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Fluorescence emission spectroscopy of a single light harvesting chlorosome from green filamentous photosynthetic bacteria

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Introduction

Green photosynthetic bacteria have a unique light harvesting antenna called chlorosome. In chlorosomes, bacteriochlorophyll(BChl)s-*c*, *d* or *e* self-aggregate to form rod-like apparatus. Light energy is absorbed by BChl aggregates, which is transferred to a reaction center *via* baseplate (BChl-*a*/protein complex in a chlorosomal membrane), converting into chemical energy available for living bacteria.

Light harvesting BChl-*c* in chlorosomes from a green filamentous photosynthetic bacterium *Chloroflexus* (*Cfl.*) *aurantiacus* is a mixture of 3^1-*R*/*S* epimers, which differ the stereochemistry of the 3-(1-hydroxyethyl) group. Such a heterogeneity of molecular structures of chlorosomal BChl-*c* might cause a spectral heterogeneity in a chlorosome. However, the difficulty in distinguishing the possible spectral properties in a single chlorosome from those among several chlorosomes prevents unravelling the detail spectral features of BChl aggregates in a chlorosome.

Recently, van Oijen *et al*. reported the fluorescence excitation spectroscopy of a single light harvesting complex 2 (LH2) from purple photosynthetic bacteria, indicating in detail the electronic structure of two circular assemblies of BChl-*a* in LH2 (van Oijen *et al*., 1998, 1999, 2000). The single-molecule spectroscopy could be useful for studying the supramolecules without ensemble averaging over the heterogeneity. In the present study, we examine the fluorescence emission spectroscopy of a single chlorosome from *Cfl*. *aurantiacus*.

Materials and methods

Chlorosomes were isolated from *Cfl. aurantiacus* Ok-70-fl according to the method of Gerola and Olson (1986). Chlorosomes were diluted to an optical density of <0.1 at a 1-cm pathlength at the Q_y peak position of *Cfl*. chlorosome with 50 mM Tris-HCl buffer (pH 8.0) containing 10 mM sodium dithionite. A 50-μL aliquot of the solution was deposited on *ca*. a 1.5×1.5 cm area of a quartz plate. After standing for 3-5 minutes, the quartz plate was thoroughly washed with the buffer and the adsorbed chlorosomes were observed by a total internal reflection and atomic force microscope.

Fluorescence emission spectroscopy of single chlorosomes was essentially the same as reported by Wazawa *et al*. (2000). 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 fluorescence emission from well-separated spots, which were emitted from 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).

For atomic force microscope (AFM) observation, a 20-50 nm thick carbon amorphous layer was deposited on the surface of a specimen in a vacuum of 10-5 Torr (Zhu *et al*., 1995). The immobilized chlorosomes were imaged by the contact mode with a commercial AFM (Nanoscope E, Digital instruments).

Results and discussion

Figure 1 shows a typical fluorescence image of *Cfl*. chlorosomes on a quartz plate immersed in a buffer solution. The fluorescence spots were appeared from a chlorosome-immobilized quartz plate with a total internal reflection fluorescence microscope. The emission from one spot (corresponding to a single chlorosome) was clearly distinguishable from other signals or background. Chlorosomes observed were gradually photobleached in about one minute at room temperature. This relatively high photostability allows us to measure the fluorescence emission spectra of *Cfl*. chlorosomes at the single-unit level. The photobleaching process was different from one-step bleaching observed at a single LH2 from purple bacteria (Bopp *et al*. 1997, van Oijen *et al*. 1998).

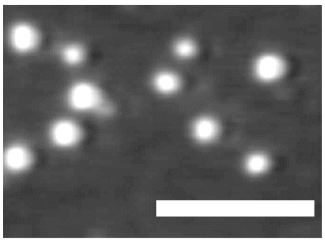

Figure 1 A typical fluorescence image of single chlorosomes adsorbed on a quartz plate. The image was integrated over 32 video frames. Bar, 5μ m.

AFM observation of a chlorosome-adsorbed quartz plate confirmed that most chlorosomes were individually situated on a quartz plate. The fluorescence signals, therefore, unambiguously came from single chlorosomes.

Figure 2 depicts the fluorescence emission spectra of chlorosomes in an aqueous buffer solution and a typical single chlorosome on a quartz plate immersed in a buffer solution. The ensemble spectrum exhibits two emission bands around 750 and 800 nm from BChl-*c* aggregates and BChl-*a* in baseplates, respectively. The BChl-*a* fluorescence is due to the excitation energy transfer from BChl-*c* aggregates predominantly absorbing 457-nm light. Fluorescence spectra of single chlorosomes also had a band from BChl-*c* aggregates around 750 nm. The fluorescence from BChl-*a* was observed in some samples examined in this study, but their intensity was much weak in many samples. The suppression of BChl-*a* fluorescence intensity might be ascribed to quenching of BChl-*a* fluorescence at the chlorosome on a quartz surface.

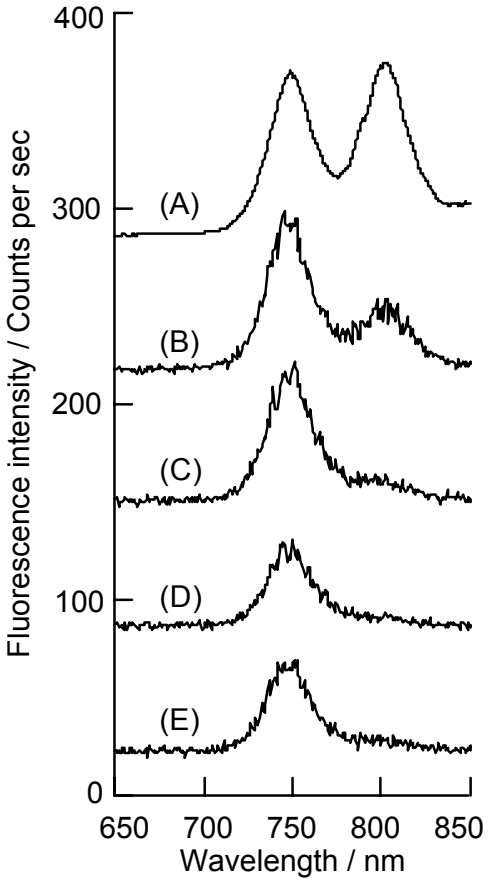

Figure 2 Fluorescence emission spectra of chlorosomes in a buffer solution (A) and single chlorosomes on a quartz plate (B)-(E).

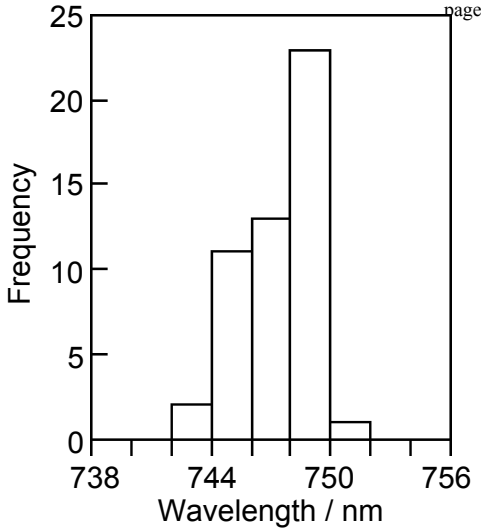

Figure 3 Distribution of estimated fluorescence peak positions from BChl-*c* aggregates in single chlorosomes.

Distribution of the emission peak position from BChl-*c* aggregates was shown in Figure 3. The fluorescence bands of BChl-*c* aggregates were estimated from fitting by a Gaussian curve. Fluorescence peaks were positioned in the narrow range between 742 and 752 nm. The estimated bandwidth of BChl-*c* emission band of a single chlorosome was also quite homogeneous. These provide the direct information on the spectral homogeneity of single chlorosomes from *Cfl. aurantiacus*.

BChl-*c* in *Cfl*. chlorosomes is an epimeric mixture at the 3^1-position (*R*/*S*=2/1), but the stereochemistry does not induce the spectral heterogeneity of BChl-*c* self-aggregates. This implies the homogeneous composition or a homogeneous association of 3^1-*R*/*S* BChl-*c* in *Cfl*. chlorosomes. Additionally, the single homolog of BChl-*c*, which has the same substituents at the 8- (ethyl group) and 12-positions (methyl group) (Fages *et al*., 1990), might be a possible reason in the spectral inheterogeneity among BChl-*c* self-aggregates in single *Cfl*. chlorosomes.

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References

Bopp MA, Jia Y, Li L, Cogdell RJ, Hochstrasser RM (1997) *Proceeding of the National Academy of Sciences of the USA* **94**, 10630–10635.

Fages F, Griebenow N, Griebenow K, Horzwarth AR, Shaffner K (1990) *Journal of the Chemical Society Perkin Transactions 1*, 2791–2797.
Gerola PD, Olson JM (1986) *Biochimica et Biophysica Acta* **848** 69–76.
van Oijen AM, Ketelaars M, Kohler J, Aartsma TJ, Schmidt J (1998) *The Journal of Physical Chemistry B* **102**, 9363–9366.
van Oijen AM, Ketelaars M, Kohler J, Aartsma TJ, Schmidt J (1999) *Science* **285**, 400–402.
van Oijen AM, Ketelaars M, Kohler J, Aartsma TJ, Schmidt J (2000) *Biophysical Journal* **78**, 1750–1757.
Wazawa T, Ishii Y, Funatsu T, Yanagida T. (2000) *Biophysical Journal* **78**, 1561–1569.
Zhu Y, Ramakrishna BL, van Noort PI, Blankenship RE (1995) *Biochimica et Biophysica Acta* **1232**, 197–207.