LIFETIME OF THE EXCITED STATE IN VIVO

II. BACTERIOCHLOROPHYLL IN PHOTOSYNTHETIC BACTERIA AT ROOM TEMPERATURE

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ABSTRACT Lifetime of the excited state (τ) of bacteriochlorophyll (BChl) in photosynthetic bacteria, measured with a mode-locked argon laser (oscillating at 488 nm; mode locked at 56 MHz) as light source, ranged from 0.3 to 2.5 nsec. These τ values are reported with a precision of ± 0.1 nsec. The value of τ at high exciting light intensity (I) was two to three times that at low intensity. For young cultures of green bacterium *Chloropseudomonas ethylicum*, τ ranged from 0.5 (low I) to 1.0 nsec (high I); for those of the purple bacterium *Rhodospirillum rubrum*, from 0.4 (low I) to 1.0 nsec (high I); and for those of the BChl *b*–containing *Rhodopseudomonas viridis*, from 1.0 (low I) to 2.5 nsec (high I). These data provide information regarding the efficiencies of the photochemical process in these bacteria. Quantum yield (ϕ) of BChl fluorescence, calculated from $\phi = \tau/\tau_0$ (where τ_0 is the intrinsic lifetime of fluorescence), ranges from 2–6% at low intensities to 6–14% at high intensities.

INTRODUCTION

The primary process of bacterial photosynthesis begins with light absorption in BChl followed by energy transfer to the reaction centers where the oxidation-reduction reactions occur (see reference 1). During these events, some energy is lost as fluorescence which competes with photochemistry. The average lifetime of the excited state, measured by the rate of decay of fluorescence, is related to the quantum yield of fluorescence, ϕ, as $\tau = \phi \cdot \tau_0$, where τ_0 is the intrinsic lifetime, which can be calculated from the absorption spectra of the pigment. Thus, fluorescence lifetime values provide a measure of the quantum yield of fluorescence, in addition to providing information regarding the rate of primary photochemistry that must compete with fluorescence. (For a discussion of work on Chl *a* in green plants see reference 2.) Rabinowitch (3) calculated τ of BChl fluorescence in vivo as 0.6 nsec by assuming ϕ to be 7×10^{-3}, and τ_0 to be 80 nsec. τ measurements on photosynthetic bacteria are available only for *Chromatium vinosum* (4) and *Ectothiorhodospira Shaposhnikovii* (5). (The latter paper came to our attention only during the

preparation of this manuscript.) In these bacteria, τ ranged from 0.8 to 2 nsec. We present here new data on τ and calculated values of ϕ of BChl in three other species (*C. ethylicum*, *R. rubrum*, and *R. viridis*) selected from different groups of photosynthetic bacteria.

MATERIALS AND METHODS

All the bacteria were grown anaerobically in culture media described by previous investigators (see reference 6 for *C. ethylicum*, reference 7 for *R. rubrum*, and reference 8 for *R. viridis*). The temperature during cultivation was 30°C. Bacteria were suspended in their culture media for fluorescence experiments. Their concentrations were adjusted to give absorbance values of about 0.1–0.3 at their BChl peaks.

The experimental procedure for measuring τ was similar to that previously described (9, 10), except that excitation was provided by an argon ion laser oscillating at 488 nm (see reference 11). Intracavity modulation by acoustical standing waves in a fused quartz block produced forced mode locking with pulse widths of less than 1 nsec (12). A small portion of the beam was used to monitor the pulse and to provide a phase reference signal, the remainder entering the sample cuvette at right angles to the axis of the fluorescence-detecting photomultiplier (Fig. 1). A lens focused the laser beam when higher intensity excitation was required, whereas the unfocused beam and a set of neutral density filters were employed for lower intensity excitation. One or two CS 2-64 red cutoff filters (Corning Glass Works, Corning, N. Y.) were used in front of the photomultiplier (RCA 7102) to eliminate the exciting light and to observe fluorescence. The direct reading phase delay meter (HP-8405A) incorporated high speed sampling techniques to convert fluorescence and reference signals to an intermediate frequency of 20 kHz. Error due to time dispersion of photoelectrons arriving at the first dynode was reduced by aperturing the photomultiplier; error introduced by nulling the phase with scattered light at the excitation wavelength (rather than at fluorescence wavelengths) is assumed to be small for the lifetimes measured here. All lifetime measurements were made at room temperature, and no attempt was made to exclude air from the bacterial suspensions during measurements.

RESULTS AND DISCUSSION

The lifetime of the excited state was calculated from the phase difference (θ) between the fluorescent and the scattered light signals; the intensities were adjusted to approximately equal amplitudes. For exponential decay of fluorescence, τ is equal to $\tan \theta/2\pi f$, where $f = 56$ MHz in our experiments. In high intensity measurements, the power of incident light was greater than 0.30 mw cm^{-2} (see footnote 1); in low intensity measurements it was smaller than 0.050 mw cm^{-2}. The τ values reported have a precision of ± 0.1 nsec.

C. ethylicum

(*C. ethylicum* contains *Chlorobium* chlorophyll and some BChl *a*.) Five different cultures of this bacterium were tested. We found that τ for young cultures (2 days old) was 1.0 nsec at high light intensities and 0.5 nsec at low light intensities. In

[1] Measurements made at 1.5–3.0 mw cm^{-2} gave the same τ as reported here.

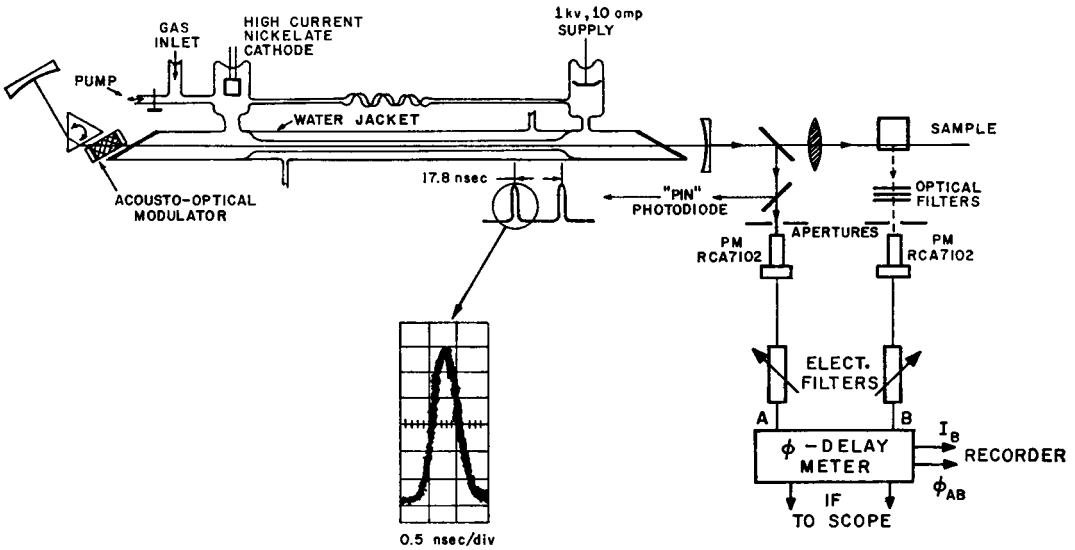

FIGURE 1 Diagram of the experimental system.

older cultures (15 days–1 month), τ decreased to 0.6 nsec for high light and 0.3 nsec for low light intensities.

R. rubrum

(*R. rubrum* contains mainly BChl *a*.) 20 different cultures of various ages were tested. We found that τ for all cultures (2–9 days old) was 1.0 nsec at high, and 0.4 nsec at low light intensities. There was no correlation between τ and the age of the culture; this was not surprising in view of the finding of deKlerk et al. (13) that, although the ratio of variable to constant fluorescence changes with age, the total fluorescence yield remains essentially the same when purple bacteria age.

R. viridis

(*R. viridis* contains BChl *b* instead of BChl *a*.) 10 different cultures of this bacterium were tested. All young cultures gave τ values of 2.5 nsec for high and 1.0 nsec for low light excitation. Chromatophores prepared from these cultures gave a τ value of 2.5 nsec at high light intensity. Contrary to our findings with *R. rubrum* (where no effect of age could be established) and with *C. ethylicum* (where τ decreased with age), we observed an increase of τ with age in *R. viridis*. For example, at medium intensities, τ, which was 1.4 nsec in a 2-day-old culture, increased to 2.4 nsec in a 15-day-old culture. The physiological significance of these differences is not yet clear. In all likelihood, they are related to changes in the rate of photochemistry that competes with fluorescence.

Table I is a summary of our τ measurements with young cultures of bacteria together with calculated quantum yields of fluorescence based on the assumption that τ_0[2] = 18 nsec for *R. rubrum* and *R. viridis* (14), and 15 nsec for *C. ethylicum*.[3] These quantum yield values cannot be properly compared with those obtained by other investigators (15, 16) because of differences in the species, the cultures, and the intensity and wavelength of exciting light. At low intensity, ϕ (calculated from τ) is about 2% in *R. rubrum*, 4% in *C. ethylicum*, and 6% in *R. viridis*. At high intensities, these values are doubled or tripled, depending upon species and culture conditions of the bacteria.

Our calculated ϕ value of 5.5% (λ excitation = 488 nm) in *R. rubrum* agrees with Wang and Clayton's (15) directly measured ϕ values of 5.5% (λ excitation, 850 nm) and 5.2% (λ excitation, 870 nm). Despite the differences in wavelength of excitation, we take this agreement as substantiation of the relationship $\tau = \tau_0\phi$ in *R. rubrum*.

The increase of τ with increasing intensity, observed in our experiments, does not agree with the data of Borisov and Godik (5) on *E. Shaposhnikovii*, who find a decrease in τ. We do not know the reason for this disagreement; however, the increase observed by us in *R. rubrum*, *R. viridis*, and *C. ethylicum* is in agreement with earlier direct measurements of Wassink et al. (16) on *Chromatium*, and of deKlerk et al. (13) on several species of *Athiorhodaceae*. These results (similar to those obtained with green plants) are easy to understand: when the chemical reactions which restore the reaction center after the primary photoprocess cannot keep pace with the influx of quanta, the yield of fluorescence, which is in competition with the primary photoprocess, rises.

Some of our calculated ϕ values (from τ measurements) appear to be higher than the directly measured values, perhaps because nonfluorescent (or weakly fluorescent) BChl may be present in these photosynthetic bacteria. Direct measurement of the quantum yield of fluorescence is difficult for several reasons, including light scattering and geometrical factors. On the other hand, ϕ computed from τ may be an "overestimate" because of possible nonfluorescent components, as noted above. If the nonfluorescent components do not contribute to photosynthesis, however, then the yield derived from τ may be more meaningful. To fully understand the difference between directly measured ϕ and ϕ calculated from τ values, simultaneous measurements on τ and ϕ on the same samples are needed.

The order of magnitude of τ (0.4–2.5 nsec) of BChl in vivo indicates clearly that the primary oxidation and reduction at the reaction centers in photosynthetic bac-

[2] $1/\tau_0 = 3 \times 10^{-9} k^2 \int \epsilon dk$, where k is the wave number (in centimeters^{-1}) for the transition, and $\int \epsilon dk$ is the area under the absorption band plotted as ϵ vs. k, and ϵ is the molar extinction coefficient (see pp. 94–95 in reference 1).

[3] Sybesma, C. Personal communication.

TABLE I

LIFETIME OF THE EXCITED STATE AND (CALCULATED) QUANTUM YIELD OF FLUORESCENCE OF BACTERIOCHLOROPHYLL IN YOUNG PHOTOSYNTHETIC BACTERIA*

Bacteria	Lifetimes		Calculated ϕ‡	
	Low *I*	High *I*	Low *I*	High *I*
	nsec	*nsec*		
R. viridis cells	1.0	2.5	0.055	0.14
R. viridis chromatophores	—	2.5	—	0.14
R. rubrum cells	0.4	1.0	0.022	0.055
C. ethylicum cells	0.6	1.0	0.04	0.07

* Wavelength of excitation, 488 nm (selected from argon laser); intensity of light, 0.30–1.5 mw cm^{-2} (high) (measurements made at 1.5–3.0 mw cm^{-2} gave the same τ as reported here), 0.010–0.030 mw cm^{-2} (low). Wavelength of measurement: all fluorescence passed by Corning CS 2-64 filter. Photomultiplier, selected RCA 7102 (S1 sensitivity). Method of measurement, phase at 56 MHz.

‡ Assuming τ_0 of 18 nsec for *R. rubrum* and *R. viridis* (reference 14) and of 15 nsec of *C. ethylicum* (footnote 3); ϕ values given here may be higher than actually measured if nonfluorescent pigments are present.

teria must be accomplished within nanoseconds in order to compete effectively with fluorescence (see Parson, reference 17). Also, the quantum yield of fluorescence at low light intensities is small (2–6%) and, thus, photosynthetic reactions are expected to be very efficient (see reference 18–20).

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