MAGNETIC FIELD EFFECT ON TRIPLETS AND RADICAL IONS IN REACTION CENTERS OF PHOTOSYNTHETIC BACTERIA

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1. Introduction

The primary step currently assumed in bacterial photosynthesis involves fast electron transfer between the electronically excited bacteriochlorophyll 'special pair' [1], ^{1}(BChl)$_2^*$, and a bacteriopheophytin, BPh, with a rate $k_R > (10 \text{ ps})^{-1}$ [2–4]. In blocked reaction centers, the radical ions thus formed live for ≅ 10 ns at 300 K [5] and decay by the back transfer of the electron. If this occurs in an overall singlet state of the radical ion pair, either the electronic ground states, (BChl)$_2$ and BPh, or, energetically uphill, the excited singlet state [6], ^{1}(BChl)$_2^*$, are populated with the rate constants k_S and k'_S, respectively. As revealed by its unusual spin polarization [7,8] in the ESR and its magnetic field-dependent yield [9,11], the triplet, ^{3}(BChl)$_2^*$, is formed with the rate k_T via another recombination channel opening when hyperfine interaction induces singlet-to-triplet transitions in the pair state (see kinetic scheme in fig.1). Such singlet-to-triplet transitions are partially supressed in an external magnetic field of some 100 Oe. From the magnetic field effect on the triplet yield the splitting of the triplet and singlet states of the radical pair, i.e., the spin exchange integral J has been estimated to be very small, $<10^{-3}$ cm^{-1}. This value for J is at least one order of magnitude too small [12] to allow for the fast experimental electron transfer rate k_R [2–4] of the forward reaction.

Such a discrepancy casts some doubt [12] on the view that the hyperfine interaction indeed develops at the site of the initially formed radicals and demonstrates that information on the forward electron transfer can be derived from the magnetic field effect on the back reaction. For the determination of the recombination rates and the electronic exchange integral J the temporal evolution of the magnetic field effect on both the decay of the radical pair and the growing-in of triplets is needed (as outlined in [12,13]).

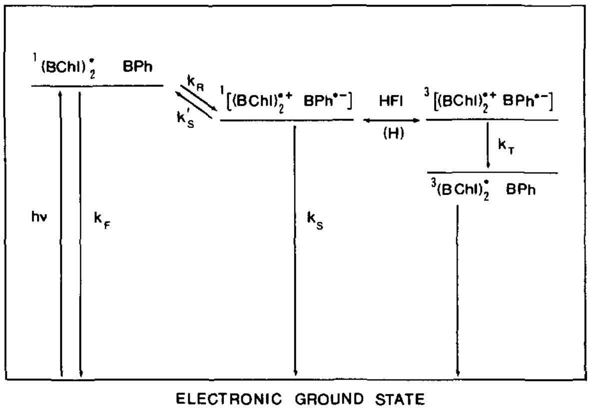

Fig.1. Kinetic scheme of primary electron transfer and recombination processes in bacterial photosynthetic reaction centers.

The appropriate time scale is the ns range since the hyperfine splittings are of the order of 10 G [1,14] implying singlet-to-triplet transitions on this time scale. In this letter we show how discrimination between the different magnetic field effects on radical pair and triplets is achieved by tuning the probing wavelength between 525 and 555 nm. The results obtained for a single delay time of 16.5 ns between excitation and probing pulse allow inference on the relative magnitude of the recombination rates in the two spin states of the radical pair.

Furthermore, the comparison of the ps absorption spectrum [15] with the spectrum derived from the dependence of the magnetic field effect on the wavelength of probing indicates how this effect can be used in the analysis of complex spectra.

2. Experimental details

The sample preparation and the experimental setup have been described [11]. We used a quartz cell (allowing for a lightpath of 2 mm) filled with 52 μM solution of reaction centers of *Rhodopseudomonas sphaeroides*, strain R-26, in 20 mM Tris buffer (pH 7.5) containing 0.1% Triton, X-100. The sample was titrated with sodium dithionite prior to measurements.

The wavelengths of both the excitation and the interrogation pulses (FWHM 3 ns) were tuned from 525 to 555 nm. In order to exclude effects due to the spectra dependence of the ground state absorption, experiments were performed under conditions of saturated absorption, i.e., the photon density incident on the sample was 1.3×10^{16} photons/cm^2. The height of the focal line as determined by scanning the beam with a pinhole was 0.2 mm.

3. Results and discussion

The upper curve in fig.2 shows the wavelength dependence of the optical density changes in zero magnetic field (ΔOD (0)) as measured 16.5 ns after the excitation pulse. The structure of the spectrum resembles the one observed for the radical pair in the ps-range (drawn line in fig.3 [15]). The spectrum in fig.2, however, is shifted to more positive values due to the superposition of the positive triplet absorption [16] which is almost independent of the wavelength in the spectral region investigated.

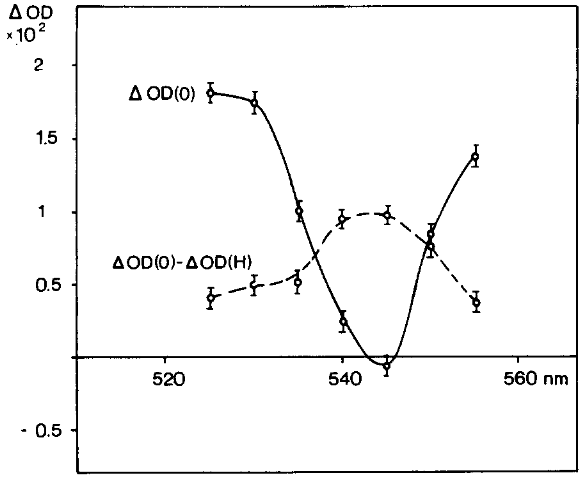

Fig.2. Optical density change in zero magnetic field (ΔOD (0)) and magnetic field effect (H = 300 Oe) thereon.

The value for the optical density change in zero field is given by:

$$\Delta OD\,(0) = [\Delta\epsilon_R c_R\,(0) + \Delta\epsilon_T c_T\,(0)]\; d \qquad (1)$$

where $\Delta\epsilon_R$ and $\Delta\epsilon_T$ denote the difference absorption coefficients of the radical pair and the triplet state (referred to the ground state species, (BChl)$_2$ and BPh) and d the length of the light path in the sample cell.

According to the spectra of radicals [15,16] and triplets [16], the ΔOD (0) values at 535 and 550 nm reflect directly the triplet concentration c_T since at these wavelengths $\Delta\epsilon_R = 0$ (see also fig.3). With $\Delta\epsilon_T = 6.9 \times 10^{-3}\ \mu\mathrm{M}^{-1}\ \mathrm{cm}^{-1}$ [16] at 550 nm follows $c_T\,(0) = 6.1\ \mu$M at the time of measurement, $t_m = 16.5$ ns. This corresponds to a triplet yield $c_T\,(t_m)/c_R\,(t_0)$ of ~12% since under the condition of saturating excitation the initial concentration of radicals, $c_R\,(t_0)$ is equal to the concentration of reaction centers, $c_{RC} = c_R = 52\ \mu$M.

As follows from the difference spectrum of the radical pair (fig.3), the concentration c_R is suitably probed at 545 nm. At this wavelength $\Delta\epsilon_R = -5.8 \times 10^{-3}\ \mu\mathrm{M}^{-1}\ \mathrm{cm}^{-1}$ [16]. Assuming a constant value for $\Delta\epsilon_T$ between 525 and 555 nm [16], the concentration of radicals still present after 16.5 ns is $c_R\,(0) = 8\ \mu$M corresponding to 15% of the initial concentration $c_R\,(t_0)$. A comparison of this value with the analogous triplet yield at 16.5 ns indicates that the concentrations of radical pairs and triplets have become approximately equal as also reflected in the almost zero ΔOD (0) value at 545 nm in fig.2. This is expected for equal concentrations of radicals and triplets since the two difference absorption coefficients are similar but opposite in sign. The balance of 12% triplets and residual 15% radical pairs at 16.5 ns implies the recombination of ~75% of the initially formed radicals via one of the singlet channels, k_S or k'_S.

A saturating magnetic field of 300 Oe causes the triplet concentration c_T to decrease by a factor of 3 [11]. Therefore:

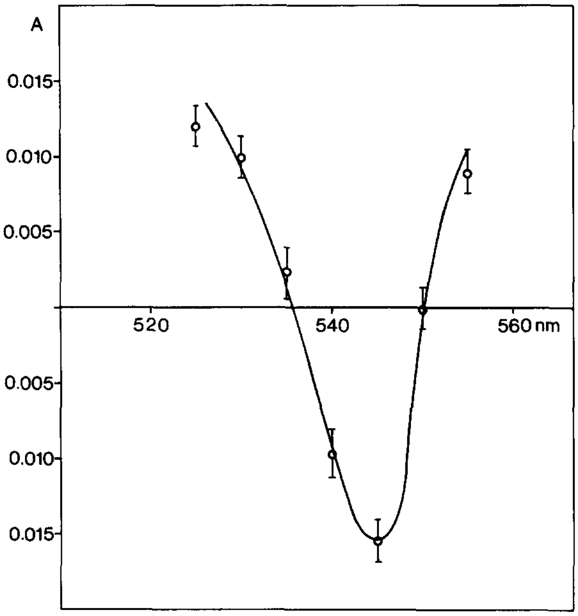

Fig.3. Difference absorption spectrum of the radical pair. The drawn curve is taken from [15], the measured points are derived from the two curves in fig.2.

$$\Delta c_T = c_T\,(0) - c_T\,(H) = 4.1\ \mu M$$

Such a magnetic field effect reflects the theoretical maximum to be expected if transitions between the singlet state and 2 of the 3 triplet sublevels of the pair are inhibited at an early stage of the spin precession.

The magnetic field effect as a function of probing wavelength ranges from $|\Delta OD\,(H)/\Delta OD\,(0)| = 0.3$ at the isosbestic points at $\lambda = 535$ and 550 nm to $|\Delta OD\,(H)/\Delta OD\,(0)| = 13$ (in theory infinity) at $\lambda = 545$ nm where $\Delta\epsilon_T \cong -\Delta\epsilon_R$. The lower curve in fig.2 shows this spectral dependence of the magnetic field effect on the optical density:

$$\Delta OD\,(0) - \Delta OD\,(H) = (\Delta\epsilon_R\,\Delta c_R - \Delta\epsilon_T\,\Delta c_T)\,d \quad (2)$$

with:

$$\Delta OD\,(H) = (\Delta\epsilon_R\,c_R\,(H) + \Delta\epsilon_T c_T\,(H))\,d$$

We make use of eq. (2) in two respects:

(i) Magnetic field effect on triplets as internal standard for the analysis of a complex spectrum;
(ii) Determination of the magnetic field effect on the radical pair.

For the simulation of the structure of the radical spectrum, $\Delta\epsilon_T$ has to be eliminated from eq. (1) and (2). Therefore, we multiply eq. (2) by the factor $c_T\,(0)/\Delta c_T = 1.5$ and subtract the resulting expression from eq. (1). This way we obtain a quantity:

$$A = \Delta\epsilon_R\,(c_R\,(0) - 1.5\,\Delta c_R)\,d$$

which depends on $\Delta\epsilon_R$ and a wavelength-independent term reflecting the actual concentration of R and the magnetic field effect thereon at the time of measurement. With the values for A derived from the two curves depicted in fig.2 we can indeed reproduce the spectrum of the radical pair in excellent agreement with the absorption spectrum measured on the ps time scale in [15].

The magnetic field effect on the radicals follows immediately from eq. (2):

$$\Delta c_R = c_R\,(0) - c_R\,(H) = -3.5\ \mu M$$

Comparing this value with the analogous one for the triplets, we obtain $\Delta c_T = -\Delta c_R$ at $t_m = 16.5$ ns. As immediately follows from the kinetic scheme in fig.1, the change of sign of the magnetic field effect on radicals and triplets indicates that k_T exceeds the rates leading back to the ground state species; that is:

$$k_S \text{ and } k_{S,\,\text{eff.}} = k'_S \cdot k_F \cdot k_R^{-1}$$

where k_F denotes the rate of the radiative decay of $^1(\text{BChl})^*{}_2$.

A smaller value of k_S as compared to k_T can be rationalized in terms of the different exothermicities [17]. The following arguments support the view that also $k_{S,\,\text{eff.}} < k_T$: In the reaction center preparations dealt with here and in [11], the halfwidth of the magnetic field effect was of the order of 50 Oe and did still depend on the time of measurement in the 10 ns time range. This implies that the inverse recombination rates, k_S, k'_S and k_T, are at least comparable to the time of probing or somewhat larger. Therefore we assume as upper bound $k'_S = 10^9\ s^{-1}$. Faster rates are expected to broaden the halfwidth [13,18] and at the same time make it insensitive to the time of probing. An effect of the exchange interaction J on the halfwidth is improbable considering the high yield of triplets (10–20% ([16] this paper) at 300 K and ≅ 90% [16] at 20 K). With the most recent [4] experimental value $k_R = (3\ \text{ps})^{-1}$, $k'_S \leqq 10^9\ s^{-1}$ and $k_F \leqq 10^{10}\ s^{-1}$ follows

$k_{S,eff} = 3 \times 10^7$ s^{-1}, a value necessarily smaller than k_T, even though the uphill rate k'_S might be similar.

The observation that the value of the magnetic field effect on triplets and ion pairs is similar can be qualitatively explained by the low triplet yield at early times. During the first nanoseconds hyperfine interaction has not yet led to an appreciable triplet character of the radical pair with the consequence that recombination occurs exclusively in one of the singlet channels. Since the superposition of spin motion and recombination rates turns out to be complicated, the product yields in singlet and triplet states do not depend on the recombination rates in a simple way [13]. The total triplet yield may be smaller than the singlet yield although $k_T > k_S$. Concomitant with the higher triplet probability at later times the magnetic field effect on the residual concentration of radicals increases to values similar to the ones measured for the triplet concentration.

In conclusion we have presented some kinetic details on the recombination of the primary radical pair deduced from time-resolved magnetic field experiments. In the ns-range, the concentration of both radicals and triplets is easily probed if the optimum spectra range is chosen. We consider this to be the region between 525 and 555 nm where a nearly constant triplet absorption is superimposed on a sharp negative peak in the spectrum of the radical pair passing through zero difference absorption at two wavelengths, 535 and 555 nm.

Acknowledgements

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