Estimation of the rate of photochemical charge separation in *Rhodopseudomonas sphaeroides* reaction centers by fluorescence and absorption picosecond spectroscopy

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Time-resolved fluorometry of reaction center (RC) preparations from *Rhodopseudomonas sphaeroides*, wild strain 1760-1, shows that the lifetime of the excited state of bacteriochlorophyll P870* is $\tau = 6 \pm 1.5$ ps and independent of temperature within the range 293–77 K. This value was found to coincide well with the time (7 ± 3 ps) of the RC porphyrin pigment transition into the ion-radical pair state P^F, as measured by picosecond absorption spectroscopy of the same preparations.

Rps. sphaeroides *Reaction center* *Charge separation* *Picosecond spectroscopy*

1. INTRODUCTION

The primary events of photosynthesis are characterized by a very high efficiency of absorption and transduction of energy of light in the reaction center (RC). The high quantum yield value of the photochemical act suggests that the primary charge separation occurs within the 10^{-10}–10^{-11} s time range [1–4].

Progress in pulsed laser spectroscopy techniques enabled direct kinetic recording of fast picosecond processes in the RC [5–11].

It was found that following the absorption of light, the bacteriochlorophyll RC special pair P870 undergoes oxidation, giving rise to the transient state P^F which appears to be a mixture of the $P870^+B800^-$ and $P870^+Bph^-$ states [11] (B800, bacteriochlorophyll absorbing at 800 nm; Bph, bacteriopheophytin of the RC). The process seems to occur within 4–7 ps [4,8–10]. Most data known on the primary photosynthetic events occurring in the RC were obtained by absorption picosecond spectroscopy. Less information is available on the decay kinetics of P870* fluorescence associated with the transition of the RC into the P^F state.

The fast time-resolved picosecond fluorescence of RCs was firstly observed by us in [4,10,13,14]. However, the time resolution in the experiments was not sufficiently high (⩾8 ps) and some of the molecular characteristics of the RC preparations used were different compared to those observed in vivo.

Following research in other laboratories also provided evidence of the existence of the fast RC fluorescence decay component, which was attributed to the transition of P870 into the P^F state. Study of the effects of pH, temperature, etc. changes on the quantum yield of the fast fluorescence have also been made. However, the time resolution in the experiments was insufficient to estimate accurately the lifetime of P870* [15].

Here, we investigated the photochemical charge separation in RC preparations from *Rps. sphaeroides*. Picosecond fluorometry was performed to measure the lifetime of P870*

Some results of the present investigation in preliminary form were published in [17]

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fluorescence and its temperature dependence. The results are compared with the kinetics of the $P^* \longrightarrow P^F$ reaction observed by picosecond absorption spectroscopy. We believe that this complex study is the first attempt to compare in parallel experiments the results of picosecond absorption and fluorescence spectroscopy made on the same RC preparation.

2. MATERIALS AND METHODS

RCs were prepared from *Rps. sphaeroides* strain 1760-1 (wild type) by solubilization of chromatophores with the detergent lauryldimethylamine *N*-oxide as in [16].

A streak camera, coupled to a vidicon and a Nokia LP-3840 pulse analyzer, were used to record the decay kinetics of RC fluorescence. The time resolution of the fluorometer was 1.5 ps. Excitation was given by single 530 nm pulses, $t_p = 2$ ps, with a pulse repetition frequency of 0.5 Hz. The pulse energy density was 10^{14} quanta/cm^2.

A programmable picosecond absorption spectrometer was used to measure the kinetics of the absorption changes associated with the $P870^* \longrightarrow P^F$ electron transfer reaction. Excitation was provided by single 24 ps laser pulses at $\lambda = 530$ nm with a pulse repetition frequency of 2 Hz. Monitoring 760 nm light was obtained from the continuum generated in D_2O. For each measurement, the number of records to be averaged was assigned by the computer to provide the specified signal-to-noise ratio. Laser pulses with a fixed duration were sorted out. This procedure, together with the mathematical method used for data processing and corrections for the apparatus function of excitation and monitoring beams, made it possible to improve the time resolution to approx. 3 ps.

3. RESULTS AND DISCUSSION

Fig.1 shows the decay kinetics of the long-wavelength (926 nm) fluorescence emitted by RC preparations with reduced or oxidized P870 (curves 2(o) and 3, respectively). An interference filter ($\lambda_{max} = 926$ nm, $\Delta\lambda = 10$ nm) was used to isolate the desired spectral band of the fluorescence to be recorded. Experimental points (●——●) approximated by the dashed line represent the apparatus function of the fluorometer.

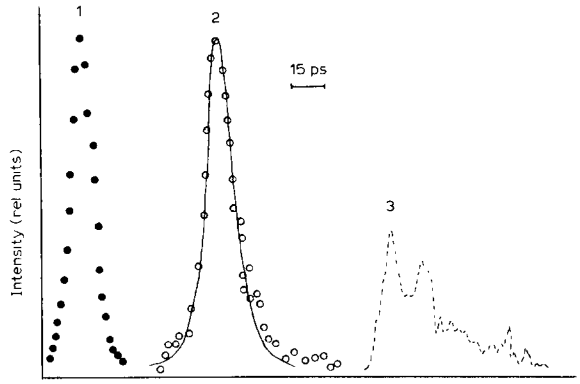

Fig.1. Decay kinetics of fluorescence emitted at 926 nm by *Rps. sphaeroides* RCs. Curves: 1 (●), apparatus function of the fluorometer; 2 (○), recorded RC fluorescence signal; 2 the solid curve obtained by convoluting the apparatus function of the fluorometer with the experimental curve $K(t) = A_0\exp^{(-t/\tau_0)}$ where $\tau_0 = 6$ ps; 3, fluorescence signal following pulsed laser excitation of oxidized RCs. Kinetic trace 1 (●) is the average of 12 records; 2 (○), the average of 40 records; 3, the average of 80 records. Excitation energy, 3×10^{14} quanta/cm^2. Concentration of P870, 28 μM; pH 8, $T = 298$ K.

The decay time of P870 fluorescence was determined by convolution of the fluorometer apparatus function with the exponential function $K(t) = A_0\exp(-t/\tau_0)$. Calculations show that the experimental points fit the function best with $\tau_0 = 6$ ps (see fig.1, curve 2 where the theoretical curve is given by a solid line). This suggests that the decay of the 926 nm RC fluorescence follows an exponential pattern with a characteristic time of 6 ps. The decay kinetics of the long-wavelength RC fluorescence also contains a nanosecond component that is readily discernible (fig.1, curve 2). Its contribution, however, is negligible compared with the fast decay component.

In the short-wavelength spectral range, the fluorescence exhibits a 2-component decay pattern: $\Phi(t) = Ae^{-t/\tau_0} + Be^{-t/\tau_1}$ with $\tau_0 = 6$ ps and $\tau_1 = 1.2$ ns. The contributions of these components depend upon the spectral interval being recorded. In the region $\lambda \geqslant 900$ nm, their relative contributions are virtually equal. In a broader spectral interval, $\lambda \geqslant 800$ nm, the slow component has much greater amplitude, masking the fast component. The long-lived decay component ($\tau = 1.2$ ns) was observed by us in earlier investigations using RC preparations obtained by a different isolation procedure [10,13,14]. This fluorescence seems very likely to be emitted by porphyrin pigments (bacteriochlorophyll, bacteriopheophytin) which are associated with the RC protein in a non-specific way. The oxidation of P870 by continuous background light causes a drastic decrease in the yield of the long-wavelength picosecond fluorescence (fig.1, curve 3) but has little effect on the nanosecond fluorescence.

Fig.2 shows the temperature dependence of the fluorescence characteristic time τ_0 of P870*. Within the temperature range 293–77 K, the τ_0 value was found to be virtually constant.

This agrees well with the data on the temperature independence of the primary charge separation in RCs as measured by picosecond absorption spectroscopy [11]. It implies an activationless character of electron transfer (probably by tunneling) within the RC.

For a better understanding of the mechanisms of light-induced formation of the ion-radical pair state P^F in the RC it seems interesting to compare the absorption and fluorescence picosecond spectroscopy data obtained on a single sample under identical conditions.

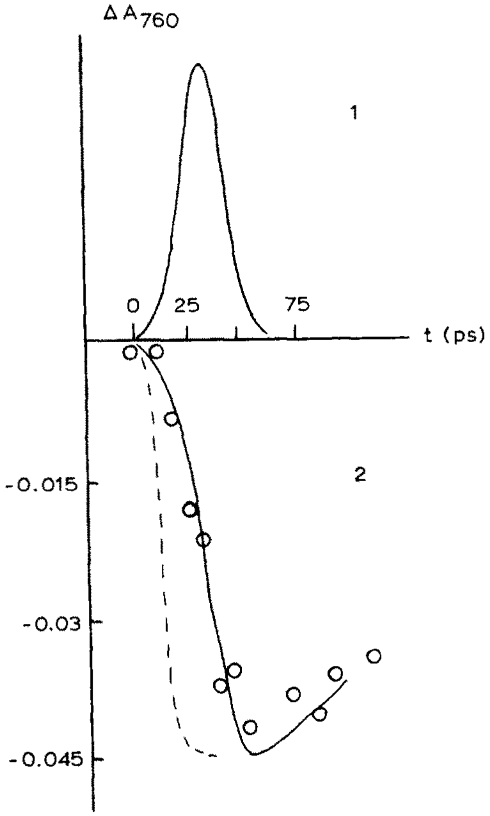

Fig.3. Kinetics of pulsed laser-induced absorption changes at 760 nm in the *Rps. sphaeroides* RCs. (1) Line shapes of the exciting and probing pulses; 2 (○), experimental points (SE = ± 3 × 10^{-3} ΔA); 2 (– – –), theoretical curve obtained by convoluting the apparatus function (1) of the absorption spectrometer with calculated kinetics (3) having a characteristic time of 7 ± 3 ps.

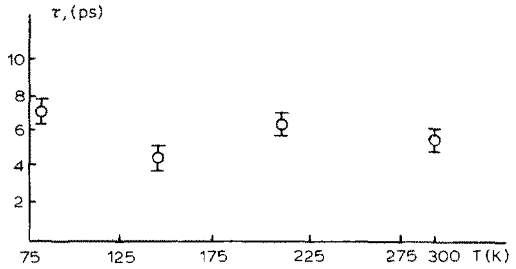

Fig.2. Characteristic decay time of 926 nm fluorescence emitted by the RC of *Rps. sphaeroides* as a function of temperature.

Fig.3 shows the kinetics of the RC absorption changes recorded at 760 nm under experimental conditions similar to those in which picosecond fluorescence had been studied. The decrease in the 760 nm absorbance is known to be associated with the formation of the P^F state [9,11,12]. The line shape of exciting and monitoring laser pulses is also presented on fig.3. Within the accuracy of registration (± 1.5 ps, apparatus function of the streak camera) the line shapes of the exciting and monitoring pulses are identical: both have a Gaussian form with a half-width of Δt = 24 ± 1.5 ps. To eliminate the laser pulse form effect on the kinetics of bleaching of the 760 nm band and to improve the time resolution we performed a mathematical simulation of the experimental data. A set of differential equations was considered to describe the sequence of states of the photoactive RC,

$$\xrightarrow{h\nu} P \longrightarrow P^* \longrightarrow P^F,$$

and solved to obtain the desired parameters. In particular, the rate constant of the $P^* \longrightarrow P^F$ reaction was found by calculating the function of the mean-square error

$$\phi = \sum_{i=1}^{N} f_i^2$$

where $f_i = A_i(x_j) - \Delta A_i^{exp}$; x_i are the parameters of the system of equations: ΔA_i^{exp} is the experimentally obtained value of the change in absorbance of the sample at point i; $\Delta A_i(x_i)$ is the value of the approximating function at point i. Fitting was accomplished by iteration using standard least-squares method.

By processing the experimental curve 2 (fig.3) as described above and by making corrections for the pulse form effects of both the exciting and probing pulses (curve 1, fig.3), we found that the 760 nm absorption band is bleached for 7 ± 3 ps. Convolution of the theoretical curve 3 (fig.3) with the apparatus function 1 yielded a theoretical curve 2 that fits, within experimental error, the experimental points.

Thus, we found that there is a good agreement between the kinetics of the formation of the P^F state and the decay of the fluorescence emitted by P870*. From these findings it is concluded that the formation of the P^F state, which is apparently a mixture of the $P870^+B800^-$ Bph and $P870^+B800$ Bph^- states, occurs in a single step from the excited state of P870. In the course of its formation, for about 6–7 ps, the electron population is localized nearly fully at the reaction center Bph.

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