ELSEVIER

FEBS Letters 340 (1994) 167–172

FEBS 13720

FEBS LETTERS

Presence and significance of minor antenna components in the energy transfer sequence of the green photosynthetic bacterium *Chloroflexus aurantiacus***

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Received 25 October 1993; revised version received 17 January 1994

Abstract

Antenna components in the energy transfer processes of a green photosynthetic bacterium *Chloroflexus aurantiacus* were spectrally investigated by time-resolved fluorescence spectroscopy at −196°C on intact cells. Besides major antenna components so far reported, three minor components were resolved; those were Bchl *c* located at 785 nm, the baseplate Bchl *a* at 819 nm and Bchl *a* in the B808–866 complex at 910 nm. The last component was assigned to a longer wavelength antenna closely associated with a reaction center. An additional Bchl *c* fluorescence component was kinetically suggested to be present, which can be an energy donor to a major Bchl *c*. Presence of these minor components was signified in terms of (1) increase in the spectral overlap integral and (2) adjustment of the direction of dipole moments in the energy transfer sequence of intact cells.

Key words: Bacteriochlorophyll; Energy transfer; Green photosynthetic bacteria; Photosynthesis; Pigment system; Time-resolved fluorescence spectrum; *Chloroflexus aurantiacus*

1. Introduction

The photosynthetic pigment system of the thermophilic green gliding bacterium *Chloroflexus aurantiacus* consists of bacteriochlorophyll (Bchl) *c*, found in membrane-associated antenna structures called chlorosomes and Bchl *a*, found in the chlorosome baseplate and in the B808–866 complex in the cytoplasmic membranes [1]. The B808–866 complex is coupled to the reaction center (RC) in the membranes. Steady-state spectroscopy [2,3] indicates an energy flow in the order of Bchl *c*, baseplate Bchl *a*, B808–866 Bchl *a* and finally to RC.

Each chlorosome contains about 10,000 molecules of Bchl *c* and about 400 molecules of Bchl *a* in the baseplate [4]. It is widely accepted that Bchl *c*'s form the self-aggregated functional structure [5,6] which corresponds to rod elements found by electron microscope observation [7]; polypeptides in the chlorosomes are not involved in the formation of the self-aggregated structure. This concept is an exceptional case for the organization of photosynthetic pigment systems where pigment–polypeptide interaction is the main force. Therefore, organization of the antenna system in green photosynthetic bacteria is a suitable target for the understanding of the antenna system as a whole.

In our previous study [6], we identified two major Bchl *c* components as based on linear dichroism (LD) and circular dichroism (CD) spectra of chlorosomes. We also suggested the presence of additional Bchl *c* components in the longer wavelength region of major Bchl *c* components in chlorosomes. The spectral inhomogeneity in the baseplate Bchl *a* has been indicated by time-resolved fluorescence spectra [8]. Furthermore the presence of a special antenna component the energy level of which is lower than that of RC is clearly shown in several species of purple non-sulfur bacteria, *Rhodobacter sphaeroides* [9–12], *Rhodospillirum rubrum* [13,14] and in the aerobic bacterium *Roseobacter denitrificance* [15] (formerly known as *Erythrobacter* sp. OCh 114 [16]). Its presence was also suggested in purple sulfur bacteria *Chromatium vinosum* [17] and *Chromatium tepidum* [18] as based on CD spectrum of chromatophores. In the case of *C. au-*

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** This paper is dedicated to Dr. Tetzuya Katoh, Kyoto University, on the occasion of his 60th birthday.

Abbreviations: Bchl, bacteriochlorophyll; CD, circular dichroism; F765, a representative expression of fluorescence component located at 765 nm; LD, linear dichroism; LH, light harvesting; RC, reaction center.

SSDI 0014-5793(94)00104-4

rantiacus, the RC complex consists of L and M subunits [19], lacking the H subunit, and the pigment composition in RC was also different from that in purple bacteria [20]. The presence of the longer wavelength antenna, therefore, is very critical in terms of antenna–RC interaction.

Kinetic analyses of the energy transfer processes of *C. aurantiacus* so far studied [8,21–27] suggest a linear energy transfer cascade. However, when all the above mentioned components are incorporated into the transfer processes, the transfer pathways are not so simple as proposed. A plural and parallel pathway is also possible, as shown in *Rhodobacter palustris* [28]. Thus it is very important to identify all spectral components participating the energy transfer. Therefore we investigated inhomogeneity of the components under a low temperature conditions, where individual antenna components will have narrower band widths, thus a higher spectral resolution is expected. We used intact cells because perturbation or modification in the transfer sequence would be minimum. We found a few spectral components in Bchl *c* in chlorosomes and Bchl *a* in the B808–866 complex. Presence and significance of minor components are discussed from spectral and structural points of view in the energy transfer sequence.

2. Materials and methods

Cells of *Chloroflexus aurantiacus* (J-10-fl) were grown under photoheterotrophic conditions in the medium of Pierson and Castenholz [29] at 55°C. Photon density was about 1.0 W/m^2. Cells cultured for 48 h were harvested and used for measurements without any kind of treatment.

Time-resolved fluorescence spectra were measured by means of a time-correlated single-photon counting method as reported previously [8,12,15]. Measuring conditions for low temperature spectroscopy were the same as reported [12].

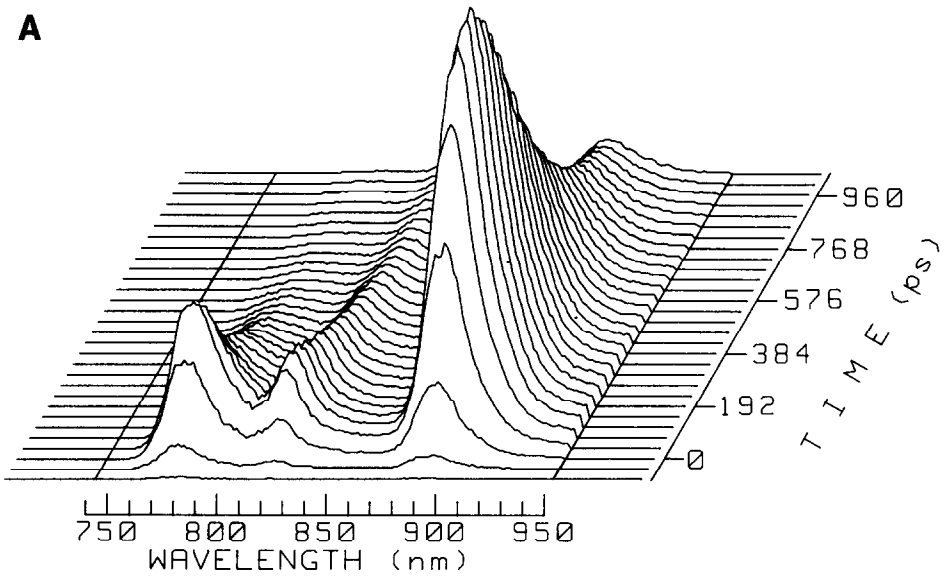
Time-resolved fluorescence spectra at particular times were resolved into components based on the least-square method [12,15]. Lifetimes of individual fluorescence components were estimated by convolution calculation with an assumption of exponential decay after separation of time-resolved spectra into component bands. The criteria for the best fit was chi-square. The channel shift treatment was not used for the estimation. Time and spectral resolution were 3 ps and less than 2 nm, respectively.

3. Results

When Bchl *c* in chlorosomes was selectively excited (715 nm), an overall energy flow was clearly shown by the time-resolved fluorescence spectra of a bird's-eye-view (Fig. 1A). A rise and decay of Bchl *c* (760–780 nm) was very fast, followed by baseplate (810–820 nm) and antenna component(s) in the membranes (888–893 nm), indicating the energy flow in this order. The times for maximum intensities were shorter than 38 ps for Bchl *c*, about 76 ps for baseplate Bchl *a* and 192 ps for B866 in the membranes, respectively.

When the time-resolved spectra were shown after normalization, a clear time-dependent shift of the emission maxima was noted in three wavelength regions; Bchl *c* (760–780 nm), baseplate Bchl *a* (810–820 nm) and B808–866 Bchl *a* (888–893 nm) (Fig. 1B). A higher intensity around 820 nm in a later time range (longer than 1 ns) was remarkable, indicating the presence of a long-lived component(s). This is consistent with the results reported by Brune et al. [2]. These time-dependent shifts clearly indicate inhomogeneity of spectral components in these wavelength regions.

Changes in the time-resolved fluorescence spectra indicated that the spectra consist of multiple components, thus the decay curve measured at a particular wavelength, in principle, should be the sum of plural fluores-



cence components. Therefore, we separated the spectra into components. As a first step of analysis, the presence of components was estimated by difference in the time-resolved spectra (Fig. 2B) between two different times (Fig. 2A). The difference spectrum clearly indicates multiple components, by a shoulder in the region of Bchl *c* (760–780 nm) and by a shift in the region of baseplate Bchl *a* (810–820 nm) and B808–866 complex (880–910 nm). Therefore, it is reasonable to assume two components for each of the three pigment complexes, which gave rise to six components in the wavelength region from 750 to 950 nm.

For the best fit, two additional bands were necessary, therefore the total number of components became eight; their maxima were located at 765, 781, 810, 819, 845, 889, 910 and 939 nm with band widths (full width at 1/e of the maximum) of 406, 494, 335, 367, 454, 353, 334 and 530 cm^{-1} (Fig. 2C) (hereafter these were shown with a prefix F indicating fluorescence component, i.e. F765).

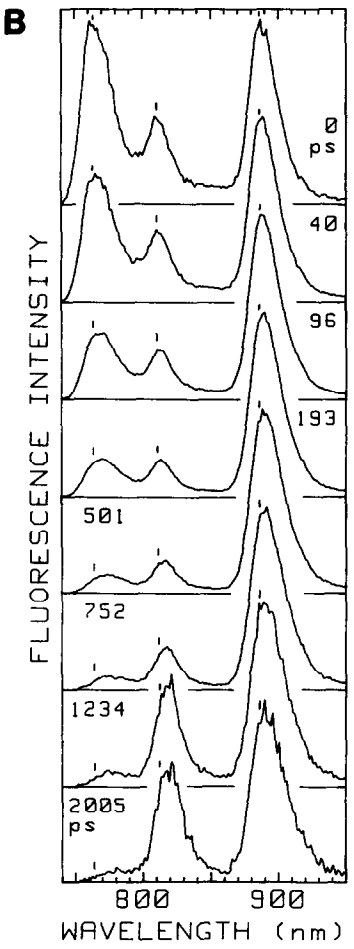


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Fig. 1. Time-resolved fluorescence spectra of *C. aurantiacus* measured at −196°C. (A) Bird's-eye-view and (B) normalized spectra at -196°C. The excitation pulse was at 715 nm wavelength with a duration of 6 ps. Zero time corresponds to the time when the excitation pulse showed maximum intensity. Small bars on the spectra indicate locations of the fluorescence maxima at 0 ps.

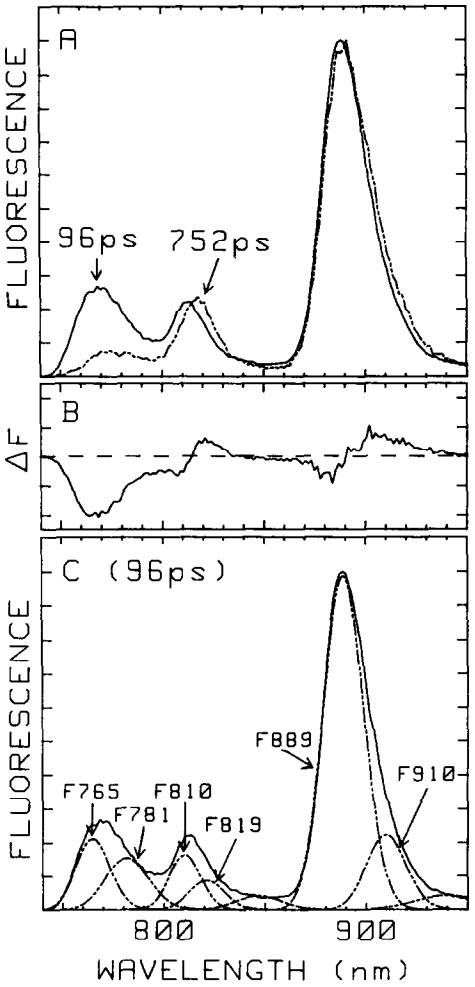

Fig. 2. Identification of component bands by difference in the time-resolved fluorescence spectra at two different times. (A) Spectra were measured at −196°C and those at 96 and 752 ps were shown after normalization. (B) Difference spectra between the two spectra. (C) Deconvolution of time-resolved spectrum at 96 ps by using the components estimated by the difference spectra shown in (B). A Gaussian band shape was assumed. Two additional components were included for the best-fit (F845 and F933).

Among these, F765 and F781 were assigned to Bchl *c*, F810 and F819 to the baseplate Bchl *a*, and F889 and F910, to the B808–866 complex. F845 and F939 were regarded as vibrational bands of some components. Locations of the absorption maxima of F765, F781, F810, F819, F889 and F910 were estimated at 743, 766, 794, 801, 867 and 889 nm at −196°C, respectively, by the Stepanov equation [30]. By using these components, the spectra were consistently fitted over the whole time range of measurements.

Changes in the time-resolved fluorescence spectra were clearly explained by changes in the relative intensities of individual component bands. In the wavelength regions where spectral inhomogeneity was observed, relative intensities of the longer wavelength components were always high in a later time, responsible for the red

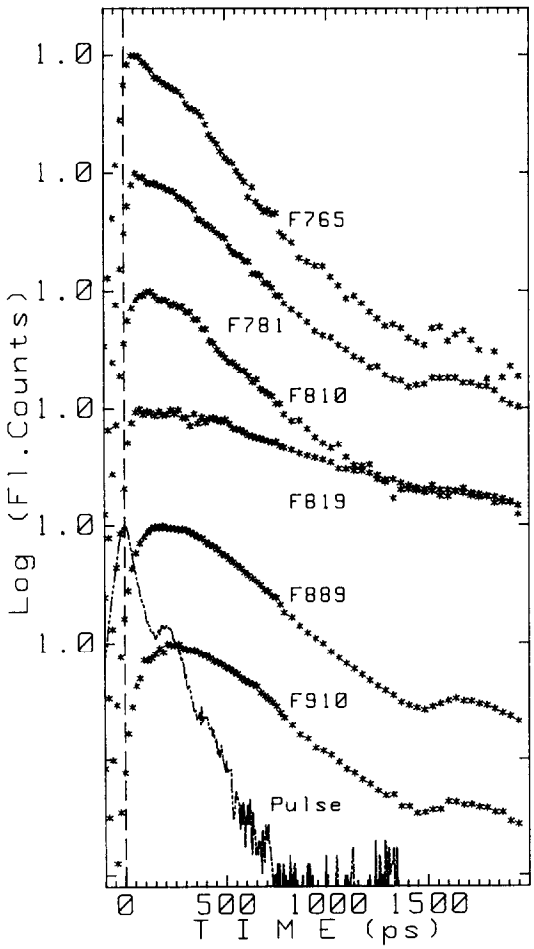

Fig. 3. Rise and decay curves of individual fluorescence components at −196°C. Asterisks show the rise and decay curves of resolved components, as shown in Fig. 2C. The pulse profile was adjusted to the decay of F889.

shift of the maxima. Based on the deconvolution pattern, the time-dependent behavior of individual components was monitored by using their relative intensities and actual photon numbers in spectra at particular times.

A fast rise and decay was found in F765 (Fig. 3; cf. Fig. 1). The rise of the F781 was very similar to that of F765, however, the decay was a little slower. The times for the maximum intensity of the baseplate Bchl *a* (F810 and F819) were around 80 ps for both, but the decay of the F819 was very slow. The rise and decay curves of the two components in B808–866 complex (F889 and F910) were similar to each other; a slow rise time indicated the presence of rise terms.

Lifetimes of individual components were estimated by convolution calculations on the resolved decay curves in the time range from −100 to 2000 ps (Fig. 3). Since we found six major components, we did not try to apply the global fit procedure [31]. Several interesting features were found in the decay parameters (Table 1). Even for the main component of Bchl *c*, F765, a rise term was resolved with 14 ps. This indicates the presence of a donor component to F765. This donor might correspond to the short-lived component resolved by global analysis [23]. The main lifetime of F765 was 28 ps, which in turn corresponds to the rise time of one of the baseplate Bchl *a*'s (F810, 30 ps) and the decay time of F819 (83 ps) was almost the same as the rise time of B866 (F889, 88 ps) and B890 (F910, 90 ps). These kinetic parameters indicate the energy transfer sequence in the order of F765, F810, F819 and either or both of F888 and F910. Finally, energy is transferred to RC (P865).

4. Discussion

4.1. Heterogeneity of antenna components

We found six fluorescence components in this study and suggested the presence of a short wavelength form of Bchl *c*. The last one may correspond to Bchl *c*727 which we observed by LD spectra [6]. Besides these, Bchl *a*806 [24] is known to be present. Thus, the number of spectral components has now been estimated to eight in *C. aurantiacus*. Two major Bchl *c*'s (Bchl *c*727 and Bchl *c*744) [6] and the third fluorescence component (Bchl *c*766 or F781, in this study) were assigned to be constituents of chlorosomes. Presence of two baseplate Bchl's *a* (F810 and F819 in this study) are consistent with our previous report [8]. The third component in B808–866, which was cryptic in absorption spectrum, was assigned to the long wavelength antenna which has been found in many species of purple sulfur and non-sulfur bacteria of the *Rhodopsuedomonas viridis* type RC; this was also the case in higher plants [32].

The presence of the long wavelength antenna (F910) was suggested by the fluorescence polarization spectrum [33] and similarly F781 was observed in the fluorescence spectrum of chlorosomes [34] and whole cells [3] measured at 4 K, even though its presence was not inferred. A long-lived baseplate fluorescence (F819) suggests a difference in function between two baseplate Bchl *a*'s.

Table 1
Lifetimes of individual fluorescence components of *C. aurantiacus* at −196°C

Components	Rise term		Decay terms					
	A	τ (ps)	A_1	τ_1 (ps)	A_2	τ_2 (ps)	A_3	τ_3 (ps)
F765	−0.878	14	0.602	28	0.393	150	0.005	736
F781	−0.767	15	0.371	(2.4)	0.619	195	0.010	1800
F810	−0.502	30	0.507	(2.4)	0.483	191	0.010	1420
F819	−0.905	68	0.808	83	0.179	662	0.013	20000
F889	−0.990	88	0.586	74	0.410	191	0.003	2460
F910	−0.999	90	0.545	65	0.449	196	0.005	1760

A and τ mean ampltitudes and lifeimtes, respectively. Numbers in parentheses have some uncertainty due to the limit of time resolution (4.82 ps per channel) used for the convolutin calculation. For explanation, see the text.

In addition to the above mentioned eight components, another Bchl *c* component is possibly present, judging from the time-resolved fluorescence spectrum of hexanol-treated chlorosomes and the steady-state spectrum of artificial aggregates (unpublished). Even though such a new component was not observed in intact cells at present, the total number of spectral component might be increasing further.

Spectral heterogeneity was evident at low temperature as shown in this study. The heterogeneity was also observed in the baseplate by time-resolved spectra even at 50°C [8]. A spectral resolution less than 5 nm is necessary to detect a small shift of the baseplate fluorescence. This might be the reason for the discrepancy between our results and those reported by Müller et al. [27].

4.2. Energy transfer sequence in C. aurantiacus

There are eight spectral components in the pigment system of *C. aurantiacus*. This large number of components are not adequate for a global analysis, because arbitrary factor(s) will increase. Our kinetic analysis on the individual decay curves will not necessarily prove the consistency in kinetic parameters for the decay of donor and rise of the acceptor, however this was the reasonable selection of an analytical method for the system consisting of such a large number of components.

The energy transfer pathway to RC including the above eight components would be different from the previous proposal of the single and one-directional energy flow model [27]. Matching of kinetic parameters (Table 1) indicates the energy flow in the order of Bchl *c*744 (F765), F810, F819, B808–866 complex (F889) and the long wavelength antenna (F910). This is assigned to the major path of energy flow in this bacterium. The function of F781 is not necessarily clear at present. A fluorescence component probably corresponding to F781 was detected in the polarized time-resolved fluorescence spectra (unpublished); its lifetime was very short, consistent with the value in Table 1. Therefore, F781 is probably incorporated into the energy flow to the baseplate Bchl *a*, however, it is not definitive at this experimental stage. The rise times for F889 and F910 were almost the same, thus the parallel energy transfer pathway from the baseplate Bchl *a* is possible. However, F910 is postulated to be tightly associated with RC, thus a sequential flow in the order of F889, F910 and RC is a probable pathway. In this sense, the main decay term of F910, 65 ps, was assigned to the energy transfer time to a closed RC at low temperature conditions. The second decay term of F910, 196 ps, coincided with the estimated transfer time to the closed RC at 50°C [27].

The rate-limiting step of energy flow in intact cells was between the baseplate Bchl *a* and the B808–866 complex in the membrane; the transfer time was about 85 ps at −196°C. This step was also rate-limiting at physiological temperature (ca. 40 ps) [8,25,27]. Spatial separation between chlorosomes and membranes may be responsible for this rate-limiting step. A prolonged transfer time under a low temperature condition is most probably due to a smaller spectral overlap. This clearly indicates the transfer mechanism between these two components to be Förster mechanism [35].

4.3. Significance of minor components in energy transfer sequences

In general, an increase in the spectral overlap integral between the fluorescence spectrum of donor and the absorption spectrum of acceptor will bring about a higher energy transfer efficiency. Therefore, at low temperature conditions, the transfer time is expected to be slower due to a narrower band width, as found in the transfer between baseplate and B808–866. When a minor component(s) is present between pigment pools, it increases the spectral overlap. For example, the spectral overlap between the major Bchl *c*744 (F765) and F810 was increased more than 10-fold due to the presence of F781. This is one of the significant points of minor components in the energy transfer sequence.

The other possibility is adjustment of the orientation of dipole moments. As shown by LD spectra of chlorosomes [6,36], the dipole moment of major Bchl *c* oriented almost parallel to the longest axis of chlorosomes, on the other hand, that of baseplate Bchl *a* aligned perpendicularly. To increase the transfer efficiency, adjustment of the orientation of the dipole moment is required. A minor component can carry out such a function in a series of energy transfers (cf. ref. [6]).

The localization of minor components is not clear. At present, rod elements in chlorosomes are postulated to be homogeneous in terms of composition, thus they consist of major Bchl *c*. If this is the case, an energy gap is the largest between chlorosomes and the baseplate. When heterogeneity is expected and one minor component, for example the F781, is included in the rod structure, the energy gap between the chlorosomes and baseplate will become smaller due to a gradient of energy levels towards the baseplate. These differences will be reflected by the kinetic behavior of spectral components. Thus, localization of minor components will become a critical subject to be studied.

Acknowledgements: The authors thank Drs. K. Shimada and K. Matsuura, Tokyo Metropolitan University for their discussions, and Prof. M. Hatano, Tohoku University for his encouragement during this work. This study was financially supported by the Grant-in-Aids for Scientific Research, from the Ministry of Education, Science and Culture, Japan to M.M.I.Y. acknowledges the Nissan Science Foundation.

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