On the Influence of Microwaves and Static Magnetic Fields on the Recombination of Radical Ions in Reaction Centers of Photosynthetic Bacteria

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Recombination of the bacteriochlorophyll dimer cation and the bacteriopheophytin anion depends on the spin multiplicity of this radical ion pair and can be studied by optical spectroscopy in an external static magnetic field in the absence (MARY) and presence of microwaves (RYDMR). Model calculations of MARY- and RYDMR-spectra are discussed.

Interaction of pigments involved in the first fast electron transfer steps in photosynthetic reaction centers can be studied by a variety of methods focussing on different electronic states as e.g. radical ion pairs. Considering the bacteriochlorophyll dimer cation, $(\mathrm{BChl})_2{}^{+}$, and the bacteriopheophytin anion, $\mathrm{BPh}^{\overline{\cdot}}$, in quinone depleted reaction centers of Rhodopseudomonas spheroides, strain R-26, it has been established [1, 2] that the recombination pathway is spin selective leading with a much higher rate k_{T} to the triplet state $^3(\mathrm{BChl})_2{}^{*}$ than via k_{S} to the singlet ground state of $(\mathrm{BChl})_2$ and BPh. Since singlet and triplet phased radical pair states are coupled by hyperfine interaction (HFI), the product yield in the two spin states can be manipulated by external magnetic fields (a static magnetic field H_0 and/or a microwave field H_1) as indicated in the kinetic scheme:

$$\begin{tikzcd}
{}^1[(\mathrm{BChl})_2^{+}\ldots\mathrm{BPh}^{\overline{\cdot}}] \arrow[r, leftrightarrow, "\mathrm{HFI}", "H_0,\,H_1"'] \arrow[d, "k_{\mathrm{S}}"] & {}^3[(\mathrm{BCHl})_2^{+}\ldots\mathrm{BPh}^{\overline{\cdot}}] \arrow[d, "k_{\mathrm{T}}"] \\
\text{singlet product} & \text{triplet product}
\end{tikzcd}$$

It is the purpose of this paper to discuss the kinetic and structural information which can be derived from the experimental dependence of the triplet yield on static magnetic fields, in the absence and in the presence of microwaves.

*MA*gnetic field dependent *R*eaction *Y*ields (MARY) of the recombined triplet states of photosynthetic reaction centers have been measured with nanosecond time resolution [2]. The observed spectra exhibit a narrow MARY line (FWHM $= 66$ G) with large peak to background ratio indicating low splitting between the singlet and triplet states. To fit these spectra only an isotropic exchange interaction J was considered, yielding the very small value of $J \leqq 5$ G associated with the kinetic parameters $k_{\mathrm{S}} = 3.9 \cdot 10^7\ \mathrm{s}^{-1}$ and $k_{\mathrm{T}} = 7.4 \cdot 10^8\ \mathrm{s}^{-1}$ [2]. The rate induced lifetime broadening of the singlet and triplet levels is comparable to their mutual splitting. Therefore, the fitting of an unambiguous set of kinetic and energetic parameters can be successful only, if both the time evolution of the product yield and the lineshape of MARY are taken into account simultaneously. Details of the interaction between the radical spins, however, remain unclear.

Recently, a new approach to this problem was made by observing *R*eaction *Y*ield *D*etected *M*agnetic *R*esonance (RYDMR) on the short-lived radical pair, $^3[(\mathrm{BChl})_2{}^{+}\ldots\mathrm{BPh}^{\overline{\cdot}}]$ [3]. The method makes use of the effect that the observed yield of $^3(\mathrm{BChl})_2^{*}$ is influenced by microwaves under specific resonance conditions.

A theoretical description of RYDMR can be accomplished starting with the total high field spin Hamiltonian in the rotating frame ($\hat{S}_x = \hat{S}_{1x} + \hat{S}_{2x}$, $\hat{S}_z = \hat{S}_{1z} + \hat{S}_{2z}$):

$$\begin{aligned}\hat{\mathscr{H}}^{(k)}_{\mathrm{rot}} = {} & \hbar\,\Delta\omega_{(k)}\,\hat{S}_z + g\,\beta\,H_1\,\hat{S}_x + a_{(k)}\,(\hat{S}_{1z} - \hat{S}_{2z}) \\ & - J\,\hat{P}^{\mathrm{s}} + D_{zz}\,(\tfrac{3}{2}\,\hat{S}_z^2 - \hat{P}^{\mathrm{r}})\,. \end{aligned} \tag{1}$$

The index k represents the different nuclear spin configurations. Since anisotropy of hyperfine inter-

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action is neglected, nuclear Zeeman interaction can be ignored, too. Thus, the nuclear configuration is a constant of motion, the index k running over a statistical distribution of initial nuclear arrangements. The z-component of the effective magnetic field in the rotating frame is given by $\hbar \Delta\omega_{(k)}/g\beta$, consisting of the residual Zeeman contribution and the hyperfine contribution:

$$\hbar \Delta\omega_{(k)} = g\beta H_0 - \hbar\omega + \tfrac{1}{2}\left(\sum_n^{(1)} A_n^{(1)} m_{n,k}^{(1)} + \sum_n^{(2)} A_n^{(2)} m_{n,k}^{(2)}\right). \quad (2)$$

Hyperfine coupling data for the various nuclei (n) on the two radicals, (1) and (2), were taken from [4].

Transitions from the initial singlet state to the T_0 sublevel of the radical pair proceed with the mixing frequency $a_{(k)}/\hbar$:

$$a_{(k)} = \tfrac{1}{2}\left(\Delta g\beta H_0 + \sum_n^{(1)} A_n^{(1)} m_{n,k}^{(1)} - \sum_n^{(2)} A_n^{(2)} m_{n,k}^{(2)}\right). \quad (3)$$

At moderately high external magnetic fields ($H_0 \approx 3$ kG) the contribution from the hyperfine interaction dominates the one from the difference of the magnetic g-factors ($\Delta g = -0.001$). Microwaves with a field strength H_1 couple T_0 and $T_{\pm 1}$ triplet substates. The exchange interaction J and the contribution D_{zz} of the spin dipolar interaction cause energy splittings thus hindering transitions between the states involved (S, T_0, $T_{\pm 1}$). D_{zz} is the component of the dipole tensor parallel to the external field ($D_{zz} = \hat{z} \cdot \bar{\bar{D}} \hat{z}$) given by

$$D_{zz}(\theta, \psi) = (\cos^2\theta - \tfrac{1}{3}) \cdot D - \sin^2\theta \cos 2\psi \cdot E. \quad (4)$$

The Euler angles θ and ψ describe the orientation of the dipolar main axis system in the laboratory frame. The operators $\hat{P}^{\mathrm{S}}$ and $\hat{P}^{\mathrm{T}}$ project out the singlet state and triplet states, respectively. The triplet yield is calculated numerically by solving the stochastic Liouville equation in the rotating frame

$$\frac{\mathrm{d}\hat{\sigma}(t)}{\mathrm{d}t} = -\frac{i}{\hbar}[\hat{\mathscr{H}}_{\mathrm{rot}}, \hat{\sigma}(t)] - \frac{k_{\mathrm{S}}}{2}\{\hat{P}^{\mathrm{S}}, \hat{\sigma}(t)\} - \frac{k_{\mathrm{T}}}{2}\{\hat{P}^{\mathrm{T}}, \hat{\sigma}(t)\}. \quad (5)$$

$\hat{\sigma}(t)$ is the density matrix in the rotating frame, [ ] and { } are the commutator and the anticommutator, respectively.

The RYDMR spectra, i.e. the relative triplet yield $\langle \Phi_{\mathrm{T}}(H_0, H_1)\rangle / \langle \Phi_{\mathrm{T}}(H_0, H_1 = 0)\rangle$ were obtained as a function of H_0 by averaging over all orientations of the reaction centers. All calculations refer to the triplet yield accumulated at $t = 500$ ns after generation of the radical pair. The microwave frequency $\omega/2\pi$ was set to 9.2 GHz.

Figure 1a shows the calculated RYDMR spectrum for the set of parameters: $J = 0$, $D = 0$, $k_{\mathrm{S}} = 3.9 \cdot 10^7\ \mathrm{s}^{-1}$ and $k_{\mathrm{T}} = 7.4 \cdot 10^8\ \mathrm{s}^{-1}$, consistent with the above mentioned MARY experiments. The RYDMR line is inverted, i.e. the triplet yield decreases by the application of microwaves. For $J = 0$ this is true for any values of H_1, D, k_{S} and k_{T}. This behaviour reflects the increase of the energy gap between the triplet sublevels and the singlet state due to dynamic Zeeman splitting in the microwave field H_1.

In contrast to the RYDMR line (a) in Fig. 1, the RYDMR spectrum measured by Bowman et al. [3] is non-inverted at low microwave powers. As our calculations show, such a spectrum can only be simulated with values of J at least comparable to the lifetime broadening of the spin states by the

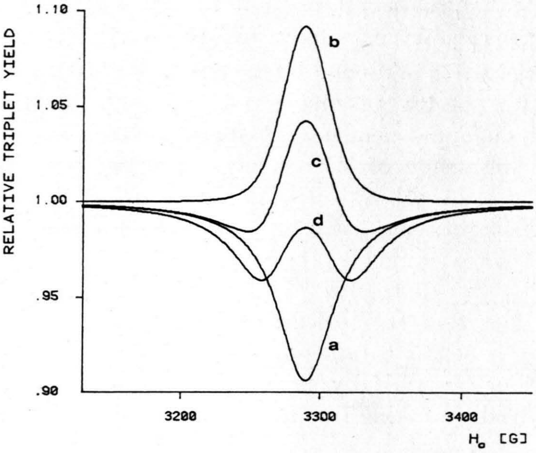

Fig. 1. RYDMR spectra for $H_1 = 10$ G and
a) $J =$ 0 G, $D =$ 0 G, $\langle \Phi_{\mathrm{T}}(H_0, 0)\rangle = 0.23$,
b) $J = 20$ G, $D = -60$ G, $\langle \Phi_{\mathrm{T}}(H_0, 0)\rangle = 0.09$, $\hat{E}_{\mathrm{exc}} \parallel \hat{E}_{\mathrm{prob}} \parallel \vec{H}_0$;
c) $J = 20$ G, $D = -60$ G, $\langle \Phi_{\mathrm{T}}(H_0, 0)\rangle = 0.14$, $\hat{E}_{\mathrm{exc}} \parallel \vec{H}_0$, $\hat{E}_{\mathrm{prob}} \perp \vec{H}_0$;
d) $J = 20$ G, $D = -60$ G, $\langle \Phi_{\mathrm{T}}(H_0, 0)\rangle = 0.21$, $\hat{E}_{\mathrm{exc}} \perp \vec{H}_0$, $\hat{E}_{\mathrm{prob}} \perp \vec{H}_0$; $\hat{E}_{\mathrm{exc}} \perp \hat{E}_{\mathrm{prob}}$.

recombination processes $(J \gtrsim \hbar(k_S + k_T)/2)$. For $D = 0$ the exchange interaction J cannot be much stronger than $\hbar(k_S + k_T)/2$. Otherwise, the RYDMR spectrum at low microwave powers as well as the MARY spectrum would consist of two distinct resonance lines in contradiction to the experimental results. For $D \neq 0$, however, J may well exceed this limit. For instance, in the case of $J = -D/3$, the triplet sublevels T_x and T_y are (approximately) degenerate with the singlet level at zero magnetic field. The MARY spectra then remain unsplit for any value of J and moreover for any orientation of the dipole tensor. For such a case of non vanishing dipolar interaction (J = 20 G, D = −60 G) the RYDMR spectrum is shown in Figure 2. The transition moments of probing and excitation were assumed to be parallel to the symmetry axis of the dipole tensor while the polarisation vectors $\hat{E}_{\text{exc}}$, $\hat{E}_{\text{prob}}$ of the exciting and probing light were chosen parallel to the external field. As to its inversion with increasing microwave power the RYDMR spectrum of Fig. 2 qualitatively resembles the spectrum observed by Bowman et al. [3]. Similar spectra, however, can be obtained for vanishing dipolar interaction and J comparable to or slightly exceeding $\hbar(k_S + k_T)/2$. The most significant manifestation of a strong dipolar interaction is expected to occur in the angular dependence of RYDMR (and also of MARY) spectra as depicted in Fig. 1, b–d for J = 20 G and D = −60 G. To clearly establish the participation of dipolar interaction in RYDMR and MARY spectra it is therefore indispensable to test their angular dependence. First attempts to measure the anisotropy of MARY have been reported by Boxer et al. [5].

The apparent discrepancy between the finding that our experiments [2] were consistent with $J = 0$, $D = 0$, $k_S = 3.9 \cdot 10^7\,\text{s}^{-1}$ and $k_T = 7.4 \cdot 10^8\,\text{s}^{-1}$ and the fact that the (calculated) RYDMR spectrum for this set of parameters does not show the behaviour observed by Bowman et al. [3] may be resolved by taking into account the wide range of reaction center quality due to preparation and aging. Such variations seem to be especially pronounced in the case of quinone depleted reaction centers. Besides the very narrow MARY spectrum published [2] we observed as well much broader spectra depending on preparation and age. This implies that results derived from experiments on different samples are not necessarily consistent.

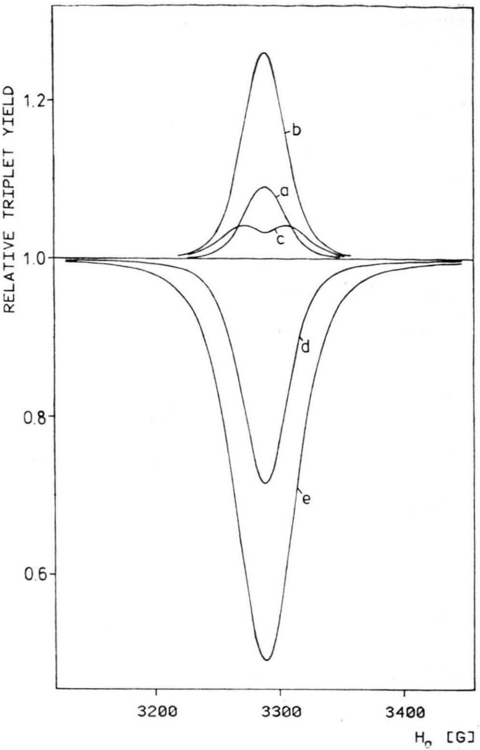

Fig. 2. RYDMR spectra for J = 20 G, D = −60 G, $\langle \Phi_T(H_0, 0) \rangle = 0.09$, $\hat{E}_{\text{exc}} \parallel \hat{E}_{\text{prob}} \parallel \vec{H}_0$ and a) H_1 = 10 G, b) H_1 = 25 G, c) H_1 = 40 G, d) H_1 = 55 G, e) H_1 = 70 G.

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