Fluorescence Emission Spectroscopy of Single Light-Harvesting Complex from Green Filamentous Photosynthetic Bacteria

Yoshitaka Saga,[†] Tetsuichi Wazawa,[‡] Toshitaka Nakada,[§] Yoshiharu Ishii,[‡] Toshio Yanagida,[‡,||] and Hitoshi Tamiaki*,[†,⊥]

Department of Bioscience and Biotechnology, Faculty of Science and Engineering, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan, Single Molecule Processes Project, International Cooperative Research Project, Japan Science and Technology Corporation, Mino, Osaka 562-0035, Japan, Department of Physical Sciences, Faculty of Science and Engineering, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan, Department of Physiology and Biosignaling, School of Medicine, Osaka University, Suita, Osaka 565-0871, Japan, and "Form and Function", PRESTO, Japan Science and Technology Corporation, Japan

Received: October 16, 2001

The fluorescence emission properties of supramolecular light-harvesting complexes (= chlorosomes) from a green filamentous photosynthetic bacterium (*Chloroflexus aurantiacus*) on a quartz plate were studied for the first time at the single-unit level with a total internal reflection fluorescence microscope. Atomic force microscope observation evidenced that most chlorosomes were independently situated on the quartz plate. All the fluorescence bands from bacteriochlorophyll-*c* aggregates of a single chlorosome had a similar peak position and also a similar bandwidth. The conservative spectroscopic properties of each *Chloroflexus* chlorosome indicate that the presence of stereoisomers at the 3^1-position of bacteriochlorophyll-*c* molecules (3^1-R/S = 2/1) does not provide any heterogeneity of chlorosomal pigment organization among *Chloroflexus* chlorosomes.

Introduction

The primary event of photosynthesis is the light absorption by light-harvesting antennas. Light energy captured by light-harvesting complexes is transferred to a reaction center, where charge separation occurs to produce high energy compounds for living organisms. The rapid and highly efficient energy transfer at photosynthetic light-harvesting complexes has stimulated considerable research interest.

Green photosynthetic bacteria have a structurally unique light-harvesting complex called chlorosome. In a chlorosome, thousands of bacteriochlorophyll (BChl)-*c* (the molecular structure is shown in Figure 1), *d*, or *e* self-aggregate to form a dozen rodlike structures (called rod-elements), surrounded by a lipid monolayer.[1−3] The self-aggregates absorb sunlight in the cell. The excited energy migrates among rod-elements, is transferred to a complex of BChl-*a* with peptide located in a chlorosomal membrane called baseplate, and is finally focused on a cytoplasmic membranous reaction center. The self-assembly of chlorosomal BChls is in sharp contrast to antenna systems of purple bacteria and higher plants in which light-harvesting pigments are embedded in proteins. Electronic properties of BChl self-assemblies without participation of any proteins in a chlorosome are hence of great interest in terms of supramolecular photochemistry. Many reports of chlorosomes are

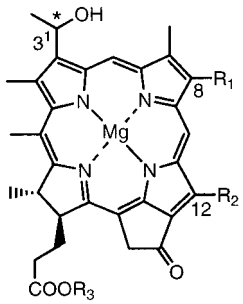

Figure 1. Molecular structures of bacteriochlorophyll (BChl)s-*c*. For BChl-*c* from green filamentous photosynthetic bacteria: R_1 = ethyl, R_2 = methyl, and R_3 = stearyl (major). In green sulfur photosynthetic bacteria, BChl-*c* is a mixture of several homologues in which R_1 = ethyl, *n*-propyl, or isobutyl, R_2 = methyl or ethyl, and R_3 = farnesyl (major).

available,[1−3] but the distribution of energy levels of rod-elements and the energy migration mechanism among rod-elements in the chlorosome have not been unraveled. One of the problems in spectroscopic studies of chlorosomes is the difficulty in distinguishing the spectral properties inside a chlorosome from the spectral heterogeneity among chlorosomes. To overcome this problem, we examine here the fluorescence emission spectroscopy of a single chlorosome from a green filamentous photosynthetic bacterium *Chloroflexus* (*Cfl.*) *aurantiacus*.

Recent developments in single-molecule imaging and spectroscopy allows us to study supramolecular functions at the single assembly level, without ensemble averaging over the heterogeneity among the supramolecules.[4,5] Typically, van Oijen et al. reported the fluorescence excitation spectroscopy of a single light-harvesting complex 2 (LH2, BChl-*a*/peptide complex) from purple photosynthetic bacteria, indicating in detail

* Corresponding author. Fax: +81-77-561-2659. E-mail: tamiaki@se.ritsumei.ac.jp.
[†] Department of Bioscience and Biotechnology, Ritsumeikan University.
[‡] Single Molecule Processes Project.
[§] Department of Physical Sciences, Ritsumeikan University.
[||] Department of Physiology and Biosignaling, School of Medicine, Osaka University.
[⊥] "Form and Function", PRESTO.

10.1021/jp0138227 CCC: $22.00
Published on Web 01/17/2002

the electronic structure of two circular assemblies of BChl-*a* in LH2.[6−8] Here we report application of the single-molecule spectroscopy to a unique antenna apparatus, *Chloroflexus* chlorosome (BChl-*c* self-aggregates).

Ligh-harvesting pigments in chlorosomes from green photosynthetic bacteria are mixtures of components that vary in the stereochemistry of the 3^1-position (epimer), as well as in the alkylation at the 8- and 12-positions (homolog).[9] Such a heterogeneity in the molecular form of chlorosomal chlorophylls is not present in the light-harvesting chlorophyllous pigment (only BChl-*a*) of LH2 from purple bacteria. A variety of spectroscopic methods examined previously provide no clear pictures to the question of how the inhomogeneous molecular structures of chlorosomal chlorophylls affect the structure and function of BChl self-assemblies in a chlorosome. This work also addresses to elucidation of distribution of 3^1-epimeric BChl-*c* molecules in a single *Chloroflexus* chlorosome; analysis of BChl-*c* extracted from large amounts of *Chloroflexus* cells showed 3^1-R/S = 2/1.[10]

Materials and Methods

Chlorosomes were prepared from *Cfl. aurantiacus* Ok-70-fl according to the method of Gerola and Olson.[11] Purified chlorosomes were diluted with 50 mM Tris-HCl buffer containing 10 mM sodium dithionite (pH 8.0) to an optical density of <0.1 at a 1 cm path length at 740 nm (Q_y peak position of *Chloroflexus* chlorosome). A 50 μL aliquot of the solution was deposited on ca. a 1.5 cm × 1.5 cm area of a quartz plate. After standing for 3−5 min, the quartz plate was thoroughly washed with the buffer solution and immediately used for observation.

Fluorescence emission spectroscopy was performed essentially according to the reported procedures by Wazawa et al.[12] The 457.9 nm line of a CW argon−krypton mixed gas laser (Innova 70C spectrum, Coherent) was used as a light source. The laser beam was totally reflected at the incident angle of 70° to the norm at the silica glass slide−sample solution interfaces. The illumination area was ca. 250 × 110 μm^2 ($1/e^2$ value) at the specimen plane. The fluorescence emission from chlorosomes was collected with an oil-immersion microscope objective. In single-molecule spectroscopic measurement, the fluorescence from well-separated spots (corresponding to single chlorosomes) was dispersed by wavelength with a spectrograph (Holospec f/2.2, Kaiser) equipped with a grating (model HFG-750, Kaiser), and was taken with a CCD camera (CCD15-11-0-232, Wright Instruments). The fluorescence intensity of single chlorosomes was uncorrected. The corrected fluorescence emission spectra of ensemble chlorosomes in aqueous buffer solution were measured with a spectrophotometer (F-4500, Hitachi).

For atomic force microscope (AFM) measurement, a 20−50 nm thick carbon amorphous layer was deposited on the surface of a chlorosome-immobilized quartz plate in a vacuum of 10−5 Torr.[13] The sample was imaged by the contact mode with a commercial AFM (Nanoscope E, Digital Instruments).

Results and Discussion

Figure 2 depicts a typical fluorescence image of chlorosomes on a quartz plate immersed in a buffer solution. The fluorescent signal from one spot was clearly distinguishable from other signals or background. Single chlorosomes examined here were gradually photobleached in about one minute at room temperature (data not shown). This bleaching process was different from that in a single LH2 from purple bacteria. The fluorescence of a single LH2 disappeared primarily in one-step like the photobleaching reactions of a single dye molecule.[6,14] The

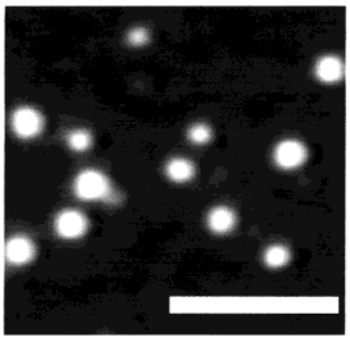

Figure 2. A typical fluorescence image of single chlorosomes immobilized on a quartz plate. The specimen was illuminated by an argon−krypton mixed gas laser at 457.9 nm with the excitation power of 1 mW at the specimen plane. The image was integrated over 32 video frames. Bar, 5 μm.

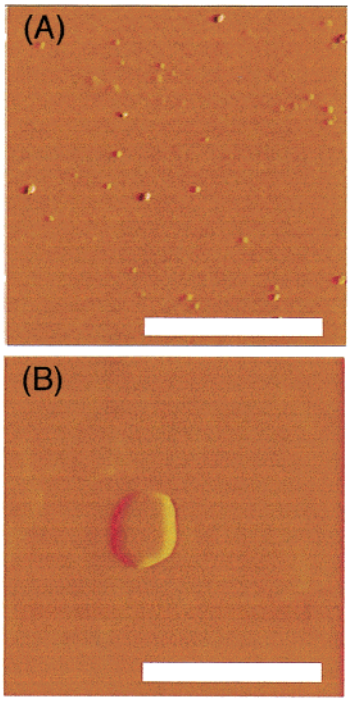

Figure 3. (A) A typical AFM image of single chlorosomes immobilized on a quartz plate. A carbon layer was applied on the surface to fix chlorosomes. The specimen was imaged by the contact mode. Bar, 5 μm. (B) A typical magnified image of a single chlorosome. Bar, 500 nm.

photobleaching of 18 BChl-*a* circular assembly in LH2 would result from the trapping of delocalized excitations by even one bleached BChl-*a* molecule.[14] Comparison of the photobleaching processes of a light-harvesting antenna between purple and green filamentous bacteria implies that the exciton is not delocalized over most BChl-*c* molecules in a chlorosome, and is probably localized on oligomers of these molecules; excitations might be localized on each rod-element.

AFM observation confirmed that most fluorescence spots imaged with a fluorescence microscope resulted from the individual chlorosomes. Typical AFM images of chlorosomes immobilized on a quartz plate by the same procedure as by fluorescent microscopic observation are shown in Figure 3.[15] Ellipsoidal particles about 200−250 nm in length and about 150−200 nm in width were individually observed. Their size was a little larger than that estimated previously by AFM[13] or electron microscope.[16] This was ascribed to the carbon coating (ca. 20−50 nm thickness) and size effect of the silicon nitride

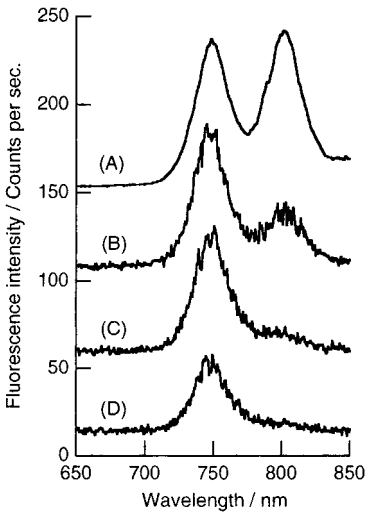

Figure 4. Fluorescence emission spectra of chlorosomes in a buffer solution (A) and individual chlorosomes on a quartz plate (B)−(D). (A) was obtained with a Hitachi F-4500 spectrophotometer (excitation wavelength, 457 nm), while (B)−(D) were obtained as described in the text.

AFM tip used, which was pyramidal with ca. a 40 nm radius of curvature at the tip apex, and a cone angle of ca. 70°. Most previous studies of single-molecule imaging and spectroscopy gave no direct evidence that the fluorophore observed was a single molecule. Here we indicated using AFM that the fluorescence came from individual chlorosomes.

In Figure 4, the fluorescence emission spectra from a typical single chlorosome on a quartz plate immersed in a buffer solution are compared with an ensemble spectrum of chlorosomes in aqueous buffer. The ensemble spectrum (Figure 4(A)) exhibited two bands at 749 and 802 nm, corresponding to the emission from BChl-*c* aggregates and BChl-*a* in baseplates, respectively. The emission of BChl-*a* is due to the excitation energy transfer from BChl-*c* aggregates predominantly absorbing 457-nm light.

The fluorescence emission spectra from all the individual chlorosomes exhibited a band from BChl-*c* aggregates around 750 nm (see Figures 4(B)−(D)). The averaged peak positions of 27 measured samples was 748.4 ± 0.9 nm (means ± s.d.), similar to the ensemble spectrum in a buffer solution (749 nm). No other fluorescence emission band (due to monomeric BChl-*c* or bacteriopheophytin-*c*, etc.) appeared below 700 nm, indicating that BChl-*c* aggregates would not be dissociated or denatured by adsorption of purified chlorosomes on a quartz plate. The fluorescence emission from BChl-*a* of a baseplate (BChl-*a*/peptide complex) located in a chlorosomal membrane was observed in some samples examined here, but the BChl-*a* fluorescence lost its intensity in many samples. The suppression of the BChl-*a* fluorescence intensity might be ascribed to low energy transfer efficiency from BChl-*c* to BChl-*a*, or to quenching of BChl-*a* fluorescence at the chlorosome on a quartz surface. The denaturation of baseplates on a quartz plate could not be ruled out as the reason for the suppression.

All the emission peaks from BChl-*c* aggregates in a single chlorosome[17] were positioned in a limited wavelength range between 746 and 750 nm. The estimated bandwidth (at 1/*e* value of the peak) of the BChl-*c* emission band of a single chlorosome was quite homogeneous (644 ± 40 cm^{-1}, means ± s.d.) and was well matched to the ensemble spectrum in a buffer solution (639 cm^{-1}). The narrow distribution provides direct information on the spectroscopic inheterogeneity of single chlorosomes from *Cfl. aurantiacus*. The spectral properties of BChl-*c* aggregates among individual *Chloroflexus* chlorosomes indicate the homogeneous supramolecular structures assembled by BChl-*c* molecules in the chlorosome, based on the local structural interactions between 3^1-hydroxyl group, 13^1-keto group, and central magnesium.[1−3]

We note a possible reason for the spectral homogeneity: the molecular structure of BChl-*c* in green filamentous photosynthetic bacteria (*Chloroflexus*). The light-harvesting BChl-*c* molecule in chlorosomes from *Cfl. aurantiacus* has the same substituents at the 8- (ethyl group) and 12-positions (methyl group), whereas chlorosomes from green sulfur photosynthetic bacteria (*Chlorobium*) has several kinds of substituted BChls at both positions (see Figure 1). The variation of alkyl chains at the 8- and 12-positions as in homologues significantly affects the spectral properties of chlorosomal self-aggregates formed by many BChl molecules.[18−22] In view of this point, a single homologue of chlorosomal pigments of *Chloroflexus* would give less heterogeneity of BChl self-assembly in the chlorosomes.

Interestingly, the BChl-*c* existing in *Chloroflexus* chlorosomes is an epimeric mixture at the 3^1-position (R/S = 2/1), but the stereochemistry would not cause the heterogeneous BChl organization among the chlorosomes. This is in shape contrast to the spectral difference between in vitro self-aggregates of epimerically pure chlorosomal BChls.[21−26] The results obtained here suggests that the 3^1-R/S epimeric ratio of BChl-*c* is almost the same in each *Chloroflexus* chlorosome, and/or that R- and S-epimers (2:1) of BChl-*c* are mixed to form a new homogeneous association in a *Chloroflexus* chlorosome, as implied elsewhere.[23,24]

To summarize, we present the first results of single-supramolecule fluorescence emission spectroscopy of light-harvesting antenna from a green filamentous photosynthetic bacterium, indicating that all the BChl-*c* aggregates in a single *Chloroflexus* chlorosome have almost the same spectroscopic property. This work demonstrates that single-molecule spectroscopy is a powerful tool to elucidate the electronic properties of photosynthetic pigment organization as well as the distribution of composed pigments in a single antenna apparatus.

Acknowledgment. This work was supported in part by a Research Grant from the Foundation Advanced Technology Institute. We thank Prof. Katsumi Matsuura and Dr. Niels-Ulrik Frigaard, Tokyo Metropolitan University, for their kind advice about culturing *Cfl. aurantiacus*. We also thank Prof. Mamoru Mimuro and Ms. Tomoko Shigemura, Yamaguchi University, for the preparation of chlorosomes.

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