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BACTERIOCHLOROPHYLL FLUORESCENCE OF PURPLE BACTERIA AT LOW REDOX POTENTIALS

THE RELATIONSHIP BETWEEN REACTION CENTER TRIPLET YIELD AND THE EMISSION YIELD

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Summary

This work describes fluorescence yield measurements in suspensions of strains of *Rhodospirillum rubrum* and *Rhodopseudomonas sphaeroides* in which the iron · quinone complex (X) was chemically reduced (state $[PIX^-]$; P is the reaction center bacteriochlorophyll dimer, I is the long wavelength bacteriopheophytin), and compares these with the fluorescence observed when all the traps are open (state $[PIX]$) and with the fluorescence observed when all the traps are closed (state $[P^+IX]$). At 77 K the amplitude and the shape of the fluorescence emission spectrum in $[PIX^-]$ are identical to those observed in $[PIX]$. This is a strong indication that all the extra fluorescence observed at room temperature in $[PIX^-]$ is, in fact, caused by an efficient back reaction $[P^+I^-X^-] \rightarrow [P^*IX^-]$. Using an equation similar to the original Vredenberg-Duysens relationship (Vredenburg, W.J. and Duysens, L.N.M. (1963) Nature 197, 355—357) but now assuming that a single reaction center has a probability p_t of trapping an excitation and $(1 - p_t)$ of re-emitting it to the surroundings, we are able to calculate p_t as a function of the temperature by measuring the fluorescence in $[PIX]$, $[PIX^-]$ and $[P^+IX]$ as a function of the temperature. The calculated p_t values agree reasonably well with triplet yields measured in isolated reaction centers. Finally, we have measured the reaction center triplet yield (P_{TR}) in intact systems and we have shown that the sum of the triplet yield and the remaining loss processes (P_L) in the antenna bacteriochlorophyll including the bacteriochlorophyll dimer (such as fluorescence, internal conversion or direct triplet formation) is approximately constant;

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if we assume that at 77 K the only process which occurs in the reaction center is the formation of a reaction center triplet, than $P_{TR} + P_L = 1$. The energy barrier between $[P^*IX^-]$ and $[P^+I^-X^-]$ was estimated to be 0.11—0.15 eV for a set of preparations.

Introduction and some theoretical considerations
(For list and explanation of symbols see page 24)

The primary photochemical reaction (see Fig. 1) in purple bacteria after a short laser flash is the formation of a radical pair composed of P^+-870 (or P^+) and I^-, the anion of the long wavelength bacteriopheophytin [2—14]. Under normal photosynthesis I^- reduces the iron-quinone complex (X) with a half time of about 0.2 ns [15,16]. If, however, X is reduced before the flash the reaction $[PIX^-] \xrightarrow{h\nu} [P^+I^-X^-]$ is still possible and occurs with a quantum efficiency as high as that observed for the formation of $[P^+IX^-]$ in non-reduced samples [6], but in this case no charge stabilization can take place, because of the recombination of the radical pair P^+I^-. We consider three possible decay pathways [6,17,18] for $[P^+I^-X^-]$ (Fig. 1).

(1) The formation of $[P^{TR}IX^-]$ with rate constant k_{TR}.

(2) The return to the singlet ground state with rate constant k_G.

(3) The return to the first excited singlet state $[P^*IX^-]$ with rate constant k_{RC}.

The emission yield in $[PIX^-]$, as measured with continuous or flash illumination is higher than that observed when all the traps are in $[PIX]$ [19—22]. This phenomenon may be explained by either of the following hypotheses:

(a) The rate k_{CS} of the formation of $[P^+I^-X^-]$ is not significantly decreased because of the prereduction of X. The higher emission might then be explained by assuming an efficient back reaction $[P^+I^-X^-] \rightarrow [P^*IX^-]$ as suggested by Holmes et al. [21]. Emission can arise from P^* or from B^* after the excitation has returned to the antenna bacteriochlorophyll.

(b) The higher emission yield at room temperature might arise from a longer lifetime of P^* due to the slower rate k_{CS} (Fig. 1) in $[PIX^-]$ than in $[PIX]$. One should, however, note that experiments done by Parson et al.

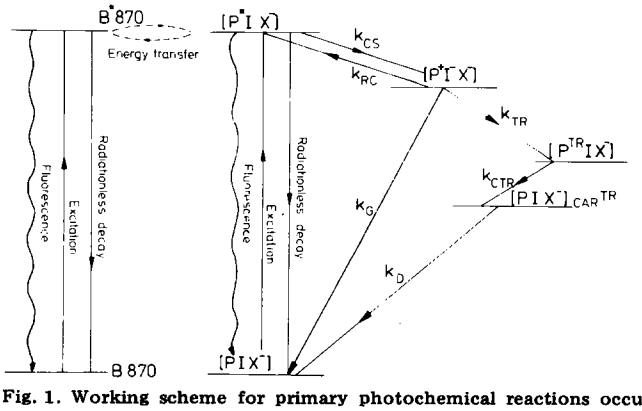


Fig. 1. Working scheme for primary photochemical reactions occurring in the antenna bacteriochlorophyll · reaction center complex. The rate constants k_{CS}, k_{RC}, k_G, k_{TR}, k_{CTR} and k_D are discussed in the Introduction and are subject of this work.

[6] suggest that the quantum yield for the reaction $[PIX^-] \xrightarrow{h\nu} [P^+I^-X^-]$ appears to be equal to that observed for the reaction $[PIX] \xrightarrow{h\nu} [P^+I^-X] \xrightarrow{0.2ns} [P^+IX^-]$.

We have tried to distinguish between these two possibilities by measuring very carefully the emission yield and emission spectra at room temperature and at 77 K of two types of preparations, one in [*PIX*], the other in $[PIX^-]$. Because it is known that at 77 K the formation of $[P^{TR}IX^-]$ occurs with a high quantum efficiency about equal to that observed for the formation of $[P^+IX^-]$ in preparations where X was oxidized before the flash [6,21,23], hypothesis (a) predicts that at 77 K the emission in $[PIX^-]$ will be equal to the emission in [*PIX*]. In that case, k_{RC} (see Fig. 1) would be much less at low temperatures resulting in a high reaction center triplet yield. It is impossible to predict the behaviour of the fluorescence as a function of the temperature of a sample in $[PIX^-]$ from the second hypothesis. One might, however, speculate that the slowing down of the forward reaction is caused by electrostatic repulsion by X^-. This repulsion would increase if the distance between *P* and X decreased going to lower temperatures as has been suggested recently [24]. In this case the fluorescence would increase upon cooling.

The results to be presented in this paper support hypothesis (a). We already mention this in order to introduce the analysis we have performed on the emission data. Thus, there exists a finite chance that an excitation once it has been trapped leads to luminescence due to the back reaction $[P^+I^-X^-] \rightarrow [P^*IX^-]$ where P^* may return the excitation to the antenna bacteriochlorophyll.

If now the probability for trapping of excitation energy upon excitation of the reaction center is p_t, then the probability for the excitation to return to the antenna is $(1 - p_t)$. We can now write an equation which describes the total probability P_F for an excitation to be finally emitted as fluorescence after a photon is absorbed by the antenna bacteriochlorophyll/reaction center system. We call the concentration of traps *T*; then the concentration of antenna bacteriochlorophyll is $(1 - T)$; p_h is the probability of excitation transfer from one bacteriochlorophyll molecule to another and p_f is the probability that an excited bacteriochlorophyll molecule emits its excitation as fluorescence. We then can write [25]:

$$P_F = p_f + (1 - T)p_h P'_F + T(1 - p_t)p_h P''_F \tag{1}$$

where P'_F is the probability after one transfer step of being finally emitted as fluorescence; P''_F is the probability of being finally emitted as fluorescence after returning from the trap. In first approximation one can write

$$1/P_F = 1/p_f - p_h/p_f(1 - p_t T) \tag{2}$$

and a similar equation for the total trapping probability P_T:

$$P_T = \frac{p_t T}{1 - p_h + p_h p_t T} \tag{3}$$

Eqn. 2 shows that there exists a linear relationship between the reciprocal of the emission yield and the trapping probability in the absence of energy transfer p_t. Eqn. 2 is analogous to the Vredenberg-Duysens relationship [1] (see also ref. 35) and, of course, reduces to this formula if we assume $p_t = 1$.

This equation has shown a good agreement with experimental results under conditions where the traps are closed by the formation of [P^+IX] [1] or [$P^{TR}IX^-$] [21].

We have measured the fluorescence of a preparation in [PIX^-] as a function of the temperature and compared this with the fluorescence of [*P*IX] and [P^+IX]. Our estimation of p_t for different strains of *Rhodospirillum rubrum* and *Rhodopseudomonas sphaeroides* have been compared with results on triplet yield in isolated reaction centers. Finally, we have measured the reaction center triplet yield in intact systems, and compared the results with the emission data.

Materials and Methods

Whole cells of *R. rubrum* WT, *R. rubrum* FR1 VI, *Rps. sphaeroides* WT, *Rps. sphaeroides* G1C and *Rps. sphaeroides* R26 were grown under conditions as described before [26]. Whole cells were harvested after 3—4 days of growth and suspended in a 50 mM morpholinopropane sulphonate (MOPS)/50 mM KCl buffer. Sometimes chromatophores were used and were prepared by procedures described elsewhere [27].

To keep the samples clear upon freezing to 77 K we used two procedures. The first of these was to add 1 M of sucrose to the cell suspension and then dilute it to a final solution of 70% glycerol/30% cells in buffer (v/v). These samples showed a normal fluorescence rise upon illumination. The fluorescence yield observed after the reduction of the FeQ complex was, however, 10—20% lower than that of samples diluted only with MOPS/KCl buffer. The reaction center triplet yield in these preparations was comparable to that of the reduced "normal" samples. To prevent this lowering of the fluorescence yield of reduced samples in the presence of 70% glycerol we also tried a freezing mixture which consisted of 50% sodium glycerophosphate and glycerol in a ratio of 3 : 1 (v/v) [28]. This mixture also contained MOPS at a concentration of 50 mM. The sample was prepared in the following way. To a centrifuged pellet a small volume of buffer was added and this suspension was mixed with the glass-forming medium up to a maximum ratio of 5% cells + buffer/95% glycerol/glycerophosphate (v/v). These samples also showed a "normal" fluorescence rise when in [*P*IX] before illumination, the fluorescence in [PIX^-] was now 0—10% lower compared to the reduced normal preparations. All strains of *Rps. sphaeroides* could easily be suspended in this medium up to rather high cell concentrations, *R. rubrum*, however, was difficult to suspend in this medium and then only to a low concentration.

All fluorescence experiments were done on a modified single beam spectrophotometer [27], the response time during these experiments was approximately 1 ms. Continuous illumination was provided via a combination of CS 4-96 and BG 38-4 filters, $\lambda = 400$—600 nm. Fluorescence was measured using an S-1 photomultiplier through a combination of UG 1/1, KV 550 and an appropriate interference filter. False light in this set-up was less than 10% of the lowest fluorescence level measured. Fluorescence emission spectra were measured using a number of interference filters in the region 850—980 nm. These spectra have been calibrated by measuring scattered light of a

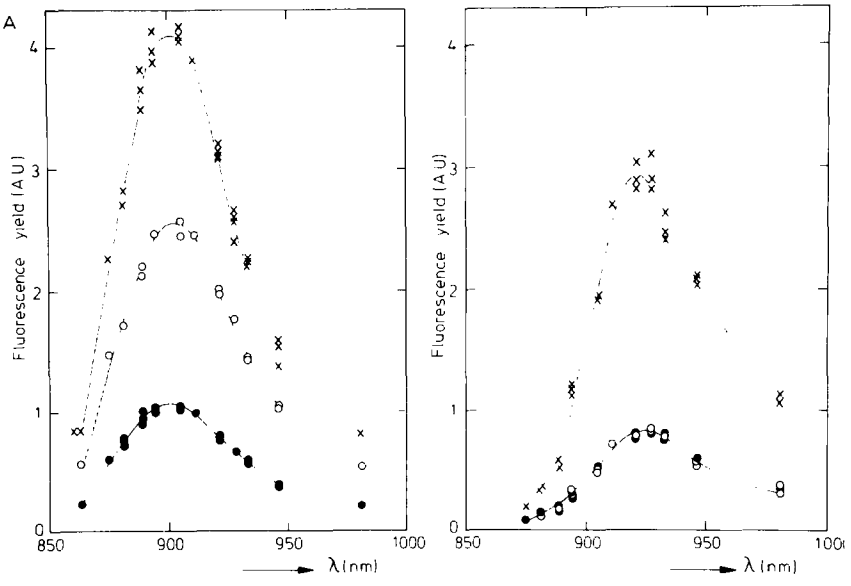
known intensity through the same combination of filters and photomultiplier.

Reaction center triplet kinetics have been measured using a single beam absorption spectrophotometer equipped with a Zeiss dye laser (pulse width 0.6 μs) or a YAG Nd^{3+} laser (pulse width 20 ns) for actinic illumination. The amplitude of the triplet signal was in the order of $\Delta A = (1 - 3) \cdot 10^{-3}$. Several traces were averaged to obtain a good signal to noise ratio. In many of these experiments we employed the Zeiss dye laser because the YAG Nd^{3+} laser directly produced a carotenoid triplet in the bulk carotenoid molecules (Rademaker et al., in preparation) in some species.

The low temperature absorption and fluorescence kinetics were measured using a special cuvette holder described before [28]. The temperature was read by means of a copper-constantan couple mounted in the holder close to the cuvette.

Results

We have measured emission spectra for chromatophore preparations with reaction centers in [*P*IX$^-$] and in [*P*IX]. Illumination of the sample in [*P*IX] produced states [P^+IX] or [P^+IX$^-$] dependent on the treatment of the sample and the temperature. In our experiments we could never find a difference in the bacteriochlorophyll emission yield observed when the reaction centers were in [P^+IX] or in [P^+IX$^-$], both on a slow and on a microsecond time scale (van Grondelle, R. and Holmes, N.G., unpublished observations). Fig. 2 shows the fluorescence emission spectra at room temperature (293 K) and at 77 K observed in a set of species when the reaction centers are in these three states. Fig. 2 (upper), shows the results for *R. rubrum* WT, Fig. 2 (middle), for *R. rubrum* FR1 VI. and Fig. 2 (lower), for *Rps. sphaeroides* G1C. At 77 K the emission spectrum in [*P*IX$^-$] is at all wavelengths equal to F_0 for all three species. Similar results were obtained for *Rps. sphaeroides* WT and *Rps. sphae-*



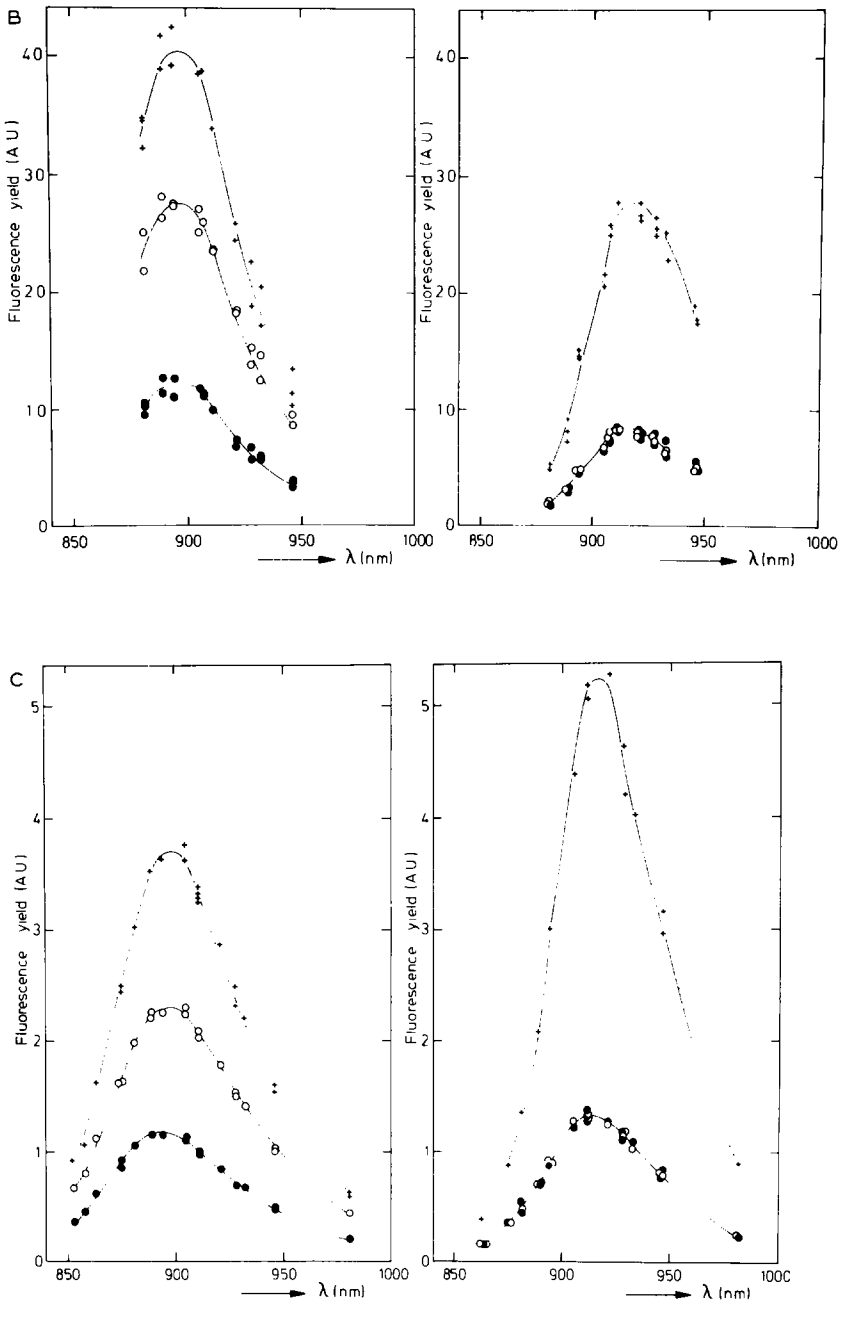


Fig. 2. A. Emission spectra measured in whole cells of *R. rubrum* WT, suspended in 3 : 1 glycerophosphate/glycerol + 50 mM morpholinopropane sulphate. ●, [*P*IX]; ○, [*P*IX$^-$]; X, [*P*$^+$IX] or [*P*$^+$IX$^-$]. State [*P*IX$^-$] was created by reducing the cells with 100 mM sodium dithionite. Left, 293 K; right, 77 K. B. Emission spectra measured in whole cells of *R. rubrum* FR1 VI, suspended in 50 mM morpholinopropane sulphate/50 mM KCl (left) or 70% (v/v) glycerol (right). ●, [*P*IX]; ○, [*P*IX$^-$], created by reduction of the cells with 100 mM sodium dithionite; +, [*P*$^+$IX] or [*P*$^+$IX$^-$]. Left, 293 K; right, 77 K. C. Emission spectra measured in whole cells of *Rps. sphaeroides* G1C, suspended in 3 : 1 glycerophosphate/glycerol (v/v) and 50 mM morpholinopropane sulphate. ●, [*P*IX]; ○, [*P*IX$^-$]; +, [*P*$^+$IX] or [*P*$^+$IX$^-$]. [*P*IX$^-$] was created by reducing the cells with 100 mM sodium dithionite. Left, 293 K; right 77 K.

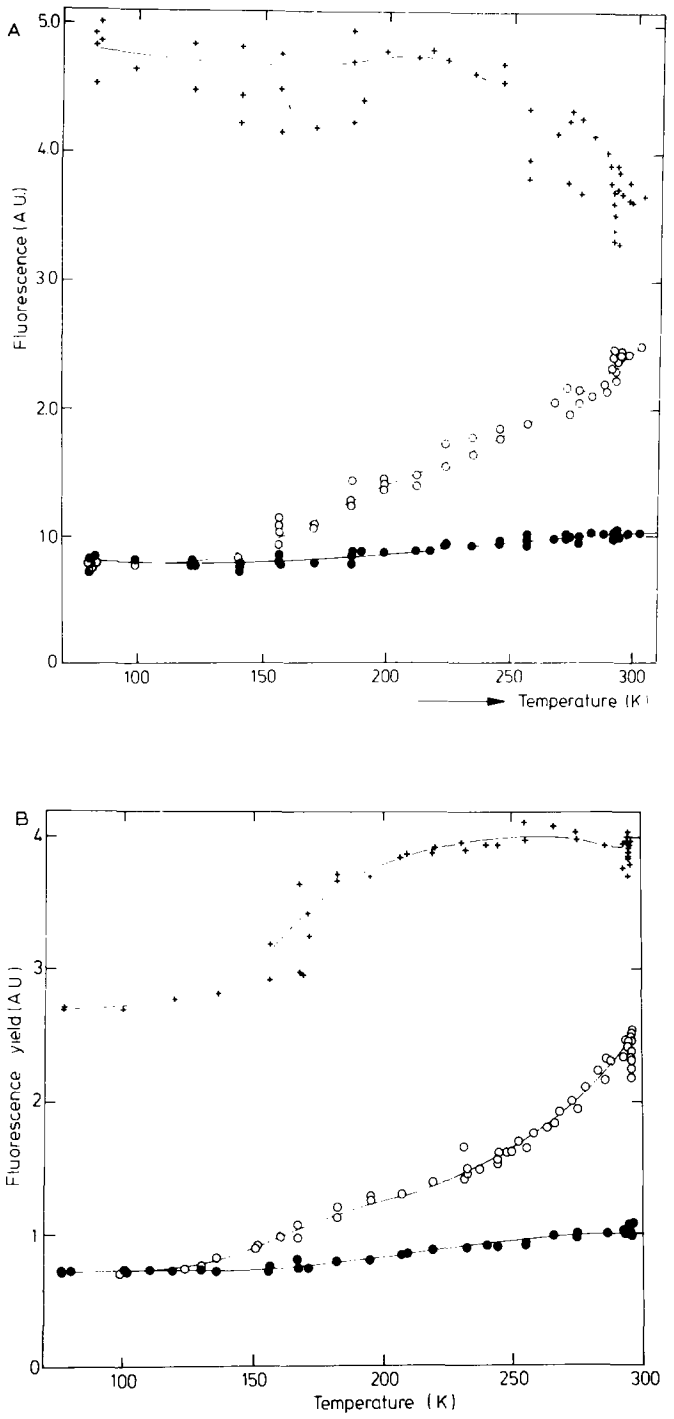


Fig. 3. A. Temperature dependency of the emission yield observed in [*P*IX] (●), [*P*IX$^-$] (X was reduced with 100 mM sodium dithionite) (○), and [P^+IX] or [P^+IX$^-$] (+), in whole cells of *Rps. sphaeroides* WT. The cells were suspended in 3 : 1 glycerophosphate/glycerol (v/v) and 50 mM morpholinopropane sulphate. The measuring wavelength was 911 nm. B. As A, but now cells of *R. rubrum* were used.

roides R26. There was no noticeable difference between chromatophores and whole cells.

Fig. 3 shows a complete temperature dependence of F_{red} compared to F_0 and F_{max} for *Rps. sphaeroides* WT (upper) and *R. rubrum* (lower). The data are normalized to give $F_0 = 1$ at 293 K. In both experiments the measuring wavelength was 911 nm. We could restrict ourselves to this wavelength because the variable emission was at all wavelengths a constant fraction of the F_0 level; only at wavelengths shorter than 890 nm a relative decrease in the ratio F_{red}/F_0 or F_{max}/F_0 was observed in the case of *Rps. sphaeroides* WT. This phenomenon has been observed before [29,30] and has been ascribed to the fact that the F_0 emission results from both the *B*-850 and *B*-870 bacteriochlorophyll molecules, whereas the variable emission might arise from *B*-870 only. We could not observe such an effect in *R. rubrum*, contrary to results published before. The reason may be that we have made special efforts to minimize false light in our system.

Fig. 4 again shows the results of Fig. 3, but now presented as the reaction center trapping probability p_t, as calculated from Eqn. 2. The two constants $1/p_f$ and p_h/p_f could be calculated by assuming that when the emission equals F_{max}, $p_t T = 0$, and when the fluorescence equals F_0, $p_t T = 1$. Also shown are the results obtained with whole cells of *Rps. sphaeroides* G1C and the reaction center triplet yield as measured by Parson et al. [6] in isolated reac-

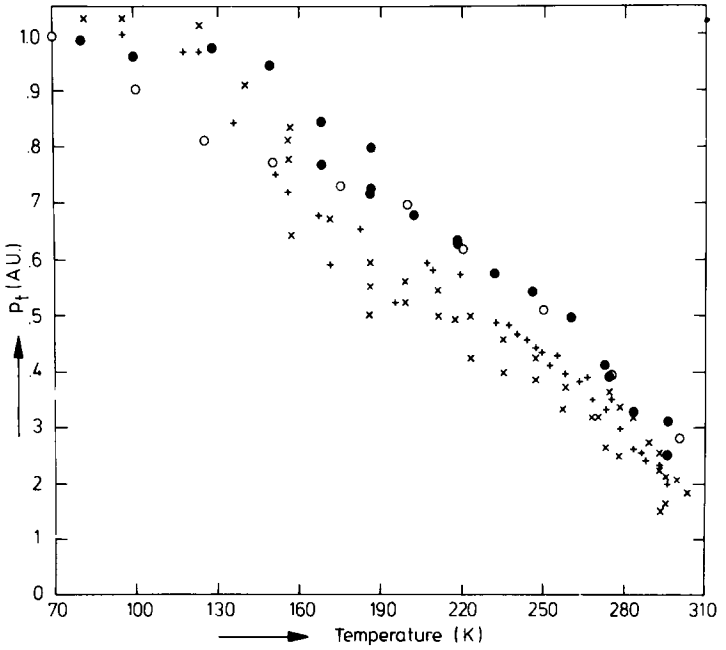


Fig. 4. Plot of p_t (see Introduction) as calculated from emission yield experiments as a function of the temperature in [PIX], [PIX$^-$] and [P^+IX$^{(-)}$] for *R. rubrum* WT whole cells (+), *Rps. sphaeroides* WT whole cells (X) and *Rps. sphaeroides* G1C whole cells (•). Also plotted are reaction center triplet yields measured as a function of the temperature in isolated reaction centers of *Rps. sphaeroides* R26 (○). Data taken from Parson et al. [6]. All triplet yields were normalized to 1 at 77 K; it should be remembered that the data taken from ref. 6 still showed an increase of 15% in the range of 100—4 K.

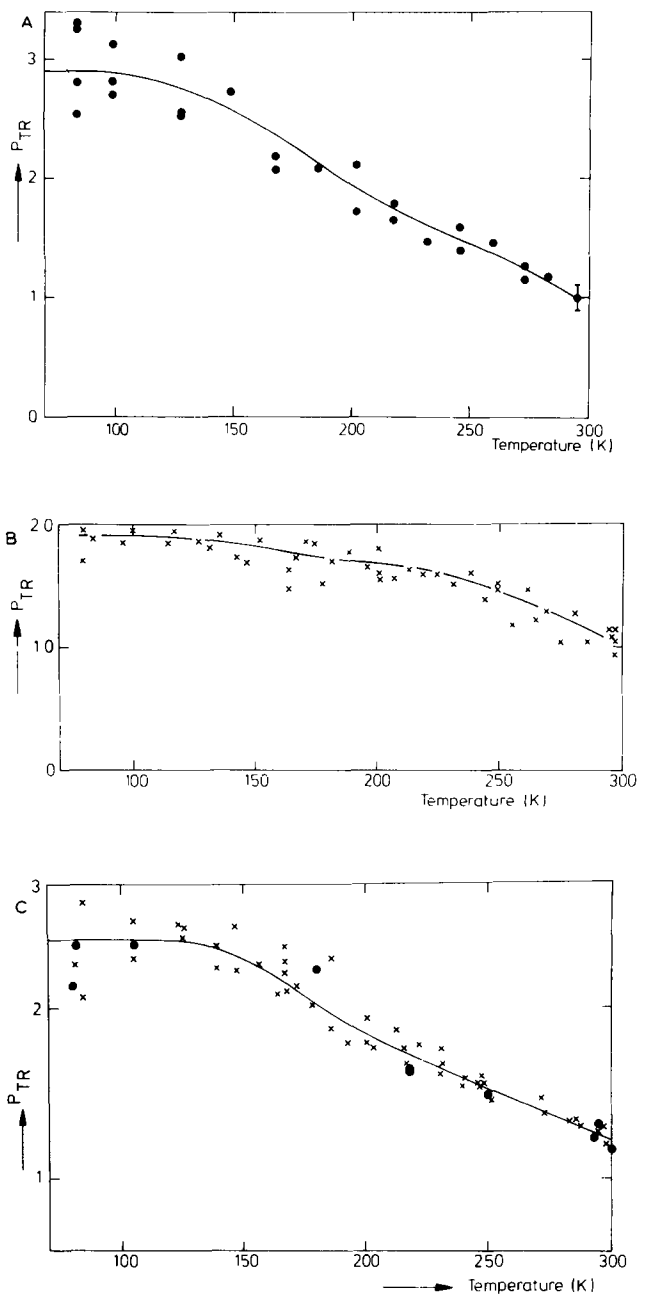


Fig. 5. A. Reaction center carotenoid triplet yield in whole cells of *Rps. sphaeroides* WT as function of the temperature. Measuring wavelength 545 nm, actinic illumination was provided by a Zeiss dye laser (λ = 612 nm, pulse width 0.3 μs). The cells were suspended in a 70% glycerol (v/v) solution and reduced with 250 mM sodium dithionite. B. As A, but now for whole cells of *Rps. sphaeroides* G1C. As actinic illumination both the Zeiss dye laser (λ = 612 nm, pulse width 0.3 μs) and Nd^{3+} laser (λ = 530 nm, pulse width 20 ns) were used. The measuring wavelength was 545 nm, the cells were reduced with 100 mM sodium dithionite. C. As A, but now for whole cells (•) and chromatophores (X) of *R. rubrum*. Both cells and chromatophores were dissolved in 70% glycerol, 30% cells + buffer (v/v). Actinic illumination as in A, measuring wavelength 580 nm. The cells and chromatophores were reduced with 100 mM sodium dithionite.

tion centers of *Rps. sphaeroides* R26. It is obvious that the p_t values as calculated from the emission yield experiments show approximately the same temperature dependence as the measured triplet yield of the reaction center.

Because we wanted to compare the temperature dependence of the bacteriochlorophyll emission with temperature-dependent processes occurring in the reaction center, we have measured the yield of reaction center triplet formation in intact systems. We used undersaturating laser flashes (20—40% saturation) and monitored the absorbance at the laser wavelength to correct for possible changes in the absorption spectrum. Results obtained by Cogdell et al. [23] showed that when the temperature was decreased the car^{TR} difference spectrum of *Rps. sphaeroides* 2.4.1 did not show a shift of the peak, however, a small sharpening of the car^{TR} absorption band might occur. Some experiments with *R. rubrum* WT indeed showed a difference spectrum which might be explained as the sharpening of the triplet-triplet absorption band (the rise in triplet yield was a factor 2.5 — 2.6 at 580 nm going from 293 K to 80 K, 2.1 at 570 nm and less than 2.0 at 560 nm). However, in the experiments shown here this effect was neglected and we used a rather wide band of the monochromator (±8 nm) around the absorption maximum to average over the triplet-triplet absorption spectrum.

Fig. 5 shows the results for the yield of car^{TR} formation after a short laser flash as a function of the temperature for cells of *Rps. sphaeroides* WT (upper), cells of *Rps. sphaeroides* G1C (middle) and chromatophores of *R. rubrum* WT (lower). All three preparations show increased triplet yield going from 273 K to 77 K, by the factors 2.8 for *Rps. sphaeroides* WT, 1.9 for *Rps. sphaeroides* G1C and 2.4 for *R. rubrum* WT. It should be noted, however, that there was some variation in these numbers even from sample to sample. In the temperature range 77 K—120 K the triplet yields are almost constant; this looks analogous to the fluorescence data (Fig. 3), but is in contrast to the data given by Parson et al. [6] which indicated that there was still some (10—15%) increase in triplet yield going from 100 K to 4 K.

Discussion

(A) The emission spectra at 293 K and at 77 K. The observation that the emission yield of $[PIX^-]$ is higher than that of [PIX] at 293 K, but equal to that of [PIX] at 77 K for a variety of species can be explained by the hypothesis that at room temperature part of the emission is in fact luminescence caused by the rapid back reaction $[P^+I^-X^-] \rightarrow [P^*IX^-]$. At low temperatures the rate of the back reaction is low and triplet formation $[P^+I^-X^-] \rightarrow [P^{TR}IX^-]$ can succesfully compete. The luminescence would have a lifetime in the same order as that observed for $[P^+I^-X^-]$, which amounts to several nanoseconds at room temperature. Recently such an emission component has been reported (Godik, V.I., personal communication; see also ref. 47), in contrast to an earlier report [31]. Our results also seem to contradict the observation by Clayton et al. [33] that the emission of isolated reaction centers in $[PIX^-]$ is not dependent on the temperature in the range 40—180 K. We want to remark, however, that such a temperature dependence should always be compared with the temperature dependence of the emission in [PIX] in the same

preparation. Nevertheless, it may be possible that this luminescence is indeed absent in these reaction center preparations, since treatment with detergents is known to change the structure [13,32] so that the back reaction may be slowed down.

(B) The temperature dependence of the bacteriochlorophyll emission yield and the reaction center triplet yield. The results presented in Fig. 4 showed that the by means of Eqn. 2 calculated p_t values reasonably agree with the reported reaction center triplet yield data as a function of the temperature. Because the reaction center triplet yield is considered to be close to 1 at low temperatures in these isolated reaction centers [6,34], this means that the lowering of the triplet yield as the temperature rises is matched by the relative increase of the emission yield. If one assumes that in Eqn. 3 P_T equals the reaction center triplet yields measured in intact systems (Fig. 5), then these data also allow a calculation of the p_t values as a function of the temperature. In case the p_t values calculated from the triplet yield data agree with the p_t values from the bacteriochlorophyll emission data, that would support our hypothesis that triplet formation is the only dissipative process occurring in the reaction center. We found a reasonable agreement in several cases.

Here we will present a more straightforward comparison of the bacteriochlorophyll emission data with the triplet yield data. Once excitations are absorbed by the pigment systems there exist at least two possible dissipative pathways leading to the dissappearance of the excitations. The first path has a probability P_L and represents the sum of all loss processes occurring directly from the first excited singlet state of the bacteriochlorophyll such as internal conversion to the ground state, fluorescence or triplet formation; this also includes loss processes occurring directly from P^*-870.

The second path has a probability P_T and represents the sum of loss processes occurring in the reaction center following charge separation, such as triplet (P_{TR}) formation or recombination to the ground state (P_G). If there are only two loss processes,

$$P_L + P_T = P_L + P_{TR} + P_G = 1 \qquad (4)$$

If the fluorescence at the various temperatures is a constant fraction of the total loss, it follows that $P_L = \alpha P_F$. If we assume that $P_L = 1$ when all the reaction centers are in $[P^+IX]$ or in $[P^+IX^-]$, and if we further assume that with the traps in $[PIX^-]$, P_{TR} is the only process, which occurs at 77 K in the reaction center [6,34], then both sets of experimental (P_F and P_{TR}, observed when the reaction centers are in $[PIX^-]$) are calibrated and we can calculate $P_L + P_{TR}$ at all temperatures. Fig. 6 shows the results obtained for *Rps. sphaeroides* WT, *Rps. sphaeroides* G1C and *R. rubrum* WT. $P_L + P_{TR}$ varies between 1.1 and 0.8 for the three preparations shown. There appears to be a small but systematic decrease of $P_L + P_{TR}$ in the region 220—270 K. We discuss three possible causes of this effect. First of all there is the already mentioned band sharpening of the triplet-triplet absorption spectrum which might lead to a slight overestimation of the triplet yield measured in the region 77—120 K where such a sharpening might occur [32]. Secondly, the regions where the lower $P_L + P_{TR}$ values were obtained were the same as those where crystallization of the sample occurred. The triplet yield experiments especially may have

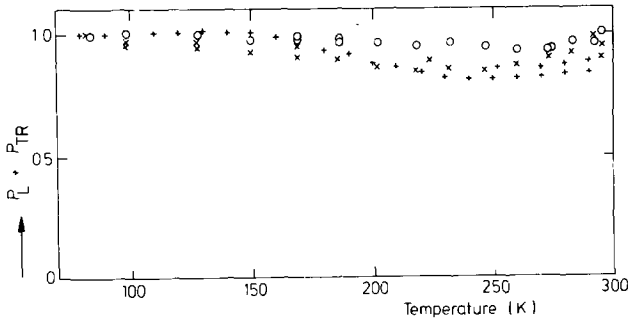


Fig. 6. $P_L + P_{TR}$ based on the assumption that at 77 K $P_L + P_{TR} = 1$ (for further explanation see text). ○, *Rps. sphaeroides* G1C; X, *Rps. sphaeroides* WT; +, *R. rubrum* WT.

suffered from this evil. Thirdly, there always was a relative depression of the emission in $[PIX^-]$ after the addition of an antifreeze medium (see Materials and Methods). The antifreeze we used (3 : 1 glycerophosphate/glycerol) still showed a 0—10% lower P_F.

The points measured in the region 270—300 K with samples in normal buffer indeed give somewhat better results for $P_L + P_{TR}$ in that temperature range. However, we want to point out here that the depression in $P_L + P_{TR}$ might be real, but even then the deviations are relatively small and we believe that the data of Fig. 6 are not inconsistent with the relation $P_L + P_{TR} = 1$. Thus, we estimate from Fig. 6, using Eqn. 4, that $P_G \leqslant 0.2$ in the temperature range 77—300 K. This means that the radiationless decay of $[P^+I^-X^-]$ to the ground state thus has a rate constant k_G which is small (see Fig. 1). This is not unlikely, since the Franck-Condon overlap factor presumably is small because of the large energy gap between $[P^+I^-X^-]$ and $[PIX^-]$.

(C) The energy difference between $[P^+I^-X^-]$ and $[P^*IX^-]$. For a simple set of reactions as those shown in Fig. 1, leaving out energy transfer to and from the antenna bacteriochlorophyll molecules, one can show that

$$\frac{1-p_t}{p_t} = k_{RC} \cdot \frac{k_L}{k_{TR}k_{CS}} + \frac{k_L}{k_{CS}} \tag{5}$$

where k_L is the rate of loss by internal conversion, fluorescence or triplet formation directly from $[P^*IX^-]$ or B^*-870. Here we have assumed, based on the foregoing that $k_G << k_{RC}$, k_{TR}. We have also simplified the complicated magnetic field effects [13] by one rate constant k_{TR}, which included the dephasing of the spins on P^+ and I^-. Thus, k_{TR} is put equal to the probability of recombination to the triplet state after $[P^+I^-X^-]$ has been formed in zero magnetic field. Especially at temperatures higher than 200 K where $(1-p_t)/(p_t) > 0.1$ it will be true that $k_{RC}/k_{TR} >> 1$. Thus, neglecting the second term on the right side of Eqn. 5 and taking logarithms, we find

$$\ln \frac{1-p_t}{p_t} = \ln k_{RC} + \ln \frac{k_L}{k_{TR}k_{CS}} \tag{6}$$

If we make the final assumption that only k_{RC} depends on the temperature we can calculate this dependence from Eqn. 6 using the p_t values calculated

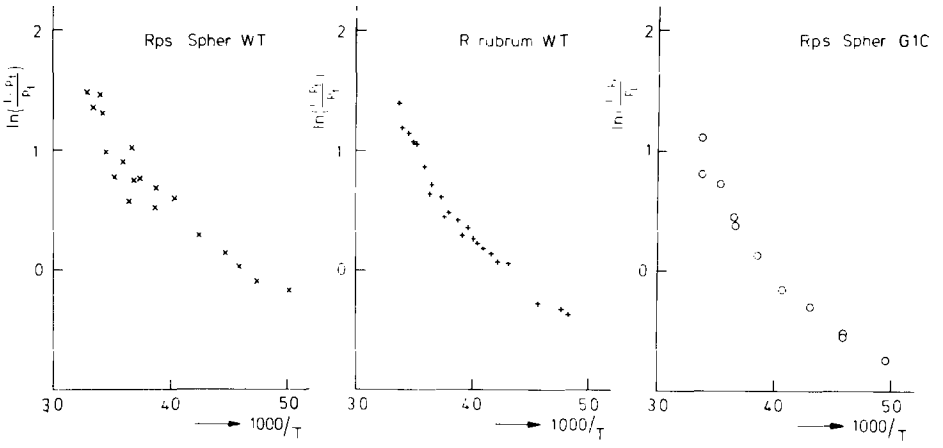


Fig. 7. Plots of ln $(1 - p_t)/p_t$ against $1000/T$ (see text for further explanation). Left, (×) *Rps. sphaeroides* WT; middle, (+) *R. rubrum* WT; right, (○) *Rps. sphaeroides* G1C.

from Eqn. 2 or 3. Fig. 7 gives this as an Arrhenius plot resulting in activation energies $\Delta E_{P^*I^-,P^+I^-}$ of 0.130, 0.131 and 0.155 eV for *Rps. sphaeroides* WT, *Rps. sphaeroides* G1C and *R. rubrum* WT, respectively, with the p_t values taken from the corresponding emission yield experiment. We have also replotted the results by Parson et al. [6] in an analogous way and these give $\Delta E_{P^*I^-,P^+I^-} \simeq 0.14$ eV; very close to our values. Using Eqn. 3 and the triplet experiments (Fig. 5) we calculated $\Delta E_{P^*I^-,P^+I^-}$ to range between 0.11 and 0.14 eV. These calculations, however, give less accurate answers because of the larger variations in the measured triplet yields.

Coincidentally, these numbers agree reasonably well with the value of 0.12 eV given by Shuvalov et al. [10], who measured the amplitude of a luminescence component with a lifetime of 10 ns as function of the temperature. This 10 ns luminescence component was absent after reduction of the bacteriopheophytin. They calculate their activation energy from an Arrhenius plot of the luminescence intensity. A simple calculation based on Fig. 1 of this paper shows that such a plot will only give the right activation energy, if one assumes that (1) k_G is very small and (2), the reaction center triplet yield approaches 1 at all temperatures. The first assumption may very well be true, however, the second assumption is certainly wrong. If we plot the numbers of Shuvalov et al. [10] in our way we find a barrier of approximately 0.5 eV. It is possible that the amplitude of their 10 ns luminescence component at temperatures lower than 270 K was too low, because a complete reduction of I was not achieved. The extent of the reduction of I is an increasing function of the rate of the reduction of P^+ by cytochrome *c*-552. This may have resulted in the too high "activation energy".

The calculated energy difference between $[P^*IX^-]$ and $[P^+I^-X^-]$ is rather small and from this a back reaction rate can be calculated. Using an energy difference of 0.13 eV and $k_{CS} = 10^{12}\ s^{-1}$, we find $k_{RC} = 6 \cdot 10^9\ s^{-1}$. With $k_{TR} = 10^7\ s^{-1}$ [23] and $k_L = 10^{10}\ s^{-1}$ [36,37] this gives a 14% triplet yield at room temperature for isolated reaction centers, whereas the efficiency for the charge stabilization $I^-X \rightarrow IX^-$ using a rate constant of $5 \cdot 10^9\ s^{-1}$ [15,16]

still gives a quantum efficiency of close to 1 for the formation of $[P^+IX^-]$, in agreement with quantum efficiencies observed in these isolated reaction centers [38]. There is some discrepancy between our results giving an energy separation of approximately 0.11—0.15 eV and the results obtained by titration of I which give an energy separation of 0.3—0.4 eV [39] and 0.1—0.2 eV [40] in *Rps. viridis*. It should be kept in mind that there is a different contribution of Coulomb energy from the interaction of P^+ and I^- and probably a different conformation of the reaction center complex in the two types of experiments, and therefore it is not easy to compare these numbers.

(D) Some concluding remarks. In the calculations shown in Fig. 7 and in the analysis discussed under (B) we used F_{max} to calibrate our bacteriochlorophyll emission data. This assumption is partly justified by the results of our analysis (as shown in Fig. 7), and partly by the following arguments. From our work [21,41] it follows that the bacteriochlorophyll emission observed under conditions that the traps are closed, is more or less the same, independent of how the traps are closed (see Table I).

This indicates that F_{max} can be used to calibrate the state in which the traps are. There appears to be one exception: $[PI^-X^-]$ has an emission yield approximately equal to F_0, both at room temperature and at 77 K (Van Grondelle, R., van der Wal, H.N. and Holmes, N.G., unpublished). A possible explanation for this anomalous behaviour is that the reduced bacteriopheophytin, which has an absorption band overlapping the fluorescence emission spectrum [12], quenches the fluorescence by trapping the excitation, thus resulting in an extremely short lifetime of $[P^*I^-X^-]$ [45].

The fact that we find F_{max} more or less the same, independent of how the traps are closed, again poses the problem of why the rise in emission yield is in most cases only 3 times F_0 instead of being much larger, as might be expected in order to explain the high quantum efficiencies of photochemistry (0.9—1.0). It is possible that a closed trap, for example $[P^+IX]$ remains a quencher of the bacteriochlorophyll emission, although a less efficient one

TABLE I

BACTERIOCHLOROPHYLL EMISSION FROM INTACT PREPARATIONS AS A FUNCTION OF THE STATE OF THE TRAPS

The emission is expressed as a fraction of F_{max}, the emission observed when the traps are in $[P^+I\,X]$.

State reaction centre	Relative emission yield (F/F_{max})	Reference
$P\,I\,X$	F_0/F_{max} (0.3—0.1)	1,29,30,41,42
$P^+I\,X$	1	per definition
$P^+I\,X^-$	1	21; van Grondelle et al., unpublished results
$P\,I\,X^-$, $p_t = 0$	1	this work
$P\,I\,X^-$, $p_t = 1$	F_0/F_{max}	this work
$P^{TR}I\,X^-$	1	21
$P\,I\,X^-\,car^{TR}$ (low temperature)	$1 \approx 1.2$	41
$P\,I^-X^-$	F_0/F_{max}	45; van Golde et al., unpublished results.
State antenna		
$Bacteriochlorophyll^{TR}$	<1 } depends on concentration	22
car^{TR}	<1 } of quencher	22,43,44

than [PIX] [46]. Heathcote and Clayton [47] suggested that traps in [P^+IX] quench the bacteriochlorophyll emission approximately 2—3-fold. This is a remarkable phenomenon, which we cannot explain yet.

List of symbols used

P = P-870 = reaction center bacteriochlorophyll dimer; P^+, P^*, P^{TR} represent respectively the oxidized state, first excited singlet state and lowest triplet state of P.

I = long wavelength bacteriopheophytin, I^- is the reduced state of I.

X = FeQ = iron · quinone complex, X^- is the reduced state of X.

c-552 = low potential cytochrome, electron donor to P^+ in *Chromatium vinosum* [14].

B-870 = antenna bacteriochlorophyll molecule absorbing around 870 nm, B^*-870 is first excited singlet state of B-870.

car = reaction center carotenoid molecule, car^{TR} is triplet state of car.

[$P^\alpha I^\beta X^\gamma$] = reaction center state where P^α can be P, P^*, P^+ or P^{TR}, I^β can be I or I^-, X^γ can be X or X^-.

F_o, F_{red}, F_{max} represent the bacteriochlorophyll emission observed when the reaction centers are in [P I X], [P I X^-] and [P^+ I X], respectively.

For the reaction rates k_{CS}, k_{RC}, k_{TR}, k_{CTR}, k_G and k_D we refer to Fig. 1.

k_L = rate of loss from B^*-870 (or P^*-870) via internal conversion, triplet formation or fluorescence.

p_f = probability for immediate emission of excitation energy as fluorescence by an excited bacteriochlorophyll molecule.

p_h = probability for excitation transfer from one bacteriochlorophyll molecule to another.

p_t = probability for trapping of excitation energy after excitation of the reaction center bacteriochlorophyll.

The following P's are quantum yields.

P_T = total probability for trapping of excitation energy in a reaction center.

P_G = fraction of P_T which represents the total probability to decay directly to the ground state.

P_{TR} = fraction of P_T which represents the total probability to form a reaction center triplet.

P_L = total probability for an excitation to get finally lost via internal conversion, fluorescence or triplet formation from B^*-870 (or P^*).

T = concentration of traps.

$E_{P^*I^-, P^+I^-}$ = energy difference between P^*IX^- and $P^+I^-X^-$.

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