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# Structural studies on the light-harvesting membrane protein complexes and cytochromes from thermophilic photosynthetic bacteria

Long-Jiang YU[1], Yu HIRANO[2], Kunio MIKI[2] and Seiu OTOMO*[1]
[1]Faculty of Science, Ibaraki University, Mito 310-8512, Japan
[2]Department of Chemistry, Graduate School of Science, Kyoto University, Kyoto, Japan

## Introduction

In purple photosynthetic bacteria, the light energy is absorbed by two types of light-harvesting complexes (LH1, LH2), and then is transferred efficiently to the reaction center (RC) where the primary charge separation takes place across the membrane and a cyclic electron transport chain occurs. The LH1 complex is located intimately around the RC with a fixed stoichiometric ratio to form the so-called core complex (LH1-RC). LH1 is a large oligomer of a basic structural unit composed of a heterodimer of two small integral membrane polypeptides (α and β, ca. 6 kDa) associated with bacteriochlorophyll (BChl) and carotenoid molecules. *Thermochromatium* (*Tch*.) *tepidum* is a thermophilic purple sulfur photosynthetic bacterium originally isolated from a hot spring in Yellowstone National Park. It grows anaerobically at optimum temperatures of 48 – 50 ºC with an upper limit of 58 ºC, and contains an unusual LH1 complex that absorbs maximally around 917 nm ($Q_y$ transition). In a series of investigations, we found that the *Tch*. *tepidum* LH1 is highly stable at room temperature when copurified with RC in a LH1-RC form, and calcium ions are involved in both the enhanced thermal stability and the large red shift of the LH1 $Q_y$ transition[1, 2].

We further examine the putative $Ca^{2+}$-binding sites of LH1 complex embedded in the native photosynthetic membrane using purified chromatophores. Preliminary results on crystallization of the LH1-RC complex are reported towards future X-ray crystal structure determination.

## Materials and Methods

Chromatophores were prepared by ultrasonicating the cells, followed by ultracentrifugation (150000 *g*, 90 min). The chromatophores were further purified by sucrose density centrifugation and resuspended in 20 mM Tris-HCl (pH 8.5) buffer.

The purified LH1-RC complex was concentrated using a Centricon centrifugal filter YM-100 (Millipore, U.S.A) and adjusted to a BChl a concentration of 1.58 mM. Crystallization was performed using the sitting-drop vapor-diffusion method at 20 ˚C. The protein solution was mixed with the same volume of precipitant solution (20 mM Tris-HCl, pH 7.5, 3 mM DPC, 50 mM $CaCl_2$, 16% w/v PEG2000).

## Results and Discussion

We made use of the topographic feature of chromatophore to identify the putative $Ca^{2+}$-binding site in *Tch. tepidum* LH1 complex[3]. Incubation of the chromatophores in the presence of EDTA revealed no substantial change in the absorption maximum of LH1 $Q_y$ transition, whereas further addition of detergents to the chromatophores-EDTA solution resulted in a blue-shift of the LH1 $Q_y$ peak from 915 nm to the final position at 892 nm. The blue-shifted LH1 $Q_y$ transition in chromatophores can be restored to its original position by addition of $Ca^{2+}$ ions. The results suggest that the $Ca^{2+}$-binding site is exposed on the inner surface of chromatophores, corresponding to the C-terminal region of LH1. The change of the LH1 $Q_y$ peak to shorter wavelengths was relatively slow compared to that of the purified LH1-RC complex. This may be attributed to the membrane environment in which the LH1 polypeptides are embedded. An Asp-rich fragment in the LH1 α-polypeptide is considered to form a crucial part of the binding network.

Crystals of the *Tch. tepidum* LH1-RC complex were obtained using PEG as a precipitant. The crystals showed a rhombic shape with typical size of 0.4 mm × 0.2 mm × 0.2 mm. We have being trying to improve the crystal quality by screening the optimum condition of buffer pH, detergents, small molecule additives, cryoprotectants and soaking with heavy metals in order to get higher-resolution diffraction and finally to identify the $Ca^{2+}$-binding sites.

## References

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*otomo@mx.ibaraki.ac.jp