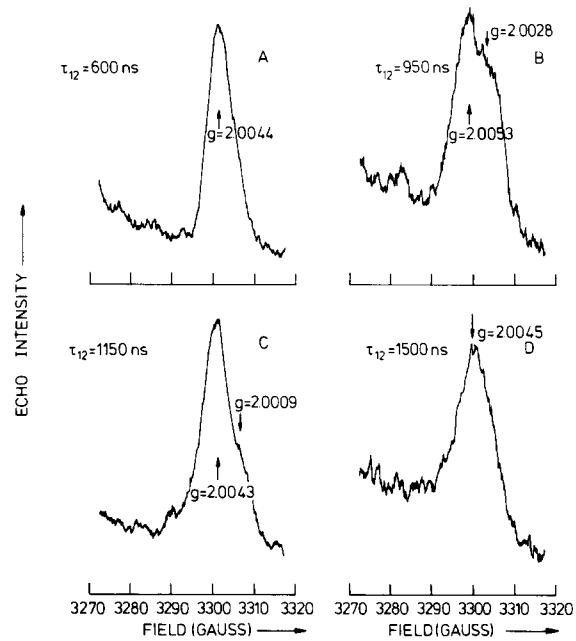

Fig. 2. Field-swept electron spin echo spectra of U^-_{pol} at 600 ns after the laser flash with varying τ_{12} (as indicated) between the two microwave pulses. Pulse power, 20 W; temperature, 4.2 K.

the two-pulse echo modulation patterns at different field positions in the ubiquinone lineshape in the dark and at a fixed time of 600 ns between the laser flash and the first microwave pulse. Some of these patterns are depicted in Fig. 3. The echo modulation of U^-_{unpol} showed no field dependence. These traces have their absolute maximum at τ_{12} = 1150 ns and a submaximum at τ_{12} = 700 ns. One of these envelopes is shown in Fig. 3A. The envelopes at 600 ns after the laser flash, however, show a clear field dependence. The envelope at 600 ns after the flash taken at g = 2.0046 (Fig. 3B) has a similar shape as that of U^-_{unpol}. The envelope taken at g = 2.0024 is clearly different (Fig. 3C). Now the envelope has its absolute maximum at τ_{12} = 700 ns while the submaximum is situated at τ_{12} = 1150 ns.

If the excitation band width exceeds the largest hyperfine coupling, then the echo modulation envelope is invariant with field position and conversely, the lineshape does not depend on τ_{12}. This situation apparently prevails for U^-_{unpol}, and, as we have shown earlier that the echo modulation envelopes of U^-_{pol} and U^-_{unpol} are identical when measured at 20 ms after the laser flash [2], also for U^-_{pol} at long delay times between the flash and the first microwave pulse. However, the fact that for U^-_{pol} at early times after the flash the lineshape does depend on τ_{12}, and the echo modulation envelope on the field position, indicates that for short delay times there are contributions to the lineshape of U^-_{pol} whose width exceeds the excitation band width (see Note added in proof). We will now turn to the time dependence of these contributions.

Time dependence

As the special lineshape was best observable with a τ_{12} of 950 ns (Fig. 2), we used this τ_{12} to study the time dependence of the shoulder. Typical electron spin echo field-swept spectra at 25 and 500 μs after the flash are shown in Fig. 4. The spectrum at 600 ns after the flash was already shown in Fig. 2B. These spectra show that at longer times after the flash the shoulder has disappeared. In fact the shoulder had already largely disappeared at 250 μs (not shown), which is in agreement with the time dependence as observed by fast continuous wave EPR (Fig. 1).

Our electron spin echo data show conclusively that the decay of the high field shoulder in the continuous wave EPR spectra is not determined by microwave-induced polarization decay. Another important feature, which is also observable in Fig. 4, is that the maximum of the lineshape at short times after the flash is shifted to a higher g-value compared to that long after the flash. At 500 μs after the flash this maximum is at the familiar 2.0043 ± 0.0008 value of U^-_{unpol}.

The shift of the g-value of the top of U^-_{pol} as a function of the delay time between the laser flash and the first microwave pulse makes it impossible to monitor the decay time of the shoulder directly. A further problem is that the presence of the triplet signal at early times after the flash results in a time-dependent sloping baseline. We therefore applied the analyzing procedure as depicted in Fig. 4. The intensity of the maximum of the lineshape (I_m) and that of a point 5.4 G upfield, approximately corresponding to the position of the shoulder (intensity I_s), was measured with respect

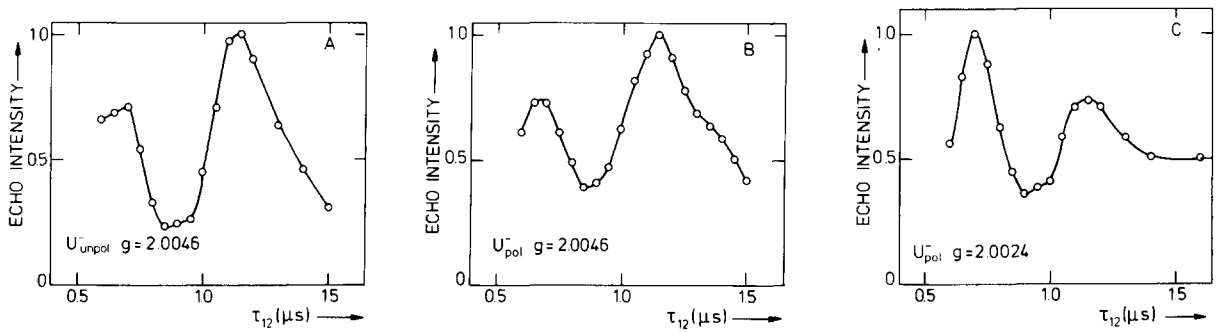

Fig. 3. Electron spin echo envelope modulation traces of (a) U^-_{unpol} at g = 2.0046; (b) U^-_{pol} at g = 2.0046 at 600 ns after a laser flash; (c) U^-_{pol} at g = 2.0024 at 600 ns after a laser flash. Temperature, 4.2 K.

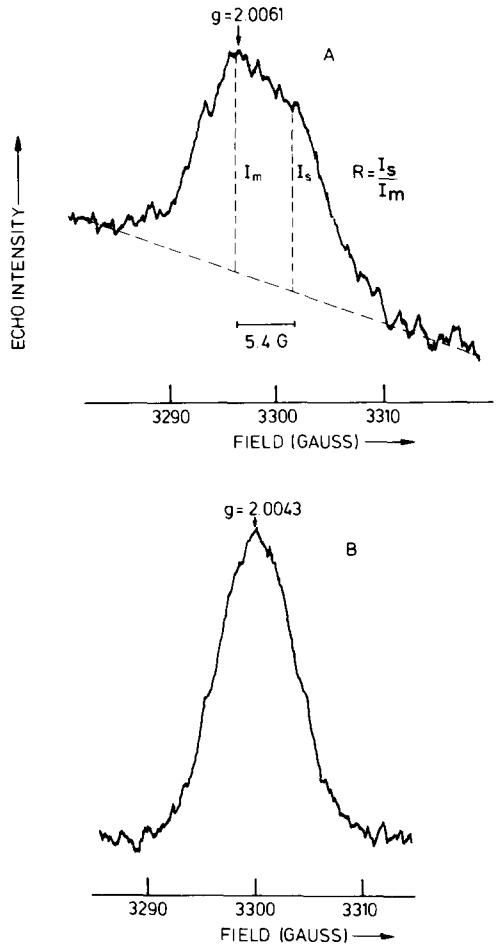

Fig. 4. Time dependence of the U^-_{pol} lineshape as measured by electron spin echo at (a) 25 μs after the flash; (b) 500 μs after the flash. Temperature, 4.2 K.

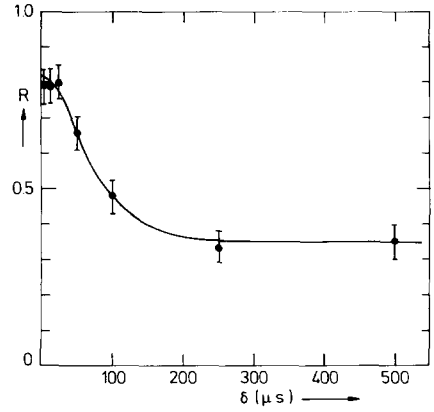

Fig. 5. The dependence of R as defined in Fig. 4 of the delay, δ, between the laser flash and the first microwave pulse. $\tau_{12} = 950$ ns; temperature, 4.2 K.

to the interpolated baseline. The time dependence of the ratio R of these two intensities is depicted in Fig. 5. It is seen that R has its maximum value at times early after the flash and decays to a steady-state value of 0.35. This value is precisely the same as that found for the electron spin echo field-swept U^-_{unpol} spectra (data not shown). From Fig. 5 the half-time of the shoulder is estimated to be 67 ± 15 μs.

Discussion

In this section we will combine the various results of the previous section to develop a model that could explain our observations. First it is important to note that our electron spin echo results reproduce the earlier continuous wave flash EPR results [5], both with regard to the lineshape and to the kinetics of the special feature. This excludes microwave-power-induced relaxation and passage effects as the cause of the unusual lineshape of U^-_{pol} at early times. From Fig. 1 it is clear that the total linewidth of all EPR spectra, including the special feature, is the same as that of U^-_{unpol}. This means that there is a splitting and a narrowing of the U^-_{pol} line shortly after the flash. This splitting and narrowing moves the maximum of the line to higher g-values at shorter interval between flash and boxcar gate.

The dependence of the U^-_{pol} lineshape on τ_{12} (Fig. 2) at a fixed, short time after the flash is different from that of U^-_{unpol}. From the comparison of the echo modulation patterns of U^-_{pol} and U^-_{unpol} it follows that the dependence on τ_{12} is not due to the fact that the excitation bandwidth is small compared to the total linewidth. This indicates that in the flash-induced polarized state at early times after the flash the contributions of the spin packets to the echo intensity are different from those in the unpolarized state.

The above observations suggest a magnetic interaction of U^-_{pol} with another paramagnetic system. Apparently, this interaction has a decay half-time of 67 ± 15 μs. This lifetime corresponds closely to the halftime of the fast decaying sublevels of the triplet state of the primary donor as measured with fluorescence-detected EPR in zero field (78 μs [14]) and low-temperature flash spectroscopy (84 μs [15]). This strongly suggests that

the peculiar lineshape of U_{pol}^- shortly after a laser flash is due to magnetic interaction between U_{pol}^- and the triplet state of the primary donor.

In the next section we will endeavour to simulate the EPR lineshape of U_{pol}^- with a model incorporating a combination of a dipolar and an exchange interaction between ^{3}P-860 and U_{pol}^-.

Simulation of U_{pol}^- ($t = 0$)

To describe the influence of the magnetic interaction between U_{pol}^- and ^{3}P-860 on U_{pol}^- we set up the spin Hamiltonian:

$$\mathcal{H} = \beta \boldsymbol{H} \cdot \boldsymbol{g}_1 \cdot \boldsymbol{S}_1 + \sum_i a_i \boldsymbol{S}_1 \cdot \boldsymbol{I}_i + J \boldsymbol{S}_1 \cdot \boldsymbol{S}_2 + \frac{(\boldsymbol{S}_1 \cdot \boldsymbol{g}_1 \beta) \cdot (\boldsymbol{S}_2 \cdot \boldsymbol{g}_2 \beta)}{r^3} - \frac{3[(\boldsymbol{S}_1 \cdot \boldsymbol{g}_1 \beta) \cdot \boldsymbol{r}][(\boldsymbol{S}_2 \cdot \boldsymbol{g}_2 \beta) \cdot \boldsymbol{r}]}{r^5} \quad (1)$$

Here the first two terms describe the electronic Zeeman and hyperfine interaction of U_{pol}^- ($\boldsymbol{S}_1$), with $\boldsymbol{g}_1$ the g-tensor of U_{pol}^- [3] and a_i the isotropic hyperfine constant of nucleus i. The third and fourth terms describe the exchange and dipolar interaction between the U_{pol}^- ($\boldsymbol{S}_1$) and the ^{3}P-860 ($\boldsymbol{S}_2$) spin systems, respectively. J is the exchange coupling constant, $\boldsymbol{g}_2$ the g-tensor of ^{3}P-860 and $\boldsymbol{r}$ the vector connecting the two point dipoles. The scalar r is the length of $\boldsymbol{r}$.

In order to simplify the exchange and dipolar parts of the Hamiltonian of Eqn. 1 the following assumptions were made:

(1) The $S_1 = \frac{1}{2}$ system as well as the $S_2 = 1$ system was assumed to be totally quantized by the external magnetic field (high-field approximation).

(2) Applying first-order time-independent perturbation theory, only the z components of the spin operators can give a correction on the energy levels. Therefore the non-secular terms were omitted. Note that, although the triplet is populated in the $m_s = 0$ sublevel and consequently $S_{2z} = 0$ regardless of the direction of the magnetic field with respect to the triplet axes, the presence of the microwave field populates the $m_s = \pm 1$ sublevels for which $S_{2z} \neq 0$ *. In the simulation we have assumed equal population of the three magnetic sublevels.

(3). In Eqn. 1, the total contribution of the dipolar term to the linewidth is of the same order of magnitude as that of the anisotropic part of the Zeeman term. The dipolar term is by far dominated by the isotropic part of the g-tensor, whereas the anisotropic part makes only a small contribution to this term. In the calculation of the dipolar interaction we have therefore approximated $\boldsymbol{g}_1$ and $\boldsymbol{g}_2$ by the free electron g-value (g_e).

With these assumptions the exchange and dipolar contributions in Eqn. 1 reduce to:

$$\mathcal{H}_{dip} = \frac{g_e^2}{r^3}(1 - 3\cos^2\gamma) S_{1z} S_{2z} \quad (2a)$$

$$\mathcal{H}_{ex} = J S_{1z} S_{2z} \quad (2b)$$

where γ is the angle between $\boldsymbol{r}$ and the field $\boldsymbol{H}$. This angle can be calculated from the angles θ, φ, α and β defined in Fig. 6, which angles give the orientation of g-tensor of U_{pol}^- with respect to the external field $\boldsymbol{H}$ and the orientation of $\boldsymbol{r}$ with respect to the g-tensor. We assume that the g-value of ^{3}P-860 is isotropic. The appropriate spin functions for the resulting Hamiltonian are the product functions $|\phi_{U_{pol}^-}, \phi^3\text{P-860}\rangle$ of the U_{pol}^- ($S_1 = \frac{1}{2}$)

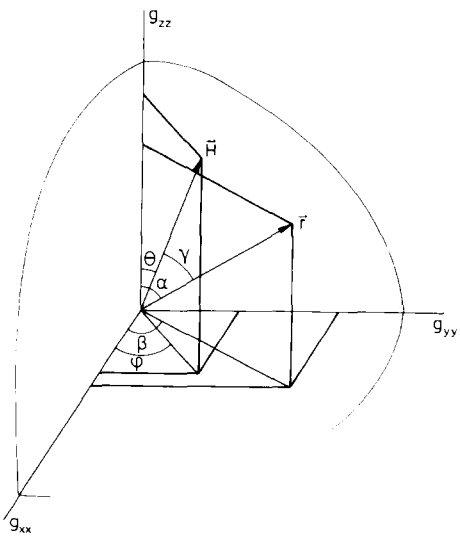

Fig. 6. Definition of the angles between $\boldsymbol{H}$, $\boldsymbol{r}$ and the principal axes of the ubiquinone g-tensor.

* For pulsed EPR this argument is somewhat modified, as the microwave pulses in EPR create a coherent superposition of the three triplet sublevels.

and the ^{3}P-860($S_2 = 1$) spin systems:

$$\begin{array}{ll} |-\tfrac{1}{2},1\rangle & |+\tfrac{1}{2},1\rangle \\ |-\tfrac{1}{2},0\rangle & |+\tfrac{1}{2},0\rangle \\ |-\tfrac{1}{2},-1\rangle & |+\tfrac{1}{2},-1\rangle. \end{array} \quad (3)$$

In the simulation the g-tensor of U_{pol}^- was fixed at the values found in [3].

Eqn. 1 treats the spin system as a static system in which the exchange interaction gives rise to a shift of the energy levels. However, exchange interaction between two radicals having an EPR signal which is inhomogeneously broadened by hyperfine interactions generally gives rise to line narrowing [18], because of the dynamic exchange process. To avoid a full dynamic treatment we have phenomenologically incorporated a reduction of the hyperfine coupling constants of U_{pol}^- by an adjustable factor equal for all a_i.

The EPR absorption lineshape was calculated at a given orientation of U_{pol}^- with respect to the magnetic field direction, keeping the orientation of U_{pol}^- with respect to ^{3}P-860 fixed. The spectra were then averaged over all possible orientations of the ubiquinone molecule with respect to the magnetic field. As a first approximation we assumed a uniform polarization, which was taken into account by multiplying the calculated absorptive lineshape by -1.

In Fig. 7 the simulated spectrum is compared with the EPR boxcar spectrum at 50 μs after the flash. It is seen that the special features of the 50-μs spectrum are quite well simulated. Not surprisingly, the simulated lineshape was strongly dependent on r. Acceptable fits were only obtained for values of r not deviating more than 0.2 nm from that of the best fit shown in Fig. 7, with $r = 1.85$ nm, $J = 9.3 \cdot 10^{-6}$ cm^{-1} and $\alpha = 20°$ ($\pm 20°$). Our best fit value of r is in agreement with the value $r \geqslant 1.55$ nm as suggested by Tiede [16] and with the value of $r \approx 2.0$ nm estimated from the X-ray stereo figures published in [19]. Comparing the U_{pol}^- lineshape of Fig. 1A with the inhomogeneously broadened near-Gaussian lineshape of U_{unpol}^- ([1], Fig. 1D) a low value for α is indeed expected, because the strong asymmetry of the U_{pol}^- lineshape at early times after the flash can only be caused by an enhancement of the g_z anisotropy, which is at its maximum when $\gamma = 0$, i.e., $\alpha = 0$.

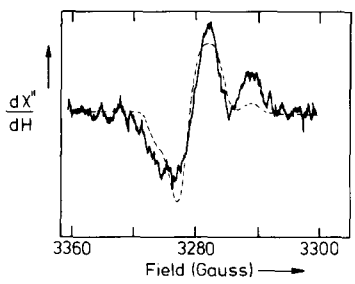

Fig. 7. Simulation (— — —) of the continuous wave EPR boxcar spectrum (———) at 50 μs after the flash. $g_x = 2.0067$, $g_y = 2.0056$, $g_z = 2.0024$, $a_{CH_3} = 2.3$ MHz, $a_{CH_2} = 1.8$ MHz, $J = 9.3 \cdot 10^{-6}$ cm^{-1}, $\alpha = 20 \pm 20$ degrees, $\beta = 80 \pm 20$ degrees and $r = 1.85 \pm 0.20$ nm.

Of course, our simulation does not reveal the orientation of the ubiquinone molecule in the photosynthetic membrane. If we assume the distance vector $\boldsymbol{r}$ to be perpendicular to the membrane surface, then the g_z axis of the ubiquinone is also approximately ($\pm 20°$) perpendicular to the membrane, hence the plane of the ubiquinone approximately parallel to the membrane. This agrees with measurements on membrane multilayers of *Rhodospirillum rubrum* by Hales and Das Gupta [17], but deviates from those of Tiede et al. [16] who found that in chromatophores of *Rhodopseudomonas sphaeroides* R-26 and *Chromatium vinosum* and in reconstituted membrane multilayers of reaction centers of *Rhodopseudomonas sphaeroides* 2.4.1. the g_z axis is parallel to the membrane.

The value of J of $9.3 \cdot 10^{-6}$ cm^{-1} that is obtained from the application of Eqs. 1–3 must be consistent with the reduction of the hyperfine constants of U_{pol}^- ($t = 0$) (Fig. 7) as compared to those of U_{unpol}^- [3] that was introduced to take into account exchange narrowing. For the simulation of Fig. 7 we have used U_{pol}^- hyperfine coupling constants that were reduced by approximately 3 MHz as compared to those found in [3]. To first approximation, this reduction corresponds to the rate of spin exchange k_x between ^{3}P-860 and U_{pol}^-. For $k_x = 3 \cdot 10^6$ s^{-1}, applying Fermi's golden rule and approximating the density of states by the inverse of J [1] we obtain $J = 2.5 \cdot 10^{-6}$ cm^{-1}, close to the value of $9.3 \cdot 10^{-6}$ cm^{-1} used for the fit of Fig. 7. Of course, the above-mentioned rea-

soning is only approximate, as for energetic reasons the exchange rate will only be important for ^{3}P-860 molecules having an orientation such that their EPR transition lies close to that of U^-_{pol}. In addition, the lineshape of U^-_{pol} at early times is dominated by the dipolar interaction, not by the exchange interaction. Both these considerations make the density of states a complicated function of orientation. However, as the main effect on the lineshape seems to be due to the dipolar interaction, a more precise calculation of the exchange rate does not seem to be warranted.

Conclusions

From the time dependence of the U^-_{pol} lineshape as measured with timeresolved continuous wave EPR and electron spin echo techniques we conclude that the U^-_{pol} signal at early times after a laser flash is split and narrowed by magnetic interactions between the ubisemiquinone and the triplet state of the primary donor.

Using a spin Hamiltonian including magnetic dipolar and exchange interactions the U^-_{pol} lineshape could be satisfactorily simulated, yielding a value of 1.85 nm for the distance between the ubiquinone and the primary donor. The z axis of the ubiquinone g-tensor appears to be approximately parallel to the distance vector between the quinone and the primary donor, i.e., the plane of the ubiquinone molecule is approximately parallel to the membrane surface.

Note added in proof (Received April 17th, 1985)

The asymmetry of the U^-_{pol} lineshape indicates that these contributions are anisotropic. Hence, the relative contribution of a particular orientation (of U^- with respect to the external magnetic field) to the echo intensity will depend on the field value. As electron spin echo envelope modulation originates from anisotropic hyperfine interactions, a field (i.e., orientation) dependence may be expected.

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