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Energy transfer processes in *Rhodopseudomonas palustris* grown under low-light conditions

Heterogeneous composition of LH 2 complexes and parallel energy flow pathways

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Excitation energy flow in the purple photosynthetic bacterium *Rhodopseudomonas palustris* grown under a low-light intensity was studied by time-resolved fluorescence spectroscopy in the ps time range. This bacterium synthesized the B824 component under this light condition. Time-resolved spectra at 20°C indicated the sequential energy flow in the order of B803, B856, B882 and B900, long wavelength antenna. An emission from B803 was not observed. A remarkable feature was the emission from B824 throughout the measuring time. After the excitation pulse of 100 ps, the spectra did not change any further, indicating the establishment of an equilibrium among components. Based on the energy distribution after equilibrium, parallel energy transfer pathways to LH 1 were suggested; one including B824 integrated in the B803–824–856 complex, and the other, from the B803–856 complex to B882. The latter was the dominant energy flow pathway in this bacterium.

Bacteriochlorophyll; Energy transfer; Picosecond spectroscopy; Pigment system; Photosynthesis; *Rhodopseudomonas palustris*

1. INTRODUCTION

In photosynthetic purple bacteria, two types of antenna complexes are well known; one is called LH 2 or the B800–850 complex, and the other LH 1 or B875 (or B880 or B890, depending on the species) [1]. In some species such as *Rhodopseudomonas acidophila* and *Chromatium vinosum*, another type of LH 2 complex, B800–820, is known to be synthesized under some growth conditions [2]. Recently, van Mourik et al. [3] reported that the B800–850 complex in low-light grown *Rhodopseudomonas palustris* contains heterogeneous form of B800 in addition to B850. They postulated that these heterogeneous forms are present in a well-defined (pure) complex, not in different complexes such as B800–825 and B800–850.

Since there have been no reports on the structural nature of complexes involving such heterogeneous spectral forms in this bacterium, the energy transfer sequence is being analyzed by spectroscopic methods at the moment. The energy migration involving such components is not well understood and is not necessarily straightforward when plural LH 2 complexes exist. In this study, we found a spectral component at around 824 nm in the peripheral light-harvesting complex (LH 2) of low-light grown cells of *Rps. palustris*, and investigated the energy flow in this bacterium by using time-resolved fluorescence spectroscopy in the ps time range.

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Abbreviations. B803 or F833, a representative expression of bacteriochlorophyll forms or fluorescence components located at 803 or 833 nm; LH, light-harvesting complex; PAGE, polyacrylamide gel electrophoresis.

2. MATERIALS AND METHODS

Rhodopseudomonas palustris cells (a strain isolated by S. Morita, cf. [4]) were grown photosynthetically in a medium containing 0.5% polypeptone, 0.1% yeast extracts and 0.4% sodium lactate [5] under low light conditions, i.e. 3×10^{13} quanta $cm^{-2} \cdot s^{-1}$. Cells of the late log-growth phase were harvested and used for measurements.

The LH 2 fraction was isolated and fractionated by preparative polyacrylamide gel electrophoresis (PAGE). In brief, membranes were solubilized with 1% octylglucoside plus 0.5% Triton X-100 and applied to a polyacrylamide gel containing 0.1% Triton X-100 [5]. After electrophoresis, all or a part of the pigmented bands were dissected, extracted [6] and, when necessary, re-electrophoresed.

Time-resolved fluorescence spectra at 20°C were measured with the same method as used for *Rhodobacter sphaeroides* [7]. A typical pulse duration of a 800-nm light was 6 ps with a frequency of 800 kHz and an intensity of ca. 10^9 photons/cm^2. Fluorescence was monitored with an S-1 type micro-channel plate photomultiplier (R-1564–05U, Hamamatsu Photonics, Japan). Time and spectral resolutions were 4 ps and better than 2 nm, respectively. Deconvolution of spectra and estimation of lifetimes were done by the procedures described previously [7–9].

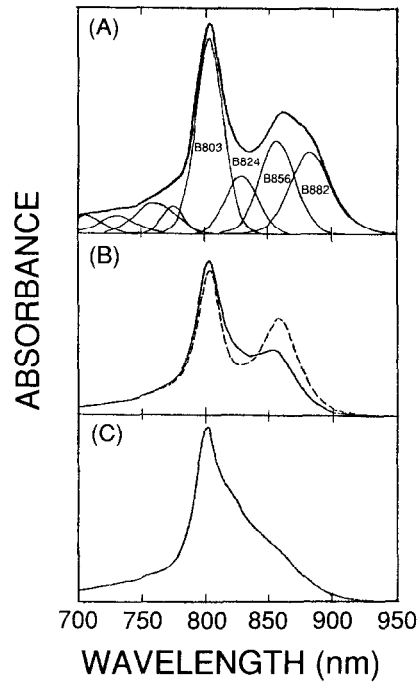

Fig. 1. Composition of antenna complexes in low-light grown *Rps. palustris* (A) Absorption spectrum of intracytoplasmic membranes and their deconvoluted pattern. (B) Absorption spectra of LH 2 complexes fractionated by PAGE. Solid line, the upper half of LH 2 band; broken line, the lower half of LH 2 band. (C) Absorption spectrum of a high 'B800/B850' LH 2 complex obtained with repeated PAGE.

3. RESULTS

3.1. *Composition of antenna complexes*

Three bacteriochlorophyll forms, B803, B856 and B882 (shoulder) were seen in the absorption spectrum of intracytoplasmic membranes of *Rps. palustris* (Fig. 1A). A remarkable feature was the presence of a component around 825 nm (B824) as shown by deconvolution of the absorption spectrum (Fig. 1A). This antenna component was detectable in a fraction of LH 2. In PAGE of the solubilized membranes, LH 2 migrated as a rather broad band. The upper part (low mobility) of the band showed a higher 'B800/B850' ratio than the lower part (Fig. 1B). Re-electrophoresis of the upper part again gave a higher 'B800/B850' fraction at the upper part of the re-electrophoresed band (Fig. 1C) in which a shoulder around 825 nm was clearly seen, although B824 was not completely separated from B856. These results suggest that two or more types of LH 2 complexes, which differ in relative content of B824 and also in size and/or net charge, are present in the low-light grown *Rps. palustris* cells. It should be noted, however, that the B803–824 complex which is completely free from B856 could not be obtained even after repeated electrophoresis.

3.2. *Time-resolved fluorescence spectra at physiological temperature*

The energy flow to B882 component was very fast. When B803 was excited, there was no emission from B803 during the entire time range, indicating a very fast energy transfer from B803. The main emission from B882 was clearly observed at 892 nm even at 0 ps. It shifted to the red by several nm with time (Fig. 2) and finally the maximum was observed at 898 nm. These results show that the main energy flow from B803 to B882 completed within our resolution time (4 ps) and the longer wavelength antenna was also observed even at physiological temperature. These are the same results as observed in *Rb. sphaeroides* [7]. In the short wavelength region of the emission band, there was a clear shoulder at 865 nm and a minor band at 832 nm. Changes in the spectra completed within 100 ps and after this time, the spectra were identical (as shown by

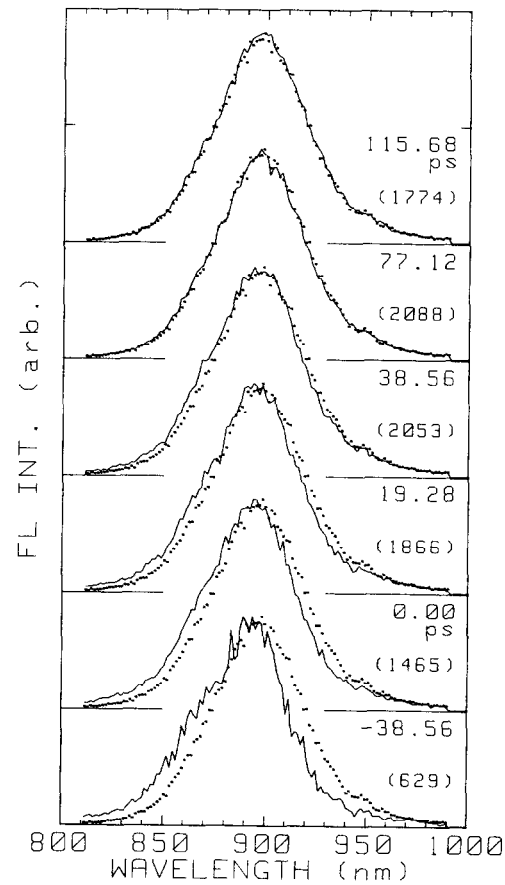

Fig. 2. Time-resolved fluorescence spectra of low-light grown *Rps. palustris* at physiological temperature. The excitation wavelength was 800 nm with a duration of 6 ps and a photon density of ca. 10^9 photons·cm^{-2} per pulse. Samples were continuously flowed between a reservoir and a cuvette. Spectra were shown after normalization to the maximum intensity in each spectrum. Numbers in parentheses are the photon counts in individual spectra. For the comparison of spectra, the spectrum at 163.9 ps was shown in each spectrum (dotted lines).

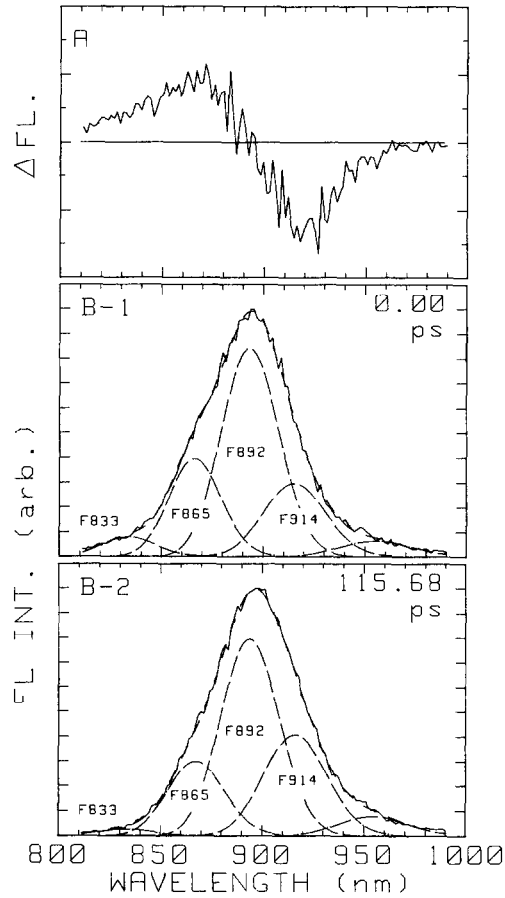

Fig. 3. Estimation of fluorescence components in low-light grown *Rps. palustris*. (A) Difference spectrum of normalized time-resolved fluorescence spectrum between at 0 ps and at 115.7 ps. (B) Deconvolution of the time-resolved spectra at 0 ps (B-1) and at 115.7 ps (B-2) by using the parameters obtained by the difference spectrum.

dotted lines). This indicates that the equilibrium of energy distribution in the antenna system was established within 100 ps.

3.3. *Rise and decay curves of individual components*

Four main emission components were resolved by the deconvolution procedure. A difference spectrum of the time-resolved spectra at two different times (Fig. 3A) indicates the presence of a few components in addition to the main emission at 892 nm; those were F833, F865 and F914. For the best fit of spectrum, parameters were obtained from the difference spectrum (Fig. 3B) and those were applied to the fit of the time-resolved spectra. In addition to the above four fluorescence components, a component at 955 nm was necessary, which was assigned to a vibrational structure of some fluorescence component(s). Based on the locations of maxima, it is reasonable to assign the F833, F865, F892 and F914 to B824, B856, B882 and the long wavelength antenna, respectively. The location of absorption maximum of the last component was estimated to be at 900 nm (B900) by the Stepanov equation [10] as in the case of *Rb. sphaeroides* (B890) [7]. Changes in relative fluorescence intensities were also discernible; in the early time (Fig. 3B-1), those of the components in the short wavelength region were higher, and the intensity of F914 increased in a later time (Fig. 3B-2). Rise and decay kinetics of individual components were calculated by the absolute photon count and the relative intensities (heights of resolved bands) at particular times (data not shown). A short lifetime component was clearly seen in F833, and the delayed maximum of F914 suggests a rise term in the kinetics (see next paragraph).

3.4. *Lifetimes and energy flow among components*

Lifetimes of individual components were estimated by a convolution calculation with the assumption of an exponential decay function (Table I). F833 decayed with a lifetime of 5 ps and the residual part had lifetimes of about 222 ps and 2.15 ns. The main decay time of F865 was 43 ps, together with two other lifetimes of 200 ps and 2.99 ns. Since the main energy flow to B882 occurred within a time shorter than our resolution time, the components with 5 ps and 43 ps lifetimes for F833 and F865, respectively, should be regarded as the residual part of the energy flow. The rise term(s) was not resolved in the kinetics of F865 and F892. The main decay of F892 was 18 ps and it corresponded to the rise of F914 (21 ps). This matching clearly indicates the energy flow between LH 1 and the long wavelength component in LH 1. This value is also consistent with the transfer time from the B875 to the long wavelength antenna in other photosynthetic bacteria [11,12]. The 200 ps and 2.6 ns lifetime components were also found in all the fluorescence components, that is, the establishment of equilibrium of energy distribution among antenna components, as shown by the invariant time-resolved spectra after 100 ps (Fig. 2).

4. DISCUSSION

A main energy flow process from B803 to B882 was not kinetically resolved due to a limit of time resolution

Table I

Lifetimes of individual fluorescence components in *Rps palustris* grown under a low-light conditions

Components	A_1	τ_1 (ps)	A_2	τ_2 (ps)	A_3	τ_3 (ps)
F833 (B824)	0.967	4.7	0.032	222	0.001	2148
F865 (B856)	0.399	42.5	0.596	200	0.005	2992
F892 (B882)	0.484	18.3	0.512	193	0.004	2600
F914 (B900)	−1.00	20.6	0.993	189	0.007	2000

Lifetimes were estimated by a convolution calculation against the pulse profile shown in Fig. 4 with the assumption of an exponential decay function [7,8]. A, amplitudes; and τ, lifetimes of individual components.

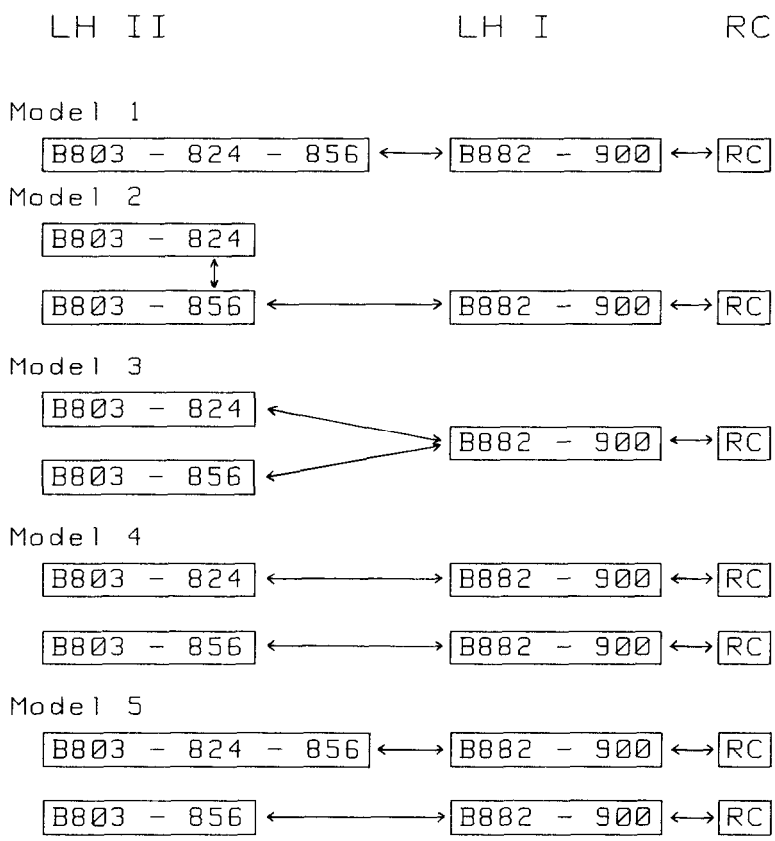

Fig. 4. Models for energy transfer pathways based on the combination of two LH complexes. Squares mean a unit of the complex. For explanation, see the text.

of our apparatus (Table I), thus it is not easy to elucidate the position of B824 in the energy transfer sequence. On the other hand, the energy distribution after the equilibration will give information on the interconnection among components. We therefore analyzed the position of the B824 in the transfer sequence by the latter method.

We made two assumptions for the analysis. One is that relative fluorescence intensities of components in an interconnected domain are determined by a relative amount of components and an energy gap between them according to the following equation [13],

$$I_A/I_B \propto (N_A/N_B)\ exp\ (-\Delta E_{AB}/kT) \quad (1)$$

where, I is the fluorescence intensity; N, number of molecules, ΔE_{AB}, energy difference; k, Boltzmann constant; and T, absolute temperature. The second is that fluorescence intensity of one equilibrated unit is proportional to an antenna size of the unit. Since the extinction coefficients for LH 2 components have not been unambiguously determined, we assumed the same values for LH 1 and the components of LH 2.

A relative amount of individual components was estimated by the areas under the deconvoluted absorption spectrum (Fig. 1A); the ratio of B803/B824/B856/B882 was 2.010:0.614:1.045:1.000. Since B882 contains a certain amount of B900, these two were separated based on the relative fluorescence yield and the energy difference between them. The estimated values were 0.836:0.164 for B882/B900. On the other hand, the relative fluorescence yields estimated from the fluorescence spectrum of the equilibrated state were 0.052:0.362:1.000:0.574 for F833/F865/F892/F914 (Fig. 3B-2). When all components were assumed to be interconnected to make a single domain (model 1, 2 and 3 in Fig. 4), the expected ratio of fluorescence yields based on eq. 1 were 0.012:0.185:1.000:0.574, which significantly disagreed with the above observation.

On the other hand, two kinds of separate domain model were also proposed: one is a parallel pathway of two independent complexes (B803–856 and B803–824) to LH 1 (model 4), and the other, the heterogeneous composition of LH 2 complexes (B803–824–856) (model 5). When model 4 was adopted, the expected relative fluorescence yield was about 0.013, 0.240 and 1.000 for F833, F865 and F892. Even if the pigment ratio of B803/B824 or B803/B856 was set to 1 or 2, the deviation of calculated values was at most 15%. Any combination of the proportion of the two pathways did

not give a satisfactory value. These values were not sufficient to explain the observed data. Significantly smaller values were found in the yield of F833 and F865.

This discrepancy can be explained by assuming the heterogeneity in LH 2 and two separate domains (model 5). For example, we divide the B856 into the proportion of 70% to 30% (0.7 vs. 0.3), and the LH 1, of 90% to 10% (the sum of B882 and 900 was 0.9 and 0.1, respectively, in the two pathways) and further set the ratios of B803/B824 and B803/B856 to 1 (0.587 vs. 0.587 and 0.3 vs. 0.3 or 0.7 vs. 0.7, respectively) as follows:

B803	—			856	→	B882	—	900	→	RC
(0.7)				(0.7)		(0.752)		(0.148)		
B803	—	824	—	856	→	B882	—	900	→	RC
(0.887)		(0.587)		(0.3)		(0.084)		(0.016)		

Numbers in parentheses show the relative amounts of individual components. The expected values for relative fluorescence intensities in this model were 0.054:0.360:1.00:0.574 for F833/F865/F892/F914. These values are very close to those observed (Fig. 3B-2). Even the stoichiometry of B803/B824 varied from 1 to 2 with a fixed ratio of B803/B856 of 1, the expected values were almost the same (deviation was less than 10%).

It seems that the fractionation of LH 1 and 2 into two separate domains is arbitrary in our simulation. Also, we are aware that there is some ambiguity in the estimation of amount of individual components and resolved fluorescence intensities; we estimated it to be about 20%. Even if we took these points into account, the heterogeneous LH 2 model can explain the observed data.

The model raised by this analysis is that the B803–856 type of LH 2 couples with LH 1, and the B824, a minor component, is incorporated into another B803–856 complex to form a B803–824–856 complex; the former pathway is the main energy flow path (about 2/3 of total) in *Rps. palustris*. There can be several other models for energy transfer (equilibration) processes (Fig. 4). However, only the above model of parallel energy transfer reproduced the observed fluorescence data.

Van Mourik et al. [3] postulated a serial energy transfer pathway in a low-light grown *Rps. palustris* (presumably model 1), based on the unsuccessful separation of two complexes biochemically and a stronger interaction between plural B800 components. Our results, in principle, gave the same indication for the presence of the LH 2 complex including B824. The most significant difference was that we proposed two separate domains different in composition of LH component and different types of LH 2, B803–856 and B803–824–856.

In contrast to the case of *Rps. acidophila* or *C. vinosum* [14,15], the separation between B824 and B856 was hardly attained in *Rps. palustris*, suggesting that the B824 is included in the B803–856 complex. The presence of the B803–824–856 complex is consistent with the model discussed above. In *Rps. palustris*, four slightly different pairs of LH 2 polypeptides are known [16]. In LH 2 of purple bacteria, the difference in two amino acid residues in C-terminal region of one of the polypeptides is considered to be responsible for the occurrence of B856 or B824 [17]. When the polypeptides for B856 and for B824 are synthesized at the same time, it is reasonable to assume that they can be incorporated into the same LH 2 complex to form B803–824–856 complex, although whether or not such complexes have fixed composition (fixed B824/B856 ratio) is not known at the moment.

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REFERENCES

[1] Zuber, H. (1985) Photochem. Photobiol. 42, 821–844.
[2] Hawthornthwaite, A.M. and Cogdell, R.J. (1991) in: Chlorophylls, (Scheer, H., Ed.), pp. 489–528, CRC Press, Boca Raton Ann Arbor.
[3] van Mourik, F., Hawthonthwaite, A.M., Vonk, C., Evans, M.B., Cogdell, R.J., Sundstrom, V. and van Grondelle, R. (1992) Biochim. Biophys. Acta 1140, 85–93.
[4] Hayashi, H., Miyao, M. and Morita, S. (1982) J. Biochem. (Tokyo) 91, 1016–1027.
[5] Fukushima, A., Shimada, K., Matsuura, K. and Satoh, T. (1988) Biochim. Biophys. Acta 933, 399–405.
[6] Shimada, K. (1985) J. Biochem. (Tokyo) 98, 465–473.
[7] Shimada, K., Mimuro, M., Tamai, N. and Yamazaki, I. (1989) Biochim. Biophys. Acta 975, 72–79.
[8] Shimada, K., Tamai, N., Yamazaki, I. and Mimuro, M. (1990) Biochim. Biophys. Acta 1016, 266–271.
[9] Mimuro, M., Tamai, N., Ishimaru, T. and Yamazaki, I. (1990) Biochim. Biophys. Acta 1016, 280–287.
[10] Stepanov, B. I. (1957) Dokl. Acad. Nauk. USSR 112, 839–841.
[11] van Grondelle, R. and Sundstrom, V. (1988) in: Photosynthetic Light Harvesting Systems; Organization and Function (Scheer, H. and Schneider, S., Eds.), pp. 403–438, Walter de Gruyter, Berlin.
[12] Visscher, K.J., Bergstrom, H. Sundstrom, V., Hunter, C.N. and van Grondelle, R. (1989) Photosynth. Res. 22, 211–217.
[13] Zankel, K.L. (1972) in: The Photosynthetic Bacteria (Clayton, R. K. and Sistrom, W.R., Eds.), pp. 314–317, Plenum Press, New York.
[14] Hayashi, H. and Morita, S. (1980) J. Biochem. (Tokyo) 88, 1251–1258.
[15] Cogdell, R.J., Durant, I., Valentine, J., Lindsay, J.G. and Schmidt, K. (1983) Biochim. Biophys. Acta 722, 247–255.
[16] Tadros, M.H. and Waterkamp, K. (1989) EMBO J. 8, 1303–1308.
[17] Fowler, G.J.S., Visschers, R.W., Grief, G.G., van Grondelle, R. and Hunter C.N. (1992) Nature 355, 848–850.