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Fluorescence lifetime of bacteriochlorophyll and reaction center photooxidation in a photosynthetic bacterium

It has been shown (see, for example, refs. 1 and 2) that the reciprocal of bacteriochlorophyll fluorescence yield, φ_{fl}, in photosynthetic bacteria is proportional to the concentration of active reaction centers. These data are in good agreement with a model, firstly advanced by VREDENBERG AND DUYSENS[1], in which several traps compete for a common pool of excitation quanta. The model predicts the $1/(1-\varphi_{ph})$-fold increase in bacteriochlorophyll fluorescence under conditions of fully inhibited photosynthesis (φ_{ph} is the quantum yield for the photooxidation of reaction centers). The latter value for purple bacteria is about 1.0 (see, for example, ref. 3). One should expect that the ratio of maximum to minimum fluorescence corresponding to closed and open traps should exceed 10. However, only 2- to 3-fold increases have been observed in experiments[1]. Three possible explanations of such a discrepancy may be considered:

1. The observed emission consists to a great extent of delayed-type fluorescence.

2. Reaction centers in the oxidized state are still functioning as traps for excitation quanta but somewhat less efficiently than in the reduced state.

3. There is some background fluorescence, *e.g.* emission with intensity which is not affected by the reaction centers' redox state.

In order to choose between the above possibilities, light-intensity dependences of φ_{fl} and fluorescence lifetime, τ_{fl}, as well as the portion of oxidized traps, $P^+/(P^+ + P)$, have been investigated (P^+ and P are reaction center concentrations in oxidized and reduced state, respectively).

The photosynthetic purple bacterium *Ectothiorhodospira Shaposhnikovii* was studied. It was grown in modified LARSEN's medium[4] and used 3–5 days after inoculation from a previous culture.

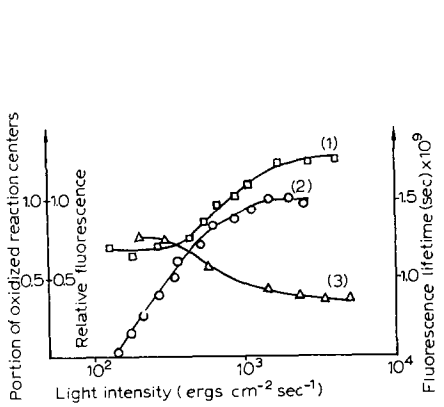
A phase fluorometer operating at the frequency of $12.3 \cdot 10^6$ Hz was used for fluorescence lifetime measurements. Time resolution of the instrument was about $5 \cdot 10^{-11}$ sec. Variation of excitation light intensity was attained by means of defocusing excitation light. Light-induced changes in absorption corresponding to photooxidation of reaction centers and the dependence of φ_{fl} on $P^+/(P^+ + P)$ were studied with an instrument with sensitivity to absorption changes of $5 \cdot 10^{-4}$ ΔA. The instrument permits the measurements of photoinduced absorption changes and fluorescence yields in the same cuvette.

An increase in fluorescence yield and decrease in fluorescence lifetime have been observed under increased light intensity (Fig. 1). Photooxidation of reaction centers is likely to be the reason for the increase in φ_{fl} observed at high light intensities. Then the dependences of $P^+/(P^+ + P)$ on light intensity and of φ_{fl} on $P^+/(P^+ + P)$ have been studied. Typical curves for aerobic suspensions are represented in Figs. 1 and 2.

Fluorescence lifetime and quantum yield are known to be related as $\tau_{fl} = \tau_0 \varphi_{fl}$, where τ_0 is intrinsic lifetime. It follows that τ_{fl} and φ_{fl} must change symbatically for one species of fluorescing molecules. And it is only when there are two species of fluorescing molecules that the measured values of τ_{fl} and φ_{fl} may change antibatically if the following conditions are satisfied:

1. One emission has approximately constant φ_{fl1} and τ_{fl1} while the appropriate values of the second emission (φ_{fl2}, τ_{fl2}) increase.

2. In the whole range of φ_{fl2} values: $\tau_{fl1} < \tau_{fl1}$.



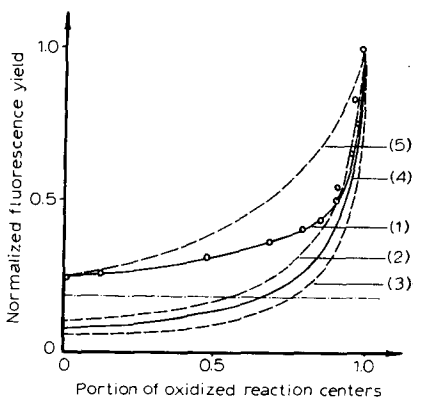


Fig. 1. Relative fluorescence yield in arbitrary units. (1), the portion of oxidized reaction centers; (2), and fluorescence lifetime of bacteriochlorophyll; (3), as a function of light intensity (actinic light of 590 nm was used). The portion of oxidized reaction centers, $P^+/(P^+ + P)$, was determined as a normalized absorption change at $\lambda = 890$ nm (characteristic maxima of long-wave bleaching for *E. Shaposhnikovii*).

Fig. 2. Normalized fluorescence yield as a function of oxidized reaction centers. (1), experimental curve for aerobic suspension of *E. Shaposhnikovii*; (2), (3), (5), theoretical curves for statistical photosynthetic unit with quantum yields of photooxidation of reaction centers 90 %, 95 %, 75 %, respectively; (4), experimental curve after substracting background fluorescence (see text). Dotted line indicates background fluorescence level.

3. The intensity of the second emission (the variable one) after increasing is of the same order of magnitude as the first one.

That is what has been observed for aerobic suspension of *E. Shaposhnikovii* (Fig. 1). Consequently, the character of light-intensity dependences of φ_{fl} and τ_{fl} suggests that at least two fluorescing species are present in the total emission of the purple bacterium investigated. Besides, φ_{fl} of the emission with large τ_{fl} does not depend on light intensity. It is likely to be the reason for a small rise in φ_{fl} observed for purple bacteria.

Two other reasons mentioned above should have required qualitatively different dependences of φ_{fl} and τ_{fl} on light intensity. If total emission had contained an appreciable component of delayed fluorescence, then τ_{fl} should have increased with light intensity much more than φ_{fl}. It is due to the fact that delayed fluorescence is characterized by lifetimes considerably exceeding 1 nsec, and this kind of emission does not contribute to the value of τ_{fl} in phase-fluorometer measurements. If photooxidized reaction centers had been traps for excitation quanta, φ_{fl} and τ_{fl} should have changed proportionally.

Some more evidence in favour of background fluorescence is the character of φ_{fl} dependence on $P^+/(P^+ + P)$ (Fig. 2). The ratio of maximum to minimum fluorescence yields corresponding to open and closed traps is about 4, which corresponds to a 75 % yield of photosynthesis (according to the formula in the beginning of this paper). But the experimental curve of φ_{fl} dependence on $P^+/(P^+ + P)$ is much steeper than the theoretical one for multicentral photosynthetic units with a quantum yield of reaction centers photooxidation, φ_{ph}, equal to 75 %. Assuming the existence of background fluorescence and subtracting it from the experimental curve, a new curve was obtained which corresponds to the theoretical one for φ_{ph} equal to 92–94 %. The criterion of the best coincidence of experimental and theoretical curves was used to determine a value of background fluorescence contribution to the total emission.

Taking into account this contribution, the lifetime of background fluorescence and that of fluorescence, whose quantum yield correlates with the state of reaction centers, were determined. The first value was 1.35 nsec, and the latter was $3–5 \cdot 10^{-11}$ sec at low light intensities and it increased to about 0.5 nsec at saturating intensities. Low τ_{fl} values for bulk bacteriochlorophyll indicate the existence *in vivo* of at least moderate molecular interaction energies. It means that excitation light energy is transferred to the reaction centers by a mechanism other than inductive resonance.

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