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Excitation energy flow in a photosynthetic bacterium lacking B850. Fast energy transfer from B806 to B870 in *Erythrobacter* sp. strain OCh 114

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Excitation energy flow in the aerobic photosynthetic bacterium *Erythrobacter* sp. strain OCh 114 was investigated at physiological temperature by the time-resolved fluorescence spectroscopy in the picosecond time range. This bacterium has two forms of bacteriochlorophyll (Bchl) on separate antenna complexes absorbing around 806 nm (B806) and 870 nm (B870); however, B850 is not detected in this species. Upon selective excitation of B806, the fluorescence from B870 was dominant even in the initial time, indicating that energy transfer from B806 to B870 complexes occurred within the time resolution of the apparatus (6 ps). This fast transfer was confirmed by the fact that no rise term was resolved by the kinetic analysis of the fluorescence from B870. The fast energy transfer suggests tight association between the different classes of antenna complexes. The antenna pigment with energy level lower than that of reaction center was also found in this species (B888) as in some purple photosynthetic bacteria. Its content was estimated to be at most 3 Bchl per reaction center, which is significantly less than that in *Rhodobacter sphaeroides*.

Introduction

In general, two types of antenna complex are found in purple photosynthetic bacteria; LH 1 (B875, B880 or B890) and LH 2 (B800-B820, B800-B850). Relative content of such complexes varies depending on growth conditions [1]. Drastic changes are usually observed in the content of LH 2. This complex has at least two spectral forms of bacteriochlorophyll (Bchl). One is absorbing around 800 nm (B800), and the other, around 850 nm (B850) or in some cases, around 820 nm (B820). B850 is found in any bacteria which can synthesize an LH 2-type complex. Excitation energy on B800 is very efficiently transferred to B850 due to close geometry of these bacteriochlorophylls in the same complex [2–4]. The energy on B850 is also known to be transferred efficiently to B875 or corresponding Bchl-form in LH 1 which tightly associates with the reaction center (RC) [2,5,6]. One of the reasons for this fast energy transfer may be the large spectral overlap between the donor emission and the acceptor absorption as stated below. The localization of B850 and B875 Bchls are considered to be near the periplasmic side of the membrane just like the special pair in RC [7]. This alignment of antenna Bchls may be also one of the reasons for the efficient energy transfer between different classes of antenna complex in typical purple photosynthetic bacteria.

The aerobic photosynthetic bacterium, *Erythrobacter* sp. strain OCh 114, is unique class of bacteria, which can synthesize Bchl only under aerobic conditions [8,9]. It contains the photosynthetic RC and the B870 antenna protein (LH 1) similar to those in typical purple photosynthetic bacteria, respectively [10]. Instead of the B800-B850 complex, this bacterium has a B806 complex as LH 2; the Bchl form corresponding to B850 is not found, even by low temperature fluorescence spectroscopy [10]. Both complexes are isolated in a stable form and optical characteristics have already been demonstrated [10].

According to the Förster mechanism, the efficiency

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Abbreviations: Bchl, bacteriochlorophyll; B806, B850 and B870, Bchl whose absorption maxima are located at 806, 850 and 870 nm, respectively; LH, light harvesting; RC, reaction center.

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of excitation energy transfer is mainly governed by three factors; distance between the donor and acceptor molecules, mutual orientation and the spectral overlap between the fluorescence of the donor and the absorption of the acceptor [11]. Compared with the spectral overlap between B850 and B875 in typical photosynthetic bacteria, that between B806 and B870 in *Erythrobacter* sp. strain OCh 114 is significantly smaller. It would be likely, therefore, that the rate of excitation energy transfer from B806 to B870 in this species is significantly lower than that from B850 to B875 in other photosynthetic bacteria. Thus, analysis of the transfer process in this species might provide critical information on the transfer mechanism between different classes of antenna complexes.

Presence of a new type of antenna whose energy level is lower than that of RC has been reported for *Rhodobacter sphaeroides* [2–5] and *Rhodospirillum rubrum* [5,6]. Such components are to be surveyed in other photosynthetic bacteria including *Erythrobacter* to investigate their common characteristics which may indicate the functional role and structural basis for the specific energy level. Thus, we investigated the energy flow in *Erythrobacter* sp. strain OCh 114 with the time-resolved fluorescence spectroscopy in the picosecond time range. We found very fast energy transfer from B806 to B870 (within 6 ps), and also detected the longer-wavelength antenna at physiological temperature.

Materials and Methods

Erythrobacter sp. strain OCh 114 was grown heterotrophically under the sufficiently aerated condition [10]. Cells at the late log-growth phase were collected, suspended in the buffer (50 mM Mops (pH 7.5)/0.34 M NaCl) to make absorbance at 806 nm to be 0.05 and used for the measurements. Membrane fragments (chromatophores) were prepared as described previously [10].

Measurements of the time-resolved fluorescence spectroscopy were carried out with the apparatus reported previously [2,12–14]. An S-1 type micro-channel plate photomultiplier (R1564-05U, Hamamatsu Photonics, Japan) [15] was used to detect fluorescence in the near-infrared region, together with the time-correlated single photon counting system. Excitation was at 800 nm with a pulse duration of 6 ps (fwhm). Photon density per pulse was in a range of 10^8 to 10^9 per cm^2, which was low enough to avoid singlet-singlet annihilation. Time resolution of this optical arrangement was 6 ps. Since the difference in the spectral sensitivity in the wavelength region used was within 9% [2], the fluorescence spectra were not corrected. Measurements were carried out at 22° C.

Deconvolution of the spectra and estimation of lifetimes by convolution calculation with reference to the excitation pulse were carried out as reported previously [2,13,14].

Results

Fig. 1 shows absorption and the steady-state fluorescence spectra of membrane fragments (chromatophores). Two prevailing absorption maxima were found at 806 nm (B806) and 870 nm (B870); their relative content was estimated to be almost 1 to 1 on a Bchl base. The component corresponding to B850 was not detected in this species [10]. In the fluorescence spectrum, two emission bands were clearly observed, arising from B806 and B870, respectively. These two complexes seem to interact loosely, because an addition of 0.1% Triton X-100 to the membrane fraction induced the uncoupling of energy transfer between them (Fig. 1.). Optical characteristics of the membrane fragments were essentially identical to those of intact cells which were used for the time-resolved spectroscopy.

The time-resolved fluorescence spectra with the preferential excitation of B806 by the 800 nm light pulse were shown in Fig. 2 after normalization. At 0 ps, the main emission was detected at 884 nm, and a minor band, around 823 nm. The former originates from B870 and the latter, B806. In the initial time of the excitation, more than 80% of the excitation energy was estimated to localize on B806, considering the absorption spectra and relative content of B806 and RC-B870 complexes [10]. Thus, it is remarkable that the emission from B870 was dominant, even in the initial time range. This clearly indicates that the energy transfer from B806 to B870 occurs within the time resolution of the apparatus (6 ps) in spite of the absence of B850.

Time-dependent changes in the spectra were not drastic; only decrease in the relative intensity of around 823 nm was observed (Fig. 2). Locations of the maxima of the two fluorescence components were constant up to 2 ns. As shown in Fig. 3A, however, the spectrum at 250 ps was different from that at 0 ps in two points; one is decrease in the intensity around 823 nm and the other,

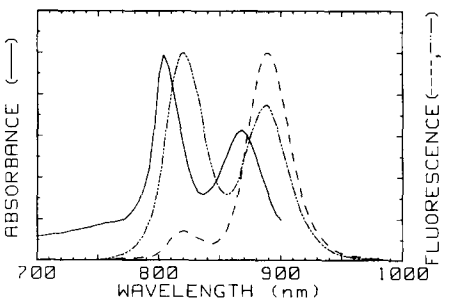

Fig. 1. Absorption (———) and steady-state fluorescence (— — —) spectra of membrane fragments (chromatophores) of *Erythrobacter* sp. strain OCh 114 at room temperature. (- - - -) shows the fluorescence spectra after the addition of 0.1% of Triton X-100 the membrane fragments.

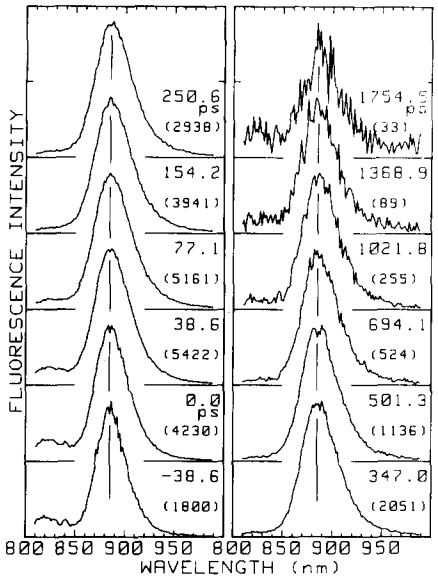

Fig. 2. Time-resolved fluorescence spectra of *Erythrobacter* sp. strain OCh 114 at 22°C. Spectra were shown after normalization to the maximum intensity of each spectrum. Numbers in the parentheses indicate the actual maximum numbers of photon in respective spectra. For details, see the text.

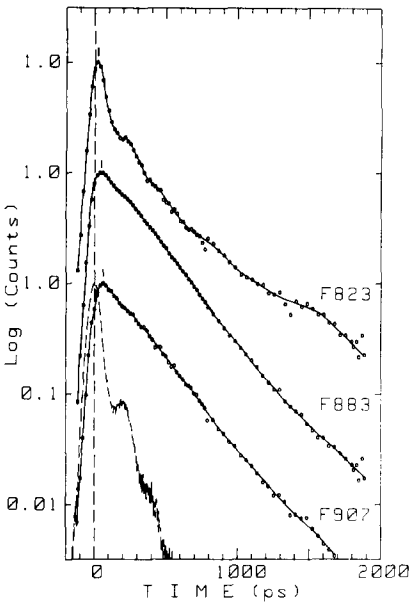

Fig. 4. Rise and decay patterns of the fluorescence components resolved by deconvolution of the spectra as shown in Fig. 2. Each point was calculated by the relative height in the spectrum and actual counts of photons. Broken line shows the pulse profile. Bars over the rise and decay curves represent the time for the maximum intensities. For details, see the text.

the increase around 910 nm. This difference strongly suggests the spectral heterogeneity of the main emission band. Although the red-shift of the main emission band seems to be smaller than that observed in *Rb. sphaeroides* (approx. 5 nm) [2], the difference spectrum (Fig. 3B) clearly indicates the presence of a component in the longer wavelength region. Based on the observed difference, time-resolved fluorescence spectra were deconvoluted into component bands with the assumption of Gaussian band shape (Fig. 3C). Three major and one minor components were resolved; those are called F823, F883, F907 and F955 hereafter, according to the locations of their maxima. The former two are interpreted to arise from B806 and B870, respectively. F955 may be assigned to a vibrational band. F907 is most probably a longer-wavelength antenna whose energy level is lower than that of RC, as found in *Rb. sphaeroides* [2–5] or *R. rubrum* [5,6]. Corresponding absorption maximum was estimated to be located at 888 nm, based on the Stepanov equation [16]. This is close to our estimation in the case of *Rb. sphaeroides* (890 nm) at physiological temperature [2]. The relative intensity of F907 to that of F883 increased only in the initial time up to about 80 ps, and after that time it was almost constant throughout the measuring time range, indicating the establishment of the equilibrium of excited molecules between these two components.

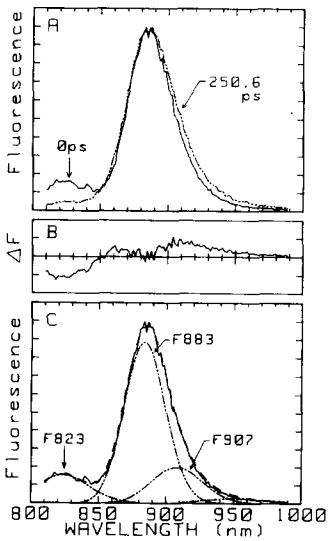

Fig. 3. Presence of three emission bands in the time-resolved fluorescence spectra. In (A), the spectrum at 0 ps (———) and at 250.6 ps (- - - -) after the excitation pulse. In (B), the difference spectrum between 0 ps and 250.6 ps. In (C), deconvolution of the time-resolved spectrum at 0 ps. (———) the observed spectra, (- - - -) component bands and (— — —) sum of the component bands. For details, see the text.

Rise and decay curves of individual components were shown in Fig. 4. A shift of the times for maximum intensities was clearly observed, indicating a sequential energy transfer among these three components. Component lifetimes were estimated by convolution calculation with the assumption of exponential decay (Table I). In the kinetics of F823, three decay components were necessary for a good fit; 19, 210 and 910 ps with the relative amplitudes of 95.0%, 4.5% and 0.5%, respectively. The presence of the 910 ps lifetime component is consistent with an increase in the relative fluorescence

TABLE I

Lifetimes of fluorescence components in Erythrobacter sp. strain OCh 114 at 22°C

Numbers in parentheses indicate corresponding absorption maxima of fluorescence components.

Components	Rise term	Decay terms					
	τ (ps)	τ_1 (ps)	A_1	τ_2 (ps)	A_2	τ_3 (ps)	A_3
F823 (B806)	–	19	0.95	210	0.045	910	0.005
F883 (B870)	–	191	0.902	385	0.098		
F907 (B888)	9	195	0.91	405	0.09		

intensity around 823 nm in a later time range (Fig. 2). No rise terms were found in the kinetics of F883. Essentially, two decay components were enough for a good fit, with the lifetimes of 191 ps (90.2%) and 385 ps (9.8%). In the rise and decay curve of F907, on the other hand, a clear rise term was resolved (9 ps). This time probably corresponds to the shift of the equilibrium between F883 and F907, as suggested by the time-resolved spectra. Two decay components were also found in the decay of F907 whose lifetimes were 195 ps (91.0%) and 405 ps (9.0%). The lifetime of 191 ps (F883) or 195 ps (F907) may correspond to the average trapping time of excited molecules with the closed RC, which are reported for many purple photosynthetic bacteria (about 200 ps) [3–5].

Inconsistency of the decay kinetics between B806 and B870 and the absence of the rise term in the acceptor molecules (B870) clearly indicate that a major part of energy is transferred within the time resolution of the apparatus (6 ps). The 19 ps decay time of the F823 (B806), therefore, may not reflect the main energy flow but the residual part. Thus, in terms of decay component, B806 cannot be homogeneous; one tightly coupled with B870 (transfer time shorter than 6 ps), and the other, loosely coupled (19 ps). The 910 ps component seems almost disconnected. Origins of the other long lifetime components (385 or 405 ps) are not clear. The 9 ps equilibration time indicates the transfer time to be shorter than 17 ps, with the assumption of a 2-fold forward transfer rate constant to the backward transfer rate constant as suggested by Zankel [17].

The content of B888 was estimated by the method proposed by Zankel [17]. After the equilibration between the F883 and F907, ratio of the fluorescence yield of each component was estimated to be 3.1 ± 0.2, by integration of area under respective fluorescence bands in the wavenumber unit. When we assume the location of respective components as 870 and 888 nm, the ratio of B870 to B888 which give rises to the above fluorescence ratio could be estimated to be 10.0 ± 0.7 on Bchl *a* basis. The B870 content per RC was 26 ± 2 Bchl *a* (Shimada et al., unpublished data), which is close to the case of other purple photosynthetic bacteria (24 Bchl *a* per RC) [18]. Thus the molar ratio of the B888 could be estimated to be about 2.3, at most 3 per RC. This number is significantly smaller than that in other purple photosynthetic bacteria (7 ± 1 molecules per RC) [2–4].

Discussion

Presence of the longer-wavelength antenna

In addition to F823 (B806) and F883 (B870), a new fluorescence component was clearly detected in *Erythrobacter* sp. strain OCh 114 at 907 nm, and its location of absorption maximum was estimated to be 888 nm at physiological temperature. This component may correspond to the longer-wavelength antenna component found in other photosynthetic purple bacteria [2–6]. The rise and decay curve of this component clearly shows that it is involved in the sequential energy flow to RC. When RC is closed, equilibration of excitation energy is attained among B870, B888 and RC. In this sense, interaction between antenna components in LH1 and RC is essentially identical to the case of other purple bacteria.

A significant difference was found in the relative content of the longer-wavelength antenna. In *Erythrobacter* sp. strain OCh 114, it was estimated to be at most 3 per RC, which is significantly lower than that in the other purple bacteria (about 7 molecules per RC). This smaller number suggests that the density of excited molecules around RC is not always high even in the equilibrated state, although accumulation of excitation energy around RC is the presumed function of the longer-wavelength antenna in other purple bacteria. B888 might have an additional function other than accumulation of the energy around RC, i.e., structural role between RC and antenna complexes, and/or functional role associating with the charge separation or stabilization process.

Energy transfer between LH 1 and LH 2

According to the Förster mechanism [11], the spectral overlap between fluorescence of the donor and the absorption of the acceptor is one of the major factors for the efficiency of excitation transfer. In *Erythrobacter* sp. strain OCh 114 which lacks B850 or corresponding Bchl-forms, the spectral overlap between B806 (LH 2) and B870 (LH 1) is considerably smaller than that in other bacteria which possess B850. The energy transfer time from B806 to B870 was, however, estimated to be shorter than 6 ps which seems to be in the same range as that between B850 and B875 in other purple bacteria [2,3], although the actual transfer time could not be estimated due to the limit of time resolution of the apparatus. The shorter transfer time from B806 to B870 than was expected suggests that the distance between

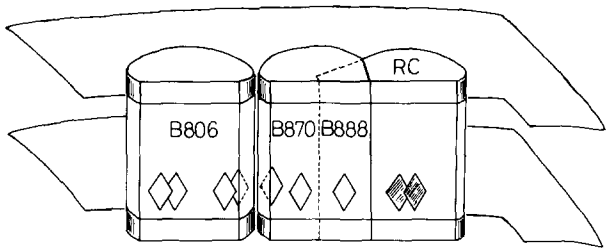

Fig. 5. Schematic model for arrangement of pigments in the membranes of *Erythrobacter* sp. strain OCh 114. B806 and B870 complexes represent assembled forms, i.e., hexamer or larger associating structure. Rhombi are Bchl, and hatched rhombi, the special pair in RC. The upper part corresponds to the cytoplasmic side and the lower, the periplasmic side of the membranes. For details, see the Discussion.

Bchls in the different complexes, B806 and B870, is short and the orientation factor is optimized enough to account for the fast transfer in spite of the small spectral overlap between them. Even if a transfer mechanism other than Förster's one is functioning, a short minimum distance and a favorable orientation between B806 and B870 Bchls must also be the primary requisite. Thus, the fast energy flow should be further discussed in two points; the location of Bchls in the respective complexes and specific interaction between B806 and B870 complexes.

In B800-850 of typical photosynthetic bacteria, the Bchl for B850 is localized near the periplasmic side of the membranes just like the Bchl in B870 and the special pair Bchl in RC [7]. On the other hand, the Bchl for B800 is thought to be located near the cytoplasmic side of the membranes [7]. B870 and RC of *Erythrobacter* sp. strain OCh 114 are very similar, respectively, to the corresponding complexes of purple photosynthetic bacteria in terms of spectroscopic properties and polypeptide compositions [10,19,20]. Therefore, the localization of Bchls in B870-RC complex (including B888) can be assumed to be also near the periplasmic side. Consequently, it is possible to speculate that B806 Bchl in *Erythrobacter* sp. strain OCh 114 is also located near the periplasmic side of the membranes (Fig. 5), which facilitates the fast energy flow from B806 to B870 with the minimum distance. If the localization of Bchl in B806 is analogous to that of B800 in the B800-850 in other purple bacteria, the distance between B806 and B870 might be too long for the fast energy transfer by the Förster mechanism [11]. B806 shows a strong dimer type CD spectrum [19] just like B850 in other purple bacteria [21]. This also supports the similar situation of B806 Bchl to that of B850 Bchl, although the primary structure of B806 polypeptide(s) and, consequently, the binding site of Bchl are not clear at this experimental stage.

From a biochemical point of view, it seems that the interaction between LH 1 and LH 2 is not strong, because coupling between these two can easily be disrupted by low concentration of detergents. This is also the case in *Erythrobacter* sp. strain OCh 114 (Fig. 1). However, the actual transfer time is very short, indicating a fixed geometry between B806 and B870 complexes. These results suggest that B806 and B870 complexes interact with each other to form a higher order structure. Considering the specific geometry of bacteriochlorophylls in the separate complexes as discussed above, the association sites between these complexes may be specific (Fig. 5). This is also suggested for the case of B850 and B875 in *Rb. sphaeroides* [2]. Hence, tight interaction between LH 2 and LH 1 can be assigned as a general feature of the photosynthetic antenna system in purple photosynthetic bacteria.

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