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SHORT-LIVED DELAYED LUMINESCENCE OF PHOTOSYNTHESIZING ORGANISMS

II. THE RATIO BETWEEN DELAYED AND PROMPT FLUORESCENCE AS STUDIED BY THE MODULATION METHOD

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Summary

The ratio between the intensities of delayed and prompt fluorescence was studied for different photosynthetic objects under different conditions by a modulation method. The method is based on excitation of luminescing objects by light, modulated harmonically, and on a combined study of phase shifts and demodulation coefficients of the luminescence as related to excitation light. The presence of intense delayed emissions was revealed in purple bacteria, *Ectothiorhodospira shaposhinokovii*, *Rhodospirillum rubrum* and *Rhodopseudomonas sphaeroides*, in the micro- and nanosecond range. Under conditions of saturating light, their proportion was several percent of the total emission.

The most striking phenomenon was observed under reducing conditions (addition of $1 \cdot 10^{-2}$ M $Na_2S_2O_4$ to whole-cell suspensions of purple bacteria) where the intensity of the delayed emissions grew dramatically and became comparable to that of prompt fluorescence.

The data obtained indicate that, at room temperature, reversal of some early stages of charge separation in bacterial reaction centres may proceed largely via the channel that includes generation of the reaction-centre bacteriochlorophyll in the excited singlet state, followed by excitation-energy migration to antenna bacteriochlorophyll.

Abbreviations used: P^+ and P^*, the reaction centres in oxidized and singlet excited states, respectively; X^-, reduced classically-defined primary acceptor; BChl, bacteriochlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

The relation of these phenomena to the efficiency of solar energy utilization in photosynthetic apparatus is discussed.

Introduction

Plants and photosynthesizing bacteria are known to emit, together with prompt fluorescence, delayed luminescence long after the cessation of illumination. It has been well established experimentally, that delayed luminescence in a millisecond time range originates from a reversal of the primary electron-transfer reactions of photosynthesis [1—3]. For purple bacteria, *Rhodopseudomonas viridis*, the kinetics of delayed luminescence decay were demonstrated to be identical to those of the reduction of the oxidized reaction centre (P^+) and to those of the oxidation of the reduced primary acceptor (X^-) under appropriate conditions [2,3]. Hence, delayed luminescence appears to result from emittive recombination of P^+ and X^- which produces the reaction-center bacteriochlorophyll dimer in the first singlet excited-state (P*). Partial migration of this state back to antenna BChl and its radiative decay there entails the occurrence of photosynthetic delayed luminescence.

The ratio between the intensities of delayed and prompt fluorescences (DL/PF) provides direct information about the relation of the portion of singlet excitations in the antenna pigment complex, arising from the reversal of photosynthetic processes, to those produced by direct light-absorption. The study of the DL/PF ratio as a function of the redox state of the reaction centres and coupled electron-transfer carriers seems to be significant in elucidating the mechanisms of the subsequent steps of energy stabilization in photosynthesis.

According to the available data (see, for example, Refs. 3 and 5), the proportion of delayed luminescence in the total emission of photosynthesizing organisms is small, particularly for purple bacteria, and is no more than $1 \cdot 10^{-2}$—$1 \cdot 10^{-3}$% even under the conditions of saturated and/or inhibited photosynthesis. This conclusion is valid for delayed luminescence with decay times (τ_{DL}) in the range $1 \cdot 10^{-4}\ s < \tau_{DL} < \infty$, since in previous works delayed luminescence was recorded within this time interval.

In the present work, delayed luminscence of purple bacteria and plants is studied by a method which permits investigation of very short-lived delayed luminescence components, beginning with lifetimes of several nanoseconds duration. The contribution of delayed luminescence components with lifetimes $\tau_{DL} \geqslant 100$ ns is determined under different conditions. The results obtained give a new insight into the mechanisms of the early stages of photosynthesis.

Materials and Methods

Purple bacteria *R. rubrum*, *E. shaposhnikovii* and *R. sphaeroides* were grown anaerobically in light as described in Ref. 6. Two—three-day-old cell suspensions were used in the experiments. Pigment-protein complexes of light-harvesting antenna devoid of reaction centres were isolated from *R. rubrum* by the

method described in Ref. 7. The technique of preparation of chloroplasts from pea seedlings is described in Ref. 8. The cell suspensions of alga *Chlorella* were kindly gifted to us by K.T. Nikitina of the Chair of Plant Physiology, Moscow State University.

Fluorescence lifetimes, τ_m, and the emission modulation coefficients, α, were measured with a phase-fluorimeter, operating at the modulation frequency (12.3 MHz) of exciting light [9]. The time resolution of the instrument was 50 ps. The fluorescence spectra were recorded by an Aminco-Bowman spectrofluorimeter equipped with a 7102 photomultiplier. The basis for discovering short-lived delayed luminescence components and determining the DL/PF ratio by a modulation method consists of the following procedure. On exposure of a luminescing object to a harmonically-modulated light beam $I(t)$:

$$I(t) = I_0[1 + m \cdot \cos \omega t)]$$

where m is the modulation coefficient of exciting light, and ω the cyclic frequency; the intensity of luminescence $F(t)$ should change in the following manner [10]:

$$F(t) = F_0[1 + m\alpha \cdot \cos(\omega t - \Psi)] \quad (1)$$

Thus, luminescence is modulated with the same frequency as the exciting light, but its modulation coefficient is α times lower, and the emission phase lags behind, as compared to the excitation phase, by angle Ψ. For exponentially-decaying emission the following equations are valid [10]:

$$\tan \Psi = \omega\tau \quad (2a)$$

$$\alpha = \frac{1}{\sqrt{1 + \omega^2\tau^2}} = \cos \Psi \quad (2b)$$

where τ is the characteristic time of luminescence decay.

Eqns. 2a and 2b are widely-used for τ-determinations in homogeneously-emitting objects with phase-type and modulation fluorimeters, respectively.

If the object under investigation emits several time-resolved luminescence components, both phase- and modulation-type fluorimeters also produce some Ψ_m and α_m, which cannot be directly related now to the lifetimes of particular emissions. Quantitative analysis of the emission, consisting of n exponents, requires determination of $2n - 1$ independent parameters, that may be obtained by measurements of Ψ_m and α_m at several modulation frequencies. However, even at a single modulation frequency, the valuable information may be obtained if both methods are simultaneously used.

First of all, such a combined study allows one to distinguish between mono- and multicomponent luminescence. In fact, in the latter case, the values of the lifetimes obtained according to Eqns. 2a and 2b will be different. Usually, it is hardly possible to have more than two emission components of comparable amplitude. For emission consisting of two exponentially-decaying emissions, $F_1(t)$ and $F_2(t)$, the overall intensity, $F_m(t)$, is given by the following equation

(see Eqn. 1):

$$F_m(t) = (F_1 + F_2) + m[\alpha_1 F_1 \cos(\omega t - \Psi_1) + \alpha_2 F_2 \cos(\omega t - \Psi_2)] = F_= + F_\sim \quad (3)$$

where $F_=$ and $F_\sim$ are the direct and alternating components of luminescence light (or of photomultiplier current) respectively.

Now let us consider a simplified example.

Example 1. Two emissions, F_1 and F_2, are of equal intensity ($F_1 = F_2$). One is short-lived ($\tau_1 << \omega^{-1}$) which means according to Eqns. 2a and 2b that $\Psi_1 \to 0$ and $\alpha_1 \to 1$. The other has a lifetime comparable with ω^{-1}. Let us assume, for the simplicity of diagrammatic representation, that $\Psi_2 = 60°$, then $\omega\tau_2 = \sqrt{3}$ and $\alpha_2 = 0.5$. Separate emission components are summed up as vectors in accordance with Eqn. 3. The situation is diagrammatically illustrated in Fig. 1a in polar coordinates. Thus, measured by the phase-fluorimeter, phase-shift, $\Psi_m \approx 19°$, and the lifetime (determined from Ψ_m (Eqn. 2a))

$$\tau_m = \frac{0.346}{\omega}$$

The measured value of the modulation coefficient is given by:

$$\alpha_m = \frac{F_\sim}{F_=} = \frac{F_m}{F_1 + F_2} = 0.662$$

and the lifetime, determined from the modulation coefficient, according to Eqn. 2b and Fig. 2, by:

$$\tau_m = \frac{1.13}{\omega}$$

Thus, the values of the lifetimes obtained via Eqns. 2a and 2b, differ more than

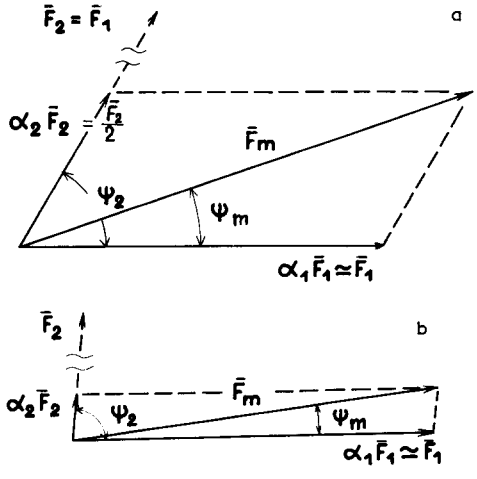

Fig. 1. Vector diagrams of alternating parts of the complex emissions (F_m), consisting of two components (F_1 and F_2). For details see text.

3-fold, which unequivocally proves the presence of two (or more) emissions.

Now let us consider an example of an emission similar to those from photosynthetic organisms.

Example 2. Suppose two emissions, F_1 and F_2, coexist, with their lifetimes differing greatly: $\tau_1 \leqslant 1.0$—1.3 ns (prompt fluorescence) and $\tau_2 > 100$ ns (delayed luminescence). Then, for our particular instrument with a modulation frequency of 12.3 MHz and $\omega^{-1} = 7.7 \cdot 10^7\ s^{-1}$, application of Eqns. 2a and 2b gives, for prompt fluorescence (see Fig. 2): $\tau_1 << \omega^{-1}$, $\Psi_1 \leqslant 5°$ and $\alpha_1 \geqslant 0.995$; and for delayed emission: $\Psi_2 \geqslant 83°$ and $\alpha_2 \leqslant 0.125$.

Besides $F_2 < F_1$, which means that the delayed luminescence quantum yield is less than those for prompt fluorescence. It can clearly be seen from the diagrammatic representation of the example in Fig. 1b that, in this case, $|\bar{F}_m| \to |\bar{F}_1|$, and the demodulation coefficient, δ_m, is given by:

$$\delta_m = 1 - \alpha_m = 1 - \frac{F_m}{F_1 + F_2} \approx \frac{F_2}{F_1 + F_2} \tag{3a}$$

This means that the demodulation coefficient is equal to the ratio of the total intensity of delayed luminescence with lifetimes $\tau_{DL} >> \omega^{-1}$ to the sum of prompt fluorescence and delayed luminescence intensities. Note, that for luminescence with $\tau_2 \sim \omega^{-1}$ (as in example 1), then:

$$\frac{F_2}{F_1 + F_2} > 1 - \alpha = \delta_m$$

Consequently, measurement of δ_m is a simple way to estimate the contribution of delayed luminescence to the total emission and hence, the DL/PF ratio, if the conditions listed in example 2 are observed. It should be mentioned that BChl prompt-fluorescence lifetimes are really shorter than 1.0—1.3 ns [11].

Luminescence demodulation, δ_m, is by definition,

$$\delta_m = 1 - \alpha_m = \frac{I_\sim/I_=|_{ex} - I_\sim/I_=|_m}{I_\sim/I_=|_{ex}} \tag{3b}$$

where $I_\sim/I_=|_{ex}$ and $I_\sim/I_=|_m$ are the ratios between the alternating and direct

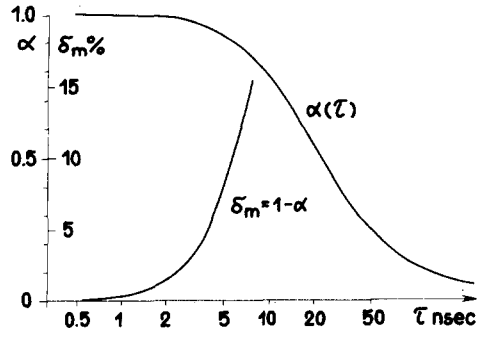

Fig. 2. The theoretical dependencies of modulation (α) and demodulation (δ_m) coefficients on the lifetime of monoexponential fluorescence, excited with harmonically-modulated light (Eqn. 2a). Modulation frequency is equal to $12.3 \cdot 10^6$ Hz as in our particular instrument.

components of photocurrent produced by exciting light and luminescence, respectively.

To determine δ_m, the ratio between the amplitude of the alternating component of photomultiplier current and the direct component, $I_{\sim}/I_{=}$ was measured, in turn, for scattered excitation light and for luminescence. The method used is similar to that described by Müller and Lumry [12].

The values of the direct currents, induced by scattered excitation light and luminescence, were usually equalized with an accuracy of about 2% for eliminating the influence of possible non-linearities of the recording system. In each series of experiments, control measurements of demodulation induced by a $1 \cdot 10^{-4}$ M solution of fluorescein in 0.01 M KOH were carried out. The lifetime of fluorescence is 5 ns under these conditions, and demodulation produced by such luminescence should be equal to 7%, with the frequency of modulation $\omega = 7.7 \cdot 10^7\ s^{-1}$, which was the case in our experiments.

Results

Purple bacteria

The simultaneous measurements of phase-shifts, Ψ_m, and demodulation coefficients, δ_m, were performed for different photosynthesizing organisms under varied conditions. The lifetimes, τ_m, determined through the measured phase-shifts, Ψ_m, from Eqn. 2a, lie in the range from 0.5 to 1.2 ns for purple bacteria, *E. shaposhnikovii*, *R. rubrum* and *R. sphaeroides* (Table I), both in low and saturating light. The demodulation coefficients, δ_m, were found to be in the range 0.5—1% in low light for chromatophore suspensions without additions, which is close to the limits of the resolution of the instrument used. For whole-cell suspensions under the same conditions, δ_m values of about 1—2% were recorded, which are slightly higher than those corresponding to monoexponentially-decaying emissions (see Eqn. 2b) and hence, the divergence between lifetimes determined from δ_m (τ_m^*) and from Ψ_m (τ_m) is displayed. After switching on the saturating light, a gradual (within several seconds) 3- to 4-fold increase in δ_m was observed, while Ψ_m, and hence, lifetimes, τ_m, decrease 1.3 to 2-fold; thereby an enormous discrepancy between the lifetimes τ_m and τ_m^* was brought about (Table I). This discrepancy inevitably proves the heterogeneous nature of the emissions investigated. The fact that; (1) under all conditions, $\Psi_m \leqslant 5^\circ$ ($\tau_m \leqslant 1.2$ ns); and (2) δ_m rise was not accompanied by an increase in Ψ_m (τ_m), which even decreases, points out that the demodulation, appearing under light-saturation conditions, is due to delayed luminescence with the lifetime exceeding 100 ns (see example 2 in Materials and Methods). Otherwise the demodulation increase ought to be accompanied by a rise in τ_m. The reason for $\Psi_m(\tau_m)$ decrease under light-saturation conditions was considered in detail elsewhere [11]. It is evoked by the heterogeneous nature of prompt fluorescence in the picosecond range. The prompt fluorescence heterogeneity of this type does not bring about any change in considerations (reported in Materials and Methods) concerning the behavior of Ψ_m and δ_m of the overall emission, consisting of prompt and delayed fluorescences.

As shown above, for emission consisting of prompt fluorescence with lifetime $\leqslant 1.2$ ns and delayed luminescence with lifetime $\tau_{DL} \geqslant 100$ ns, the values

TABLE I

THE CHARACTERISTICS OF THE EMISSIONS OF PURPLE BACTERIA AS STUDIED BY THE MODULATION METHOD

Culture	Intensity of exciting light	Conditions	Measured phase-shift ($\psi_m \pm 0.2°$) (°)	Lifetime determined from ψ_m ($\tau_m \pm 0.1$) (ns)	Demodulation coefficient δ_m (%)	Lifetime determined from δ_m [a] (τ_m^*) (ns)
E. shaposhnikovii						
cells	low ($2 \cdot 10^2$ erg $\cdot$ cm^{-2} $\cdot$ s^{-1})	aerobiosis, no additions	5	1.1	(1 ± 0.5)	1.9
cells	high ($1 \cdot 10^4$ erg $\cdot$ cm^{-2} $\cdot$ s^{-1})	aerobiosis, no additions	3	0.7	(4.5 ± 0.5)	4.5
cells	low and high	anaerobiosis, $1 \cdot 10^{-2}$ M $Na_2S_2O_4$	3.3	0.75	(25 ± 2)	12.0
chromatophores	low	aerobiosis, no additions	2.7	0.6	(1 ± 0.5)	1.9
R. rubrum						
cells	low	aerobiosis, no additions	4.8	1.0	(1.5 ± 1)	2.0
cells	high	aerobiosis, no additions	2.7	0.6	(3.5 ± 0.5)	3.9
cells	low and high	anaerobiosis, $1 \cdot 10^{-2}$ M $Na_2S_2O_4$	5.3	1.2	(33 ± 3)	15.0
chromatophores	low	aerobiosis, no additions	2.3	0.5	(0.5 ± 0.5)	1
chromatophores	high	aerobiosis, no additions	1.9	0.4	(5 ± 0.5)	4.6
R. sphaeroides						
cells	low	aerobiosis, no additions	3	0.7	(2 ± 0.5)	2.8
cells	high	aerobiosis,	1.5	0.35	(3.5 ± 0.5)	3.9
		anaerobiosis	5	1.1	(6 ± 0.5)	5.0

[a] For the frequency of exciting light modulation of $7.7 \cdot 10^7$ s^{-1} the accuracy of τ_m^* determination was 30—40% for lifetimes 1—5 ns, and 15—20% for lifetimes exceeding 7—8 ns.

of δ_m are equal to the proportion of delayed luminescence in the total emission (i.e., DL/(DL + PF) ratio). It can easily be derived from the data of Table I that the DL/PF ratio comes to 3—6% for purple bacteria in saturating light. Clayton [5] also measured a DL/PF ratio for similar organisms under similar conditions, but with the time range being $1 \cdot 10^{-4}\ s \leqslant \tau_{DL} < \infty$, and found this ratio to be in the range $1 \cdot 10^{-4}$—$1 \cdot 10^{-5}$. It follows from the comparison of our data to those of Clayton that rather intense short-lived delayed luminescence components exist in the time range $1 \cdot 10^{-7}\ s \leqslant \tau_{DL} < 1 \cdot 10^{-4}$ s. The intensity of these delayed luminescence components was found to grow enormously under reducing conditions. The addition of sodium dithionite ($Na_2S_2O_4$) at a concentration of $1 \cdot 10^{-2}$ M to the cell suspensions of *E. shaposhnikovii* and *R. rubrum* brought about a sharp rise of up to 25—30% in δ_m, which corresponds to a DL/PF ratio of 35—40%.

This effect was only observed for young-cell suspensions. Reduction of aged-cell suspensions with saturating concentrations of $Na_2S_2O_4$ resulted in an increase of up to 3 ns in τ_m, with δ_m being practically unchanged, i.e., exactly as it had been reported earlier for chromatophores [13]. This difference in the behaviour of whole cells and chromatophores is associated with different membrane permeability for $Na_2S_2O_4$, since further lowering of redox potential by the addition of $1 \cdot 10^{-5}$ M neutral red, besides $Na_2S_2O_4$, resulted in the same effects, whichever was used: cells and chromatophores both exhibited a sharp rise in τ_m with insignificant changes in δ_m. It was shown [13,14] that emission of purple bacteria under these conditions consisted of two components with approximately equal intensities: prompt picosecond fluorescence and nanosecond (τ_{DL} = 4—6 ns) recombination luminescence, which most likely appeared as a result of radiative decay of the primary ion-radical pair, P^F.

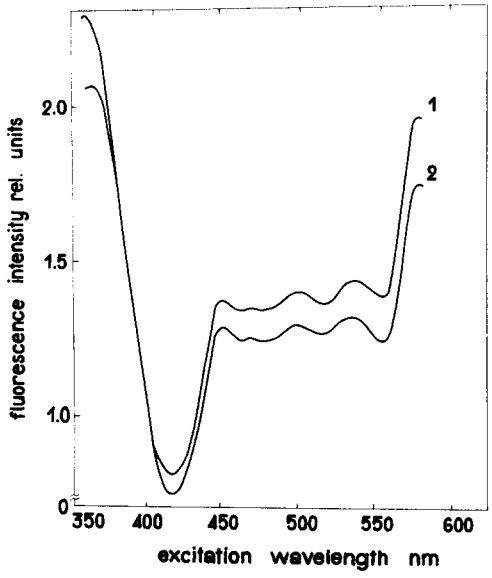

Fig. 3. Spectra of fluorescence excitation for *R. rubrum* cells: (1) no additions (2) after addition of $Na_2S_2O_4$ ($1 \cdot 10^{-2}$ M). Fluorescence was detected at 910 nm, absorbance at 870 nm was 0.4 in a 1 cm cell.

In order to clarify the nature of the delayed luminescence of whole cells with lifetimes of $1 \cdot 10^{-7}\,s < \tau_{DL} < 1 \cdot 10^{-4}\,s$, the luminescence spectra of *R. rubrum*, as well as the luminescence-excitation spectra, were studied under normal (no additions) and reduced (addition of $1 \cdot 10^{-2}$ M $Na_2S_2O_4$) conditions. No marked difference in the luminescence spectra was observed, which agrees well with the data of previous work [15]. Spectra of luminescence excitation for intact and reduced cells also practically coincide in the range 360—600 nm (Fig. 3). The differences in the spectra, due to light absorption of $Na_2S_2O_4$, were observed only at wavelengths shorter than 360 nm. These two observations indicate that it is the antenna bacteriochlorophyll, rather than extraneous pigments, which emits this intense delayed luminescence. Phosphorescence of protoporphyrin IX, observed by Arata et al. [16] for *Chromatium vinosum* and by Carithers and Parson [17] for *R. sphaeroides* at very low potentials, was several orders weaker than prompt fluorescence, while in our case, the intensities of prompt fluorescence and delayed luminescence are of the same order. Besides, we succeeded in simulating the dithionite effect on the emission of *R. rubrum* chromatophores at a moderate redox potential using the electron-transfer inhibitor, *o*-phenatroline [14].

If delayed luminescence with a lifetime of $1 \cdot 10^{-7}\,s < \tau_{DL} < 1 \cdot 10^{-4}$ s, as well as the nanosecond delayed luminescence, is emitted by BChl and originates from the reversibility of charge-separation in the reaction centres, then it should disappear under the conditions when this separation is blocked, or simply in the absence of functional reaction centres. For this purpose, pigment-protein complexes from *R. rubrum*, devoid of reaction centres and containing only BChl, and the PM-8 mutant of *R. spheroides*, which has no functional reaction centres, were investigated. In the latter case, the lyophilized cells, kindly gifted to us by Professor R.K. Clayton, were dispersed in 50 mM Tris-HCl buffer (pH 7.8) and used in the experiments. Delayed luminescence of the type described was absent in both cases, as none of the treatments ($Na_2S_2O_4$, *o*-phenantroline plus sodium ascorbate, saturating light, etc.) had any effect on τ_m and the demodulation of the emissions. These observations provide evidence that it is the functioning of the reaction centres that initiates this delayed luminescence. For nanosecond delayed luminescence, a direct kinetic correlation was observed [13] between the delayed luminescence generation and the portion of the reaction centres capable of primary charge separation. Lowering the temperature from 293 to 170 K removed the effects of $Na_2S_2O_4$ on the modulation characteristics of the emissions described above. In the case of reduced cells a decrease in both the emission yield (approx. 40%) and demodulation (from 20—30% at 293 K to 1—2% at 170 K) was observed. Reduced chromatophores under these conditions displayed an approx. 2-fold decrease in the emission yield, and a decrease in τ_m from 3 ns to approx. 100 ps. The latter value is close to the lifetime of the prompt fluorescence.

Plants

The emission lifetimes, τ_m, of pea chloroplasts and *Chlorella* cells were found to be less than 1 ns in low light (Table II), but an increase in light intensity produces a rise in both τ_m and emission demodulation, δ_m. Nevertheless, the

TABLE II

THE CHARACTERISTICS OF PLANT EMISSIONS AS STUDIED BY THE MODULATION METHOD

DCMU; 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

Culture	Intensity of exciting light	Conditions	Measured phase-shift ($\psi_m \pm 0.15$) (°)	Lifetime, determined from ψ_m ($\tau_m \pm 0.1$) (ns)	Demodulation coefficient ($\delta_m \pm 0.5$) (%)	Lifetime, determined from δ_m (τ^*_m) (ns)
Chlorella cells	low ($2 \cdot 10^2$ erg $\cdot$ cm^{-2} $\cdot$ s^{-1})	no additions	3.3	0.8	1.5	2.0
	high	—	7.8	1.8	10	6.6
	—	DCMU $1 \cdot 10^{-4}$ M	10.8	2.5	2	2.8
	—	K_3FeCN_6 $1 \cdot 10^{-4}$ M	3.5	0.8	8	5.9
Pea chloroplasts	low	no additions	2.7	0.6	0.8	1.5
	high	—	7.8	1.8	7	5.3
		DCMU $1 \cdot 10^{-5}$ M	12.5	2.85	2	2.8
	—	K_3FeCN_6 $1 \cdot 10^{-5}$ M	3.8	0.85	9	6.2

disproportion between emission lifetimes, τ_m and τ_m^*, determined via Ψ_m and demodulation, δ_m, respectively (Eqns. 2a and 2b), is clearly seen. This manifests a heterogeneous nature of the emission investigated. However, unlike in the case of purple bacteria, the demodulation value itself cannot be used here as the quantitative ratio of DL/PF, since afterglows with lifetimes in the range of dozens of nanoseconds cannot be excluded. A detailed study of delayed luminescence of plant organisms by the modulation method has been presented elsewhere [8].

Discussion

Lavorel was the first to stress the exceptional importance of short-lived delayed luminescence studies, in particular the DL/PF ratio, for photosynthetic organisms [18]. Such investigations may provide unique information about the mechanisms of the earliest steps of charge separation and stabilization in the reaction centres. According to the currently-accepted scheme of the primary processes of bacterial photosynthesis [19], charge separation that results in formation of a rather stable primary oxidant, P^+, and a reductant, X^-, proceeds as follows:

(1) $h\nu + \text{BChl} \rightarrow \text{BChl}^*$ (light absorption)

(2) $\text{BChl}^* \xrightarrow{\text{approx. 100 ps}} P^*$ (excitation energy migration)

(3) $P^* \xrightarrow{5-10\ \text{ps}} P^F$

(4) $P^F \xrightarrow{150-250\ \text{ps}} P^+X^-$ (charge separation and stabilization)

where P^F is the primary ion-radical pair, including P^+ and reduced intermediate acceptor, probably bacteriopheophytin. Prompt fluorescence is generated in process 1, while delayed luminescence results from the reversal of steps 3 and 4, i.e., from a radiative recombination of photo-produced charges. Processes 3 and 4 are the subsequent steps of excitation-energy stabilization, and energy losses that accompany this stabilization are believed to be approx. 0.4 eV for each of steps [19].

The findings of this work and those of others [13—15,20], that, under certain conditions, the intensities of prompt fluorescence and delayed luminescence may be of the same order, contradict the above scheme. Such a situation can never take place unless the rates of nonradiative recombination of states P^F and P^+X^- are lower than $1 \cdot 10^3$—$1 \cdot 10^4\ s^{-1}$ and approx. $1 \cdot 10^{-2}\ s^{-1}$, respectively, which is absolutely inconsistent with the experiment evidence [19]. According to our data [13,14], the data of Shuvalov et al. [20] and of van Grondelle et al. [15], the energy barrier between P^F and P^* states is 0.05—0.15 eV, rather than 0.4 eV.

The above simplified scheme cannot explain the occurrence of delayed luminescence in the microsecond time range, either. Recombination of P^+ and X^-, measured by the characteristic differential-absorption changes in a visible range, proceeds within about 100 ms [21]; that is why the existence of more short-lived delayed luminescence components is considered to be improbable.

However, Carithers and Parson [17] observed in *R. sphaeroides* that delayed luminescence decayed much more rapidly than P^+X^-. Their observations, and ours, of very intense delayed luminescence components in the range of $1 \cdot 10^{-7}\,s < \tau_{DL} < 1 \cdot 10^{-4}\,s$, as well as the fact that under certain conditions the intensity of delayed luminescence components becomes comparable with that of prompt fluorescence, provide evidence that some additional levels may be included in the process of charge separation between P^F and P^+X^- equilibrium states. The theoretical treatment by Vorotyntsev and Itskovich [22] suggests the existence of such energy levels, representing different conformational states of the pigment-protein complex of the reaction centre; these states are produced as a result of a gradual relaxation of the system as a whole along the conformational degrees of freedom. The latter process not only stabilizes the photo-produced charges, but also stores part of the energy of the absorbed photon in a useful form.

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