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BRIEF COMMUNICATION

Lifetime of the Excited States *in Vivo* IV. Bacteriochlorophyll and Bacteriopheophytin in *Rhodospirillum rubrum**

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Abstract

Lifetime of excited states (τ) for bacteriopheophytin is higher than for bacteriochlorophyll in reaction center preparation from *Rhodospirillum rubrum*, and this may be the reason why τ with 890 nm interference filter is slightly lower than with *RG-10* filter. τ with 488 nm excitation is higher than with 633 nm excitation. As this difference exists also in the carotenoidless mutant of *R. rubrum* it cannot be due to the transfer time from carotenoids to bacteriochlorophyll. Its possible reason is the difference in energy $\Delta E(= W\text{-}h\nu$, where $W =$ photocathode work function, and $h\nu =$ excitation photon energy) as the phase shift was measured from the difference between the scattering (at 488 nm) and fluorescence (~900 nm).

Reaction center preparations from photosynthetic bacteria are being used in various laboratories for the study of primary reactions in these organisms (see CLAYTON 1973; SAUER 1975). An understanding of the primary photochemical events requires that we know the nature of the excited states delivering energy from the antenna pigments to special converting centers. Some information can be obtained from the measurements of lifetime of excited states (τ) of intact cells (see review by BORISOV and GODIK 1973). GOVINDJEE *et al.* (1972) showed that τ for bacteriochlorophyll, upon excitation with 488 nm, was 1.0 ns for *R. rubrum* and C. *ethylicum* cells, and 2.5 ns for *R. viridis* cells and chromatophores at high excitation intensity. Several questions including the following remained unanswered: (*1*) whether the use of sharp cut-off red filters, employed in the above study, passed only bacteriochlorophyll fluorescence or also passed bacteriopheophytin or other fluorescence; (*2*) whether the use of 488 nm light gave higher lifetimes due to the fact that transfer times from carotenoids to bacteriochlorophyll were included in these measurements (carotenoids absorb significantly at 488 nm). To answer the first question, we have measured lifetimes by collecting fluorescence with *RG-10 Schott* filters and an 890 or 908 nm interference filter ($F =$ 890 or 908 nm) in *R. rubrum* cells, a carotenoidless mutant of *R. rubrum* cells, *R. spheroides* cells, and large reaction center preparation from *R. rubrum* (SMITH 1972) that has a much higher ratio of bacteriophytin to bacteriochlorophyll than the intact cells. In the latter case, a 788 nm interference filter was also used to collect bacteriopheophytin fluorescence. To answer the second question, we measured lifetimes by exciting, with 488 nm and 632.8 nm light (that does not excite carotenoids), cells of *R. rubrum*, a carotenoidless mutant of *R. rubrum*, *R. viridis* and *R. spheroides*.

All the bacteria were grown as described previously by other authors (see references in GOVINDJEE *et al.* 1972). However, the cells used here were of different physiological age; comparisons

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are reported for identical samples. Reaction center preparations were made as described by SMITH (1972). The experimental procedures and the apparatus have been described by GOVINDJEE *et al.* (1972). The frequency of modulation of the actinic laser beams was 75 Mhz, the photomultiplier was *RCA 7102*, and the measurements were made at room temperature. [BChl] was 5—30 μg ml^{-1} suspension. The incident radiant energy was about 500 W m^{-2} (488 nm) and 10 000 W m^{-2} (633 nm); both these irradiances were high and there was no change in τ when the 633 nm irradiance was decreased to 500 W m^{-2}. All the reaction center preparations were reduced by added sodium dithionite ($Na_2S_2O_4$). Thus, all measurements, reported here, are for τ max.

Table 1

Lifetime of excited states in photosynthetic bacteria at room temperature. Photomultiplier: *RCA 7102*; modulation frequency: 75 Mhz (for both 488 and 633 nm excitation); filters: *2RG10 Schott* (*RG10*) or interference filter (F = 890 or 908 nm) used to block scattered laser light from the photomultiplier; average of five experiments.

Organism	τ[ns ± 0.15 ns]		
	λ_{ex} = 488 nm (*RG10*)	λ_{ex} = 633 nm (*RG10*)	λ_{ex} = 633 nm (F = 890 or 908 nm
R. rubrum	1.26	1.06	0.96
R. rubrum (carotenoidless mutant)	1.71	1.25	0.92
R. viridis	1.59	1.49	—
R. spheroides	1.16	0.96	0.98

For intact bacterial cells of the species tested, τ with 633 nm irradiance is always lower than for τ with 488 nm irradiance (Table 1). In changing from *RG-10* filter to 890 nm interference filter, τ decreases for *R. rubrum* and its carotenoidless mutant; in *R. spheroides* no significant change is observed. Hence the difference in τ obtained with 488 nm or 633 nm excitation cannot be ascribed

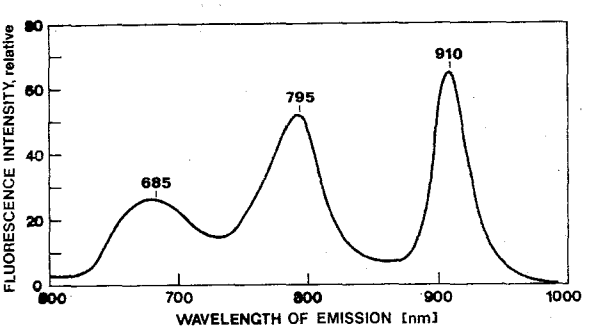

Fig. 1. Fluorescence emission spectrum of dithionite-reduced "large" reaction center preparation from *Rhodospirillum rubrum* (for the method of preparation see SMITH 1972) at 77 K. Actinic illumination: broad-band blue light, 10 W m^{-2}; measuring conditions: *C.S.2—58* filter; halfband width, 6.6 nm; photomultiplier, *RCA 7102*. The peaks at 910, 795, and 685 nm are due to reaction center bacteriochlorophyll, bacteriopheophytin, and solubilized bacteriochlorophyll (?), respectively.

to energy transfer time from carotenoids to bacteriochlorophyll. It could be due to the difference in the energy, ΔE, between the photocathode work function, W, and the excitation photon energy $h\nu$, as scattering is measured at 488 nm and at 633 nm whereas fluorescence at ~900 nm. This difference may be especially true for S1 photocathodes since they are of multi alkali compositions. A fluorescent component (perhaps bacteriopheophytin; see later) contributes to higher τ in *R. rubrum* when it is measured with *RG-10* filters than with 890 nm interference filters.

Table 2

Lifetime of excited states in reduced large reaction center preparations for *R. rubrum*. Photomultiplier: *RCA 7102*; modulation frequency: 75 Mhz; λ exciting = 633 nm; average of five experiments.

Reaction center preparation	Fluorescence	τ [ns ± 0.15 ns]
Without cytochrome	Bacteriopheophytin + bacteriochlorophyll (Filters *RG10* + *RG8*)	1.8
	Bacteriopheophytin (Filter: *F 788*)	2.1
	Bacteriochlorophyll (Filter: *F 890*)	1.5
With cytochrome	Bacteriopheophytin + bacteriochlorophyll (*RG10* + *RG8*)	1.8
	Bacteriopheophytin (*F 788*)	2.1
	Bacteriochlorophyll (*F* 890)	1.5

In order to test the latter point, we used large reaction center preparations that contain a higher proportion of bacteriopheophytin to bacteriochlorophyll than the intact cells. Fluorescence spectrum of a reaction center preparation at 77 K, as excited with broad band blue light (Fig. 1), allowed us to select the appropriate filters to measure bacteriopheophytin (788 nm) and bacteriochlorophyll fluorescence (890 or 908 nm). τ values for two reaction center preparations (one of them without the cytochromes) with 890 nm and 788 nm filters were 1.5 ns, and 2.1 ns, respectively (Table 2). (All measurements, reported for 890 nm, were confirmed at 908 nm, although the values at 908 nm were lower by 0.1 to 0.2 ns.) Thus, τ of bacteriopheophytin in such preparations was higher (2.1 ns) than of bacteriochlorophyll (1.5 ns). This is why when *RG-10* filters, that transmit fluorescence from both bacteriopheophytin and bacteriochlorophyll, show an intermediate value of τ (1.8 ns). If the reaction center preparations were stored in the dried state after lyophilization, and then resuspended in buffer before use, they showed a slightly larger τ but the general effect mentioned above was confirmed as τ with 890 nm filter was 1.7 ns, with 788 nm filter 2.7 ns, and with *RG* filters 2.1 ns. These data further allow us to stress the point that storage does change the physical state of the pigments, and caution must be exercised in their use for biochemical studies.

Finally, we wish to point out that it is difficult to measure the τ of the reaction center bacteriochlorophyll because such preparations have to be extremely pure (1 BChl bulk: 10 000 or more reaction center BChl). τ for reaction center BChl is suggested to be of the order of 10^{-11} to 10^{-12} s (see ZANKEL *et al.* 1968; BORISOV and GODIK 1973). A simple calculation shows that measured τ will be dominated by that of "bulk" BChl unless the preparation contains only reaction center bacteriochlorophyll molecules. As we did not use such pure preparations, we will not comment on the τ of reaction center BChl.

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