The effect of a membrane potential on the kinetics of the nanosecond recombination luminescence and the reaction center triplet yield in *Rhodospirillum rubrum* chromatophores

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1. INTRODUCTION

The primary charge separation in the reaction center of a photosynthetic purple bacterium consists of the transfer of an electron from the reaction center bacteriochlorophyll dimer (P) to the electron acceptor bacteriopheophytin (I) within 5 ps. Under normal conditions this charge separation is followed by electron transfer from I to a secondary acceptor Q which is a quinone [1]. If Q is already reduced before the charge separation, this electron transfer step is inhibited. The lifetime of the state P^+I^- then is 6–8 ns [2,3,4].

The radical pair P^+I^- is assumed to be created from the singlet excited state. Due to different hyperfine interactions and *g*-values of the unpaired electron spins on P^+ and I^-, recombination of P^+I^- may result in the excited singlet state (P^*I) the ground state (P I) or in one of the triplet states (P^TI). The latter decays to the triplet state of

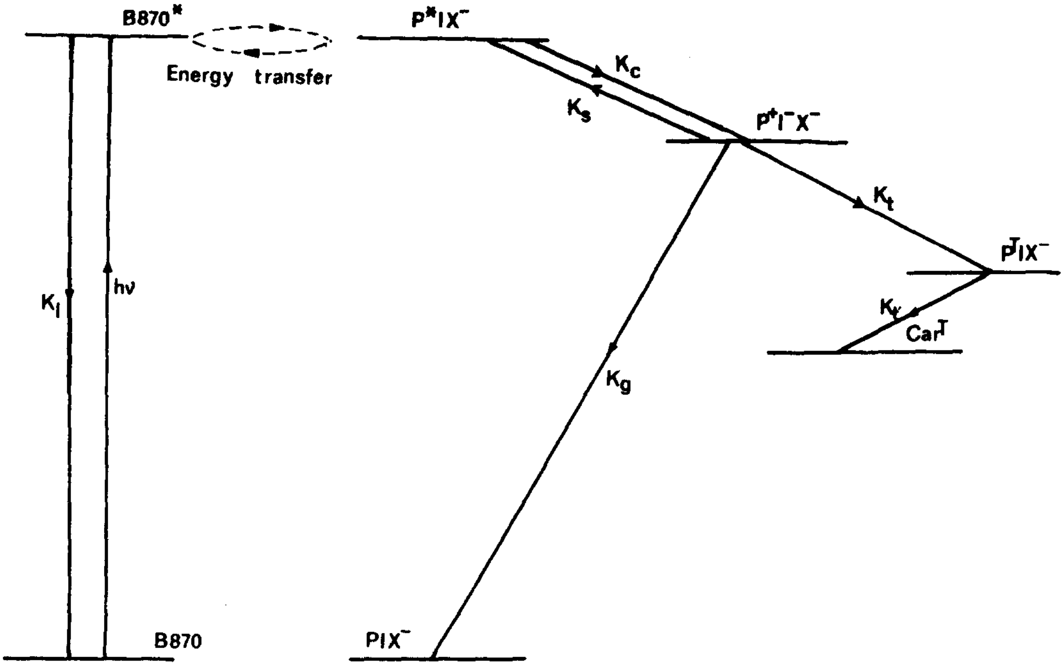

Fig.1. Reaction scheme used to illustrate the relevant pathways in the reduced reaction center of *R. rubrum*. The ratio of B870 to P is 40.

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the carotenoid of the reaction center; the state P^*I decays either by transfer of the excitation back to the antenna, which is the cause of the recombination luminescence, or by again producing a charge separation. The possible pathways are summarized in fig.1; their relative importance is still a controversial matter [3,5,6].

One can follow the decay of the state P^+I^- by the recombination luminescence [3,6] and the carotenoid triplet [6,7]. Borisov et al. and Kotova et al. [8,9], using a phase fluorometer, have found that the average lifetime and the yield of the luminescence increased when an electrical potential ($\Delta\Psi$) was applied across the membrane in which the reaction center is embedded. In their experiments the potential was created by adding pyrophosphate or ATP to their sample of chromatophores of *Rhodospirillum rubrum* or by giving light in the presence of the inhibitor *o*-phenanthroline. They explained the effect on the luminescence by a stimulation of the rate constant k_s (fig.1).

We studied this phenomenon by looking at the kinetics and the yield of the recombination luminescence and the triplet yield of the reaction center carotenoid. Moreover, it is well described that the luminescence yield and the triplet yield are affected by the presence of a magnetic field [10], and therefore we used this technique too. Our results show that the increase of the luminescence induced by an electric field occurs with a decrease of the luminescence lifetime and a decrease of the triplet yield. The luminescence change induced by a magnetic field is also diminished while the magnetic field strength, which is necessary for half saturation, increases. Referring to fig.1, it seems that almost all of the decay of P^+I^- occurs via the luminescence, (k_s), or the triplet formation, (k_t). Almost nothing decays via the radiationless path (k_g) to the ground state P I, in agreement with earlier suggestions [3,6].

2.METHODS

The chromatophores of *R. rubrum* strain S1M were prepared as in [11]. In the time resolved luminescence measurements, the sample was excited by a 30 ps pulse of a frequency doubled, mode-locked Nd-YAG laser (λ = 532 nm) and the luminescence measured through a Kodak Wratten filter (87C) with an avalanche photodiode with a response time of 0.2 ns as described in [4]. The reaction center carotenoid triplet was induced by a 300 ns flash from a Zeiss dye laser tuned at 600 nm and measured by the absorption at 430 nm as described in [7]. The magnetic field-induced luminescence changes were measured as in [12]. All measurements were performed at room temperature, in a sample containing 50 mM Tricine buffer (pH 7.8), 5 mM $MgCl_2$ and, when reduced, 5 or 25 mM $Na_2S_2O_4$. *A* at 880 nm was 1. The membrane potential was produced by the addition of 2 mM adenosine triphosphate (ATP).

3. RESULTS

Fig.2 shows the luminescence decay in a sample of chromatophores of *R. rubrum*, reduced by 25 mM $Na_2S_2O_4$ after a flash in the presence and absence of 2 mM ATP. The first 3 ns of the kinetics are deformed due to the high fluorescence peak preceding the luminescence. If these kinetics are fitted using single exponentials, decay times of 6.2 and 4.4 ns are obtained. By extrapolating the single exponentials to the time of the flash we found that the initial amplitude of the emission increased by 75% on addition of ATP which means that the

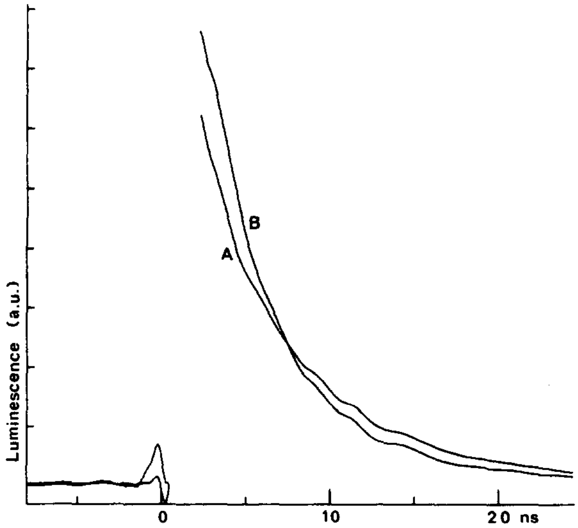

Fig.2. Kinetics of the luminescence decay after a short flash on chromatophores of *R. rubrum*. Trace A shows the decay in a reduced sample. Trace B after adding 2 mM ATP to the reduced sample.

integrated emission increased by 24%. As it is rather speculative to extrapolate these kinetics over an interval of 4 ns we also measured the luminescence yield in continuous illumination to obtain an independent estimate. To this end we first measured the yield of prompt fluorescence in an oxidized (not reduced) sample andthen reduced the sample to measure the total emission, which was 2.5 times higher. The increase of the total emission on addition of ATP was 17% which means that the fraction of the emission ascribed to recombination luminescence increased by 28%. This value could be compared with the 24% obtained by the flash method. Although the difference is not large, it indicates that the luminescence decay is not a single exponential which was also found by Van Bochove et al. [4].

Fig.3 shows flash-induced absorption changes measured at 430 nm in a reduced sample in the presence and absence of ATP. The decay time is 3 μs in both cases. Measuring and averaging 9 of these traces we found that the initial triplet yield decreases by 34% on addition of 2 mM ATP. Using an extinction coefficient for triplet formation at 430 nm of 65 mM^{-1} cm^{-1} [5,13], about 0.3 triplet/RC was formed in the absence of ATP.

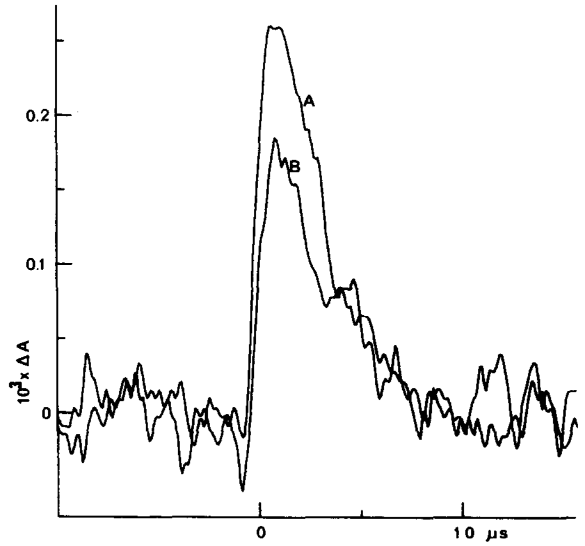

Fig.3. Kinetics of absorption change after a short flash in chromatophores of *R. rubrum* measured at 430 nm. The traces show the carotenoid triplet. A and B as in fig.2.

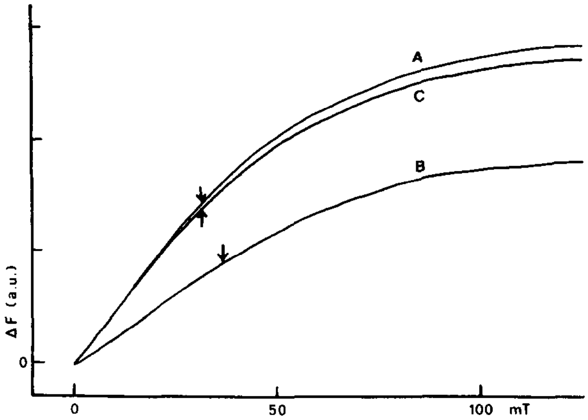

Fig.4. Magnetic field-induced fluorescence change in chromatophores of *R. rubrum*. The three traces were measured in one sample by changing the magnetic field and simultaneously monitoring the emission. The maximum of the magnetic field-stimulated emission (+ dithionite, B = 140 mT) was ~9.5% of the total emission without a magnetic field. A and B as in fig.2. To obtain trace C, valinomycin and nigericin were added, 1 μM of both. Arrows indicate the points of half maximal effect ($B_{1/2}$).

Fig.4 shows the dependency of the emission yield on a magnetic field. Addition of 2 mM ATP in the presence of 5 mM dithionite decreased the emission change (ΔF) by ~33% while the value of the magnetic field at which the stimulation of the emission was half maximum ($B_{1/2}$) shifted by 4.5 mT from 33.5 to 38.0 mT. Both $B_{1/2}$ and ΔF were restored by the addition of valinomycin and nigericin.

The increase of the luminescence decay time, the stimulation of the luminescence yield and the decrease of the yield of the reaction center triplet by the addition of ATP were restored to their values without ATP by valinomycin in the presence of KCl but not by nigericin alone, indicating an effect of $\Delta\Psi$.

4. DISCUSSION

The kinetic scheme of fig.1 is the minimal model required to understand the effect of the transmembrane electric field, $\Delta\Psi$, on the luminescence decay kinetics, the luminescence yield and the triplet yield.

Table 1

Rate constants, decay times, triplet yield (T.Y.) and luminescence yield (L.Y.) from a numerical solution of the rate equations compared with the experimental values

						Theoretical			Experimental		
	k_c	k_s	k_t	k_g	k_l	decay time of P^*IX^-	T.Y. (relative)	L.Y. (relative)	decay time of lumin.	T.Y. (relative)	L.Y. (relative)
		$\times 10^9\ s^{-1}$									
$-\Delta\Psi$	100	0.37	0.09	0	0.7	6.2 ns	100%	100%	6.4 ns	100%	100%
$+\Delta\Psi$	100	0.74	0.09	0	0.7	4.3 ns	70%	125%	4.6 ns	63%	124%

The choice of the rate constants k_c, k_s, k_t, k_g and k_l is discussed in the text. The columns on the left give the numerical results, those on the right the experimental ones.

Table 1 gives the values for the various rate constants of fig.1 which we used in our calculations. The values of k_l and k_c were chosen to give a decay time of the fluorescence of 1.4 ns when all the reaction centers are closed (i.e., $k_c = 0$), and a decay time of 300 ps if all reaction centers are in the active state P I X (X not reduced). This choice of parameters yields a fluorescence increase by a factor of 4 or 5 on closing the reaction centers, which is in agreement with the experimental values found in chromatophores of *R. rubrum* (R. van Grondelle, unpublished results). We were not able to simulate these effects by assuming the alternative models of [5] and [15] in which it is assumed that the fluorescence is substantially quenched by the reaction centers in the inactive state.

Several combinations of the rate constants k_s, k_g and k_t yielded the right luminescence decay time and realistic luminescence and triplet yields. Especially the triplet yield allows some freedom in the choice of parameters since the absorption coefficient of the reaction center carotenoid triplet has not been estimated accurately, and therefore the experimental triplet yield is also subject to this uncertainty. In all combinations k_g had to be chosen one order of magnitude smaller than k_s and k_t to be able to simulate both the $\Delta\Psi$ effect on the luminescence and the triplet yield. The choice of $k_t = 9 \times 10^7\ s^{-1}$ and $k_s = 3.7 \times 10^8\ s^{-1}$ then gives a triplet yield of 40% and a luminescence decay time of 6.2 ns, in reasonable agreement with the experiments. From this set of values we calculated an energy difference of 0.12 eV between P^*I and P^+I^-. The effects of a $\Delta\Psi$ of ~60 mV on the triplet yield, the luminescence yield and the luminescence decay time are very well simulated by doubling the rate constant k_s and the set of rate constants give an energy difference of 0.10 eV. Applying the equations of Godik and Borisov [3] to these sets of rate constants gives approximately the values of effective lifetime and emission yield as found by the phase fluorometry method in [8].

The effect of $\Delta\Psi$ on the magnetic field dependent fluorescence can be calculated by solving the Schrödinger equation as given in [10,14] taking into account the rapid recombination k_s. This calculation is difficult and beyond our present goal, and we shall discuss the effect in a qualitative way.

The decrease of the magnetic field-induced luminescence change is in agreement with the increased rate of the back reaction, k_s. The larger $B_{1/2}$ value can be explained from the broadening of the triplet sublevels due to the shorter decay time of P^+I^- in the presence of an electric field. The broader the triplet levels, the larger the magnetic field necessary to disconnect the $|T_+>$ and $|T_->$ levels from the $T_0>$ and $S>$ levels [10,14]. The shift in $B_{1/2}$ of ~4.5 mT can be accounted for by interpolating fig.8 of [10] for various values of k_s. It should be noted, however, that the absolute $B_{1/2}$ values of [10] are based on P^+I^- decay times obtained in isolated reaction centers, which are 2–3 times longer than in chromatophores. If the external electric field had a large effect on k_g, this would not markedly affect the obtained $B_{1/2}$ value (fig.5 of [10]).

If finally we assume that the electric potential generated in the membrane by the addition of 2 mM ATP is 0.06 V, this would mean that the pair of charges on P^+ and I^- 'feel' ~30% of the transmembrane field. If the transmembrane field is homogenous and the membrane thickness 50 Å, a distance of 17 Å between the charges on P^+ and I^- is obtained.

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