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MAGNETIC FIELD-STIMULATED LUMINESCENCE AND A MATRIX MODEL FOR ENERGY TRANSFER

A NEW METHOD FOR DETERMINING THE REDOX STATE OF THE FIRST QUINONE ACCEPTOR IN THE REACTION CENTER OF WHOLE CELLS OF *RHODOSPIRILLUM RUBRUM*

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In whole cells of *Rhodospirillum rubrum* the light-induced absorbance difference spectrum of the reduction of the first quinone electron acceptor Q_1 was determined in order to relate the emission yield ϕ and the magnetic field-induced emission increase $\Delta\phi$ to the redox state of Q_1. It was found that $\Delta\phi/\phi^2$ is a linear function of the number of reaction centers, in which Q_1 is reduced, independent of the fraction of reaction centers in the oxidized state. The emission yield is a hyperbolic function of the fraction of reaction centers closed, either by reduction of the acceptor Q_1 or by oxidation of the primary electron donor P. Apparently, in whole cells of *R. rubrum* a matrix model for energy transfer between various photosynthetic units can be applied. A model is presented, which is a generalization of theoretical considerations reported before (Duysens, L.N.M. (1978) in Chlorophyll Organization and Energy Transfer in Photosynthesis, Ciba Found. Symp. 61 (New Series), pp. 323–340, Elsevier/North-Holland, Amsterdam) and which is in excellent agreement with the experiments. From simultaneous measurements of $\Delta\phi$ and ϕ the redox state of the reaction center can relatively easily be determined. So far, this is the only method for simultaneously measuring the fractions P^+ and Q_1^- in intact cells under steady-state conditions.

Introduction

If the quinone acceptor Q_1 of the reaction center complex of purple bacteria is in the oxidized state before illumination, electron transport mainly proceeds towards Q_1 and the second quinone acceptor Q_1 [1–5] (see Fig. 1). Upon reduction of Q_1 both the light-induced bacteriochlorophyll emission and the reaction center triplet yield increase [6]. The increase in emission (by a factor of about two [7–9]) can be ascribed to luminescence caused by charge recombination of the singlet state of the radical pair $(P^+I^-)^S$ (P is the primary donor, a bacteriochlorophyll dimer and I is bacteriopheophytin [6,10,11,29]).

A weak magnetic field (H) increases the luminescence yield and decreases the reaction center triplet yield [12–15]. In a previous paper [16] this is discussed in terms of a magnetic field-dependent steady-state distribution between singlet and triplet states of the radical pair P^+I^-. It was shown that upon excitation of bacteriochloro-

Abbreviations: TMPD, *N,N,N',N'*-tetramethyl-1,4-phenylenediamine dihydrochloride; DAD, 2,3,5,6 tetramethyl-*p*-phenylenediamine; Tricine, *N*-tris(hydroxymethyl)methylglycine.

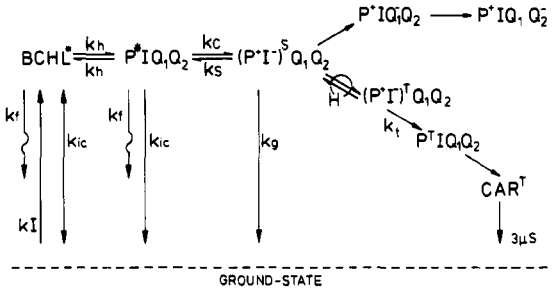

Fig. 1. Scheme of energy and early electron transfer in *R. rubrum* S1. BCHL, antenna bacteriochlorophyll; P, the reaction center bacteriochlorophyll; I, bacteriopheophytin; Q_1 and Q_2, ubiquinones; CAR, reaction center carotenoid; k_f, k_{ic} and k_c are the respective rate constants for fluorescence, internal conversion and charge separation; k_s, k_g and k_t are the rate constants for charge recombination of the radical pair to P*, P and P^T, respectively. See text.

phyll, this magnetic field-induced emission increase (ΔF) only occurred when the acceptor Q_1 was prereduced.

Here we show that the magnetic field-induced emission increase can be used as a monitor specific for the concentration of PIQ_1^-. This was done by correlating the emission yield increases with the light-induced absorbance changes due to the reduction of Q_1 in whole cells of *R. rubrum*. In many cases the magnetic field-induced emission increase will provide a more simple and reliable probe for determining the redox state of the reaction center than the small absorbance changes in the near-ultraviolet region of the spectrum due to the reduction of Q_1.

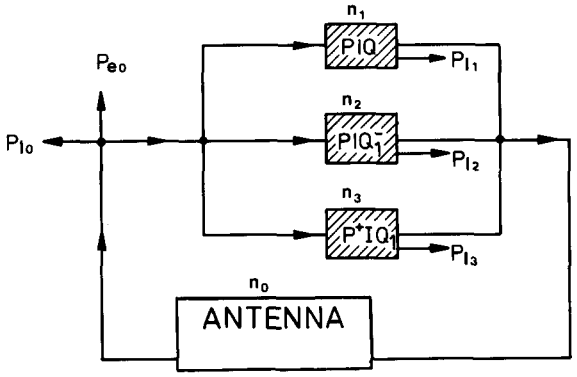

Fig. 2. Schematic representation of the model used for computer simulation based upon the matrix model. A light quantum absorbed by the antenna molecules (n_0, number of antenna molecules per reaction center) has a probability P_{e0} of emission, $P_{\ell 0}$ of losses and $(1 - P_{e0} - P_{\ell 0})$ of transfer to the reaction center in the various states. n_1, n_2 and n_3 indicate the fraction of traps in the state PIQ_1, PIQ_1^- and P^+IQ_1, respectively. After trapping, excitation energy is either lost with a probability $P_{\ell k}$ ($k = 1, 2, 3$) due to photochemistry, triplet formation, and other losses, or energy is transferred back to the antenna.

The yield of bacteriochlorophyll emission not only depends on the concentration of Q_1^- and the intensity of a magnetic field, but also on the type of energy transfer in the antenna pigment system (lake or matrix model vs. separated units). Fig. 2 pictures a model based upon energy transfer in a matrix of pigment molecules with 10 or more reaction centers, which describes very well the results presented here.

Theoretical

In this section we will derive a set of equations which describe the relation between the emission yield and the fractions of reaction centers in various states for a matrix of antenna molecules and reaction centers. Let n_k be the average number of molecules of the type or the state k per reaction center. By n_0 ($k = 0$) the number of antenna molecules per reaction center is designated, by n_1 the fraction of reaction centers in the photoactive state PIQ_1 and by n_2 and n_3 the fractions of reaction centers in the states PIQ_1^{-1} and P^+IQ_1, respectively, with:

$$n_1 + n_2 + n_3 = 1 \tag{1}$$

P_{ik} is the probability that a process (e.g., emission) occurs on a molecule of type k before energy transfer to any other molecule takes place. $P_{\ell k}$ is the probability that energy is lost or converted and not transferred after arriving on a molecule of type k. If the probability of loss of energy for each excited molecule is low compared to the probability for transfer, and if the probabilities are equal for all antenna molecules, then the excitation will visit a representative part of the molecules of the matrix with equal probability. The quantum yield for the process i is then given by:

$$\phi_i = \sum_{k=0}^{3} n_k P_{ik} \Big/ \sum_{k=0}^{3} n_k P_{\ell k} \tag{2}$$

All terms in the numerator of Eqn. 2 are small compared to $n_0 P_{e0}$ for the process of emission (i ≡ e), because $n_0 \simeq 50$ and $n_1 + n_2 + n_3 = 1$, and $P_{ek} \ll P_{e0}$ for $k = 1$, 2 or 3. P_{ek} is defined as the proability of emission of an excited molecule of type k. By taking the inverse of both sides of Eqn. 2 we find:

$$\frac{1}{\phi_e} = \frac{n_0 P_{\ell 0} + n_1 P_{\ell 1} + n_2 P_{\ell 2} + n_3 P_{\ell 3}}{n_0 P_{e0}} \quad (3)$$

Only the states PIQ_1, PIQ_1^- and P^+IQ_1 may be expected to be present because of the relatively high rate of the back-reaction $P^+IQ_1^- \rightarrow PIQ_1$ in moderate continuous light [8]. This proved to be consistent with the experiments (see below). A saturating magnetic field causes a decrease $\Delta P_{\ell 2}$ in the probability $P_{\ell 2}$ of loss processes occurring in reaction centers in the state PIQ_1^- and thus results in an increase of $\Delta\phi_e$ in emission. By taking the differential of Eqn. 3 we obtain in first approximation ($\Delta\phi \ll \phi$):

$$\frac{\Delta\phi_e}{\phi_e^2} = -\frac{\Delta P_{\ell 2}}{n_0 P_{e0}} n_2 \quad (4)$$

In a similar way as was done for Eqn. 3, an expression for the reaction center triplet yield is found:

$$\frac{1}{\phi_t} = \frac{n_0 P_{\ell 0} + n_1 P_{\ell 1} + n_2 P_{\ell 2} + n_3 P_{\ell 3}}{n_2 P_{t2}} \quad (5)$$

where P_{t2} represents the probability of triplet formation in the reaction center in the state PIQ_1^- ($P_{t0} = P_{t1} = P_{t3} = 0$; $\Delta P_{t2} \simeq \Delta P_{\ell 2}$). The form of Eqns. 2 and 3, giving the emission yield as a function of the concentrations of the various states of the reaction center, does not depend on the type of emission, whether it is fluorescence or luminescence. Eqns. 3–5 have been derived using the condition that the rate constant of transfer of excitation energy k_h is so large that the excitation energy visits a representative part of the matrix or $P_{\ell 1} < 0.3$. Probably the linear relation between the fractions n_k and the increase of ϕ_e remains approximately valid if the condition of very rapid transfer is relaxed, provided that the excitation energy can visit 10 or more units and the ratio $P_{\ell 1}/P_{\ell 3}$ not too high (Den Hollander et al., unpublished data).

Materials and Methods

Whole cells of *R. rubrum* strain S_1 were grown in a continuous culture at a light intensity of about 5 mW/cm^2 (incandescent light). The absorbance (880–960 nm) of the suspension was kept constant at 0.5 by a feed-back system. After centrifugation cells were washed twice in buffer containing 50 mM tricine, 5 mM K_2HPO_4, 5 mM $MgCl_2$ (pH 8.0) and resuspended in the same buffer. Samples were then immediately prepared as follows. The cells ($A_{880-960} = 0.5$) were aerated and put in the dark for 5 min to prevent accumulation of reduced acceptors. Then *o*-phenanthroline was added up to a final concentration of 2.0 mM to block the electron transport between Q_1 and the next quine acceptor Q_2 [17,18]. After 5 min of flushing with nitrogen in the dark to obtain anaerobiosis, ascorbate and DAD were added to final concentrations of 0.20 and 10.0 mM, respectively, as an efficient donor system to prevent accumulation of the state P^+IQ_1 or $P^+IQ_1^-$ upon illumination. Gramicidin (200–250 μM) was added to prevent the formation of a membrane potential. The relatively high concentration of gramicidin as compared to that ususally applied to chromatophores (approx. 1 μM) was found to be necessary to eliminate completely electrochromic absorbance changes upon illumination as judged from the absorbance difference spectrum of $P^+ - P$ at high light intensities. Samples in which Q_1^- was completely reduced were prepared by adding sodium dithionite (final concentration 5.0 mM) after 5 min of dark adaptation and flushing with nitrogen. During all experiments the temperature was controlled at 288 ± 1 K.

The magnetic field-induced emission and the absorbance change associated with the increase in Q_1^- were measured simultaneously by means of the apparatus described previously [16]. To improve the resolution at low measuring light intensity, this apparatus was altered slightly. A beam splitter was placed in the measuring light beams, so that small changes in intensity could be detected using a photodiode. By means of a differential amplifier a correction was made for instabilities of the measuring light. By applying a sinusoidal 50 Hz-modulated magnetic field with an amplitude of maximally 130 mT, it was possible to

compute the stimulated emission saturation curve from half a period, averaged at low light intensities up to 270 000-times to increase the signal-to-noise ratio. Excitation occurred through appropriate interference filters with light of 603 nm, that excited the bacteriochlorophyll but not the carotenoids. The emission was detected at 905 nm by means of an S1-type photomultiplier with a Kodak Wratten 87C absorbance filter and a Schott AL 905 interference filter. Simultaneously, absorbance changes were detected by means of an S20-type photomultiplier with a Balzers interference and a Corning CS 7-59 filter. The wavelengths of the beams for the absorption measurements were selected by a monochromator (bandwidth 3.2 nm). During these experiments, no actinic effect of these beams, which were only admitted during a short period, was detected. The absorbance difference spectrum of the acceptor Q_1 was determined in a chopped single-beam spectrophotometer described elsewhere [19]. The absorbance change due to the decay of the reaction center carotenoid triplet (see scheme of Fig. 1) and the magnetic field-induced decrease of the reaction center carotenoid triplet yield were measured at 432 nm upon excitation with a 602 nm dye laser flash (half-time 10 ns). The light-induced absorbance difference spectrum of the reaction center carotenoid triplet shows a maximum at 432 nm, where no absorbance change due to the formation of an antenna carotenoid triplet is observed [20]. The 432 nm absorbance increase also reflects the oxidation/reduction of P, but can be distinguished from the 3.2 μs carotenoid triplet decay kinetically. The oxidation of P occurs within approx. 10 ps [21,22], the reduction of P^+ by recombination of the radical pair in approx. 3 ns [24] and the reduction by DAD or cytochrome in several milliseconds.

Results

Light-induced absorbance difference spectrum for the reduction of Q_1

Fig. 3A shows the kinetics of the light-induced absorbance changes at 330, 350 and 450 nm upon illumination of whole cells of *R. rubrum*. Three traces are depicted, measured at an excitation light intensity of 3 mW/cm^2 at 603 nm, which saturated the changes at the wavelengths shown. At light intensities below 5 mW/cm^2 the absorbance changes showed similar simple kinetics with decay times which were independent of the light intensity. At higher intensities the kinetics became more complex and the difference spectrum (not shown) indicated the accumulation of the oxidized primary

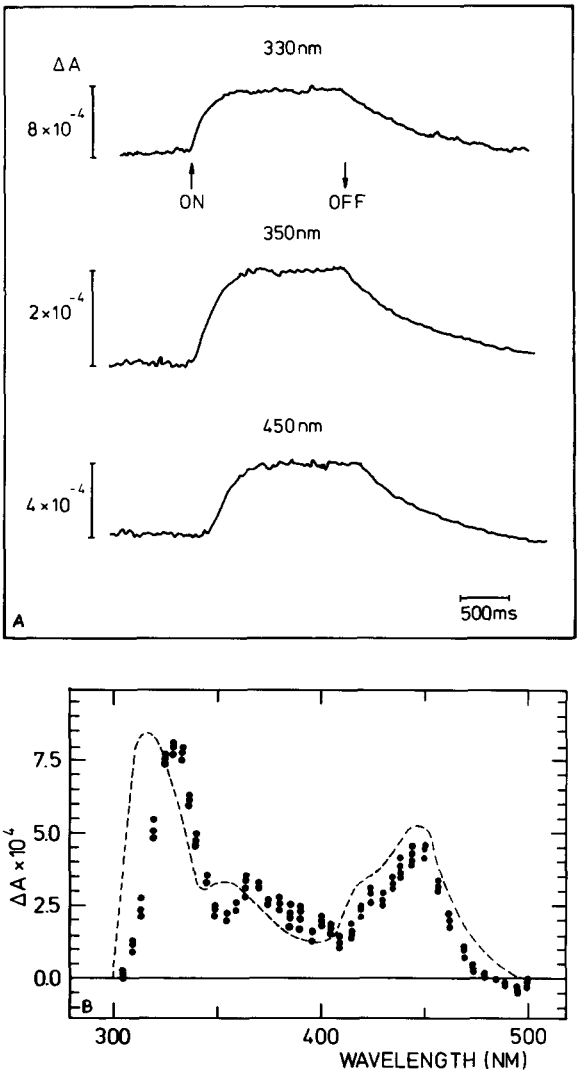

Fig. 3. (A) Kinetics of the absorbance changes at 330, 350 and 450 nm in whole cells of *R. rubrum* upon 603 nm excitation (3 mW/cm^2) in the presence of 10.0 mM DAD, 2.0 mM *o*-phenanthroline, 0.20 μM sodium ascorbate and 250 μM gramicidin. (B) Light-induced absorbance difference spectrum in whole cells of *R. rubrum* (●). Absorbance at 880 nm, after correction for the absorbance at 960 nm, 0.5. Samples were excited at 603 nm (3 mW/cm^2). The dashed line represents the absorbance difference spectrum of ubisemiquinone-10 minus ubiquinone-10 in methanol (- - - - -) [25].

donor P-870. For the spectrum shown in Fig. 3B a saturating excitation light intensity of 3 mW/cm^2 was used. Although the kinetics showed some variation, the same difference spectrum was obtained with different samples. To obtain reproducible kinetics the sequence of additions and the dark-adaptation times had to be rigorously standardized. The same spectrum was found with TMPD as electron donor instead of DAD. This indicates that the spectrum was not affected by absorbance changes due to oxidation of the electron donor. In the experiments discussed below, the 450 nm absorbance change was measured in order to relate the magnitude of the magnetic field-induced emission change to the fraction of the acceptor Q_1^-.

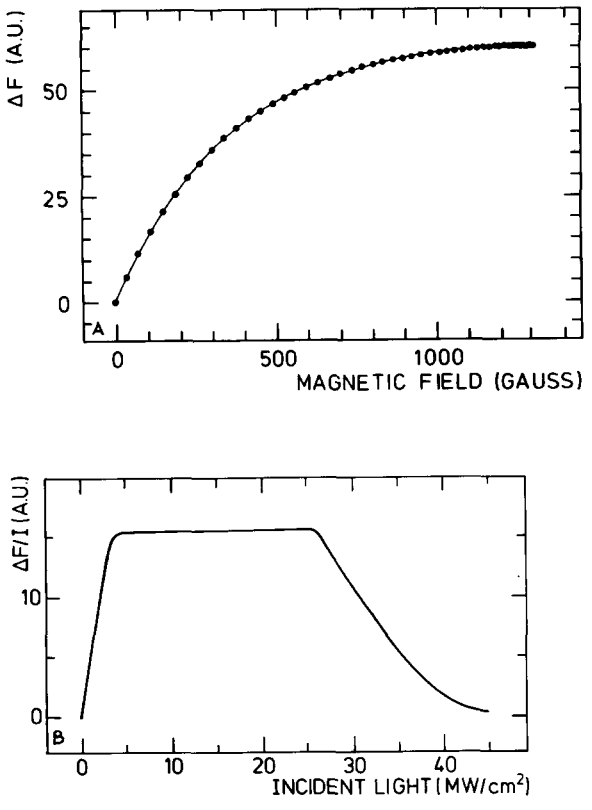

Fig. 4. (A) The emission change ΔF detected at 905 nm upon excitation at 603 nm (4 mW/cm^2) as a function of the magnetic field strength. Conditions as in Fig. 3A. (B) The magnetic field-induced emission increase ΔF_{max} detected at 905 nm in a saturating field normalized for the excitation light intensity, as a function of the 603 nm excitation light intensity I. Conditions as in Fig. 3A, (A.U.) = arbitrary units.

Magnetic field-induced emission change ΔF

Fig. 4A shows the increase in emission at 905 nm in whole cells of *R. rubrum* induced by a magnetic field. The addition of gramicidin did not affect the shape or amplitude and similar curves were obtained at various light intensities and after chemical reduction by dithionite. The absorbance change at 450 nm during continuous 603 nm excitation was simultaneously detected. By varying the light intensity I of the actinic light more than

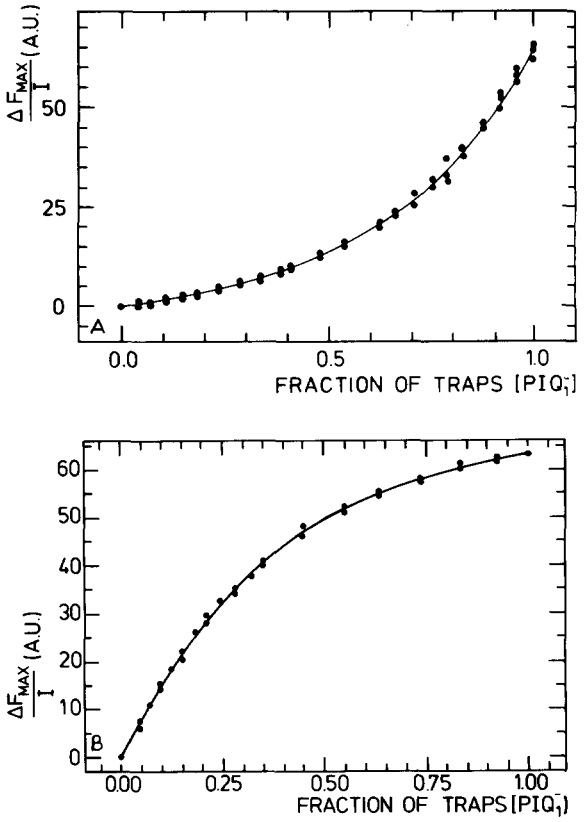

Fig. 5 (A) The normalized magnetic field-induced emission increase of $\Delta F_{max}/I$ in a saturating field, monitored at 905 nm, as a function of the fraction n_2 of traps PIQ_1^- ($n_3 = 0$). Excitation at 603 nm (35 μW/cm^2–5 mW/cm^2). n_2 is calculated from the 450 nm absorbance change due to the reduction of Q_1. Conditions as in Fig. 3A. The curve through the experimental points is calculated using Eqn. 3 (see discussion). (B) The normalized magnetic field-induced emission increase $\Delta F_{max}/I$ in a saturating field at high excitation light intensity (25–45 mW/cm^2) as a function of n_2 ($n_1 = 0$). Conditions as in Fig. 3A. The curve through the experimental points is calculated using Eqn. 3 (see discussion), for which n_2 and n_3 are calculated from the absorbance changes at 432 and 450 nm.

1000-times from 35 $\mu W/cm^2$ to 45 mW/cm^2, different steady-state concentrations of reduced acceptor Q_1 were established. For a number of intensities I we determined the relation between emission yield increase and magnetic field strength resulting in curves similar to the one shown in Fig. 4A. From these curves we obtained the maximum change in (saturating) high field, ΔF_{max}, and the field strength, $H_{1/2}$, at which the emission increase was half maximum. In each sample the light-induced absorbance changes at 330, 380 and 450 nm were measured to check the condition of the sample. Fig. 4B shows the relation between $\Delta F_{max}/I$ and the light intensity I. At intensities below 5 mW/cm^2 $\Delta F_{max}/I$ increases due to the accumulation of Q_1^- ($n_3 = 0$) as is indicated by the absorbance difference spectrum. At intensities above 25 mW/cm^2, $\Delta F_{max}/I$ decreased. At these light intensities the absorbance difference spectrum indicated that the state P^+IQ_1 was accumulated and that Q_1^- became oxidized.

Fig. 5A shows $\Delta F_{max}/I$ as a function of n_2, i.e., the fraction of traps in the state PIQ_1^- keeping $n_3 = 0$; n_2 was calculated as the ratio of the 450 nm absorbance change induced by the light applied to determine ΔF_{max} and the maximum absorbance change of a saturating light intensity of 5 mW/cm^2 after chemical reduction of the sample by addition of 5 mM dithionite. The light-induced absorbance change at 450 nm at an intensity of 5 mW/cm^2 was about 95% of the chemically induced absorbance change, indicating that almost all reaction centers were in the reduced state PIQ_1^- at this light intensity and that no contributions of other chemically induced absorbance changes were present at 450 nm. The maximum relative magnetic field-induced emission change $\Delta F_{max}/F$ measured 1.5%. The curve through the experimentally obtained points was calculated by using Eqn. 3, assuming that the magnetic field-induced emission increase can be ascribed to a decrease of P_{f2} (see Discussion). In chromatophores of *R. rubrum* similar results were obtained. The curve in Fig. 5A is markedly nonlinear, which can be explained by energy transfer in a matrix consisting of a number of reaction centers and associated antenna pigments (units); for separate units the curve would be linear [28]. Fig. 5B shows $\Delta F_{max}/I$ as a function of n_2, keeping $n_1 = 0$. Excitation light intensity varied from 25 to 45 mW/cm^2 at 603 nm. The fractions n_2 and n_3 were calculated from the absorbance changes at 432 and 450 nm. The curve through the experimental points is calculated by using Eqn. 3 (see Discussion).

Fig. 6 shows $H_{1/2}$ as a function of the fraction of traps n_2. At low light intensities the resolution is relatively poor due to a low signal-to-noise ratio, caused by small signals.

Fig. 7 shows the reaction center carotenoid

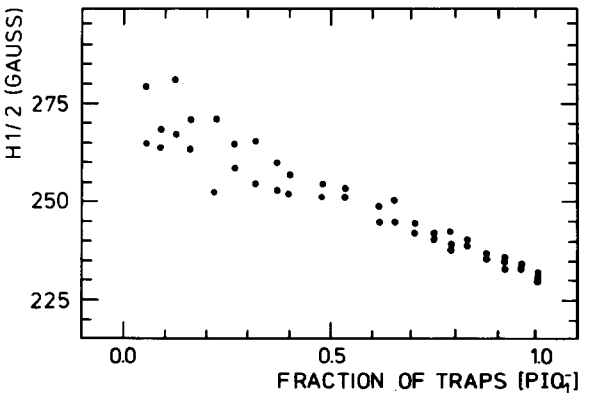

Fig. 6. The magnetic field strength $H_{1/2}$, at which the emission increase is half maximum (see Fig. 4A) as a function of n_2. Conditions as in Fig. 3A. n_2 is calculated from the absorbance change at 450 nm.

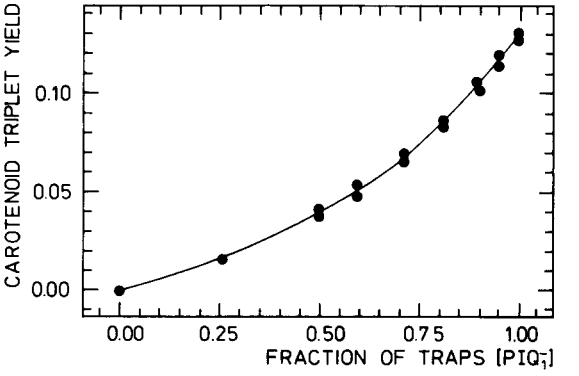

Fig. 7. The reaction center carotenoid triplet yield ϕ_t in a nonsaturating 10 ns dye laser flash (602 nm) as a function of n_2 ($n_3 = 0$). The triplet yield is monitored at 432 nm ($\Delta\epsilon = 63$ mM^{-1}). n_2 is varied by using different intensities (35 $\mu W/cm^2$–5 mW/cm^2) of the continuous excitation light and calculated from the 450 nm absorbance change. Conditions as in Fig. 3A. The curve through the experimental points is calculated by using Eqn. 5 (see text).

triplet yield (fraction of carotenoid triplets per reaction center) induced by a nonsaturating 10 ns dye laser flash (602 nm) as a function of the fraction of traps n_2 varied by different intensities of the continuous background illumination (603 nm). The absorbance increase at 432 nm due to the formation of the carotenoid triplet decays with a single exponent of 3.2 μs. At 432 nm no absorbance change due to antenna carotenoid triplet formation is observed. The oxidized primary donor is completely reduced by the artificial donor system within 3 ms [16]. The curve drawn through the experimental points is calculated by using Eqn. 5 (see Discussion). The carotenoid triplet yield decreased by about 10% in high magnetic field (1300 G).

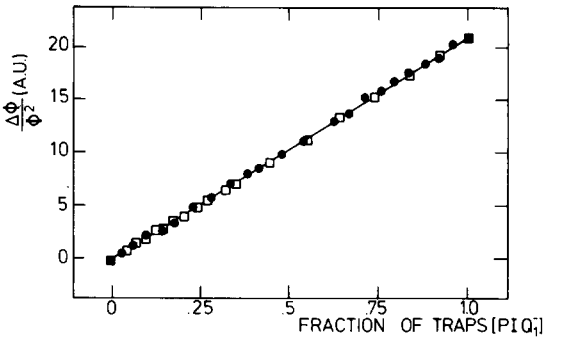

Fig. 9. The Magnetic field-induced emission increase $\Delta\phi$ in a saturating field divided by the square of the relative emission yield ϕ^2 as a function of n_2 at low 603 nm excitation light intensities (●———●, $n_3 = 0$) and at high light intensities (□———□, $n_1 = 0$). Conditions as in Fig. 3A (see text).

Relative emission yield of the states PIQ_I, PIQ_I^- and P^+IQ_I

For the interpretation of the curves shown in Figs. 5 and 7 we also determined the relative emission yield of suspensions with reaction centers in the states PIQ_1, PIQ_1^- and P^+IQ_1. In Fig. 8 the inverse of the relative emission yield is plotted as a function of the fraction of traps, either by accumulation of the reduced acceptor Q_1^- (×) keeping $n_3 = 0$, or by accumulation of P^+ (●———●), keeping $n_2 = 0$. The fraction of reaction centers in the oxidized state P^+IQ_1, n_3, was determined for $n_2 = 0$, by measuring the absorbance increase at 432 nm due to the oxidation of the primary donor. As predicted by the trapping-limited matrix model (see Introduction), the inverse of the emission yield is a linear function of the fraction of traps closed. The slope of the straight line is a measure of the relative trapping rate of a trap in the state P^+IQ_1 or PIQ_1^-. Because of the short lifetime of the reaction center bacteriochlorophyll triplet ($\tau_{1/2} \simeq$ 10 ns), the fraction of traps in the triplet state ($P^TIQ_1^-$) is negligible in the weak actinic light used. The open symbols (○——○) represent the relative emission yield as a function of the traps P^+IQ_1 accumulated in the light at intensities above 25 mW/cm^2.

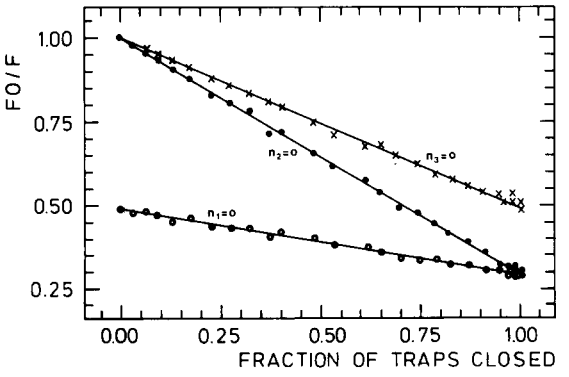

Fig. 8. The increase of the relative yield, F_0/F monitored at 905 nm, plotted as a function of n_2 keeping $n_3 = 0$ (603 nm excitation light intensity 35 μW/cm^2–5 mW/cm^2, (×———×) and $n_1 = 0$ (603 nm excitation light intensity 25–45 mW/cm^2, ○———○). Conditions as in Fig. 3A. The closed symbols (●———●) show F_0/F as a function of n_3 keeping $n_2 = 0$ (603 nm excitation light intensity 35 μW/cm^2–45 mW/cm^2). Samples prepared in the presence of 250 μM gramicidin only. n_2 and n_3 were calculated from the absorbance changes at 450 and 432 nm, respectively (see text).

In whole cells of *R. rubrum*, we also determined the emission yield for a number of redox states of the quinone acceptor Q_2, $n_3 = 0$ (unpublished data). These experiments indicate that the emission yield does not change upon the reduction or protonation of Q_2.

Discussion

The absorbance difference spectrum induced by 603 nm continuous light (Fig. 3B) is, apart from a red shift of the 320 and 365 nm bands, very similar to the absorbance difference spectrum of ubisemiquinone-10 minus ubiquinone-10 in methanol as reported by Bensasson and Land [25], and confirms the conclusion in an earlier report

concerning the identity of the first quinone acceptor in isolated reaction centers of *Rhodopseudomonas* sphaeroides by Slooten [3]. The spectrum of Fig. 3B presumably contains no contributions from redox change of quinones other than Q_1, because the secondary electron transport from Q_1 to Q_2 was blocked by *o*-phenanthroline. In a previous paper we concluded that presumably the magnetic field-induced emission only occurred if the acceptor Q_1 was reduced [16]. Indeed, a magnetic field-induced emission change was detected only if the absorbance changes shown in the spectrum of Fig. 3B were observed, which shows that both the absorbance changes at 450 nm and the emission change ΔF can be used to establish the redox state of Q_1. At excitation light intensities beyond 25 mW/cm^2 (see Fig. 4B) the absorbance difference spectrum indicates the accumulation of the oxidized primary donor while the reduced quinone acceptor Q_1 becomes oxidized. In contrast with the experiments performed at intensitites below 25 mW/cm^2, the reduction of P^+ seems to become the limiting reaction at high light intensities.. The explanation for this phenomenon, in which accumulation of P^+ and of Q_1^- depends upon the excitation light intensity, is not trivial and may be due to a secondary reaction which does not depend upon the concentration of Q_1^-.

If the pigment system were to consist of identical 'separate' units, i.e., units each containing a single reaction center and its associated antenna molecules without the possibility of energy transfer between the units, then a linear relationship would be attained between the emission change and the number of reaction centers in the state PIQ_1^-. The nonlinear curves shown in Fig. 5A and B thus show that energy transfer between units occurs. This can be explained by means of the model of Fig. 2, where no barriers exist between the various photosynthetic units.

The $H_{1/2}$ values of the saturation curves of the stimulated emission decrease as the number of traps PIQ_1^- increases (Fig. 6). In an experiment under similar conditions, in which the emission decay after a weak 30 ps flash was measured in the nanosecond range, it has been found that the lifetime of the recombination luminescence increases as a function of the fraction n_2 of traps PIQ_1^- (Van Bochove, A.C., Kingma, H. and Van Grondelle, R., unpublished results). Both these effects thus indicate an increase of the lifetime of the radical pair [23]. A decrease of the rate constant k_s (Fig. 1) by the formation of a light-induced membrane potential will induce such an increase of the lifetime of (P^+I^-) [24]. However, all experiments were done in the presence of gramicidin preventing the build up of a significant membrane potential.

A second possibility occurs if a significant fraction of the radical pair $(P^+I^-)^S$ decays via the singlet excited states (P* and BChl*) (see Fig. 1). The decay rate of the radical pair will then be significantly smaller and the lifetime longer if most of the traps are in the state PIQ_1^- when compared with most of the traps in the state PIQ_1. A calculation of the increase in lifetime, as a function of k_s and other parameters,will be given elsehwere (Van Bochove, A.C., Kingma, H. and Van Grondelle, R., unpublished data). In chromatophores of *R. rubrum* in the presence of dithionite, upon addition of ATP a membrane potential is induced, while $\Delta F_{max}/F$ decreases and $H_{1/2}$ increases [24]. Addition of gramicidin restored the initial shape and amplitude of the saturation curve of the magnetic field-induced emission change. The absorbance difference spectrum of the reduction of Q_1 (Fig. 3B) was affected by electrochromic shifts if no gramicidin was added. However, upon addition of gramicidin, neither in the samples chemically reduced by 5 mM dithionite nor in the samples with blocked secodnary electron transport by *o*-phenanthroline, a change of $\Delta F_{max}/F$ or $H_{1/2}$ is detected. Apparently, the electrochromic shift present in our samples without gramicidin is not correlated with a large membrane potential which affects both $\Delta F_{max}/F$ and $H_{1/2}$ as was observed in the presence of ATP [24].

The linear relation between the inverse relative emission yield and the fraction of traps in the state PIQ_1^- or P^+IQ_1, respectively (Fig. 8), supports the hypothesis that the antenna system in reduced whole cells of *R. rubrum* is organized as a matrix [28]. In the state PIQ_1^- the emission is for an appreciable part delayed fluorescence (luminescence), and its yield appears to be lower than the prompt fluorescence in the state P^+IQ_1, because apparently the losses (probably mainly caused by triplet formation) are higher than those caused by

fluorecence quenching by P^+. For an estimation of the ratios of the probabilities P_{ek} in Fig. 2 the value of the emission yield of the state P^+IQ_1 ($n_3 = 1$) suffices. The emission yield in *R. rubrum* is about 40–50% (data not given) of the yield of *Rps. sphaeroides*, which is about $0.25/18 = 0.014$ (the lifetime of fluorescence in the state P^+IQ_1 in vivo is 0.25 ns [26] and the natural lifetime is about 18 ns [27]). From Eqn. 3 we find for $n_3 = 1$:

$$\frac{1}{\phi_e} = (n_0 P_{\ell 0} + P_{\ell 3})/n_0 P_{e0} = \frac{1}{0.007} = 143$$

Furthermore, we can write:

$$P_{e0} = \frac{k_f}{k_f + k_\ell + k_h}$$

where k_f, k_h and k_ℓ are the respective rate constants of emission, energy transfer and loss processes of an excited antennna bacteriochlorophyll molecule. Further, assuming that $k_\ell = 5k_f$, $k_h \gg k_\ell$, $n_0 = 50$ and $k_f = 1/18$ ns^{-1} we find:

$$P_{\ell 3} = 138 n_0 k_f/k_h = 138 \cdot 50 \cdot 5.6 \cdot 10^7/k_h = 3.9 \cdot 10^{11}/k_h$$

So, if the reaction center in the state P^+IQ_1 is an irreversible trap, the rate constant of trapping would be about $3.9 \cdot 10^{11}$ s^{-1}. Since ϕ_e ($n_3 = 1$) $\simeq$ $3.4\phi_e$ ($n_1 = 1$), we find $P_{\ell 1} \simeq 3.4 P_{\ell 3} = 1.3 \cdot 10^{12}/k_h$ ($k_h > 10^{12}$ s^{-1}; Van Grondelle et al., unpublished data). In a similar way we find $P_{\ell 2} = 8.0 \cdot 10^{11}/k_h$. Assuming that $P_{\ell 0} = 5k_f/k_h = 2.8 \cdot 10^8/k_h$ and using the experimental result of Fig. 7 that ϕ_t ($n_2 = 1$) = 0.13, we find using Eqn. 5 $P_{t2} = 0.13 \times (n_0 P_{\ell 0} + P_{\ell 2}) = 1.1 \cdot 10^{11}/k_h$. The relative magnetic field-induced emission change $\Delta\phi_e/\phi_e$ measures $1.5 \cdot 10^{-2}$ which, in our model, is ascribed to a decrease $\Delta P_{\ell 2}$ of $P_{\ell 2}$ and with Eqn. 3 results in:

$$\Delta P_{t2} = \Delta P_{\ell 2} \simeq 0.015 \times (P_{\ell 2} + n_0 P_{\ell 0}) \simeq 1.3 \cdot 10^{10}/k_h$$

Similarly, we find that $\Delta\phi_t/\phi_t = \Delta P_{t2}/P_{t2} = 0.12$, which agrees reasonably with the experimental result of 0.10. We are now able to calculate the emission and triplet yield as a function of the redox state of the reaction center, which results in the curves in Figs. 5 and 7–9. Apparently, application of the matrix model used yields a good agreement with the experimental findings. The fluorescence yield of the state PIQ_1 ($n_1 = 1$) is $1/3.4 \cdot 0.017 \simeq 0.25\%$. The total loss in the antenna will then be $5 \cdot 0.25\% + 0.25\% = 1.5\%$ ($k_\ell = 5k_f$). So 98.5% of the excitation energy may be trapped in the reaction center. A calculation of the efficiency of Q_1 reduction, based upon the initial slope of the light-induced change at 330 nm, results in an efficiency of $(92 \pm 6)\%$. Because carotenoid excitation induces a secondary magnetic field emission change in the antenna, the model presented can only be applied if excitation of bacteriochlorophyll is established (unpublished data).

Fig. 9 shows $\Delta\phi/\phi^2$ as a function of n_2 under reducing conditions ($n_3 = 0$) and under oxidizing conditions ($n_1 = 0$). For both conditions identical linear relationships are found, as predicted by Eqn. 4.

From relative measurements of $\Delta\phi$ and ϕ for a certain suspension, n_1, n_2 and n_3 are determined as follows. First, four calibration measurements are performed. All reaction centers are forced into the state $n_2 = 1$ by one of the methods described [16], and ϕ and $\Delta\phi$ are determined. Similarly, ϕ is determined for the states $n_1 = 1$ and $n_3 = 1$. For the suspension in an unknown state, n_2 is directly found from the following equation, obtained from Eqn. 4:

$$n_2 = \frac{\Delta\phi/\phi^2}{(\Delta\phi/\phi^2)_{n_2=1}} \tag{6}$$

In order to obtained the unknown n_1, n_3 is eliminated from Eqns. 1 and 3:

$$1/\phi = a + bn_1 + cn_2 \tag{7}$$

in which a, b and c are constants. The three calibration measurement of ϕ for $n_1 = 1$, $n_2 = 1$ and $n_3 = 1$ result in three equations, from which the three unknowns a, b and c are easily obtained. Eqn. 7 now is solved for the only remaining n_1, and n_3 is then obtained from Eqn. 1.

Conclusion

Within the relatively high precision of the experiments our results indicate that in whole cells of *R. rubrum* a matrix type of energy transfer between various photosynthetic units occurs. The model presented here, which is a generalization of several theoretical considerations reported earlier

[28], is in excellent agreement with the experimental findings. In a suspension of cells or chromatophores, n_2 can be found from Eqn. 4 by simultaneous measurements of $\Delta\phi$ and ϕ. Subsequently, n_1 and n_2 can be determined from Eqns. 1 and 3. The method is especially suited to determine the concentration of Q_1^- under steady-state conditions in continuous light. Moreover, the emision yield is less sensitive to scattering changes than are measurements of absorbance changes. Experiments to be described elsewhere indicate that the emission yield ϕ and $\Delta\phi$ do not depend on the redox state of the second quinone acceptor Q_2.

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