EXCITATION TRAPPING BY DIFFERENT STATES OF PHOTOSYNTHETIC REACTION CENTRES

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1. Introduction

Fluorescence of light-harvesting bacteriochlorophyll (BChl) of photosynthetic bacteria displays a variable yield, the reciprocal being dependent linearly on the portion of active reaction centres (RC) [1,2]. If the RC is in a photochemically active state, it traps singlet (S) excitation energy from antenna BChl in some tenth of a picosecond [3]. S–S type of energy migration to the RCs predicts the following relation between the quantum yield of primary energy trapping by active RC (φ_{ph}) and maximal increase in fluorescence yield (φ_{fl}) under transition from active (all RCs are open) to saturated (all RCs are closed) photosynthesis (see, e.g., [3]):

$$\frac{\varphi_{fl}^{max}}{\varphi_{fl}} = \frac{1}{1-\varphi_{ph}} \qquad (1)$$

where φ_{fl} and φ_{fl}^{max} are fluorescence yields for active and saturated photosynthesis, respectively.

The relation (1) is correct only for RCs in the closed states to be absolutely nonquenching, as was specified in the model of Vredenberg- Duysens [1]. A striking discrepancy is observed between the values of φ_{ph}, determined from relation (1), and measured ones. The ratio $\varphi_{fl}^{max}/\varphi_{fl}$ usually observed for photosynthetic bacteria is equal to 2–3, which corresponds to φ_{ph} = 0.5–0.67, but measured values of φ_{ph} are much higher [4]. It has previously been pointed out [5] that underestimation of φ_{ph} from relation (1) may arise from the heterogeneous nature of fluorescence emission, i.e., the existence of some background, non-sensitive of RC redox state fluorescence, together with variable one.

This paper will show that this is not the only reason for the above discrepancy. Experimental evidence will be presented that unequivocally proves efficient excitation quenching by RCs in the closed states.

The model of Vredenberg-Duysens will be revised to accomodate the data obtained.

2. Materials and methods

Non-sulfur purple bacteria *Rhodospirillum rubrum* of wild type were grown semi-anaerobically in the medium of Cohen-Bazire et al. [6]. The chromatophores were isolated by ultrasonic treatment of washed bacterial cells as described in [7] and suspended in 50 mM potassium phosphate (pH 7.0).

For isolation of pigment–protein complexes of light-harvesting chlorophylls free of RCs (antenna complexes) the chromatophore suspensions were subjected to successive 4-fold solubilization with 0.25–0.30% lauryl-dimethylamine oxide. All operations were performed at 0°C in the dark. A detailed description of the whole procedure and the flow diagram is presented in [8]. The concentration of RCs in the isolated antenna complexes was negligible (not more than one per 10^3 bacteriochlorophyll).

For the measurements under reducing conditions the chromatophore suspensions were reduced with $\simeq$ 50 mM of sodium dithionite. Maintaining of the redox potential of the medium within the range of 500–100 mV was performed in a special cuvette with

the use of platinum and silver chloride electrodes.

The number of dark oxidized RCs and those produced by light saturation under different conditions was determined with the instrument, described in [9].

Fluorescence lifetime measurements were performed with a home-made phase-type fluorimeter [10]. The time resolution of the instrument was 30–50 ps. For measurements in picosecond time scale a special method (time-lever method) was applied [10,11]. Fluorescence spectra were recorded with an Aminco-Bowman spectrofluorimeter supplied with an 7102 photomultiplier.

3. Results and discussion

Possible states of the reaction centres of photosynthetic bacteria can be classified in the following manner:

I. PA_1 – active state of RC

II. P^+A_1

III. $P^+A_1 \ldots\ldots A_n^-$

IV. $P^+A_1^-$

V. PA_1^-

} in active states of RC

P, P^+ are RC bacteriochlorophyll in the normal and oxidized states; A_1, A_n ($n \geqslant 2$) are successive electron carriers of nonporphyrine nature.

The problem to be solved can be formulated as follows: when RCs turn from photosynthetically active state I into one of the closed states II–V (by light saturation, changing of redox conditions etc.), whether the decay of photoinduced excited states occurs mainly in antenna pigment complex by trivial radiative and nonradiative mechanisms or alternatively excitation quenching continues to proceed in the closed RCs (or in their close vicinity).

In order to choose between these possibilities the measurements of fluorescence lifetime (τ_{fl}) and yield (φ_{fl}) were performed for *Rh. rubrum* chromatophores with RCs maintained in one of states I–V and also for antenna pigment complexes devoided of RCs. It was expected that the kinetics of excitation decay would be similar for chromatophores with all types of closed RCs and antenna complexes, if the first possibility is realized in vivo. On the contrary for the second case of excitation quenching by closed RCs, excitation decay constants for chromatophores with closed RCs of any type should be different from those for antenna complexes.

Aerobic suspensions of *Rh. rubrum* chromatophores, prepared as described in Materials and methods, with no additions, have nearly 80% of RCs in state II (P^+A) in the dark and the corresponding electron transport chains are devoided of electrons. In order to convert the main part of RCs to the active state PA redox agents should be added in order to have E_m of the medium within 250–350 mV. Measurement of τ_{fl} under these conditions by time-lever method [9,10] gives the values of 30–50 ps at low light intensity. Transition to the saturated light in this case converts RCs to one of the closed states, III($P^+.A_1 \ldots A_n^-$), or IV ($P^+A_1^-$). What states III or IV are realized depends on the length of the working part of electron transport chain, number of electrons in it and localization of the narrow site. Usually light saturation of chromatophores poised at moderate potentials produces RCs in state III. Under these conditions, τ_{fl} of *Rh. rubrum* chromatophores was found to be approximately equal to 0.5 ns (table 1). Slightly different values of τ_{fl} and relative fluorescence yield φ_{fl} were registered for chromatophores with RCs in state P^+A, the latter being produced in dark by prolonged (about half an hour) blowing of oxygen through chromatophore suspension.

One of the ways to maintain RCs in state $P^+A_1^-$ at saturating light is to shorten the cyclic chain and increase the number of electrons in it. Just the lowering of the redox potential would produce state PA_1^- rather than $P^+A_1^-$. TMPD in the oxidized form is known to shorten drastically the cyclic chain of photosynthetic bacteria by shunting it between ubiquinone and RC bacteriochlorophyll. Addition of 10^{-4} M TMPD together with an excess of electron donors (1 mM ferrocyanide) made it possible to maintain RCs in $P^+A_1^-$ state. The other ways of converting RC to closed state IV are photoexcitation of chromatophores under conditions of low (77°C) temperature and addition of 1 mM *O*-phenantroline to previously washed chromatophore suspensions. Fluorescence life-times of chromatophores under all these conditions were measured to be $1–1.5 \cdot 10^{-10}$ s. This

Table 1

RC state		τ_{fl} ns[a]	φ_{fl} rel. units	K_{tr} s^{-1}
I	PA	0.03–0.05	0.05	$2–3.5 \cdot 10^{10}$
II	P^+A	0.5	0.25	$2 \cdot 10^9$
III	$P^+ \ldots A_n^-$	0.4	0.21	$2.5 \cdot 10^9$
IV	$P^+A_I^-$	0.12–0.15	0.10	$6–8 \cdot 10^9$
V	$P A_I^-$	3.0	0.06	$1.5–2 \cdot 10^{10}$
Antenna complex		1.0 –1.5	1.0	$0.65–1 \cdot 10^9$

[a] τ_{fl} and φ_{fl} for closed states II–V were measured in saturating blue light ($5 \cdot 10^4$ erg/cm^2·s)

Fluorescence was measured with the combination of filtres, cutting off the range of $\lambda \leqslant 760$ nm. τ_{fl} and φ_{fl} values, presented in the table, are mean values of five independent measurements

is the shortest lifetime recorded for *Rh. rubrum* chromatophores with closed RCs. The results of the measurements of fluorescence yields are in full accord with the lifetime data (table 1).

Antenna complexes (pigment–protein complexes of light-harvesting BChl devoided of RC) with fluorescence spectra indistinguishable from chromatophores have their τ_{fl} and φ_{fl} exceeding by far those for chromatophores with any type of closed RCs. None of the treatments used (changing of light intensity, addition of TMPD, dithionite, K_4FeCN_6 etc.) has any effect on their τ_{fl} or φ_{fl}, the latter being 1–1.5 ns.

It is appropriate to mention here the puzzling observation of Clayton [12] that a mutant strain of *Rhodopseudomonas sphaeroides* PM-8 dpl lacking RCs has φ_{fl} more than twice the yield from wild type Ga even under light saturation, which is in line with the conclusions of the present work. The recent direct measurements of τ_{fl} in this organism by impulse fluorometry [13] give the value of 1.1 ns in close agreement with our measurements of τ_{fl} for antenna complexes from *Rh. rubrum*.

State PA^- needs special consideration. It is obtained under conditions of low potential, for example, after reducing the suspensions with excess of solid $Na_2S_2O_4$. The values of $\tau_{fl} \simeq 3$ ns recorded in these conditions are abnormally great even in comparison with antenna complexes. Light excitation of RCs in state PA^- has recently been shown [14, 15] to be followed by a short-lived state P^F, indicating charge separation in BChl–BPh complex. Long-lived fluorescence, recorded in these conditions represents a radiative decay of the state P^F (see forthcoming publication). This fluorescence of delayed type, amounts to about one half of the total emission. The value of φ_{fl} presented in table 1 for state PA^- corresponds to the yield of prompt fluorescence and is nearly the same as that for active RCs.

For S–S mechanism of excitation transfer to the RCs τ_{fl} of antenna Chl gives the mean interval between light absorption and excitation trapping by RC and hence, its reciprocal represents the mean macroscopic rate constant of excitation trapping (K_{tr}). In the last column of table 1 the values of K_{tr} are presented for each type of RC state and antenna complex. For the latter case K_{tr} evidently designates the mean rate constant of trivial quenching of excitations by BChl antenna molecules. The data obtained indicate that RCs even in the closed states serve as traps for excitation quanta. Each individual type of RC I–V is shown to be characterized by its own K_{tr}, different from the rate constant of excitation quenching in antenna pigment complex. The extent of quenching qualitatively correlates with the magnitude of local electrical potential, which is inherent in all states II–V. The most pronounced quenching is characteristic of state $P^+A_I^-$, which is known to produce electrical potentials up to $7 \cdot 10^6$ V/cm [16].

Consequently, the reason for the quenching properties of RCs in the closed states lies, in all probability, in perturbance action of local electrostatic fields on non-radiactive transitions of nearby BChl molecules.

Taking into consideration the quenching properties of the closed RCs, the relationship of Vredenberg and Duysens [1] between φ_{fl} and RC redox state:

$$\frac{1}{\varphi_{fl}} = A + B\,[P_a]$$

should be changed into:

$$\frac{1}{\varphi_{fl}} = A + B\,[P_a] + C[P_i]$$

where P_a, P_i are RC concentrations in active and inactive states correspondingly. A,B,C are constants. As a result, the above relation (1) (see Introduction) between φ_{ph} and increase in φ_{fl} should also be changed to:

$$\frac{\varphi_{fl}^{max}}{\varphi_{fl}} = \frac{1}{1 - \varphi_{ph} + \dfrac{K_{tr}}{K_{\Sigma} + K_{ph}}} \simeq \frac{1}{1 - \varphi_{ph} + \dfrac{K_{tr}}{K_{ph}}} \qquad (2)$$

where K_{Σ} is the rate constant of overall energy losses in antenna comples; K_{tr} the rate constant of excitation quenching by closed RCs. Application of (2) to the different states of closed RCs achieved by light saturation of *Rh. rubrum* chromatophores (table 1) gives for $\varphi_{ph} = 0.9$, 2–5-fold increases in φ_{fl} that are in close agreement with experimental evidence.

The existence of excitation quenching by RCs in the closed states may account for the absence of significant fluorescence increases in PS-I of plants under transition to light saturation. The complex time behavior of fluorescence yield in *Chlorella*, excited by a single saturating flash, observed by Mauzerall [17], with several rises and drops, may reflect φ_{fl} quenching by different RC states produced by successive electron movement through the photosynthetic chain of electron carriers.

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Note added in proof

While this manuscript was in preparation the paper of P. Heathcote and R. K. Clayton appeared in Biochim. Biophys. Acta 459 (1977) 506–515, where the quenching properties of closed RCs were established for *Rhodopseudomomas sphaeroides.*

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