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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 $c_z$) which contains one heme *c* has been determed.

## 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 DDPC, 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*

From a wide-range search for the screening conditions, crystals of the *Tch. tepidum* LH1-RC complex were obtained from the conditions using PEG as a precipitant. Typically, the crystals showed a rhombic shape with clear edges, and the sizes ranged from 1.0 mm × 0.3 mm × 0.3 mm to 0.3 mm × 0.1 mm × 0.1 mm. Formation of the crystals were found to be sensitive to the PEG concentration and the crystal growth was almost completed in two weeks. The diffraction pattern from these crystals was recorded only to a low resolution (around 6 Å resolution), but clearly indicating crystal lattices for the protein crystals [1].

*Crystal structure of the C-cyt $c_z$*

We have determined the crystal structure of the oxidized C-cyt $c_z$ by Fe-SAD method and refined to 1.3 Å resolution. 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 mediate 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.

## Reference

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