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Energy Transfer to the Reaction Centres in Bacterial Photosynthesis.

II. Bacteriochlorophyll Fluorescence Lifetimes and Quantum Yields for Some Purple Bacteria

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Abstract

From a combined study of (1) bacteriochlorophyll fluorescence lifetimes, (2) relative yields and (3) differential absorption changes corresponding to the reaction centres photooxidation, the absolute values of fluorescence lifetimes and quantum yields for two bacteriochlorophyll fractions have been calculated. The main bacteriochlorophyll fraction (~80-90%) serving as a light-gathering antenna for reaction centres P_{890} is characterized by dark values of fluorescence lifetimes of the order of 10^{-11} sec and fluorescence yields of 10^{-3}. The remaining part of the bulk pigment, not associated with P_{890} as far as excitation energy transfer is concerned, has an approximately constant fluorescence yield of about 5-8% and lifetime of about 10^{-9} sec. Basing on these results, excitation jump times and intermolecular coupling energies were estimated to be 10^{-13} sec and 10^{-2} ev respectively. The conclusion is made that excitation energy transfer in the main part of bacteriochlorophyll occurs by the exciton mechanism at moderate intermolecular energies.

Introduction

In the previous paper [1] evidence was presented in favour of background fluorescence existence in some purple bacteria. This emission is difficult to separate from photosynthetic fluorescence, the emission whose quantum yield correlates with the reaction centres functional state, as their spectral distributions nearly coincide. In the conditions of active photosynthesis (all the reaction centres are open)

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the fluorescence emissions of the three purple bacteria investigated largely consist of the background component. The main purposes of the present investigation were: (a) to obtain the quantitative fluorescent characteristics of photosynthetic and background emissions; (b) basing on these results, to consider the mechanisms of energy transfer from the bulk pigment to the reaction centres.

Materials and Methods

Purple bacteria *Ectothiorhodospira shaposhnikovii, Chromatium minutissimum* and *Rhodopseudomonas spheroides* were grown anaerobically in light as described elsewhere [1,2]. Bacteria were suspended in their culture media. All experiments were carried out with 2-3 day-old aerobic cell suspensions. Aerobiosis was provided by flowing oxygen through the cell suspensions for 10-15 min. The light-induced changes in relative fluorescence yields and the absorption changes, corresponding to photooxidation of the reaction centres, were registered as described elsewhere [1-3]. The bacteriochlorophyll fluorescence lifetimes were measured with a phase-type fluorometer (a modified version of the instrument described in [4]) operating at the frequency of 12.3×10^6 Hz. Time resolution of the instrument is 0.03 nanosecond in strong light when fluctuation noises may be neglected. In our experiments with purple bacteria, the instrument sensitivity decreased three times because of low values of fluorescence yields measured. Besides the quantum yields of photoeffect of photomultipliers used in the optical region around 900 nm (where purple bacteria fluorescence) are rather low. Nevertheless, it was possible to measure the lifetimes with the accuracy of 0.05 nanosecond making 4-5 separate measurements. The excitation was achieved with 404 nm line of a high-pressure 250 W mercury lamp. Time constant of the phasemeter used was 1.5 sec.

Results

It was shown in the previous publication [1] that in our particular case the measured value of the fluorescence lifetime (τ_m) of two component emission can be expressed as:

$$\tau_m \simeq \frac{J_{bg}\tau_{bg} + J_{ph}\tau_{ph}}{J_{bg} + J_{ph}}, \qquad (1)$$

where J_{bg}, J_{ph} are fluorescence intensities of the background and photosynthetic emissions respectively, τ_{bg}, τ_{ph} are respective lifetimes.

It can be easily shown that, according to the model of Vredenberg and Duysens [5], τ_{ph} is given by:

$$\tau_{ph} = \frac{1}{k_f + k_d + k_{ph}\left(1 - \frac{P^+}{P^+ + P}\right)} = \frac{\hat{\tau}_{ph}}{1 + \alpha\left(1 - \frac{P^+}{P^+ + P}\right)}, \tag{2}$$

where

$$\hat{\tau}_{ph} = \frac{1}{k_f + k_d}$$

is the lifetime of photosynthetic emission in the conditions of saturated photosynthesis when a portion of the photooxidized traps $P^+/P^+ + P$ is nearly equal to unity;

$$\alpha = \frac{k_{ph}}{k_f + k_d}$$

is the ratio of the rate constant for singlet excitation trapping (k_{ph}), to the sum of those for the radiative (k_f) and nonradiative (k_d) self-quenchings.

It can be derived from equations (1) and (2) that

$$\tau_m \simeq \frac{J_{bg}\tau_{bg} + J_{ph}\check{\tau}_{ph} \cdot \frac{1}{\left(1 - \frac{\alpha}{\alpha+1} \cdot \frac{P^+}{P^+ + P}\right)}}{J_{ph} + J_{bg}}, \tag{3}$$

where

$$\check{\tau}_{ph} = \frac{\hat{\tau}_{ph}}{\alpha + 1}$$

is the photosynthetic fluorescence lifetime in the conditions of active photosynthesis

$$\left(\frac{P^+}{P^+ + P} \simeq 0\right).$$

Relation (3) was used by us to obtain the separate values of τ_{ph} and τ_{bg}. It follows from equation (3), that two measurements of fluorescence lifetimes at different light intensities (i.e. different concentrations of closed, in our case oxidized [1], traps) should be carried out together with the determination of parameter α and the ratio of the photosynthetic and background fluorescence intensities. The best accuracy of τ_{ph}, and τ_{bg} determinations can be achieved at the extreme points of active and saturated photosynthesis. From equations (3) it can be easily derived:

$$\check{\tau}_m = \frac{\tau_{bg} + \frac{J_{ph}}{J_{bg}}\check{\tau}_{ph}}{1 + \frac{\check{J}_{ph}}{J_{bg}}} \tag{5}$$

$$\hat{\tau_m} = \frac{\tau_{bg} + \frac{\hat{J_{ph}}}{J_{bg}} \hat{\tau_{ph}}}{1 + \frac{\hat{J_{ph}}}{J_{bg}}}, \tag{6}$$

where $\check{\tau_m}$ and $\hat{\tau_m}$ are the measured lifetime values under active and saturated photosynthesis respectively; $\check{J_{ph}}/J_{bg}$ and $\hat{J_{ph}}/J_{bg}$ are the respective ratios of photosynthetic and background fluorescence intensities. As $\check{\tau_{ph}} = \hat{\tau_{ph}}/(\alpha+1)$, equation (6) can be transformed.

$$\hat{\tau_m} = \frac{\tau_{bg} + \frac{\check{J_{ph}}(\alpha+1)}{J_{bg}} \cdot \check{\tau_{ph}} \cdot (\alpha+1)}{1 + \frac{\check{J_{ph}}(\alpha+1)}{J_{bg}}}. \tag{7}$$

The system of two equations, (5) and (7), with two unknown quantities allows determinations of lifetimes τ_{ph} and τ_{bg} to be made. All the necessary experimental investigations were carried out and the results obtained are summarized in Table 1. The procedures of parameter α determination, or the quantum yield of singlet excitation trapping, φ_{ph}, as

$$\alpha = \frac{\varphi_{ph}}{1-\varphi_{ph}},$$

as well as the ratio of the photosynthetic and background fluorescences at different light intensities were described in detail elsewhere [3].

The lifetimes of the photosynthetic emissions are shown to be of the order of 10^{-11} sec in the conditions of active photosynthesis. At saturating light both the fluorescence lifetime and yield of this emission rise 10 times and more. The lifetimes of background emissions are determined to be 0.7-1.4 nanoseconds. The absolute quantum yields of both emissions, estimated from the relation

$$\varphi = \frac{\tau}{\tau_0}$$

(τ_0-bacteriochlorophyll (BChl) intrinsic lifetime is considered to be 18 nsec according to [6]), are given in Table II. In the conditions of active photosynthesis, the fluorescence yields of photosynthetic emission do not exceed 10^{-2} and those of background emission amount to several per cent.

The relative portion of the molecules emitting background and photosynthetic fluorescences was determined from the BChl fluorescence increase under transition to the saturated photosynthesis. The main assumptions made are that the molar extinctions of the two bacteriochlorophyll forms in the range of excitation light wavelength

TABLE I

Culture	φ_{ph}	$\check{\tau}_m$ nsec	$\hat{\tau}_m$ nsec	$\check{J}_{ph}/J_{bg}$	$\hat{J}_{ph}/J_{bg}$	$\check{\tau}_{ph}$ sec	$\hat{\tau}_{ph}$ sec	τ_{bg} nsec
E. shaposhnikovii	0.92 ± 0.03	1.25 ± 0.05	0.9 ± 0.05	0.08 ± 0.02	0.95 ± 0.05	$(4 \pm 1{,}5) \times 10^{-11}$	5×10^{-10}	1.35 + 0.1
Chr. minutissimum	0.90 ± 0.03	0.70 ± 0.05	0.35 ± 0.05	0.35 ± 0.02	3.5 ± 0.05	$(2 \pm 1) \times 10^{-11}$	2×10^{-10}	0.9 ± 0.1
Rh. spheroides	0.90 ± 0.03	0.65 ± 0.05	0.35 ± 0.05	0.13 ± 0.02	1.3 ± 0.05	$(7 \pm 3) \times 10^{-12}$	7×10^{-11}	0.8 ± 0.1

TABLE II

Culture	$\check{\varphi}_{ph}$	$\hat{\varphi}_{ph}$	φ_{bg}	Δ_{bg}	Δ_{ph}	$\check{\varphi}_m$	$\hat{\varphi}_m$
E. shaposhnikovii	2×10^{-3}	2.5×10^{-2}	0.07	0.10	0.90	1×10^{-2}	3×10^{-2}
Chr. minutissimum	8×10^{-4}	8×10^{-3}	0.05	0.15	0.85	0.8×10^{-2}	1.5×10^{-2}
Rh. spheroides	6×10^{-4}	6×10^{-3}	0.04	0.09	0.91	0.6×10^{-2}	1.2×10^{-2}

(404 and 590 nm) 'are approximately equal as well as the intrinsic lifetimes. As spectral distributions of the background and photosynthetic fluorescences nearly coincide in above-mentioned conditions, the fluorescence intensities registered are proportional to the amount of the appropriate molecules and their fluorescence yields. The values obtained for the portion of the background molecules, Δ_{bg}, for the three purple bacteria are presented in Table II. Although the portion of the background molecules does not exceed 10-15%, their fluorescence emissions are predominant at low light intensities when all the reaction centres are open.

Consequently according to our data, the measured values of the purple bacteria fluorescence yields at the active ($\check{\varphi}_m$) and saturated ($\hat{\varphi}_m$) photosynthesis are equal to:

$$\check{\varphi}_m = \Delta_{bg}\varphi_{bg} + \Delta_{ph}\check{\varphi}_{ph} \tag{8}$$

$$\hat{\varphi}_m = \Delta_{bg}\varphi_{bg} + \Delta_{ph}\hat{\varphi}_{ph}. \tag{9}$$

The values of $\check{\varphi}_m$ and $\hat{\varphi}_m$ calculated according to (8) and (9) are presented in Table II. They are two-three times lower than the values obtained by Wang and Clayton [7] for the absolute fluorescence yields of some purple bacteria.

Discussion

The most important conclusion of the present investigation is the fact that the main part of the light-harvesting BChl of purple bacteria is characterized by extremely low fluorescence yield and lifetime. The values reported in the previous works [8,9] seem to refer mainly to the background component. In contrast to our data, Govindjee *et al.* [10] observed an increase in τ_m values for two purple bacteria with increasing excitation light intensity. We can suggest some reasons for the divergence between these and our data. BChl does not practically absorb light at $\lambda = 488$ nm used in [10], and its main part is likely to be absorbed by carotenoids. But their extinction coefficients are several times lower than that of BChl and besides the efficiency of energy transfer from carotenoids to BChl is known to be not high [11,12]. So, excitation light intensities, providing equal energy trapping, may differ greatly in our and Govindjee's experiments. Besides the light absorbed by carotenoids is known to induce an anomalous behaviour of BChl fluorescence in purple bacteria [13].

The experimental data obtained enable us to elucidate the mechanisms of energy transfer in the main BChl part of purple bacteria.

Singlets or triplets provide energy transfer to the reaction centres?

The discovery of Vredenberg and Duysens [5] that the purple bacteria fluorescence increases as the reaction centres become photooxidized led to the conclusion that excitation energy is transferred to the reaction centres via singlet states. However, as it was pointed out in [1], the increase reported in a number of works was not more than 2-3 times; thus the possibility could not be excluded that 33-50% of energy reached the reaction centres via triplet states. The data presented above (see also [1,3]) show that the fluorescence of the main part of light-harvesting pigment increases 10 times and more under transition from active ($P^+ \gg P$) to saturated ($P^+ \ll P$) photosynthesis. According to the model of Vredenberg and Duysens, in such a case the quantum yield of singlet excitation trapping by P_{890},φ_{ph}, should exceed 0.9 (see refs. [1] equation (1)). The latter fact indicates that the maximal value of the quantum yield of triplet formation is less than 0.1, and, consequently, it may be neglected in the net balance of energy transfer processes in purple bacteria.

The Mechanism of Singlet Excitation Energy Transfer

It is currently assumed that the migration of singlet excitation energy occurs by the inductive resonance (or Förster-type) mechanism. However, according to the main criterion of this mechanism applicability, excitation jump times (τ_j), i.e. the time intervals between two successive excitation transfers, should considerably exceed energy relaxation time. The latter value is of the order of $10^{-11}-10^{-13}$ sec, for organic systems at room temperature [14]. We estimated τ_j for bacteriochlorophyll *in vivo* using the fluorescence lifetime values τ_{ph}° for active photosynthesis and random walk calculations for energy trapping in photosynthetic units by Robinson [15]. According to these calculations the mean value of excitation jumps (n), preceding excitation trapping by the reaction centres is equal to (if the efficiency of excitation trapping is considered to be 100%):

$$n = 0.72\, N \log N + 0.26\, N,$$

where N is the mean number of bulk BChl molecules per active reaction centre. As excitation energy is mainly transferred to the reaction centres via singlet states, the following relationship is valid:

$$\tau_j = \frac{\tau_{ph}}{0.72\, N \log N + 0.26\, N} \qquad (10)$$

The dark values of N amount to 180-220 for aerobic cultures of *E. shaposhnikovii, Chr. minutissimum* and *Rh. spheroides.* Such values of N are in close agreement with those obtained by Thornberg [16], with

Chromatium D, but somewhat higher than those obtained by Clayton [17] for the blue-green mutant of *Rh. spheroides.* The excitation jump times, estimated from equation (10), are of the order of 10^{-13} sec for all bacteria investigated. We also calculated intermolecular coupling energies (W) from the uncertainty principle:

$$W\tau_j \simeq \hbar,$$

$\hbar$ is Planck's constant divided by 2π.
The values of W obtained are of the order of 10^{-2} ev. If the efficiency of trapping is less than 100%, τ_j should decrease and W increase correspondingly. For the 1% efficiency of excitation trapping suggested by Robinson, τ_j should be $\simeq 10^{-15}$ sec and $W \simeq 1$ ev. These values are inconsistent with the spectral characteristics of BChl *in vivo*. Hence, the efficiency of excitation trapping is not less than several dozens of per cent.

The data presented above show that the rates of excitation energy transfer in the main part of purple bacteria BChl are too high to fit Förster's mechanism. Energy transfer to the reaction centres of purple bacteria is likely to be performed by the exciton mechanism at moderate intermolecular coupling energies [18].

The same conclusion appears to be valid for pigment system I (PS-I) of plants which is known to fluoresce with an extremely low quantum yield. It should be mentioned that this system and that of photosynthesizing bacteria are similar in some functional and structural aspects. Although, according to the previous data, the BChl fluorescence yield of purple bacteria by far exceeded that of PS-I chlorophyll of plants [7, 19]. The present investigation indicates that these pigment systems also resemble one another with respect to their fluorescent characteristics.

References

1. A. Yu. Borisov and V. I. Godik, *J. Bioenergetics,* 3 (1972) 211.
2. A. Yu. Borisov and V. I. Godik, *Biochim. Biophys. Acta,* 223 (1970) 441.
3. E. L. Barsky and A. Yu. Borisov, *J. Bioenergetics,* 3 (1972) 275.
4. A. Yu. Borisov and L. A. Tumerman, *Izvestiya Akademii Nauk SSSR, ser. fiz.* 23 (1959) 97.
5. W. J. Vredenberg and L. N. M. Duysens, *Nature,* 197 (1963) 355.
6. K. L. Zankel, D. W. Reed and R. K. Clayton, *Proc. Natl. Acad. Sci.,* 61 (1968) 1243.
7. R. T. Wang and R. K. Clayton, *Photochem. Photobiol.,* 13 (1971) 215.
8. A. B. Rubin and L. K. Osnitskaya, *Mikrobiologiya* 32 (1963) 200.
9. L. A. Tumerman and A. B. Rubin, *Dokladi Akademii Nauk SSSR,* 145 (1962) No. 1.
10. Govindjee, J. H. Hammond and H. Merkelo, personal communication.
11. L. N. M. Duysens, *Thesis,* Utrecht, 1952.

12. T. G. Ebrey, *Biochim. Biophys. Acta,* 253 (1971) 385.
13. J. C. Goedheer and Van Der Tuin A. K., *Biochim. Biophys. Acta,* 143 (1967) 399.
14. T. Förster, Comparative effects of radiation, *Rept. Cons. San Juan,* 1960 (1960) 300.
15. G. Robinson, *Brookhaven Symp. Biol.,* 19 (1967) 16.
16. J. P. Thornber, *J. Biochemistry,* 9 (1970) 268.
17. R. K. Clayton, *Photochem. Photobiol.,* 1 (1962) 201.
18. W. T. Simpson and D. L. Peterson, *J. Chem. Phys.,* 26 (1957) 588.
19. L. N. M. Duysens, *Proc. Roy Soc. B,* 157 (1963) 301.