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

Yu HIRANO¹, Long-Jiang YU¹, Makoto HIGUCHI¹, Hirozo OH-OKA², Kunio MIKI³ and Seiu OTOMO*¹

¹Faculty of Science, Ibaraki University, Mito 310-8512, Japan;
²Department of Biological Sciences, Graduate School of Science, Osaka University, Osaka, Japan;
³Department 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. In green sulfur photosynthetic bacteria, the RC consists of five subunits: PscA containing a special pair, PscB containing Fe-S clusters A and B (F₅/F₆), PscC containing a heme c (cyt c₅₅), PscD binding to the FMO, and the BChl-a protein FMO. Two molecules of cytochrome c₂ bind to the RC and each of them has been reported to directly transport an electron from cytochrome bc₁ to the P840. Cytochrome c₂ is supposed to consist of an N-terminal transmembrane domain and a C-terminal periplasmic domain which contains one heme c.

Preliminary results on crystallization of the LH1-RC complex are reported towards future X-ray crystal structure determination. The crystal structure of C-terminal periplasmic domain (C-cyt c₅₅) in which a single heme c is bound has been determined.

Materials and Methods

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 CaCl₂, 16% w/v PEG2000).

The C-cyt c₅₅ was over-expressed in Escherichia coli and purified by an anion-exchange chromatography (TOYOPEARL DEAE-650S, TOSOH) followed by gel filtration (Sephacyrl S-200 HR, GE Healthcare). Crystallization was performed using ammonium sulfate as a precipitant.

Results and Discussion

LH1-RC complexes

Crystals of the Tch. tepidum LH1-RC complex were obtained using PEG as a precipitant [1]. The crystals showed a rhombic shape with typical size of 0.4 mm × 0.2 mm × 0.2 mm. Thermal stability of the Tch. tepidum LH1-RC is much higher than that of its mesophilic counterparts and the enhanced thermal stability was shown to require Ca²⁺ as a cofactor [2]. We are in progress in improving the crystal quality in order to get higher resolution diffraction and to identify the Ca²⁺-binding sites.

Crystal structure of the C-cyt c₅₅

We have determined the crystal structure of the oxidized C-cyt c₅₅ at 1.3 Å resolution. The N-terminal 20 residues of C-cyt c₅₅ are disordered and additional 8 residues form a loop structure. This feature may explain the flexibility between the transmembrane and the periplasmic domains of cytochrome c₅₅, which makes it possible to facilitate the direct electron transfer between cytochrome bc₁ and RC. C-cyt c₅₅ shows structural similarities with cytochrome c₅₅₁ from Pseudomonas aeruginosa and cytochrome c₇ from Monoraphidium braunii. Despite of the overall structural similarities with the class I cytochrome proteins, the coordination pattern of the heme c iron is different between C-cyt c₅₅ and other members in this class. On the other hand, unusual paramagnetic NMR shifts were observed for the oxidized form of C-cyt c₅₅. This may be attributed to the unique coordination environment of the heme c as revealed from the crystal structure. All of these results are going to be submitted.

References


*otomo@mx.ibaraki.ac.jp