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

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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 (F_A/F_B), PscC containing a heme *c* (cyt c_z), PscD binding to the FMO, and the BChl-*a* protein FMO. Two molecules of cytochrome c_z bind to the RC and each of them has been reported to directly transport an electron from cytochrome bc_1 to the P840. Cytochrome c_z 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 c_z) 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 $CaCl_2$, 16% w/v PEG2000).

The C-cyt c_z was over-expressed in *Escherichia coli* and purified by an anion-exchange chromatography (TOYOPEARL DEAE-650S, TOSOH) followed by gel filtration (Sephacryl 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. 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 Ca^{2+} as a cofactor [1]. We are in progress in improving the crystal quality in order to get higher resolution diffraction and to identify the Ca^{2+}-binding sites.

Crystal structure of the C-cyt c_z

We have determined the crystal structure of the oxidized C-cyt c_z at 1.3 Å resolution[2,3]. The N-terminal 20 residues of C-cyt c_z 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 c_z, which makes it possible to facilitate the direct electron transfer between cytochrome bc_1 and RC. C-cyt c_z shows structural similarities with cytochrome c_{551} from *Pseudomonas aeruginosa* and cytochrome c_6 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 c_z and other members in this class. On the other hand, unusual paramagnetic NMR shifts were observed for the oxidized form of C-cyt c_z. 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

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