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SHORT-LIVED DELAYED LUMINESCENCE OF PHOTOSYNTHETIC ORGANISMS

I. NANOSECOND AFTERGLOWS IN PURPLE BACTERIA AT LOW REDOX POTENTIALS

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Summary

A combined study of emissions of purple bacteria *Rhodospirillum rubrum*, *Ectothiorhodospira shaposhnikovii* and *Thiocapsa roseopersicina* was performed under conditions of low potential. It has been shown that a considerable part of the emission represents a delayed luminescence with a lifetime of about 5 ns and an activation energy $\Delta E = 0.05 \pm 0.03$ eV. Intensity of this delayed luminescence is approximately equal to that of prompt fluorescence. It diminishes as temperature decreases and also as the intermediate acceptor I becomes reduced after prolonged illumination under low potential conditions.

This luminescence represents a radiative decay of the intermediate state, P^F, and the luminescence activation energy, ΔE, reflects the energy barrier between P^*-890 and P^F. The value of this barrier determined in the present work is much lower than those obtained previously [3,4,26] for the free-energy release during the primary act of charge separation, basing on redox potential techniques. The reason for this discrepancy is discussed. Delayed luminescence in the picosecond time range is predicted to exist under conditions of active photosynthesis as a result of a small (approx. 0.05 eV) energy barrier between P^F and the excited singlet state of reaction center bacteriochlorophyll.

Abbreviations: P-890, P^+-890, P^*-890, a special pair of reaction centre bacteriochlorophylls in a normal, oxidized and singlet excited states, respectively; I, an intermediate acceptor, most probably bacteriopheophytin or a complex between P-800 and bacteriopheophytin; X, a primary stabilized electron acceptor, which is shown to be an ubiquinone-iron complex; A, subsequent diffusible acceptors of the photosynthetic chain; TMPD, tetramethyl-p-phenylenediamine; BChl, bacteriochlorophyll; BPh, bacteriopheophytin.

Introduction

It has not been established as yet if any delayed luminescence accompanies charge separation in the porphyrine complex of bacterial reaction centres under conditions, when all acceptors of nonporphyrine nature, including X, are in a reduced state before illumination. This charge separation is known to produce a short-lived state P^F which represents a radical-ion pair $P^{+}\text{-}890^{\cdot}I^{-\cdot}$ [1,3,4]. During active photosynthesis P^F decays with a half-time of 120--240 ps [2,3] to produce P^{+}-890 and reduced X. When X is prereduced, the lifetime of P^F increases up to 6--10 ns [1,5]. Fluorescence yield of purple bacteria under these conditions is 2 or 3 times as high as that under normal conditions [6,7]. It is not clear, however, why this is so, as the primary charge separation was shown [5] to proceed in this case with approximately the same quantum yield as in active photosynthesis. Parson et al. [1,5] suggested that the electrostatic field of X^- changes the rate constant of the primary process ($K_{e\downarrow}$) with the fluorescence yield increasing. Another possibility is that the increase in fluorescence is due to delayed luminescence resulting from emissive recombination of the P^{+}-870 and I^- radicals. Existence of such delayed luminescence was suggested recently by Shuvalov et al. [9] and Grondelle et al. [13,28] on the basis of the temperature-dependent changes in fluorescence yield of purple bacteria during illumination at low redox potentials. Clayton [29] has found, however, the fluorescence yield of the reaction centres to be independent of temperature between 40 and 180°K.

In order to elucidate this question, a study of prompt and delayed emissions of purple bacterium chromatophores was performed under reducing conditions.

Materials and Methods

Purple sulfur bacteria *Ectothiorhodospira shaposhnikovii* and *Thiocapsa roseopersicina* were grown in a modified Larsen medium [10] and nonsulfur *Rhodospirillum rubrum* in the medium of Çohen-Bazire et al. [11]. The chromatophores were isolated by ultrasonic treatment from washed bacterial cells as described in [12] and suspended in 50 mM Tris (pH 7.5).

For measurements to be carried out under reducing conditions, the chromatophore suspensions were reduced with solid sodium dithionite in argon atmosphere. For low temperature measurements, glycerol was added to the chromatophore suspensions in a 4 : 1 ratio, v/v. Samples with absorbances of 0.4—0.6 at 870 nm in a 1-cm cell were usually employed.

The fluorescence spectra were measured in an Aminco-Bowman spectrofluorimeter supplied with a 7102 photomultiplier.

The photoinduced absorption changes were measured in a double-beam differential spectrophotometer [14]. The measurements of the fluorescence lifetimes τ were mainly performed in a phase-type fluorometer [15] with a time resolution of about 50 ps. Fluorescence was excited by 404 and 436 nm lines from a high-pressure mercury lamp. The exciting light was modulated harmonically with a frequency of 12.3 MHz. Fluorescence was isolated with a combination of glass filters cutting off the $\lambda \leqslant 760$ nm wavelength range. Some lifetime measurements were performed with a commercial single-photon counting pulse-fluorometer 'Ortec' equipped with a spark gap lamp and RCA 8852 photomultiplier. Excitation pulse profile, measured with the use of a latex suspension, had a half-life ranging from 1.2 to 1.6 ns. Fluorescence was excited and analyzed with the same combination of filters as in the phase-fluorometer measurements. Essential difference in the conditions of the lifetime measurements by the pulse- and phase-fluorometers consisted in exciting light intensities. In the pulse-fluorometer measurements it was approximately 10^2—10^3 times lower.

During the phase-fluorometer measurements, besides phase-shifts (ψ), another modulation characteristic of the emission was recorded, i.e. modulation coefficient, α. Exponentially decaying emission, excited by a harmonically modulated light may be characterized by two main parameters: ψ and α that are related to τ by a well-known formula (see, for example, Ref. 8):

$$tg\psi = \omega\tau \tag{1}$$

$$\alpha = \frac{}{\sqrt{1+\omega^2\tau^2}} = \cos\psi \tag{2}$$

where ω is cyclic frequency.

Eqns. 1 and 2, applied simultaneously to the emissions investigated, make it possible to distinguish between mono- and multi-component character of emission decay.

For luminescence consisting of two (or more) independent components, the measured values of ψ_m and α_m, produced by a phase-fluorometer, have no clear-cut physical meaning. They can be expressed, however, by the values of phase-shifts ψ_1 and ψ_2 that would be observed, if the two components were measured separately [15]:

$$tg\psi_m = \omega\tau_m = \frac{\tilde{F}_1 \sin\psi_1 + \tilde{F}_2 \sin\psi_2}{\tilde{F}_1 \cos\psi_1 + \tilde{F}_2 \cos\psi_2} \tag{3}$$

$$\alpha_m = \frac{\tilde{F}_m}{mF_m} = \frac{F_1 \cos^2\psi_1 + F_2 \cos^2\psi_2}{\cos\psi_m(F_1 + F_2)} \tag{4}$$

where $\tilde{F}_m$, $\tilde{F}_1$, $\tilde{F}_2$ are alternating components of the total emission and individual emissions, respectively; F_m, F_1, F_2 are the same for direct components; m, modulation coefficient for exciting light.

If the values of ψ_m and α_m are measured at two different modulation frequencies, then the values of ψ_1, ψ_2, (and, hence, the lifetimes τ_1 and τ_2) as well as the ratio of intensities of the two luminescing components, F_1/F_2, can be determined from Eqns. 3 and 4. Such determination is also possible at a single modulation frequency, if one of the two components is analyzed independently (or the range of its lifetime is specified).

Results

Quantum yield and lifetime of the emission of purple bacteria chromatophores under conditions of low potential

Fig. 1 demonstrates the effect of dithionite addition on φ_m and τ_m of the emission of *R. rubrum* chromatophores. The value of φ_m is stabilized under

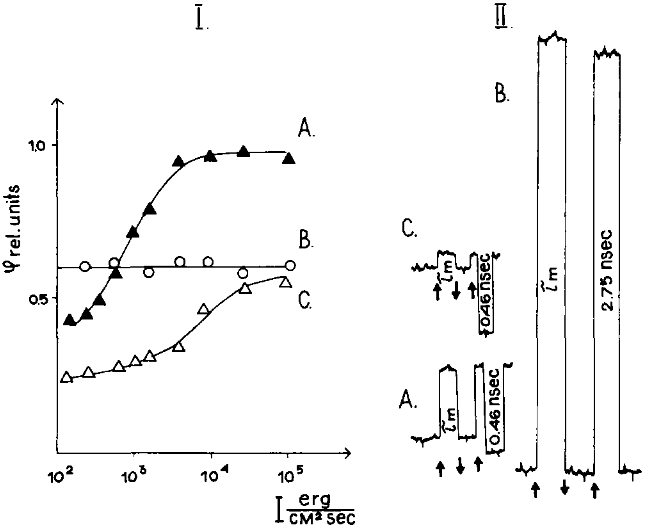

Fig. 1. I — Measured overall emission yield, φ_m, of *R. rubrum* chromatophores as a function of exciting light intensity. A, normal conditions; B, reduction with $5 \cdot 10^{-3}$ M $Na_2S_2O_4$; C, addition of $5 \cdot 10^{-5}$ M TMPD and $5 \cdot 10^{-4}$ M $K_4Fe(CN)_6$ (which was suggested to produce the reaction centres in the closed state P^+X^- at light saturation [19]. II — The lifetime of the emission of *R. rubrum* chromatophores under conditions similar to A, B and C of fluorescence yield measurements. Exciting light was saturating. Absorbance at 870 nm was 0.5. Light path, 1 cm. A typical experiment on fluorescence lifetime measurement by a phase-shift method consists in registration of a phase-shift, produced by some scattering sample, (latex suspension in our case), which is assumed to be zero, then the object under investigation is placed in the light (↑) and a phase-shift increase (upward deflection) is measured by comparison with calibrated phase-shifts (0.46 ns and 2.75 ns in the case given). Substitution of latex suspension for luminescence object, shown by ↓, restores the phase-shift to its original level.

these conditions at an approximately constant level, that is independent of the light intensity. This level is approx. 2 times as high as the dark φ_m level for chromatophores under normal conditions. The ratio between the values of φ_m, obtained on light saturation and that of dithionite-treated chromatophores, depends on the type of closed reaction centres realized under light saturation conditions [19]. Fluorescence yield of chromatophores with closed reaction centres of P^+XA^-* type (curve A) is 35—50% as high as that for the state P^+X^-A (curve C) or PX^-A^-, (curve B) the latter being realized under reducing conditions. The first possibility of dithionite-induced increase in the dark fluorescence yield, caused by diminishing of the rate constant of the primary charge separation (see Introduction), implies that this increase should be accompanied by a proportional rise in fluorescence lifetime, τ_m. In fact, in the case of homogeneous fluorescence, the quantum yield and the lifetime should change in parallel according to the well-known equation:

$$\varphi_m = \tau_m / \tau_0 \qquad (5)$$

where τ_0 is a constant.

The results of φ_m and τ_m measurements, represented in Fig. 1, and Tables I

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* The usual type of reaction centre states formed on light saturation in the chromatophores under normal conditions.

TABLE I

OVERALL EMISSION YIELD AND LIFETIME OF PURPLE BACTERIA CHROMATOPHORES AS A FUNCTION OF THE TYPE OF REACTION CENTRE STATE

A detailed description of the conditions that are necessary to maintain chromatophores with reaction centres of a definite type is given in Ref. 19.

State of reaction centre	φ_m ± 0.1 (rel. units)	τ_m (ps)	Reference
PXA (active state)	0.5	60 ± 30	this work and 19,16
	—	100 ± 50	17
	—	200	18
$PX^- A^-$	1.0	2500—2900	this work and 19
P^+X^-A or $P^+X^-A^-$	0.95	120 ± 50	this work and 19
P^+XA^- or P^+XA	2.0	450 ± 100	this work and 19

and II contradict this hypothesis. Although φ_m of chromatophores with reaction centres in the PX^-A^- state (reduction with dithionite) is about two times as high as that at active photosynthesis, fluorescence lifetimes differ by more than 30—40 times (Table I). Emission yields, φ_m, of chromatophores with reaction centres in the state P^+X^-A and PX^-A^- are approximately the same (see curves B and C of Fig. 1) but τ_m differ by more than 10—20 times. Finally, φ_m for the PX^-A^- state is even 40—50% as low as that for the P^+XA^- state, while τ_m in the former case is approx. 6 times as high as that in the latter (Table I). This peculiar behaviour of the measured values of τ_m and φ_m of the emissions investigated excludes the possibility of trivial dithionite effect on bacteriochlorophyll fluorescence, and can even hardly be explained by the coexistence of several fluorescence emissions, the descrepancies in φ_m/τ_m ratios being tremendous.

TABLE II

DITHIONITE EFFECT ON LIFETIME, τ_m, YIELD, φ_m, AND DEMODULATION, δ_m, OF THE EMISSIONS OF PURPLE BACTERIA CHROMATOPHORES IN SATURATING LIGHT AT ROOM TEMPERATURE

Conditions	φ_m ± 0.1 (rel. units)	τ_m (ns)	δ_m (%)
R. rubrum			
no additions	1.0	0.45 ± 0.1	(4.0 ± 1.0)
$5 \cdot 10^{-5}$ M TMPD + $5 \cdot 10^{-4}$ M $K_4Fe(CN)_6$	0.50	0.12 ± 0.05	(4.5 ± 1.0)
$5 \cdot 10^{-3}$ M $Na_2S_2O_4$	0.55	2.6 ± 0.1 *	(5.0 ± 1.5)
E. shaposhnikovii			
no additions	1.0	0.5 ± 0.1	(4.5 ± 1)
$5 \cdot 10^{-5}$ M TMPD + $5 \cdot 10^{-4}$ M $K_4Fe(CN)_6$	0.58	0.2 ± 0.1	(4.0 ± 1)
$5 \cdot 10^{-3}$ M $Na_2S_2O_4$	0.63	2.9 ± 0.1	(3.5 ± 1.0)
Thiocapsa roseopersicina			
no additions	1.0	0.1 ± 0.05	(3.0 ± 1)
$5 \cdot 10^{-3}$ M $Na_2S_2O_4$	0.60	2.5 ± 0.1	(4.0 ± 1.5)

* The value of luminescence lifetime of dithionite-treated chromatophores was the same at saturating ($5 \cdot 10^4$ erg · cm^{-2} · s^{-1} and low (10^2 erg · cm^{-2} · s^{-1} exciting light.

The same conclusion is inferred from the observations of the extremely long lifetimes, 2.5—3.0 ns (Tables I and II), observed for the chromatophores under reducing conditions, while purple bacterium fluorescence lifetimes do not exceed 1.5 ns for any type of open or closed reaction centres [15—18], and even for pigment-protein complexes of light-harvesting antenna, free of the reaction centres [19], or reaction centre-free mutant of *Rhodopseudomonas sphaeroides* PM-8 [17]. If, however, the emission appearing under low potential conditions, is of a delayed luminescence type, the above φ_m/τ_m changes are readily explicable. Indeed, as for delayed emission, their lifetime, τ_{DL}, and yield, φ_{DL}, never obey Eqn. 5; moreover, the opposite situation is observed: the longer τ_{DL} is, the smaller φ_{DL} is. As emissions with lifetimes, exceeding 100 ns, do not contribute to the value of τ_m measured by the phase-shift technique, the lifetime of the delayed luminescence, appearing in the dithionite reduced chromatophores, should lie in the nanosecond range.

Modulation characteristics of the emissions of reduced chromatophores

As in Methods, analysis of the modulation characteristics of heterogenous emission consisting of two components, (in particular, prompt fluorescence and delayed luminescence) may be of use in determining the decay parameters of individual components. With this purpose the simultaneous measurements of τ_m and α_m were performed for reduced chromatophores of *Rhodospirillum rubrum*, *Ectothiorhodospira shaposhnikovii* and *Thiocapsa roseopersicina* (Table II). For all bacteria investigated, addition of dithionite both at low and saturating light brought about a sharp rise in τ_m up to 2.5—3 ns, with α_m changes being insignificant.

Assuming that the emission of chromatophores under reducing conditions is of a two-component character (i.e. prompt fluorescence with lifetime varied in the range from 50 to 1200 ps [15—19] plus delayed luminescence), it may be inferred from Eqns. 3 and 4 of the Methods and the data of Table II that the delayed luminescence intensity, for all bacteria investigated, is almost the same as that of prompt fluorescence (delayed luminescence/prompt fluorescence = 0.7—2), and τ_{DL} is equal to 4—6 ns.

Unlike chromatophores, reduction of whole cell suspensions of *E. shaposhnikovii* was found to be accompanied by a pronounced rise in the emission demodulation coefficient ($\delta_m = 1 - \alpha_m$) up to 25—30%, while τ_m was practically unchanged. This means, according to Eqns. 3 and 4, that delayed luminescence with a lifetime longer than 100 ns and 30—35% amplitude appeared (a detailed analysis of the phenomenon will be presented elsewhere).

The difference in the behaviour of chromatophore and whole cell suspensions in response to reduction with saturating concentrations of dithionite is most likely associated with different membrane permeability for $Na_2S_2O_4$ of these objects. Redox potential, achieved in chromatophores by dithionite addition is obviously lower than that in whole cells. In general, it may be expected that the lower E_m is of a state and the closer it is to P^*-890, the higher the probability of its radiative decay is, and the shorter the lifetime of the appearing luminescence is. Further lowering of redox potential by addition of 10^{-5} M neutral red, besides dithionite, to cell suspensions, eliminated the difference between cells and chromatophores, i.e. they exhibited a sharp rise in

τ_m with insignificant changes in δ_m. The same was observed for aged aerobic cell suspensions which behaved, on $Na_2S_2O_4$ addition, like chromatophores.

Kinetics of luminescence of reduced chromatophores studied by a single-photon counting fluorometer

Although it follows from the modulation characteristics of the overall emission that delayed luminescence, appearing in reduced chromatophores, accounts for at least a half of the total emission intensity, the spectra of luminescence and luminescence excitation were the same for chromatophores under normal and reducing conditions. This is indicative of spectral identity of the delayed and prompt fluorescences. In this case a phase-fluorometer cannot ensure direct measurements of individual components. With this purpose, kinetics of the emission decay of dithionite-treated chromatophores was analyzed with a pulse-fluorometer Ortec. As the excitation pulse duration was 1.2—1.6 ns, short-lived decay components of fluorescence with shorter lifetimes were hard to quantitatively analyze, especially in the case of a great difference between excitation (≈400 nm) and fluorescence (≈900 nm) wavelengths [30]. The fluorescence decay of *R. rubrum* chromatophores, without additions, represented in Fig. 2, practically reproduced the excitation profile. There was no evidence of a contribution, exceeding 2%, from the components with decay times longer than 1.5 ns. Addition of TMPD with $K_4Fe(CN)_6$, which converted most of the reaction centres to the active PXA state in low light, produced no changes in the decay kinetics (in the aerobic suspensions of chromatophores without additions about 80% of the reaction centres were in the P^+XA state [19]. In the presence of dithionite the decay kinetics lengthened drastically (Fig. 2). The digitized response curves, handled by the approximate method of moments [8], showed that in the latter case a large

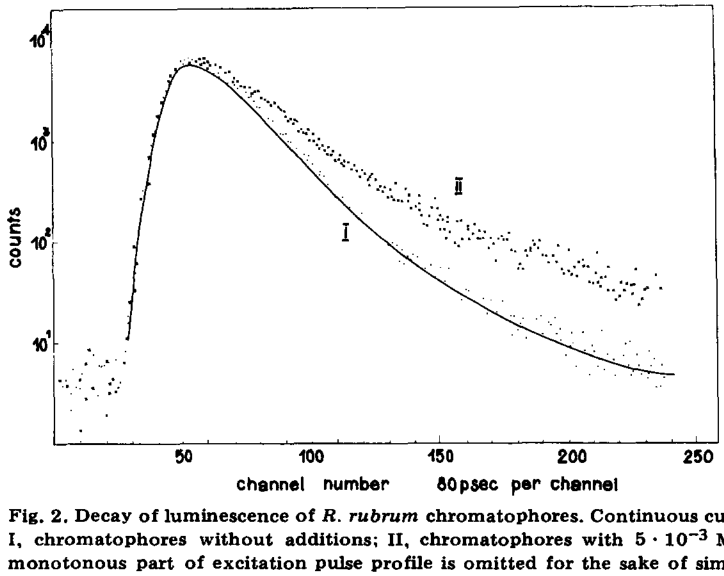

Fig. 2. Decay of luminescence of *R. rubrum* chromatophores. Continuous curve is lamp excitation profile: I, chromatophores without additions; II, chromatophores with $5 \cdot 10^{-3}$ M $Na_2S_2O_4$. The initial non-monotonous part of excitation pulse profile is omitted for the sake of simplicity of graphic representation.

component (38–40%) with the lifetime of 5.4 ± 0.2 ns appeared together with a short-lived fluorescence component (the lifetime shorter than excitation duration).

This data are in a good agreement with those obtained from a modulation study of the emission.

Temperature-dependence of the nanosecond delayed luminescence

If the ns emission component is of a delayed luminescence type, then it should be temperature-sensitive with an activation energy ΔE, reflecting the difference in energy between P^*-890 and the state the delayed luminescence is generated from. Temperature decrease resulted in disappearance of this long-lived component from the investigated emission. At 77 K τ_m of the reduced samples measured by the phase-shift technique was found to be from 200 to 600 ps for different chromatophore preparations, and the luminescence yield, φ_m, was approx. 2 times as low as that at room temperature (Table III). Subsequent warming of the samples up to room temperature restored completely the intensity and the lifetime of the emission. The whole cycle of freezing-warming may be repeated many times, still giving reproducible values of τ_m and φ_m. In the case of chromatophores under normal conditions, the temperature decrease had no pronounced effect on τ_m or φ_m (Table II), if the type of closed reaction centres was the same at low and room temperatures.

The relationship between the nanosecond delayed luminescence and the primary charge separation

Hence, when the primary charge separation takes place mainly in the porphyrine complex of the reaction centre, the temperature-sensitive luminescence appears. It is likely that it represents a delayed luminescence, resulting from emissive recombination of charges produced in the primary act of photosynthesis. In this case, it should disappear under conditions when the primary charge separation is prevented. In purple bacteria containing low-potential ‘fast’ cytochrome *c* this can be attained by prolonged illumination under reducing conditions. Such an illumination was shown [3,9,20] to trap an intermediate acceptor (probably BPh) in the reduced state at the expense of cytochrome oxidation. Fig. 3 shows the kinetics of BPh^- accumulation under prolonged illumination, measured by ΔA (bleaching) at 545 nm, and concomitant disap-

TABLE III

EMISSION LIFETIME AND YIELD OF *RH. RUBRUM* CHROMATOPHORES AT ROOM AND LOW (77K) TEMPERATURE

Chromatophore suspensions contained 50 mM potassium phosphate (pH 7.0), $5 \cdot 10^{-5}$ M TMPD, $5 \cdot 10^{-4}$ M $K_4Fe(CN)_6$, glycerol, 1 : 4, v/v. Intensity of exciting light, $I = 5 \cdot 10^4$ erg $\cdot$ cm^{-2} $\cdot$ s^{-1}.

Additions	T (K)	τ_m (ns)	$\varphi_m \pm 0.1$ (rel. units)
—	293	0.2 ± 0.05	1.0
—	77	0.15 ± 0.1	0.95
$5 \cdot 10^{-3}$ M dithionite	293	2.9 ± 0.1	1.1
$5 \cdot 10^{-3}$ M dithionite	77	0.2–0.6	0.5

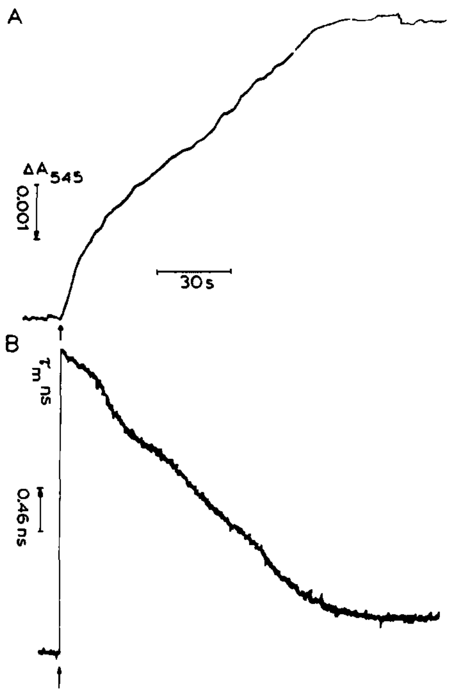

Fig. 3. Kinetics (A) absorbance changes (bleaching) at 545 nm of *Thiocapsa roseopersicina* chromatophores induced by saturating ($5 \cdot 10^4$ erg $\cdot$ cm^{-2} $\cdot$ s^{-1}) light in the presence of $5 \cdot 10^{-3}$ M $Na_2S_2O_4$ and (B) luminescence lifetime changes under the same conditions. Absorbance at 890 nm was 0.6. Light path, 1 cm; ↑ light on.

pearance of the nanosecond luminescence component for reduced chromatophores of *Thiocapsa roseopersicina* measured by luminescence lifetime decrease. It can be seen that the more reaction centres are converted to the $PBPh^-$ state, the less is the value of emission lifetime, so that the lifetime, recorded for the maximal level of BPh^- accumulation, is equal to 350—400 ps, which is close to the life-time of prompt fluorescence [15—19]. The identity of these kinetics strongly indicates that the appearance of the 5 ns component in the emission of reduced chromatophores is a result of emissive recombination of P^+-890 and BPh^- which are produced in the primary act of charge separation.

Determination of activation energy (ΔE) for the nanosecond luminescence component

Accurate determination of ΔE from temperature dependence of luminescence quantum yield is hard to make because of the heterogeneous nature of the total emission investigated. Sufficiently accurate separation of a single emission component in a wide temperature range is hardly possible at present. A much better way of determination of ΔE is a kinetic study of delayed luminescence decay.

The sequence of events in the bacterial reaction centers under reducing conditions (see Fig. 3) can be described by the following linear differential equations

$$\frac{\mathrm{d}P^*}{\mathrm{d}t} = -(K^{P}_{\Sigma} + K_{e\downarrow})P^* + K_{e\uparrow}P^{F} \tag{6}$$

$$\frac{\mathrm{d}P^{F}}{\mathrm{d}t} = -(K^{P}_{T} + K^{P}_{ST} + K_{e\uparrow})P^{F} + K_{e\downarrow}P^* \tag{6a}$$

Designations are explained in the legend to Fig. 4. Eqns. 6 and 6a are quite similar to those used in the theory of luminescence decay of organic compounds [27], except that P^F should be replaced by T, where T is the molecular triplet state.

It can be derived from Eqns. 6 and 6a that the rate constant of the delayed luminescence (K_{DL}) that should appear as a result of reversibility of the primary charge separation

$$P^*I \underset{K_{e\uparrow}}{\overset{K_{e\downarrow}}{\rightleftarrows}} P^+I^-$$

in the reaction centres is equal to:

$$K_{DL} = \frac{1}{\tau_{DL}} = K^{P}_{T} + K^{P}_{ST} + K_{e\uparrow}\frac{K^{P}_{\Sigma}}{K_{e\downarrow}}$$

$$K_{e\uparrow} = K_{e\downarrow}\,\mathrm{e}^{-\Delta E/kT} \tag{7}$$

where ΔE is difference in energy between the P^* and P^F states. In the case of energy migration between the reaction centres and antenna bacteriochlorophyll, Eqn. 7 takes the following form:

$$K_{DL} \simeq K^{P}_{T} + K^{P}_{ST} + \frac{K_{e\uparrow}K'_{M}}{K_{e\downarrow} + K'_{M}}\cdot\frac{K_{\Sigma}}{K_{M}} \tag{8a}$$

for the case

$K_{M} < K_{e\downarrow}$,

or

$$K_{DL} \simeq K^{P}_{T} + K^{P}_{ST} + \frac{K_{e\uparrow}K'_{M}}{K_{e\downarrow} + K'_{M}}\cdot\frac{K_{\Sigma}}{K_{e\downarrow}} \tag{8b}$$

for a rapid energy transfer $K_{M} > K_{e\downarrow}$.

The rate constants of Eqns. 7 and 8 are either known or their range is specified. Application of Eqn. 8 to chromatophore emission under reducing conditions with rate constants varying in the ranges of

$K_{e\downarrow}$ 10^{11}–10^{13} s^{-1}	[1,2,3,25]
K_{M} 10^{10}–10^{11} s^{-1}	[16–18]
K'_{M} $(2–5)\cdot 10^{10}$–$(2–5)\cdot 10^{11}$ s^{-1}	[21]
K_{Σ} $5\cdot 10^{8}$–10^{9} s^{-1}	[19]
K^{P}_{ST} $5\cdot 10^{7}$–10^{8} s^{-1}	[1,5]
$K^{P}_{T} \leqslant 10^{8}$ s^{-1}	[1,5]

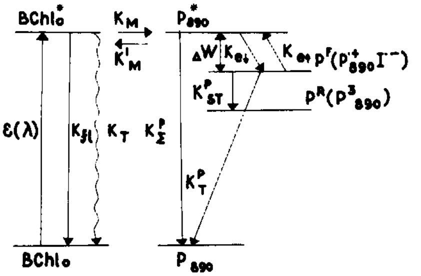

Fig. 4. Energy scheme of the primary processes at bacterial photosynthesis. $BChl_0^*$, light harvesting bacteriochlorophyll in normal and excited singlet states respectively; P-890, P^*-890, is the same for the reaction centre bacteriochlorophyll: K_M, K'_M are the rate constants for reversible energy transfer from $BChl_0$ to P^*-890 and in the opposite direction, respectively; $K_\Sigma = K_{fl} + K_T$ is the rate constant of the overall trivial energy losses in the antenna pigment complex equal to the sum of radiative (K_{fl}) and nonradiative (K_T) losses; K^P, the same for reaction centre bacteriochlorophylls; K^P_{ST}, K^P_T, are the rate constants of P^F decay via transition to the triplet state, P^R, and direct nonradiative transition to the ground state, respectively; $K_{e\downarrow}$ is the rate constant of primary charge separation; $K_{e\uparrow}$ that of the reverse process.

gives ΔE values that do not exceed 0.05 ± 0.03 eV. It was assumed that out of all rate constants, only $K_{e\uparrow}$ is temperature-dependent.

Hence the energy barrier between P^* and P^F corresponds to 1—3 kT at room temperature.

Discussion

Application of several different approaches to the case of the emission of purple bacteria chromatophores, made it possible to show that the process of charge separation in bacterial reaction centres under conditions when X is in a chemically reduced state, results in the appearance of a delayed luminescence, whose intensity is comparable with that of fluorescence. This fact naturally explains the 2—3-fold increases in the fluorescence yield, observed earlier on addition of $Na_2S_2O_4$. The contribution from the nonradiative channel of direct recombination of P^+-870 and I^-, producing the ground state P-870 (corresponding rate constant K^P_T), is hardly significant since the energy to be dissipated in this process exceeds 1.35 eV. Hence at room temperature and low redox potentials, P^F dissipation by two main channels: a radiative one (it includes P^* generation, subsequent partial excitation transfer to antenna bacteriochlorophyll accompanied by the decay of the excitations via trivial mechanisms) and a nonradiative channel, producing the triplet state of reaction centre (P^R) (see also Ref. 28).

Activation energy, ΔE, of the nanosecond delayed luminescence reflects a difference in energy between P^*-890 and P^F, since no activation barrier exists in the primary charge separation, according to the data of Clayton and Yamamoto [22]. These authors demonstrated that the charge separation yield is temperature-independent from 300 to 4 K. The value of ΔE = 0.05 ± 0.03 eV, determined in this work, is significantly lower than those determined previously [3,4,26] for the free-energy release (ΔW = 0.4—0.36 eV) during the primary act of photosynthetic energy conversion, basing on redox titration of I. But our data agree well with determination of ΔE from the temperature dependence of the luminescence yield for *Rhodopseudomonas viridis* chromatophores where this value was found to be 65 meV [23].

The discrepancy in the ΔW values obtained by the two different methods may arise from the fact that the interaction energy of charge pair $P^+ \ldots I^-$ is taken into account only in ΔW determinations, based on delayed luminescence data. Redox potential technique gives an E_m of I only in the absence of P^+-890, and also E_m of P-890 in the absence of I^-, which is quite different from the real situation, taking place during the course of the primary photosynthesis events (see also Refs. 1, 28). Besides, low potential titration in the range of $E_m < -0.4$ V entails a number of technical difficulties, greatly reducing the reliability of the results obtained [26].

According to our data, the P^F state, being only 1—3 kT lower than P^*-890, is not properly stabilized at the expense of the reverse process, $P^+I \overset{K_{e\uparrow}}{} P^*$, but it serves as a bridge in the electron transfer from P^* to X. Only the last process renders the photosynthetic charge separation practically irreversible.

Rather a slight energy difference between P^* and $I^- \approx 50$ meV is bound to entail delayed luminescence in the picosecond range, even under conditions of active (not saturated) photosynthesis. Actually, $\Delta E \approx 50$ meV gives for $K_{e\uparrow} = K_{e\downarrow} e^{-\Delta E/kT}$ a value approx. 10 times as low as $K_{e\downarrow}$, the latter being 10^{11} s^{-1} or higher [25]. Hence $K_{e\uparrow}$ is approximately equal to 10^{10} s^{-1} at room temperature, while the rate constant of P^F photosynthetic decay is equal to approx. $5 \cdot 10^9$ s^{-1} [2,3]. This means that a considerable part of electrons would be oscillating between P^* and I^- before reaching the 'primary' acceptor X. This oscillation should produce delayed luminescence with a lifetime of about 200 ps (determined almost exclusively by the rate constant of P^F decay through the photosynthetic channel). Actually, fluorescence lifetimes in the 100—300 ps range were registered for chromatophores [17,18] and even a reaction centre preparation [18].

Existence of an intermediate energy step(s) of $\lesssim 0.1$ eV between P^*-890 and a primary stabilized electron acceptor was suggested by us previously (see e.g. Refs. 16, 24) as a necessary requirement for ensuring high quantum and energy yields of photosynthesis.

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References

1 Parson, W.W. and Cogdell, R.S. (1975) Biochim. Biophys. Acta 416, 105—149

2 Rockley, M.G., Windsor, M.W., Cogdell, R.S. and Parson, W.W. (1975) Proc. Natl. Acad. Sci. U.S. 72, 2251—2255

3 Dutton, P.L., Prince, R.C., Tiede, D.M., Petty, K., Kaufmann, K.J., Netzel, T.L. and Rentzepis, P.M. (1976) Brookhaven Symp. Biol. N 28, 213—237

4 Fajer, J., Davis, M.S., Brune, D.C., Spaulding, L.D., Borg, D.C. and Forman, A. (1976) Brookhaven Symp. Biol. N 28, 74—104

5 Cogdell, R.S., Monger, T.G. and Parson, W.W. (1975) Biochim. Biophys. Acta 408, 189—199

6 Clayton, R.K. (1966) Photochem. Photobiol. 5, 679—688

7 Clayton, R.K., Fleming, H. and Szutz, E.Z. (1972) Biophys. J. 12, 46—51

8 Birks, S.B. and Monro, S.H. (1967) Progress in Reaction Kinetics Vol. 4, Pergamon Press, Oxford
9 Shuvalov, V.A. and Klimov, V.V. (1976) Biochim. Biophys. Acta 440, 587—599
10 Kondratieva, E.N. (1963) Photosyntheziruyuschie Bakterii. Izd, Akademii Nauk U.S.S.R., Moscow
11 Cohen-Bazire, G., Sistrom, W.R. and Stanier, R.Y. (1957) J. Cell Comp. Physiol. 49, 25—68
12 Nazarenco, A.V., Samuilov, V.D. and Skulachev, V.P. (1971) Biokhimiya 36, 780—782
13 Van Grondelle, R., Holmes, N.G. and Duysens, L.N.M. (1977) Abstracts of 4th Int. Congress on Photosynthesis Reading, U.K., p. 394.
14 Borisov, A.Yu., Ivanovsky, R.N. and Larionov, V.N. (1969) Prib. Techn. Exp. (Soviet) 5, 200—205
15 Borisov, A.Yu. and Godik, V.I. (1972) J. Bioenerg. 3, 211—220
16 Borisov, A.Yu. and Godik, V.I. (1973) Biochim. Biophys. Acta 301, 227—248
17 Campillo, A.J., Hyer, R.C., Monger, T.G., Parson, W.E. and Shapiro, S.L. (1977) Proc. Natl. Acad. Sci. U.S. 74, N 5
18 Paschenko, V.Z., Protasov, S.P., Rubin, A.B., Rubin, L.B. and Uspenskaya, N.Yu. (1977) Biochim. Biophys. Acta 461, 403—412
19 Godik, V.I. and Borisov, A.Yu. (1977) FEBS Lett. 82, 355—359
20 Van Grondelle, R., Romijn, J.C. and Holmes, N.G. (1976) FEBS Lett. 71, 187—192
21 Knox, R.S. (1978) in Primary Processes, Vol. 2, Chapt. 2, Photosynthesis (Barber, J., ed.), Elsevier, Amsterdam
22 Clayton, R.K. and Yamamoto, T. (1976) Photochem. Photobiol. 24, 67—70
23 Klimov, V.V., Shuvalov, V.A., Krahmaleva, I.N., Klevanik, A.A. and Krasnovsky, A.A. (1977) Biokhimia 42, 519—530
24 Borisov, A.Yu., Godik, V.I. (1975) in Biophysics of Photosynthesis (Rubin, A.B., ed.), Chapt. 2, Izd. MGU
25 Moskowitz, E. and Malley, M.M. (1978) Photochem. Photobiol. 27, 55—59
26 Prince, R.C., Leigh, J.S. and Dutton, P.L. (1976) Biochim. Biophys. Acta 440, 622—636
27 Sveshnikov, B.Ya. (1948) JETP (Russian) 18, 878—881
28 Grondelle, R., Holmes, N.G., Rademaker, H. and Duysens, L.N.M. (1978) Biochim. Biophys. Acta 503, 10—25
29 Clayton, R.K. (1977) Photosynthetic Organelles, Special Issue of Plant. Cell Physiol. 87—96
30 Hartig, P.R., Sauer, K., Lo, C.C. and Leskovar, B. (1976) Rev. Sci. Instr. 47, 1122—1129