DETERMINATION OF THE DECAY RATES OF THE TRIPLET STATE OF *RHODOPSEUDOMONAS SPHAEROIDES* BY FAST LASER-FLASH ESR SPECTROSCOPY

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Received 2 October 1977

1. Introduction

Since the discovery of the triplet state in photosynthetic bacteria, in which the primary acceptor was chemically reduced [1] much work has been devoted to elucidate the properties of this state and its mode of generation. Optical experiments in reduced reaction centers of *Rhodopseudomonas sphaeroides* [2,3] revealed a state, called P_R, that rose in 10–15 ns and decayed in 3–6 μs at room temperature. At 80°K and lower it decayed in about 110 μs. On the basis of its absorption difference spectrum it was concluded that the state P_R coincided with the triplet state as found [1,4,5] by ESR spectroscopy. It was suggested [6] that the triplet state was generated via a back reaction between the oxidized primary donor (a bacteriochlorophyll dimer) and a reduced intermediate reactant, which was suggested to be a bacteriopheophytin [7–10]. Thus at low temperatures the reaction scheme will be:

$$(\mathrm{Bchl})_2\ \mathrm{Bph}\ \mathrm{X}^- \xrightarrow{<5\ \mathrm{ps}} (\mathrm{Bchl})_2^+\ \mathrm{Bph}^-\mathrm{X}^- \xrightarrow{10\ \mathrm{ns}}$$

$$(\mathrm{Bchl}_2)^{\mathrm{T}}\ \mathrm{Bph}\ \mathrm{X}^- \xrightarrow{110\ \mu\mathrm{s}} (\mathrm{Bchl})_2\ \mathrm{Bph}\ \mathrm{X}^-$$

The rate constants (expressed as half times) of the above scheme were measured by fast optical spectroscopy. The rise kinetics of the ESR signal, corresponding to P_R, could not be measured; for its decay, at ~ 10°K, half times of about 5 μs were found [1,4,5]. ESR experiments in zero field (zero field resonance, ZFR) [11,12] at about 2°K have yielded decay rates for the x, y and z components of the triplet state. Unfortunately, the reported values for the x and y components differed considerably, yielding for the average decay half time (which obtains at higher temperatures above say 80°K) values of 287 μs [11] and 106 μs [12]. In order to resolve the discrepancies between the optical and ESR measurements of the decay rates, and between the conflicting values obtained by the ZFR method we have carried out fast laser-flash ESR spectroscopy on the triplet state of *Rhodopseudomonas sphaeroides* at a temperature of 5°K. Using a simple model, incorporating the spin-lattice relaxation and depopulating rates, we have arrived at a value for the decay rate of the x and y components, k_x and k_y, and the z component, k_z, of 6500 s^{-1} and 1800 s^{-1} (± 20%), respectively. These values compare favorably with the ZFR results [12] and with the optical low temperature measurements [13]. An average decay half time of 140 μs (± 20%) is found, in reasonable agreement with the optically-determined value of 110 μs at 80°K.

2. Materials and methods

2.1. *Experimental*

The measurements were carried out with chromatophores of *Rhodopseudomonas sphaeroides*. Bacteria were grown as described [14], chromatophores were prepared by sonification during 10 min. The chlorophyll concentration was 0.1 mg/ml. Reduction of the primary acceptor was obtained by adding excess solid sodium dithionite under nitrogen atmosphere. ESR

spectra were recorded with a slightly modified Varian E9 spectrometer, having an instrumental response time of 20 μs [15]; field modulation was 20 G at 100 kHz. The excitation light source was a Zeiss dye laser type FL3B giving a light pulse of 500 ns half width (about 10 mJ), employing rhodamine R6G tuned at 590 nm. Low temperatures were obtained by an Oxford Instruments helium-flow cryostat, equipped with a temperature control unit. Control measurements with a calibrated thermistor inserted in a dummy sample indicated that the sample temperature was to within a few tenth of a degree around 4.2°K. Temperature rise due to the laser flash proved to be negligible. The kinetic response was averaged with a Data Lab DL 102, (dwell time 5 μs or 10 μs) triggered by the laser flash. The number of scans varied from 16–512 depending on the microwave power. Computations and simulation of the depopulation of the triplet levels were carried out with a PDP 9 computer employing the program 'SIMULA' written by Mrs Lies Huyser.

2.2. *Theoretical*

The triplet state in photosynthetic bacteria is strongly spin polarized [4]. The polarization is currently thought to be brought about by the so-called radical pair mechanism [6,16,17]. In the state $(Bchl_2)^+Bph^-X^-$ the spins of the oxidized bacteriochlorophyll dimer and the reduced bacteriopheophytin oscillate between the singlet and the triplet state:

$$(Bchl_2{}^+Bph^-)^S\ X^- \leftrightarrows (Bchl_2{}^+Bph^-)^T\ X^-$$

The energy difference between the $m = \pm 1$ levels and singlet level in high magnetic field being much larger than between the $m = 0$ level and the singlet level, only the $m = 0$ level is populated in the triplet state of the radical pair; this spin polarization is conserved in the back reaction:

$$(Bchl_2{}^+Bph^-)^T\ X^- \rightarrow (Bchl_2)^T\ Bph\ X^-$$

If one admits that not only S–T_0 mixing but also some S–$T_{\pm 1}$ mixing is allowed (some indications for this have been found in experiments on the dependence of the triplet yield on magnetic field [16]) the initial signal amplitudes will be decreased, but for not too large an admixture the kinetic response is found to be

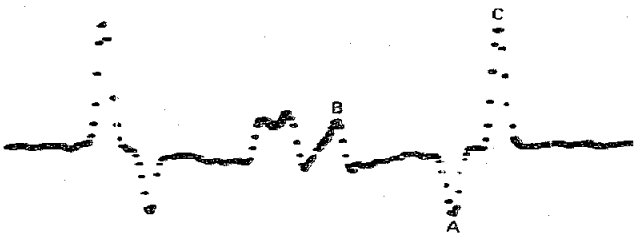

Fig.1. Light minus dark ESR spectrum of chemically-reduced *Rps. sphaeroides* at 5°K. Modulation amplitude 20 G, microwave power 1 mW. A, B and C indicate the field positions at which flash experiments were performed.

the same. Predominant S–T_0 mixing explains the unusual pattern of the ESR spectrum of the bacterial triplet [6] (fig.1).

Monitoring the kinetic response of the triplet ESR signal at the peaks A, B and C of fig.1 permits extraction of the molecular decay rate constants k_u $(u = x, y, z)$. Since the populations of the triplet levels in high field are so strongly polarized, the decay of the intensity of a flash-induced ESR signal is to a large extent determined by stimulated emission or absorption rather than by the spin-lattice relaxation time, at the levels of microwave power usually employed [18]. This effect has to be taken into account explicitly.

In fig.2 the various processes involved in the

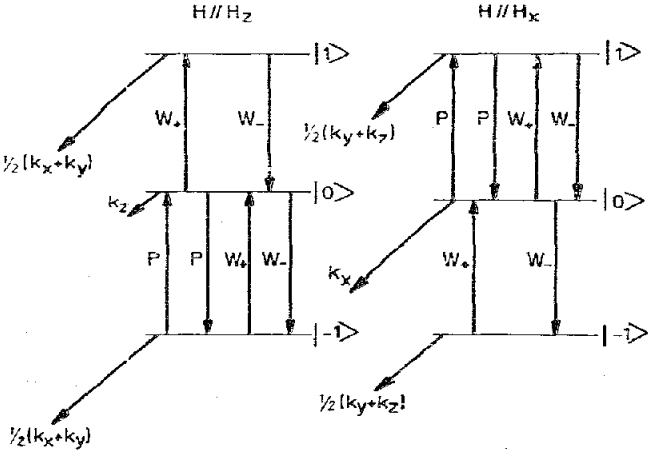

Fig.2. Schematic representation of relaxation and depopulation of the triplet states at high field for $H//x$ and $H//z$. The depopulation for $H//y$ is analogous to $H//x$.

depopulation of the triplet levels are depicted for the magnetic field H parallel to the molecular z and x axis ($H//z$ and $H//x$, respectively). Note that the main difference between these two situations lies in the different mixing of the molecular rate constants k_u upon the transformation from the zero field states $|T_x>$, $|T_y>$, $|T_z>$ to the high field states $|+1>$, $|0>$, $|-1>$. The differential equations governing the depopulation processes for $H//z$ and $H//x$ (the set for $H//y$ is analogous to that for $H//x$) are as follows:

$$H//z \quad \frac{dN_+}{dt} = W_+N_0 - W_-N_+ - \tfrac{1}{2}(k_x + k_y)N_+$$

$$\frac{dN_0}{dt} = W_+(N_- - N_0) + W_-(N_+ - N_0) + P(N_- - N_0) - k_zN_0$$

$$\frac{dN_-}{dt} = P(N_0 - N_-) + W_-N_0 - W_+N_- - \tfrac{1}{2}(k_x + k_y)N_-$$

$$H//x \quad \frac{dN_+}{dt} = P(N_0 - N_+) + W_+N_0 - W_-N_+ - \tfrac{1}{2}(k_y + k_z)N_+$$

$$\frac{dN_0}{dt} = W_+(N_- - N_0) + W_-(N_+ - N_0) - P(N_+ - N_0) - k_xN_0$$

$$\frac{dN_-}{dt} = W_-N_0 - W_+N_- - \tfrac{1}{2}(k_y + k_z)N_-$$

where N_+, N_0 and N_- are the populations of the $|+1>$, $|0>$ and $|-1>$ states, respectively; P is the probability of the transition induced by the microwave field; and W_+ and W_- are defined by $1/T_1 = W_+ + W_-$ and $W_+/W_- = e^{-\Delta E/kT}$. T_1 is the spin-lattice relaxation. For the high field transition labeled A in fig.1 ($H//z$) the ESR signal is proportional to $N_0 - N_-$, for transition labeled B in fig.1 ($H//x_z$) it is proportional to $N_0 - N_+$. The energy differences between the $|0>$ and $|+1>$ levels has been taken equal to that of the $|0>$ and $|-1>$ levels. As starting condition we assumed that the triplet state is instantaneously populated in the $|0>$ level only; k_y was taken to be equal to k_x.

3. Results

Figure 3 shows the measured decay times τ for the high field transitions $H//z$, $H//y$ and $H//x$ after a laser flash at 5°K as function of the microwave power incident on the cavity. The drawn line represents the decay times obtained by solving the differential equations; best fit was obtained taking $P = 10\ s^{-1}$ at 50 dB, $k_x = k_y = 6500\ s^{-1}$, $k_z = 1800\ s^{-1}$ and $1/T_1 = 500\ s^{-1}$. The values of P and T_1 are not unreasonable. At 4°K the spin-lattice relaxation time for triplets is of the order of a few milliseconds [19] whereas P must be much smaller than $1/T_1$ at 50 dB because we are then far from saturation. The ratio

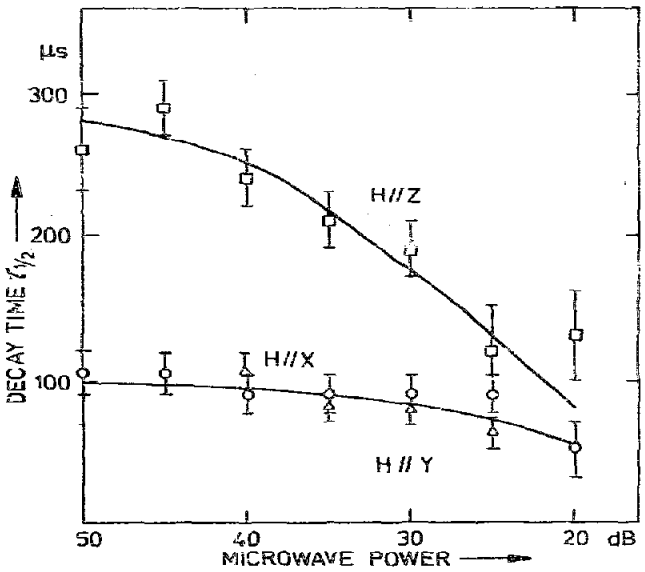

Fig.3. Triplet decay half times at 5°K as a function of microwave power for $H//x$ (–○–), $H//y$ (–△–) and $H//z$ (–□–); 20 dB corresponds to 2 mW. Drawn curves represent computer fit as calculated from the rate equations with parameters $p = 10\ s^{-1}$ at 50 dB, $k_x = k_y = 6500\ s^{-1}$ $k_z = 1800\ s^{-1}$ and $1/T_1 = 500\ s^{-1}$.

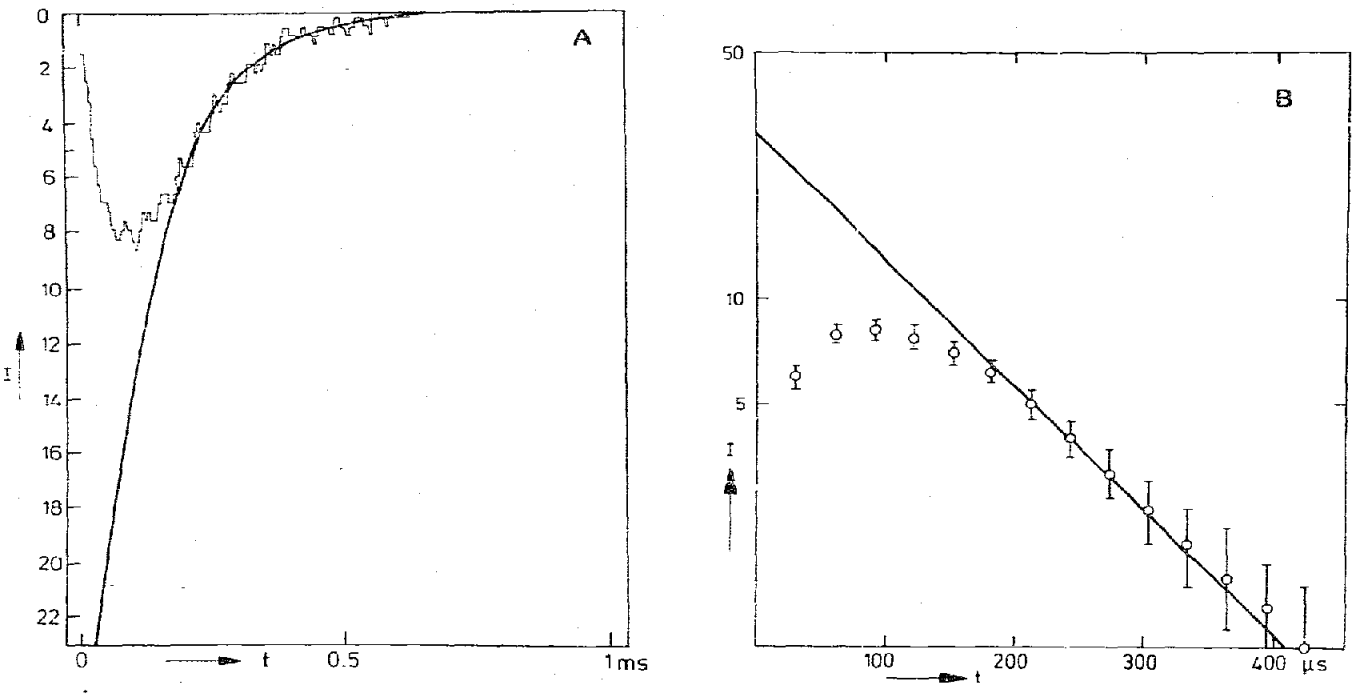

Fig.4 A. Rise and decay of the flash-induced triplet ESR signal at field position A. The risetime is instrument limited. Microwave power 0.2 mW, temp. 5°K, modulation amplitude 20 G. Accumulation of 16 laser flashes at 590 nm. Drawn line is calculated with values for P, T_1 and k_u ($4 = x, y, z$) determined from the computer fit to the data of fig.3. Fig.4 B. Semi-log plot of the curves from fig.4A.

between P and $1/T_1$ is about 1 at the microwave power corresponding to the point where the curve τ versus microwave power starts to bend down (best seen in the case $H//x$). The intersection of the curves with the $P = 0$ axis give approximate values of $k_u + 1/T_1$ (not exactly because spin-relaxation couples all three levels).

The goodness of the fit can be judged by comparing simulated and measured decay-curves. In fig.4 a typical example is displayed. It is seen that the simulated decay curve can be very well fitted with a single exponential and that it fits well with the measured decay for times exceeding the instrumental response time. For very high power ($\geqslant$ 20 dB) the decay can no longer be fitted with a single exponential, due to the fact that stimulated emission is much faster than spin-lattice relaxation and radiation less decay. Most of the decay then proceeds in a time shorter than our instrumental response time.

4. Discussion

The decay rate of the triplet ESR signal of photosynthetic bacteria is governed by stimulated emission, by spin-lattice relaxation and by the decay of the excited state to the ground state. In order to measure the molecular de-excitation, it is imperative to work under conditions of very low microwave power, and at temperatures where the triplet sublevels are effectively isolated from one another. Both these conditions were not satisfied in [4], which explains the fast decay rates found for all lines in the triplet ESR spectrum.

Our results show that following flash excitation, the decay rates of the ESR signals, as measured with very low microwave power at 5°K are very much longer. The rates for the x and y lines are faster than for the z component, in agreement with zero field resonance results [12]. The average half time for the

x and y components (106 μs) is somewhat longer than that found [12] (78 μs) and the half time (84 μs) of the fast decay component at 4.2°K found [13]. This difference may be caused by the magnetic field. It should be stressed that picosecond laser spectroscopy and the present technique, both in which the triplet decay is followed after flash excitation, are quite different from the ZFR technique which monitors changes in fluorescence caused by resonant microwaves under continuous illumination. It is well known that in the latter case the measured decay rates depend on the intensity of the exciting light [20]. At low levels of light intensity this dependence becomes even more important, a fact that is not always appreciated [21]. Thus, although the laser flash ESR technique is inherently somewhat less sensitive, it is more direct than the ZFR method with fluorescence detection. We note that our present results are in fair agreement with the ZFR work [12] but deviate considerably from the decay rates found [11] who employed the same ZFR technique. It has been suggested [21] that the discrepancy between the ZFR results of [12] and [11] resulted from differences in the light intensity employed. This possibility has been examined in subsequent work [22], in which the decay of the triplet state was measured by ZFR under conditions of very low light intensity. Again, values of k_x and k_y were found that closely agreed with our present results and with those found [13]. We conclude that the values for k_x and k_y found [11] are substantially too low.

The decay rates of the triplet state of the primary donor are much faster than those of monomeric bacteriochlorophyll in glassy solution [21]. This finding is consistent with ZFR results on dimeric model systems consisting of two planar aromatic molecules, like benzene or naphtalene, which are rigidly kept together by two $-CH_2-CH_2$ bridges [23,24]. Adding suitable substituents, these so-called biphanes can be made into charge-transfer complexes, whose triplet states show much-reduced zero field splittings and decay times compared to the monomers. Since it is known that in the oxidized primary donor the unpaired electron is fully delocalized, this suggests that it is not sufficient to take into account only the exciton character of the bacterial triplet as in the adaptation of the exciton model [25] treated [21], but that also ionized states should be taken into account. We feel, therefore, that it is premature to use the theory outlined in [21] with our values of the molecular decay rates to obtain information on the angle between the dimer constituents.

Acknowledgements

The authors are indebted to Mrs M. F. Onderwater and Mr A. H. M. de Wit for technical assistance. This work was supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organisation for the Advancement of Pure Research (ZWO).

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