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Time-resolved tryptophan fluorescence in photosynthetic reaction centers from *Rhodobacter sphaeroides*

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Tryptophan fluorescence of reaction centers isolated from *Rhodobacter sphaeroides*, both stationary and time-resolved, was studied. Fluorescence kinetics were found to fit best a sum of four discrete exponential components. Half of the initial amplitude was due to a component with a lifetime of $\simeq$ 60 ps, belonging to Trp residues, capable of efficient transfer of excitation energy to bacteriochlorophyll molecules of the reaction center. The three other components seem to be emitted by Trp ground-state conformers, unable to participate in such a transfer. Under the influence of intense actinic light, photooxidizing the reaction centers, the yield of stationary fluorescence diminished by $\simeq$ 1.5 times, while the number of the kinetic components and their life times remained practically unchanged. Possible implications of the observed effects for the primary photosynthesis events are considered.

Reaction center; Tryptophan; Stationary and time-resolved fluorescence; Protein conformation; *Rhodobacter sphaeroides*

1. INTRODUCTION

Recent experimental and theoretical evidence shows that the protein moiety of the photosynthetic reaction centers could be of fundamental importance in the course of the primary charge separation controlling the rate and reversibility of this process [1–6]. Protein fluorescence, mainly that of tryptophan residues (Trp), is known to be a useful intrinsic probe of the local polarizability and conformation changes in proteins [7,8].

The protein moiety of *Rb. sphaeroides* reaction centers contains 39 Trp residues, mainly in L and M subunits, which carry the cofactors of the primary charge separation [9,10]. Most of these aromatic residues are conserved between different bacterial strains [9–11]. This conservation points to their possible functional role which could be probed, in particular, via Trp fluorescence. Such studies have not yet been undertaken for photosynthetic systems.

In the present work, stationary and time-resolved UV fluorescence of the reaction centers from purple bacterium *Rhodobacter sphaeroides* was investigated. This fluorescence was shown to be sensitive to exposure of the reaction center suspensions to intense visible light, photooxidizing the BChl special pair.

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Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin; FWHM, full-width at half-maximum; Trp, tryptophan; Q^-, reduced quinone; DAS, decay associated emission spectra; P^+, photooxidized bacteriochlorophyll special pair; χ^2, chi-square

2. MATERIALS AND METHODS

Reaction centers were isolated using the published procedures [12] and stored in 15 mM Tris-HCl buffer, pH 8, 0.025% lauril dimethylamine oxide and 1 mM EDTA. All measurements were performed in a flowing cuvette at room temperature.

Steady-state fluorescence and absorption spectra were measured with commercial instruments, specially adopted for observation of photoinduced fluorescence and absorption changes. The time-resolved fluorescence measurements were performed using time-correlated single-photon counting with an instrumentation, employing 3.8 MHz repetition rate laser excitation and microchannel plate registration, described in detail elsewhere [13]. FWHM of the instrument response function was 30–40 ps. To photooxidize the reaction centers in the kinetic measurements, a part of 530 nm light from a pumping Nd:YAG laser was employed. The light intensity incident upon the sample was maintained in the range of 1–5 mW with neutral glass filters. The data were analyzed as a sum of discrete exponential components using both single and global analysis programs [13–15]. The quality of fit was judged by the weighted residuals inspection and by the statistical χ^2 criterion.

3. RESULTS

Stationary absorption and fluorescence spectra of the reaction centers (the latter for the near UV range) are shown in Fig. 1. On the basis of the known absorption properties of BChl, BPh and Trp, about half of absorption at 295 nm (fluorescence excitation wavelength) may be attributed to Trp. The steady-state fluorescence has its maximum at 330 nm and FWHM of the correspond-

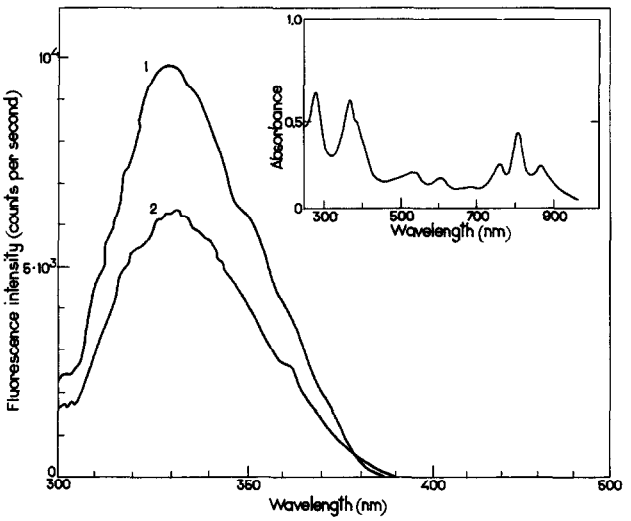

Fig. 1. The stationary fluorescence spectrum of the reaction center preparations from *Rb. sphaeroides*, wild type: (1) no treatment, reaction centers open; (2) reaction centers photooxidized by saturating red light from an incandescent lamp (two glass filters, cutting off the wavelength range shorter than 700 nm, were used). Excitation at 295 nm. Insert: the absorption spectrum of the reaction center preparations used.

ing spectrum is 45–46 nm. Both parameters are typical of Trp molecules in a very hydrophobic environment [16].

Exposure of the reaction center preparations either to saturating red light from an incandescent lamp in the stationary measurements (Fig. 1) or to light of 530 nm from a He-Ne laser in the time-resolved ones, resulted in a $\simeq$ 1.5-fold decrease in the total fluorescence intensity, which was fully reversible after switching off the actinic light.

Decay kinetics of the reaction center fluorescence, taken within the spectral range of 320–370 nm with 10-nm steps, were found to fit best a sum of four exponential components. The data of single deconvolution analysis are given in Table I. The values of χ^2 and inspection of the weighted residuals show quite a good fit to the experimental data.

The results of global analysis of the set of spectrally- and time-resolved data are given in Fig. 2 in the form of emission decay-associated spectra (DAS). It follows that each of the four components is characterized by its own emission spectrum, whose FWHM is within 45–48 nm and position of maxima varies from 325 to 335 nm, the shortest lived component being the narrowest and most blue-shifted one.

Comparison of the DAS for the dark adopted preparations (Fig. 2A) with those exposed to the green light, photooxidizing BChl special pair (Fig. 2B), shows that under the latter conditions a 20–25% increase in the relative contribution of the shortest lived component and an about 5–10 nm shift of the spectral distributions to the blue range took place.

4. DISCUSSION

4.1. *The nature of decay components of Trp fluorescence*

According to the X-ray studies of *Rb. sphaeroides* reaction centers [18,19], Trp residues are arranged randomly in space, a considerable part being at distances not larger than 15–20 Å from any BChl (BPh) molecule. Under these conditions, Trp fluorescence should be efficiently quenched by excitation transfer to the cofactors. The Förster radius for such a transfer was obtained using the relation [19]:

$$R_o^6 = 8{,}785 \cdot 10^{17} \cdot k^2/n^4 \int F(\nu) \cdot \varepsilon(\nu) \cdot d(\nu)/\nu^4,$$

where $\varepsilon(\nu)$ is the molar extinction coefficient of BChl (BPh) molecules of the reaction centers as a function of wave number; $F(\nu)$ is the normalized spectral distribution of Trp fluorescence; k^2 is the orientational factor, taken to be equal to 2/3; n is the refractive index of the medium.

To determine R_o, fluorescence and absorption spectra of the reaction center preparations were analyzed in spectral range of 280 to 450 nm, taking into consideration that six BChl and BPh molecules contributed to the Soret band. A value of R_o equal to 45–50 Å was thus obtained.

Table I

The data of a single deconvolution analysis of UV fluorescence of *Rb. sphaeroides* reaction centers: lifetimes τ_i (ns) as a function of emission wavelength

Flowing sample, reaction centers open					
Emission wavelength	τ_1	τ_2	τ_3	τ_4	χ^2
320 nm	0.075	0.42	1.5	4.6	1.12
330 nm	0.065	0.42	1.6	5.4	1.10
340 nm	0.070	0.47	1.8	6.7	1.20
350 nm	0.065	0.48	2.0	8.5	1.24
360 nm	0.065	0.49	2.1	9.2	1.21
370 nm	0.070	0.47	2.0	8.6	1.15

Green illumination, reaction centers photooxidized					
Emission wavelength	τ_1	τ_2	τ_3	τ_4	χ^2
320 nm	0.055	0.36	1.4	6.0	1.31
330 nm	0.060	0.37	1.5	6.8	1.10
340 nm	0.070	0.45	1.8	10.0	1.14
350 nm	0.070	0.44	1.7	9.5	1.25
360 nm	0.060	0.43	1.8	10.3	1.21
370 nm	0.060	0.42	1.7	9.2	1.12

A triple-exponential fit gave 40–50% higher χ^2-values and evidently non-random distribution of the weighted residuals. In the calculations the contribution from the corresponding Raman band of water at 328 nm was taken into account. The sample signals were accumulated up to 10,000 counts in the peak channel. The monochromator bandpass was 8 nm.

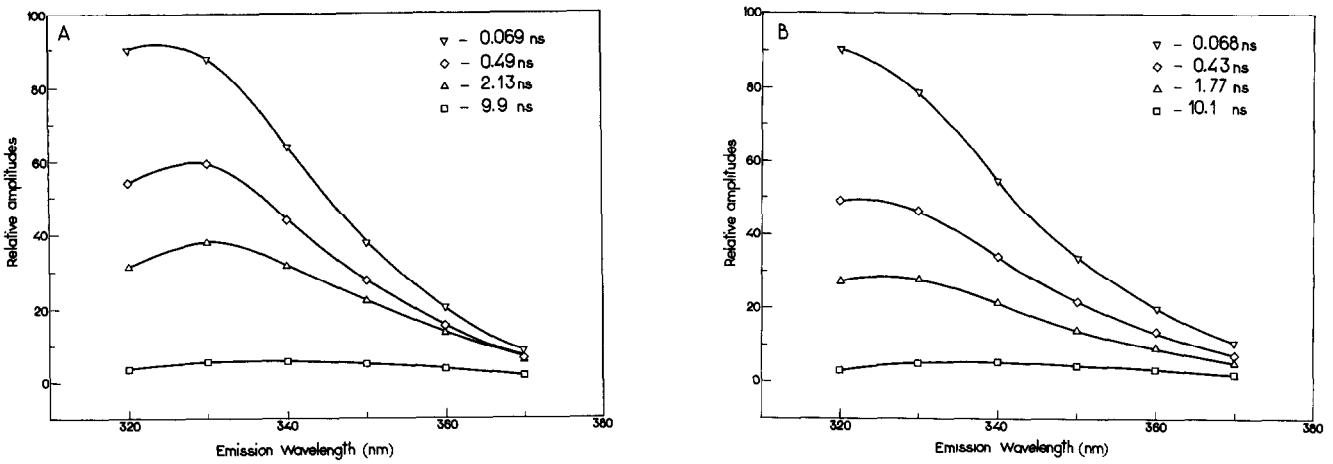

Fig. 2. The decay-associated emission spectra of *Rb. sphaeroides* reaction center preparations: a four-exponential global fit of the spectrally and time-resolved data. (A) Reaction centers open; (B) reaction centers photooxidized. For details, see section 2 and comments to Table I.

The time constant of 60 ps of the main component of Trp fluorescence decay corresponds to the mean Trp–BChl (BPh) distance of 20 Å, as determined from relation [19]: $\tau_j = \tau_o \cdot (R/R_o)^6$, where τ_o is the radiative lifetime of Trp, taken to be 20 ns [16]. This time interval corresponds, however, not to a single act of irreversible excitation transfer from some Trp to BChl molecule, but rather to the mean value averaged for a number of such residues, separated from the cofactors by distances not larger than about 20 Å. Judging from the realtive contribution of this component (Fig. 2), the number of such residues is about 20.

The other decay components seem to belong to the residues located unfavorably for such a transfer. Their time constants are close to those observed for tryptophyls in proteins without specific quenchers. The multiexponential decay of Trp fluorescence in such proteins, including single Trp-containing ones, is explained by the existence of three types of Trp rotamers around the C_α–C_β bond of the tryptophyl side chain [20–22]. The values of the conformer lifetimes (which are usually in the range of 0.3–0.4, 1–2 and 4–8 ns) and their relative amplitudes depend on the nature and proximity of an adjacent electrophile. It follows that in photosynthetic reaction centers Trp–BChl excitation transfer competes efficiently (at least for half of the residues) with fluorescence quenching produced by interaction between Trp and other protein groups.

Judging by the position of maxima and FWHM of the DAS (Fig. 2), the environment of residues, responsible for the 60 ps component, is the most hydrophobic. The longest lived component seems to be emitted by Trp located in a more hydrophilic environment. In addition, the dependence of the latter component lifetime on the emission wavelength (Table I) could be a manifestation of the nanosecond conformation mobility of the reaction center protein initiated by a change in the dipole moment of the photoexcited Trp.

4.2. *Effect of illumination on Trp fluorescence*

The most prominent effect of exposing the reaction centers to saturating light on Trp fluorescence was a decrease in the overall intensity by about 35%. At the same time, the lifetimes of all four components remained the same in the limits of experimental uncertainty. An about 25% decrease in the relative contribution of the shortest lived component (accompanied by the concomitant increase in that of the long-lived ones) could account only partly for this effect.

Another conceivable reason for this decrease could be a change in Trp τ_o value under the influence of the electric field induced by P^+ and Q^- charges. Possible influence of electrostatic charges on the electronic transition of indole ring resulting in change in molecular geometry of photoexcited Trp, and, thereby, in the value of τ_o, was suggested recently by a number of authors [22–24]. A shift of Trp absorption spectrum due to the electrostatic field of P^+ and Q^- or to a local heating of the protein environment, produced by intense illumination, could also be responsible for the observed fluorescence decrease.

One of the purposes of this study was to explore the possibility of using time-resolved Trp fluorescence measurements as a probe of the protein conformation and polarizability changes, accompanying primary events in photosynthetic reaction centers. It should be mentioned in this respect, that relatively minor changes were observed both in the time constants and in the spectral distributions of Trp fluorescence decay components. The main reason is that only the mean τ values, averaged over a large number of Trp residues, were determined. Nevertheless, 25% increase in the relative

contribution of the shortest lived component and 5–10 nm blue shift of its spectrum indicate that some rearrangement of the reaction center protein could take place, stabilizing photoproduced charges. Employment of technique of femtosecond resolution is necessary for further clarification of this problem.

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