TIME-RESOLVED MAGNETIC FIELD EFFECT ON TRIPLET FORMATION IN PHOTOSYNTHETIC REACTION CENTERS OF *RHODOPSEUDOMONAS SPHAEROIDES* R-26

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Received 14 January 1979

1. Introduction

The primary events in bacterial photosynthesis have been studied since it became possible [1] to isolate reaction centers of photosynthetic bacteria, e.g., of *Rhodopseudomonas sphaeroides*. Within $\leqslant$10 ps [2,3] after excitation of bacteriochlorophyll to its first excited singlet state, the radical pair state P^F is formed consisting of a bacteriochlorophyll dimer cation $(B\dot{C}hl)_2^+$ and a bacteriopheophytin anion, BPh^- [4]. Then, within 200 ps, the electron is further transferred to an ubiquinone nonheme iron complex. However, if this pathway is blocked, either by pre-reducing the ubiquinone chemically [5] or by its elimination [6,7], the lifetime of P^F increases to ~10 ns [5]. The radical pair P^F decays to form either the state P^R, i.e., the lowest excited triplet of the system $^3(BChl)_2$, or the ground states of the bacteriochlorophyll dimer and the bacteriopheophytin (see the kinetic scheme in fig.1).

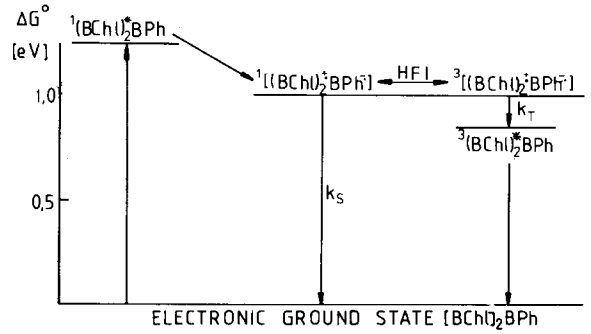

Fig.1. Kinetic scheme of primary electron transfer steps in photosynthetic reaction center.

The yield of P^R has recently been reported [8,9] to be magnetic field dependent and it was suggested that the change of multiplicity from the initially formed singlet to a triplet radical pair is induced by hyperfine interaction. This mechanism, usually known as 'radical pair mechanism' of chemically induced dynamic nuclear polarization (CIDNP) [10,11], provides also a straightforward explanation [12,13] for the unusual spin polarization of the bacteriochlorophyll triplet [12,14].

If governed by the radical pair mechanism, triplet formation should occur on a time scale determined by the hyperfine splittings of the two radicals. These are of the order of 10 G [15,16] what corresponds to the ns time domain (1 G $\simeq$2.8 MHz).

We investigate here the influence of weak magnetic fields on the triplet yield. The work in [8,9] involves measurements at long times (μs). Here we study the triplet–triplet absorption on the ns time scale in order to follow the spin precession within the pair's lifetime. This is determined by the recombination rates to the singlet ground state, k_S, and to the triplet state, $^3(BChl)_2^*$, via k_T. The magnetic field dependence of the triplet yield shows that the halfwidth of the magnetic field effect at $\geqslant$20 ns decreases with increasing delay between creation of the radical pair and probing of the triplet product. The effect is attributed to the time–energy uncertainty and it unambiguously confirms the radical pair mechanism invoked as the origin of triplet formation. Moreover, this finding indicates that spin dephasing does not play any significant role on this time scale what is certainly interesting with respect to the ubiquinone–iron complex

present in the reaction center. Obviously, this is not inducing spin relaxation.

In previous transient absorption measurements on either the sub ns [2,3] or μs [8,9] time scales, only the radical pair P^F or only the triplet P^R was present but not both. In the present ns experiment, both species are encountered and overlap in their absorption spectra.

Accordingly, the present magnetic field effect on P^R is best measured at wavelengths corresponding to zero absorbance change in the difference spectrum of P^F measured in the ps time domain [2,3]. This way one can exclude any significant contribution of P^F to the triplet absorption signal of interest. As will be demonstrated separately [17], the magnetic field effect on the triplet yield can also be used as an internal standard to determine its time evolution. This point is of special experimental interest since a variable optical delay necessarily leads to geometry changes in the course of readjustment of the probing laser beam and hence to an appreciable uncertainty of the value of the transient absorption.

2. Experimental

2.1. *Preparation of samples*

Reaction centers from the carotenoidless strain R-26 of *Rps. sphaeroides* were prepared as in [1]. After ammonium sulfate fractionation, the detergent LDAO was exchanged against Triton X-100 by dialysis under nitrogen. Stock solutions (~52 μM in 5 M Tris buffer (pH 7.5)) were kept under nitrogen in the dark. Prior to measurements they were titrated with sodium dithionite. Indigotrisulfonate (150 μM) was added to ensure redox potentials below −150 mV. By comparative runs samples treated with indigotrisulfonate were shown to yield similar results to the untreated ones.

2.2. *Apparatus*

For both, excitation and absorption measurements, we used a dye laser which was transversally pumped by a nitrogen laser (~1 MW, Lambda Physik) as illustrated in fig.2. The dye laser was tuned to the wavelengths between 525 nm and 560 nm by a diffraction grating. The pulsewidth was ~2 ns. Two beams of weak intensity (<1%) were split off the main beam

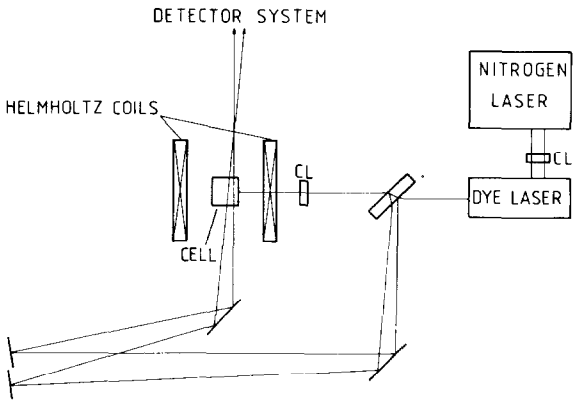

Fig.2. Apparatus.

by means of a quartz wedge. They provided a probing and reference beam. The main beam was focussed by cylindrical lenses onto the sample. 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 2 mm. After a suitable optical time delay the probing pulse was adjusted onto the focus of excitation for optimum overlap while the reference pulse was directed 5 mm above the probing beam through the dark volume. Having passed the sample cell, the intensities of the two beams were registered and 30 pulses were sampled by a laser photometer (Molectron LP 20) to give the intensity ratio. Great care was taken to avoid scattered light from the excitation volume to reach the detector unit. The magnetic field was provided by a pair of Helmholtz coils with a field homogeneity in space and time better than 1 $^0/_{00}$. An optical cuvette with 2 mm lightpath was used as sample cell. In order to minimize time effects in the sample, the measurements were performed in a statistical manner. Errors resulted essentially from the slow formation of Schlieren in the sample volume. Laser power fluctuations were <5%.

3. Results and discussion

The magnetic field dependence of the triplet yield is shown in fig.3 for two different delay times, 10.5 ns and 16.5 ns. The triplet yield has been normalized since a change of the delay time requires

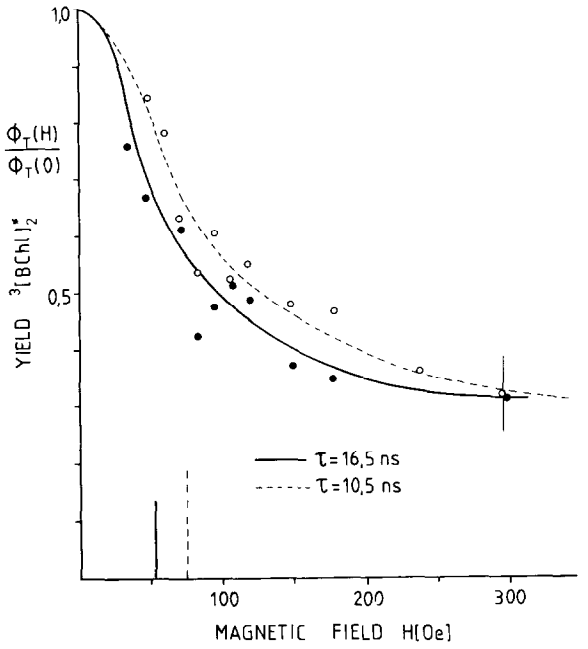

Fig.3. Time dependence of the magnetic field effect on the triplet yield in blocked reaction centers of *Rps. sphaeroides* R-26. Excitation and probing at 530 nm.

readjustment of the laser beam. This implies geometry changes which do not allow a direct comparison of the triplet yield at different times. Moreover, in the context of this paper the relative triplet yield at different times is not important.

Qualitatively, the magnetic field dependence of the triplet yield is similar to the one measured on the μs time scale [8,9]. The triplet yield decays monotonously and saturates at a field strength of ⩾300 G. The modulation between high field and zero field value is in our experiment about 1:3 which is somewhat stronger than that of the previous studies.

Comparing the two curves at measuring times τ_m = 10.5 ns and τ_m = 16.5 ns, there is a significant decrease of the halfwidth towards longer times. To understand this effect, let us briefly review the radical pair mechanism generally invoked as the origin of the magnetic field effects in bacterial reaction centers. After the initial electron transfer steps, the radical ions $(BChl)_2^{\cdot+}$ and $BPh^{\cdot-}$ should be considered essentially without any mutual interaction. Nevertheless, since spin relaxation is much slower than the processes studied here, the spin correlation between the two radicals is still preserved in the beginning. This means that the two unpaired electrons are still antiparallel, i.e., in the initial singlet state, S, and could, in a reverse electron transfer step recombine to form $(BChl)_2$ and BPh favourably in their singlet ground state. This singlet recombination channel determined by the rate k_S is, however, slow due to its large exothermicity [18-21]. Therefore, the radical pair lives for ~10 ns, and during that time the spin state of the electrons changes because of the hyperfine interaction in each of the separate radicals. There develops a finite probability of finding the pair in one of the three triplet sublevels T_m with the z component $m = -1,0,+1$. Since the hyperfine interaction is rather weak, typically 10 G, such a transition to the T_{+1} and T_{-1} states is impeded if these are energetically split far enough off the singlet state S by Zeeman interaction. Considering that the T_0 state is still accessible, this explains why at high fields the triplet product yield may decrease to 33% of its zero field value (maximum magnetic field effect).

Figure 4 shows for the simplest radical pair with only one spin 1/2 nucleus the magnetic energy of the radical pair as a function of the field strength. The triplet states T_{+1} and T_{-1} are split off to higher and

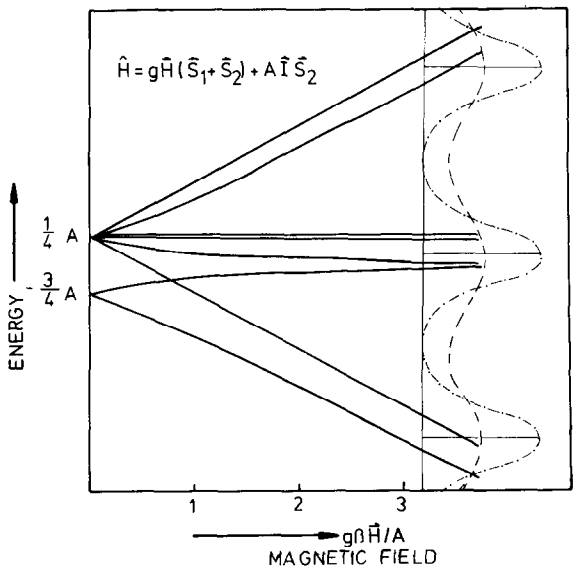

Fig.4. Magnetic energy of the radical pair as a function of field strength. Linewidth at short (- - -) and long (-.-.-) times.

lower energies, respectively, with increasing fields where each of them is split into two levels due to the interaction with the nucleus. The four remaining states are, even at high field, a mixture of S and T_0. At low field all 8 states are mixed.

The halfwidths in fig.3 correspond to field strengths where the $T_{\pm 1}$ states become disentangled from S and T_0. The reason for this dependence of the halfwidth on the time delay is that all the levels in fig.4 should be thought to be broadened due to their finite lifetime. If we see a triplet state at τ_m = 10.5 ns in the T–T absorption spectrum, it must have been created from a radical pair which lived for a time $\tau \leqslant \tau_m$ and thus the levels have an energy width:

$$\Delta E = h/\tau > h/\tau_m$$

A very short τ is unlikely because immediately after the creation of the radical pair the probability to find a triplet pair is still very small. Therefore, radical pair lifetimes not much shorter than τ_m will predominate. This energy level broadening is schematically shown in fig.4 for two different measuring times. While at the shorter time, the levels are sufficiently broad to overlap at the chosen field strength, this same field strength is sufficient to separate the narrower lines corresponding to a larger τ_m. Upon increasing the measuring time we approach the situation that no radical pairs remain. In an absolute absorption measurement, this would mean that no further increase of the triplet absorption is observed. Since accurate measurements on the ns time scale are difficult for the experimental reasons mentioned, the observation of the shape (i.e., the halfwidth) of the magnetic field dependence of the triplet yield provides a simple method of investigating the decay of the radical pair. As soon as the lifetime of the radical pair determines the halfwidth, it becomes independent of the delay of the probing pulse. Such a method can be understood as an alternative to the one proposed in [17] which uses the wavelength dependence of the magnetic field effect with the triplet modulation value as internal standard. Due to the different rates for singlet and triplet formation, the decay of the radical pair is not expected to follow a simple exponential function. A more detailed study along this line is in progress.

Acknowledgements

The strain *Rhodopseudomonas sphaeroides* R-26 was kindly placed at our disposal by Dr R. K. Clayton. Financial support by the Deutsche Forschungsgemeinschaft and NATO is gratefully acknowledged.

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