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# Energy Transfer in Bacterial Photosynthesis

## I. Light Intensity Dependences of Fluorescence Lifetimes

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*Abstract*

Four possible explanations are offered to account for low fluorescence increase observed for purple bacteria under transition from active to inhibited photosynthesis. The increase observed is inconsistent with high ($\simeq$1.0) yield of primary photosynthetic process of $P_{890}$ photooxidation. The dependences of fluorescence yield and lifetime on the portion of active reaction centres have been analysed for each case. Experimental investigation carried out favours the existence of background fluorescence together with fluorescence, whose quantum yield correlates with the reaction centre functional state. The important conclusion is made that lifetime of photosynthetic fluorescence is much lower than 1 nsec and energy is transferred to the reaction centres by a mechanism other than inductive-resonance.

It is currently accepted that chlorophyll (Chl) fluorescence lifetimes $\tau$ for plants [1-3], green bacteria [3], and purple bacteria [4, 5] are of the order of 1 nsec. Taking into account that a single reaction centre serves for 50-300 light-harvesting Chl molecules, one can derive that *in vivo* energy transfer is of inductive resonance type [6]. Förster's theory of inductive resonance [7] was applied to photosynthesis by Duysens [8] and developed by Bay and Pearlstein [6, 9] and others.

On the other hand, basing on the *in vivo* spectral characteristics of chlorophylls, Robinson [10] suggested that energy transfer in photosynthetic units was of excitation type. To account for the discrepancy between experimental lifetime values and the ones calculated for the exciton transfer he suggested that the efficiency of exciton energy trapping in reaction centres is low ($\simeq$ 1%).

In 1963 Vredenberg and Duysens [11] showed for *Rh. rubrum* cells that the inverse value of BChl fluorescence yield depends linearly on the portion of photooxidized reaction centres ($P^+$). It meant that the active

reaction centres compete for the common pool of photoinduced singlet excited states. Then similar dependences were obtained by Clayton [12] for a number of purple bacteria and by Sybesma and Vredenberg [13] for green bacterium *Chl. ethylicum*.

It can be easily shown [14] that the model of Vredenberg and Duysens predicts the following relationship to exist between the quantum yield of primary electron transfer $\varphi_e$ and the fluorescence yield increase induced by reaction centre photooxidation:

$$\varphi_e = \frac{\varphi_{max} - \varphi_{min}}{\varphi_{max}} \quad (1)$$

where $\varphi_{min}$, $\varphi_{max}$ are fluorescence yields corresponding to all excitation traps open and closed respectively.

In the above-mentioned contributions [11-13] two-three-fold increases were observed, thus giving $\varphi_e$ values equal to 0.5-0.65. These values are somewhat lower than those obtained in some recent works by measuring directly the initial rates of reaction centre photooxidation [15, 16].

In order to clarify the reason for the divergence in the values of photosynthetic yields obtained by two different methods we performed a combined study of fluorescence yield and lifetime and changes in optical densities corresponding to reaction centre photooxidation. Such approach allowed us to obtain new data on the primary photosynthetic processes.

## *Materials and Methods*

Aerobic and anaerobic suspensions of purple bacteria *Ectothiorhodospira shaposhnikovii, Chromatium minutissimum* and *Rhodopseudomonas spheroides* and green bacterium *Chloropseudomonas ethylica* were studied. They were grown in modified Larsen's medium [17] and used 3-5 days after inoculation from a previous culture.

The procedure of fluorescence yield and lifetime measurements, as well as light-induced changes in absorption are described elsewhere [16].

### *Theoretical Part*

Four different explanations may be proposed to account for the above-mentioned divergence in the values of $\varphi_e$ obtained by two different methods. It should be noted that each of the cases considered below, is characterized by the same character of $\varphi$ dependences on $P^+/(P + P^+)$, while the appropriate dependences of $\tau$ differ greatly.

1. It is noteworthy that the determination of $\varphi_e$ from Eq. (1) is correct if singlet electronic excited states are the only excited states providing energy transfer. Therefore the first explanation is that the

triplets provide additional 25-45% of light energy to the reaction centres.

If this is the case fluorescence yields and lifetime should be expected to increase in the similar way as the reaction centres become inactive (Fig. 1, curve 1).

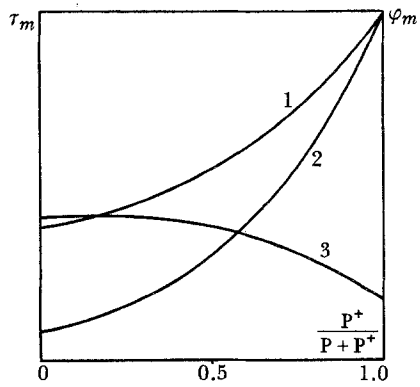


Figure 1. Theoretical dependences of fluorescence yield, $\varphi$ (curve 1) and lifetime, $\tau$ (curves 1, 2, 3) on the portion of photooxidized reaction centres (see text).

2. The second explanation is to assume that reaction centres may trap excitation quanta with an efficiency 2-3 times lower than in the reduced state. Here, too, $\varphi_e$ values, calculated from Eq. (1), would be understated and fluorescence yield and lifetime must increase proportionately, as in the first case (Fig. 1, curve 1).

3. It has not been shown that the emission of purple bacteria as a whole is prompt fluorescence and, hence, a part of it may be of delayed fluorescence type. If its lifetime considerably exceeds the inverse values of cyclic frequency of light modulation in phase-fluorometer, then it will be practically demodulated and will not contribute to the measured lifetime values. Therefore, in this case fluorescence lifetime increase must exceed considerably that of fluorescence yield (curve 2, Fig. 1).

4. Equation (1) will give understated values for $\varphi_e$ if a part of emission observed is background fluorescence whose quantum yield, $\varphi_{bg}$, and lifetime $\tau_{bg}$, does not correlate with reaction centre functional state. Let us consider this most complicated case in greater detail.

Let the exciting beam in fluorometer be as usual:

$$S = S_o\ (1 + m \cos \omega t)$$

where $m$ is light modulation coefficient and $\omega$ is cyclic frequency.

Then $S$ would induce two separate emissions from two fluorescent species of molecules which, in their turn, would produce two currents

from photocathode. In our particular experiments the following conditions were fulfilled:

(a) Two spectral forms of bacteriochlorophyll were excited in Soret band (and sometimes around 590 nm) where their extinction coefficients are nearly equal, i.e. $\epsilon \cong \epsilon_{bg}$

(b) Fluorescence spectra of the forms are approximately coincident, i.e. $F(\lambda) \cong F_{bg}(\lambda)$.

6. The values of intrinsic lifetimes ($\tau_0$) of the two forms are the same. It means, that

$$\frac{\tau}{\varphi} = \frac{\tau_{bg}}{\varphi_{bg}} = \tau_o ,$$

where $\tau, \tau_{bg}$ and $\varphi, \varphi_{bg}$ are fluorescence lifetimes and yields of two bacteriochlorophyll forms respectively.

Then the two photocurrents will be as follows:

$$i = S_o [1 + m' \cos (\omega t - \psi)] (1 - 10^{-D}) \frac{c}{c + c_{bg}} \varphi \int_{\lambda} F(\lambda) T(\lambda) P(\lambda) d\lambda$$

$$= A \frac{c}{c + c_{bg}} \varphi [1 + m' \cos (\omega t - \psi)] \tag{2a}$$

similarly

$$\tau_{bg} = A \frac{c_{bg}}{c + c_{bg}} \varphi_{bg} [1 + m'' \cos (\omega t - \psi_{bg})] , \tag{2b}$$

where $m'$, $m''$ are coefficients of respective fluorescent modulations; $\psi$ and $\psi_{bg}$ are phase shifts between alternative components of respective emissions and that of exciting light; $D$ is optical density of cell suspension investigated; $c$, $c_{bg}$ are respective concentrations of the two fluorescing forms; $F(\lambda)$ is spectral distribution of both emissions; $T(\lambda)$ is spectral dependence of transmittence of optical filters used; $P(\lambda)$ is spectral sensitivity of photomultiplier, $A$ is easy to derive by comparing two parts of formula (2a). It is well known from the usual theory of phase fluorometry that

$$\begin{aligned} m' &= m \cos \psi; & m'' &= m \cos \psi_{bg} \\ \tau &= \frac{tg\psi}{\omega}; & \tau_{bg} &= \frac{tg\psi_{bg}}{\omega} \end{aligned} \tag{3a}$$

Lifetimes, not exceeding 1.5 nsec, give phase shifts not more than 7°30′ in our phase-fluorometer, operating at the frequency of 12.3 Mhz. It means that with accuracy better than 0.4%:

$$\begin{aligned} &\cos \psi = \cos \psi_{bg} = 1; \quad \text{or} \quad m' = m'' = m; \\ &\sin \psi = tg\psi = \psi; \qquad \sin \psi_{bg} = tg\psi_{bg} = \psi_{bg} \end{aligned} \tag{4}$$

It is evident that alternative components of the photocurrents are summed as vectors, i.e.

$$\bar{J}_\Sigma = \bar{i} + \bar{i}_{bg} = J_{\Sigma o}\, m \cos(\omega t - \psi_m), \tag{5}$$

where

$$J_{\Sigma o} = \sqrt{|i|^2 + |i_{bg}|^2 + 2|i||i_{bg}| \cos(\psi - \psi_{bg})}, \tag{5a}$$

$$tg\,\psi_m = \frac{|i| \sin\psi + |i_{bg}| \sin\psi_{bg}}{|i| \cos\psi + |i_{bg}| \cos\psi_{bg}} \simeq \frac{|i|\psi + |i_{bg}|\psi_{bg}}{|i| + |i_{bg}|} \tag{5b}$$

where $i$ and $i_{bg}$—are respective amplitudes of alternative currents; $\psi_m$ is measured phase shift between $\bar{J}_\Sigma$ and $\bar{S}$. This phase angle $\psi_m$ may be referred to the measured lifetime $\tau_m$ depending on amplitudes and lifetimes of both emissions or, better to say, both photocurrents:

$$\tau_m = \frac{tg\,\psi_m}{\omega} \tag{6}$$

By combining Eqs. (2a), (2b), (5b) and (6) it is easy to obtain:

$$\tau_m = \frac{\varphi \dfrac{cm'}{c + c_{bg}}\, \psi + \varphi_{bg} \dfrac{c_{bg} m''}{c + c_{bg}}\, \psi_{bg}}{\omega\left(\varphi \dfrac{cm'}{c + c_{bg}} + \varphi_{bg} \dfrac{c_{bg} m''}{c + c_{bg}}\right)}$$

Taking into account Eqs. (1) and (4) it can be written:

$$\psi \simeq tg\,\psi \simeq \omega\tau \simeq \omega\varphi\tau_o$$

$$\psi \simeq tg\,\psi_{bg} \simeq \omega\tau_{bg} \simeq \omega\varphi_{bg}\tau_o .$$

Hence

$$\tau_m \simeq \frac{\tau_o (c\varphi^2 + c_{bg}\varphi_{bg}^2)}{c\varphi + c_{bg}\varphi_{bg}} \simeq \tau_o \varphi_{bg} \frac{c\left(\dfrac{\varphi}{\varphi_{bg}}\right)^2 + c_{bg}}{c\dfrac{\varphi}{\varphi_{bg}} + c_{bg}} \simeq \tau_{bg} \frac{1 + \alpha x^2}{1 + \alpha x} \tag{7}$$

where

$$\alpha = c/c_{bg} \quad \text{and} \quad x = \frac{\varphi}{\varphi_{bg}}$$

On the other hand the direct components of two photocurrents $i$ and $i_{bg}$ [(2a), (2b)] are summed as scalar values. It is easy to write a formula for the measured fluorescence yield ($\varphi_m$):

$$\varphi_m = \frac{\varphi \dfrac{c}{c + c_{bg}} + \varphi_{bg} \dfrac{c_{bg}}{c + c_{bg}}}{\dfrac{c}{c + c_{bg}} + \dfrac{c_{bg}}{c + c_{bg}}} = \varphi_{bg} \frac{1 + \alpha x}{1 + \alpha} \tag{8}$$

In the general case,

$$F(\lambda) \neq F_{bg}(\lambda); \qquad \epsilon \neq \epsilon_{bg}; \qquad \tau_o \neq \tau_{o\,bg}$$

parameter $\alpha$ depends on a great number of functions and it can be varied by changing optical filters or the type of photomultiplier.

Dependences of $\varphi_m$ $(x)$ and $\tau_m$ $(x)$ for different values of parameter $\alpha$, constructed according to Eqs. (7) and (8), are shown in Fig. 2. Following $\varphi/\varphi_{bg}$ increase $\tau_m$ even decreases at first, while $\varphi_m$ increases linearly all the time. Only after certain $X$ values have been reached:

$$X_{\min} = \frac{\sqrt{1+\alpha}-1}{\alpha},$$

$\tau_m$ begins increasing too. The decrease in $\tau_m$ is more pronounced for large values of $\alpha$, i.e. when concentration of the species with variable fluorescence yield exceeds greatly that of with constant yield.

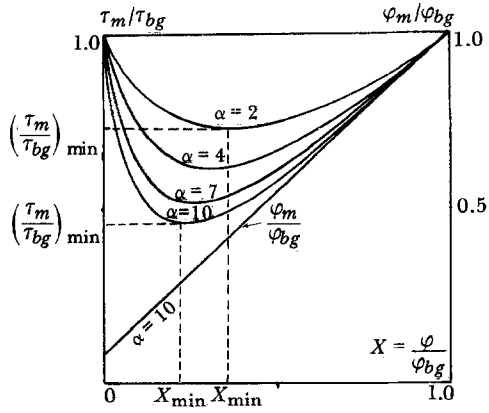


Figure 2. Theoretical dependences of the measured fluorescence lifetime and yield of heterogeneous emission on the ratio of fluorescence yields of variable and background fluorescing components $\varphi/\varphi_{bg}$ ($\alpha = c/c_{bg}$ – the ratio of respective concentrations).

Therefore, $\tau_m$ and $\varphi_m$ would change in antibatic manner in our case (Fig. 1, curve 3), if fluorescence yield of background emission exceeds considerably the initial yield of variable fluorescence.

Besides possibilities 1-4, mentioned above, it should be kept in mind that conformational changes, arising in lipoprotein matrix under the influence of powerful light, may alter some intermolecular distances in pigment complexes *in vivo*. This case is rather difficult to consider quantitatively but it appears that $\tau_m$ should increase simbatically with $\varphi_m$.

*Results and Discussion*

In order to make a choice between the above possibilities we have studied light-intensity dependences of $\tau_m$, $\varphi_m$ and the portion of oxidized reaction centres $P^+/(p^+ + p)$. The latter value was determined as normalized absorption decrease associated with $P_{890}$ photobleaching.

Excitation light intensity in the phase-fluorometer used was changed 25 times (it was achieved by means of focusing of light beam in civette plane). Initial intensity was about 500 erg/cm$^2$. sec. It corresponded for all bacteria investigated to active photosynthesis, when light practically did not oxidize $P_{890}$. Final intensity was up to 12,000 erg/cm$^2$. sec and caused maximal $P_{890}$ photobleaching.

Experimental light-intensity dependences for aerobic suspension of purple bacteria *E. shaposhnikovii, Chr. minutissimum, Rhodospirillum rubrum* and *Rh. spheroides* and green bacterium *Chloropseudomonas ethylica* are shown in Fig. 3. For all cultures investigated the decreases in $\tau_m$ were observed.

Light-intensity dependences of fluorescence yield and $P^+(P^+ + P)$ for aerobic culture of *Rh. spheroides* are shown in Fig. 4. The same character of these dependences were observed for *E. shaposhnikovii* and *Chr. minutissimum*. According to data, presented in Fig. 4, light intensity

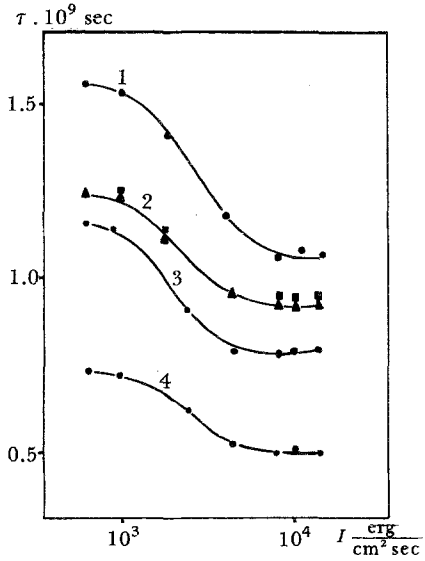


Figure 3. Fluorescence lifetime as a function of exciting light intensity (λ 404 and 590 nm). 1—cell suspension of *Rh. rubrum*; 2 triangles—*E. shaposhnikovii*; 2 squares—*Rh. spheroides*; 3—*Chl. ethylica*; 4—*Chr. minutissimum*; aerobic conditions.

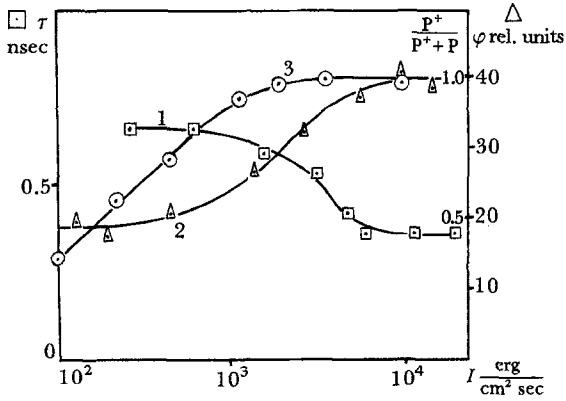


Figure 4. Fluorescence lifetime (1); relative fluorescence yield (2) and the portion of photooxidized reaction centres (3) as a function of the exciting light intensity (λ404 and 590 nm). *Rh. spheroides*; aerobic conditions.

increase converts the reaction centres $P_{890}$ to the oxidized state. At light intensities of $10^4$ erg/cm$^2$ sec practically all the reaction centres are in non-active, oxidized, state. $P_{890}$ photooxidation is accompanied by increase in $\varphi_m$. The latter fact agrees with the currently accepted fact that fluorescence and photosynthesis compete for the energy of singlet excited state. Under the same conditions the measured value of $\tau_m$ decreases. Antibatic behavior of $\tau_m$ and $\varphi_m$ undoubtedly favours hypothesis 4. According to this hypothesis fluorescent emission of photosynthetic bacteria is heterogeneous. Together with fluorescence, whose intensity correlates with $P_{890}$ functional state (here and below it will be called as photosynthetic fluorescence), it contains some background emission. The character of $\tau_m$ light-intensity dependence indicates that background fluorescence lifetime $\tau_{bg}$ exceeds that of photosynthetic fluorescence in the whole range of exciting light intensities. The relative decrease in $\tau_m$ observed is about 30% as a rule (Fig. 3). It means that $\alpha \geqslant 2$ (Fig. 2) and quantum yield of background fluorescence exceeds that of photosynthetic one more than 15-20 times at low light intensities. Therefore fluorescence lifetimes of photosynthetic emission in the range of active photosynthesis are much lower than the currently accepted value of 1 nsec. The method of calculation of true lifetimes from the measured values $\tau_m$ at different light intensities will be presented in the subsequent publication.

Such low fluorescence lifetime values are inconsistent with the idea of the inductive resonance mechanism of energy transfer in bacterial photosynthesis. Using the formula of Pearlstein [9] and Robinson [10] for the number of jumps preceding energy trapping in homogeneous

two-dimensional pigment complex, one receives for jump times the values comparable with lattice relaxation times. So the main condition, on which Förster's theory may be applied, is not maintained.

One more important conclusion is that the time of the reaction centres photooxidation is shorter than $10^{-10}$ sec. It means that $P_{890}$ interaction with diffusible primary electron acceptor may be ruled out when considering possible mechanisms of primary charge separation.

The same conclusion is likely to hold for pigment system I of plants, where chlorophyll fluorescence life-times were shown not to exceed 0.07 nsec [18].

In pigment system II (PS-II) of plants chlorophyll fluorescence lifetime varies from 0.35 nsec to 2 nsec with exciting light intensity [3]. For green alga Chlorella changes in $\tau_m$ were observed to be accompanied by a proportional increase in fluorescence yield (Table I).

TABLE I. Light-intensity dependence of fluorescence lifetime and relative yield for *Chlorella pyrenoidosa*

| exp (Nr) | $\tau$ nsec | $\varphi$ relative units | $J \frac{\text{erg}}{\text{cm}^2 \text{ sec}}$ |
|---|---|---|---|
| 1 | 0.8 | 22.5 | $10^2$ |
| | 1.9 | 56 | $10^4$ |
| 2 | 0.9 | 19 | $10^2$ |
| | 1.5 | 29 | $10^4$ |
| 3 | 0.65 | 22.5 | $10^2$ |
| | 1.3 | 46 | $10^4$ |

Besides, proportional changes in $\tau_m$ and $\varphi_m$ were reported [19] for Chlorella and pea chloroplasts during induction period.

Hence, PS-II fluorescence is likely to represent a homogeneous emission and the criterion of Förster's theory applicability, mentioned above, is observed.

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