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ON THE MAGNETIC FIELD DEPENDENCE OF THE YIELD OF THE TRIPLET STATE IN REACTION CENTERS OF PHOTOSYNTHETIC BACTERIA

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Summary

The yield of the triplet state in reaction centers of *Rhodopseudomonas sphaeroides* is dependent on the strength of an applied magnetic field. Reaction centers of the wild type that lack a functional iron complexed to the primary acceptor ubiquinone show a dependence similar to that of reaction centers of the mutant R-26 in which the iron-ubiquinone complex is intact. Apparently, the iron of the iron-ubiquinone complex is not essential to the effect, but it does exert an influence on its extent. In chromatophores, the effect is about 2-fold decreased; the value of the magnetic field at which half the effect is found is about 500 G, in contrast to this value for reaction centers, which is 50—100 G. The magnetodependence of the triplet yield is discussed in terms of the Chemically Induced Dynamic Electron Polarization mechanism (CIDEP).

Recently, Blankenship et al. [1] and Parson [2] observed that the yield of the triplet state generated in bacterial reaction centers in which the "primary" acceptor iron-quinone is reduced, is dependent on the strength of an applied magnetic field. At room temperature, the triplet yield was about 50% lower in high magnetic fields and the field at which half the effect was observed, $B_{1/2}$, was about 500 G (50 mT). The authors explained their observation using the theory for Chemically Induced Dynamic Nuclear Polarization (CIDNP) as developed by Kaptein [3]. In a high magnetic field, the energy difference between the $T_{\pm}$ and T_0 components of the triplet state of the radical pair P^+I^- (P is the primary donor, I the first identifiable acceptor, probably bacteriopheophytin [4—7]) is much larger than the energy difference between T_0 and the singlet state of the pair. Therefore, in a high magnetic field, the singlet state in which the pair is created mixes only with the T_0 triplet state, the interaction being proportional to $(E_S - E_T)^{-2}$, whereas without a magnetic field the triplet components T_0, T_+ and T_- are equally populated. One can visualize the mixing of the states by a dephasing of the spins of the electrons [8]. Although immediately after charge separation, the spins $\vec{S}_1$ and $\vec{S}_2$ are uncoupled ($J \cong 0$), they are still correlated, i.e. in a singlet configuration with $\vec{S}_1 + \vec{S}_2 = 0$. In a magnetic field, $\vec{S}_1$ and $\vec{S}_2$ perform a Larmor precession around the magnetic field vector $\vec{H}$. Because of different local magnetic fields due to hyperfine interactions, and because of different electronic *g*-values, the precession frequency of $\vec{S}_1$ and $\vec{S}_2$ is not quite the same, and the spins get out of phase, i.e. at a certain time after the separation, $\vec{S}_1$ and $\vec{S}_2$ are aligned in the same direction and the resultant spin $\vec{S} = \vec{S}_1 + \vec{S}_2 = 1$, giving rise to a triplet state. Without other external dephasing fields, the phase relationship of $\vec{S}_1$ and $\vec{S}_2$ will oscillate between pure singlet and pure triplet state.

According to this mechanism, triplet formation must be completed within the lifetime of the state P_F, which is about 10 ns. Mixing in this short time could be produced by assuming a magnetic interaction between I^- and a third component, such as X^-, the reduced iron-ubiquinone (Fe • UQ) complex. In order to test this hypothesis, we have measured the field dependence of the triplet yield in chromatophores, in reaction centers with intact Fe • UQ complex (RC_m), and in reaction centers in which only ubiquinone acts as the primary acceptor (RC_{wt}). We have found that the magnetic field effect is present in all preparations, but not to the same extent. $B_{1/2}$ also showed a marked dependence on the preparation used. In chromatophores, the triplet yield in high magnetic field is 15% lower than that in zero-field and $B_{1/2}$ was about 500 G. In RC_m, the triplet yield is 25% lower in a magnetic field, the total extent of the effect is already obtained in fields as low as 100 G. In a RC_{wt} preparation, the triplet yield in field is 35% lower, the effect being fully developed at about 50 G. The iron-chelating agent *ortho*-phenanthroline has a marked influence on the magnetic field effect.

Our findings lead to the conclusion that the magnetic field effect is critically dependent on the value of the exchange parameter J, which measures the exchange interaction between members of the pair P^+I^-. Apparently, the iron of the Fe • UQ complex is not essential to the effect, but it does exert an influence on the extent of it. It seems that the magnetic field effect on the triplet yield is a more sensitive test of the "nativeness" of reaction center preparation than the decay time of the back reaction $P^+IX^- \rightarrow PIX$ [9].

Chromatophores of *R. sphaeroides* wild type and its mutant R-26 (kindly provided by Dr. G. Feher) were prepared by sonication of cells in growth medium and differential centrifugation. Reaction centers were isolated as described [10]. Briefly, a reaction center fraction was obtained by treatment of chromatophores with sodium dodecyl sulphate. This fraction was purified further by gradient centrifugation in the presence of urea and Triton X-100 at pH 10 (RC_{wt}) or lauryl dimethylamine oxide (LDAO) (RC_m), followed by dialysis against a solution of 0.05 M Tris (pH 8) containing 0.01 M $MgCl_2$. The reaction centers thus prepared are fully photoactive in all respects [10—13] as measured by differential absorption spectroscopy, except for the characteristic time of the back reaction $P^+X^- \rightarrow PX$ in RC_{wt} which is at 77 K the same as for chromatophores (30 ms), but is slower by about a factor of two at room temperature (200 ms vs. 90 ms for chromatophores). The EPR spectrum of the P^+X^- state of RC_{wt} shows a slightly asymmetric gaussian line, with width $\Delta H = 10$ G and g-value $g = 2.0037$ (measured with a P-doped Si marker). The state PX^- shows a narrow line, $\Delta H = 8.7$ G, at $g = 2.0042$, which has some structure. The line shape resembles the one obtained by Feher et al. [14] for the state PX^- of iron-depleted reaction center particles. At 10 K, no signal similar to the $(Fe \cdot UQ)^-$ EPR spectrum [15,16] could be

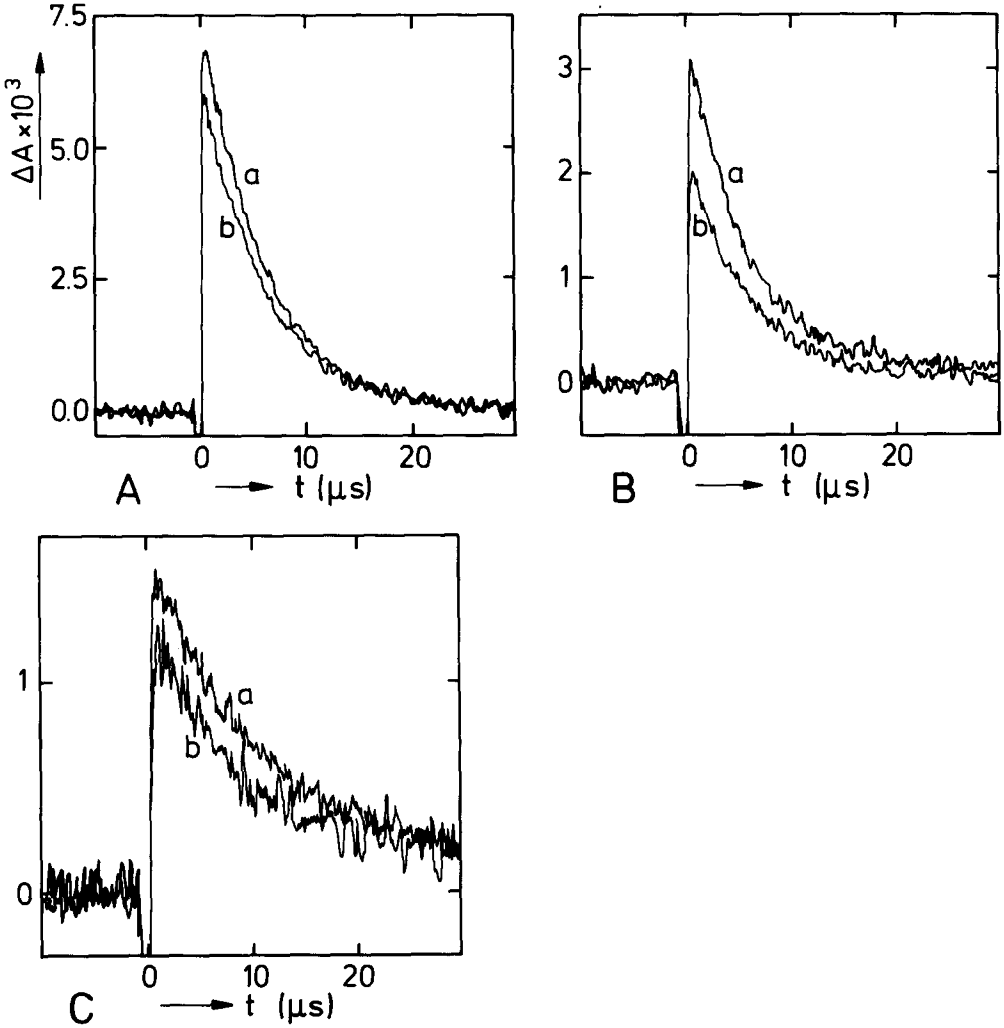

Fig. 1. Kinetics of the triplet absorption, monitored at 545 nm, after a flash for (A) chromatophores of *R. sphaeroides* wild type, (B) reaction centers of same, (C) reaction centers of *R. sphaeroides* R 26. Accumulations of 40, 64 and 192 flashes respectively. Chlorophyll concentration (A) 30 μM, (B) and (C) 8 μM. The excitation light source was a Zeiss dye laser, utilising Rhodamine 6 G as a dye (602 nm, pulse width, 0.3 μs; output 5 mJ per pulse). The measuring light from a 250 W tungsten-iodine lamp was filtered by a AL 547 filter. The photomultiplier (EMI type 9658 R) was protected from the excitation light by AL 549, AL 552 and B 40-544 filters. The detection system consisted of a Biomation 8100 transient recorder, which was read out by a PDP-9 computer for accumulation and further data processing.

detected, only the triplet spectrum [16] being present. On the basis of the EPR evidence, we believe that our reaction centers RC_{wt} do not possess a functional Fe bound to the ubiquinone, i.e. the ubiquinone as such serves as the acceptor. They are thus similar to the particles obtained by Loach [17] and by Feher et al. [14] which were also obtained by SDS treatment. Contrary to the RC_{wt}, the RC_m particles show the normal ESR signal for the state P^+X^- (ΔH = 9.7 G, g = 2.0026) indicating that in these centers the Fe • UQ complex is intact.

Absorption difference spectroscopy was carried out in a single beam spectrophotometer mounted in a Varian 9-inch magnet. No effect of the mag-

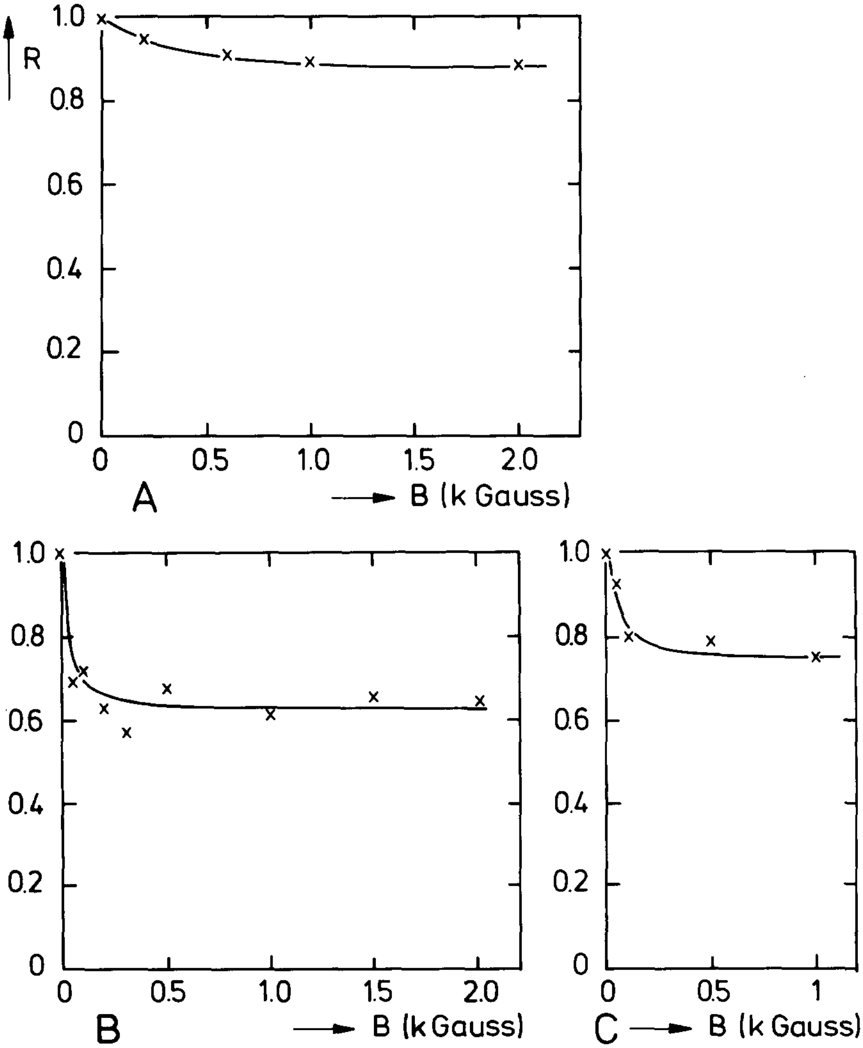

Fig. 2. Relative triplet yields vs. magnetic field strength for (A) chromatophores of *R. sphaeroides* wild type, (B) their reaction centers, (C) reaction centers of *R. sphaeroides* R 26. The magnetic field was measured by an AEG NMR gaussmeter. Values below 300 G were calibrated by extrapolation from a plot of field dial values against measured magnetic field strength. Remanent magnetization was made small by cycling the magnetic field prior to the experiment.

netic field (up to 7 kG) on multiplier efficiency was noticeable. Typical kinetic traces with, and without magnetic field are shown in Fig. 1. The decay curves are single exponentials, with a decay time of 5 to 6 μs. Relative triplet yields are displayed in Fig. 2 as a function of the magnetic field. It is seen that the reaction center preparations all show a field-induced depression of the triplet yield. Apparently, the absence of a functional iron in the Fe • UQ complex does not impair the effect of the magnetic field. The maximum value of the depression is 35% for the RC_{wt} and 25% for the RC_m. Surprisingly, the maximum field effect was already obtained at very low magnetic field, viz. 50—100 G. Chromatophores of *R. sphaeroides* wild type, which were reduced with 100 mM dithionite, initially gave almost no field effect. When *o*-phenanthroline was added, the triplet yield in zero-field remained the same, but the field effect became much stronger, up to 15%.

The finding that an exchange interaction between the iron of the iron-ubiquinone complex is not essential for the magnetic field effect has prompted us to reexamine the application of the radical pair theory as discussed by Parson [2]. In the following we will show that hyperfine fields seem to be sufficient to generate large triplet contributions.

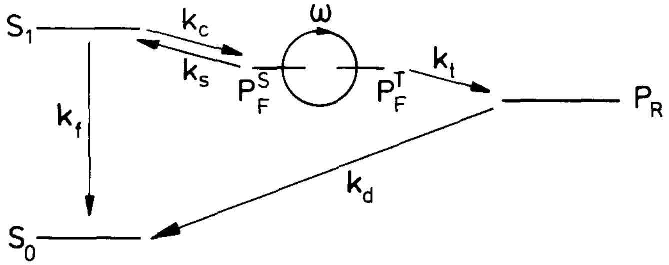

Fig. 3. Energy level scheme of primary reactants (after ref. 18). The intermediary state P_F is a combination of a singlet state P_F^S, and triplet state P_F^T, which in a magnetic field are periodically converted into each other with (angular) frequency ω. In zero magnetic field, they are assumed to be degenerate. Reaction rates k_f, k_c and k_d are about 1 ns^{-1}, 1000 ns^{-1} and 0.2 μs^{-1}, respectively. The lifetime of P_F is about 10 ns. k_f includes fluorescence, energy transfer and radiationless decay.

The various reactions between the primary reactants after a flash can be represented by the scheme of Fig. 3. This scheme is an extension of the one given by Holmes et al. [18], where no distinction between singlet and triplet character of the state P_F was made. These authors introduced the back-reaction from P_F to S_1 to account for the variation in the fluorescence yield after a flash in reaction centers with reduced acceptor. The scheme deviates in this respect from the one presented by Blankenship et al. [1] and Parson [2], who assumed a reaction from P_F directly to the ground state S_0. It seems logical that the back reaction occurs between the singlet state of P_F, P_F^S and S_1, whereas P_R is populated solely via the triplet state of P_F, P_F^T. The frequency of oscillation between P_F^S and P_F^T, ω, is given by Kaptein [3]: $\omega = (a_n^2 + J^2)^{1/2}$, where a_n is given by the difference in local magnetic field as seen by the two electrons:

$$a_n = \tfrac{1}{2}(g_a - g_b)\beta\hbar^{-1}H + \sum_{j}^{a} A_j M_j - \sum_{k}^{b} A_k M_k \tag{1}$$

Here, a and b denote the members of the radical pair, $A_{j,k}$ and $M_{j,k}$ are the hyperfine coupling constants and nuclear quantum numbers of atoms j and k in radical a and b respectively, n stands for one particular spin configuration, and the other symbols have their conventional meaning.

The yield of the triplet P_R can now be calculated in principle by solving the set of differential equations describing the various reactions. The solution, however, is a complex function of the rate parameters, and will not be reproduced here. Instead, we will approximate the yield by introducing an average lifetime for P_F, τ, given by $\tau^{-1} = k_f k_s (k_f + k_s + k_c)^{-1} + mk_t$ or $\tau^{-1} = k_l + mk_t$, where m, the accessible multiplicity of the triplet state of P_F, is 3 and 1 for zero and high magnetic field, respectively. k_l follows from the reaction

$$S_0 \xleftarrow{k_f} S_1 \underset{k_s}{\overset{k_c}{\rightleftarrows}} P_F^S,$$

and represents the "leak" from P_F to S_0. On averaging over the lifetime τ, the average probability of finding P_F^T in high magnetic field is [4—7]:

$$P_t = \frac{2a_n^2\tau^2}{1 + 4\omega^2\tau^2} \tag{2}$$

Implicit in the above approximation is the assumption $k_c, k_s >> \omega > k_f, k_t$. Consequently, the triplet yield can be written as:

$$\phi_t = \frac{P_t' k_t}{(1 - P_t')k_l + P_t' k_t} \tag{3}$$

where P_t' is given by Eqn. 2 for high magnetic fields, and $P_t' = 3/4$ for zero magnetic field. The ratio of the yield in high magnetic field, to that in zero magnetic field is then:

$$R = \phi_t^H/\phi_t^0 = \frac{P_t}{1 - P_t}\left[3 - \phi_t^0\left(3 - \frac{P_t}{1 - P_t}\right)\right]^{-1} \tag{4}$$

We are now in a position to calculate R as a function of H, using Eqns. 1 and 2. When we identify I^- with the anion of bacteriopheophytin [4—7], $|\Delta g| = 0.0009$ (Feher, G., personal communication), and we obtain a contribution to a_n of $4.5 \cdot 10^3\ H$ rad s^{-1}. The sum over $A_k M_k$ cannot be calculated exactly for I^- for want of values of A_k. We may approximate the A_k values by the values for monomeric bacteriochlorophyll [19]. We take into account only the largest contributions to the sums, i.e. the contributions from the four β protons of the hydrogenated pyrrole rings of I^- and the eight β protons of the dimer P^+. Assuming their hyperfine interaction to be equal, being 5.7 and 2.9 G for I^- and P^+, respectively [19], and considering all 4096 possible nuclear spin configurations, we obtain for the rms value of the hyperfine contribution to a_n $0.6 \cdot 10^8$ rad s^{-1}. It is seen that the hyperfine term is dominant up to fields of 10 kG. The lifetime of P_F is estimated to be about 10 ns [20]. Substituting the values for a_n and τ in Eqn. 2, we obtain $P_t \cong 0.3$. Eqn. 4 then yields $R \cong 0.2$ when ϕ_t^0 is taken to be 0.15. From our simple calculation, it is seen that large magnetic field effects can be obtained without having recourse to exchange interactions with a third species. In fact a more sophisticated calculation, based on the exact solution of the kinetics scheme of Fig. 3, and using the exact distribution of nuclear spin configurations yields an even higher value for the hyperfine term of a_n. If the exchange interaction between I^- and X^- which has been reported for low temperatures [21] would persist at room temperature, it would increase ω and lower P_t. It is possible that *o*-phenanthroline, by complexing to the primary acceptor, enhances such an exchange interaction, but as yet we have no firm evidence for this.

From Eqn. 2, it follows that P_t can never exceed the value 0.5, i.e. according to our high field approximation keeping $\phi_t^0 = 0.15$, the upper limit for R is 0.37. We find consistently much higher values for fields up to 7 kG. It is not very probable that this is caused by spin-lattice relaxation between $T_\pm$ and T_0. For small radicals in solution, this relaxation time is of the order of 10 ns [22]. It is doubtful, however, that the modulation of the electron-electron dipolar interaction which is the dominant relaxation perturbation, is as effective in the densely packed reaction centre as it is in solutions of low viscosity. Alternatively, the assumption that only $S \to T_0$ mixing occurs has to be relaxed, and $S \to T_{\pm 1}$ mixing is also to be taken into account. This may be caused by a relatively large exchange interaction between P^+ and I^-. If $J \neq 0$, the singlet level is shifted towards either T_+ or T_-, depending on the sign of J, and the transition probability $S \to T_\pm$ is increased. If J has an appreciable value, the dipolar interaction between the two radicals has also to be taken into account, shifting the $T_\pm$ levels, and making one, or both, more accessible to mixing with the singlet state. Also the low field (50—100 G) at which reaction center preparations already exhibit the full field effect cannot be obtained with simple $S \to T_0$ mixing.

At such low fields, the hyperfine fields become important. This raises the question whether the axis of quantization then still is that of the external magnetic field, or that the local fields determine its direction. In the latter case, the discrimination between T_0 and $T_\pm$ looses its meaning and the theory outlined above is no longer applicable. It may well be that the abrupt onset of the magnetic field effect on R in certain preparations reflects the transition from a situation with many individual axes of quantization to one in which all spins are quantized along the direction of the external magnetic field. Since the hyperfine fields must be the same for all preparations with different $B_{1/2}$, the other contributor to the local field, the spin-spin dipolar interaction, must vary between these preparations, and concomitantly the exchange interaction. This suggests that the different behaviour of chromatophores and reaction centers is caused by different values of J, J being smaller for reaction centers. Since J depends on the relative orientation between the members of the pair P^+I^- as well as on their distance, rather subtle changes in the geometry may cause dramatic effects on the magnetic field dependence of the triplet yield. We are currently exploring these effects in greater detail, with the hope to gain some insight into the molecular structure of the complex containing the primary reactants.

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Note added in Proof: (Received March 23rd, 1977)

We have learned that Blankenship et al. have also found that the extend of the magnetic field effect on the triplet yield is dependent on sample preparation. They have similarly concluded that exchange interaction between the state P_F and the primary acceptor is not the sole determining factor in the magnetic field effect.

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