

Structural studies on the light-harvesting and electron transport protein complexes 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. *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 °C with an upper limit of 58 °C, 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, and calcium ions are involved in both the enhanced thermal stability and the large red shift of the LH1 Q_y transition [1, 2]. Preliminary results on crystallization of the native LH1-RC complex are reported towards future X-ray crystal structure determination.

We have determined crystal structures of two soluble electron transport proteins, cytochrome (Cyt) c' and flavocytochrome c , from *Tch. tepidum*. The structural features provide insights into the mechanism of their high thermostabilities and spectroscopic properties.

Materials and Methods

Crystallization of the native *Tch. tepidum* LH1-RC complex was performed using 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 PEG3000).

Crystals of the Cyt c' and flavocytochrome c were obtained by sitting-drop vapour-diffusion method at 20 °C using 30% (v/v) PEG400 and 18% (w/v) PEG3000 as precipitants, respectively. Structure determination was performed by molecular replacement method.

Results and Discussion

Crystals of the native *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 continue 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 Ca²⁺-binding sites.

The Cyt c' and flavocytochrome c exhibit high structural similarities to their mesophilic counterparts from *Alc. vinosum*, and the different residues between the corresponding proteins are mainly located on the surface and exposed to the solvent. The enhanced stabilities can be interpreted on the basis of the structural and sequence information: increased number of hydrogen bonds formed between main chain nitrogen and oxygen atoms, more compact structures and reduced number of glycine residues. Many residues with large side chains in *Alc. vinosum* Cyt c' are substituted by alanines in *Tch. tepidum* Cyt c' . Water molecules are found in the heme vicinity of *Tch. tepidum* Cyt c' and form hydrogen bonds with the heme ligand and C-terminal charged residues. Similar bound waters are also found in the vicinity of one heme group in the diheme subunit of *Tch. tepidum* flavocytochrome c . Electron density map of the *Tch. tepidum* flavocytochrome c clearly revealed the presence of disulfur atoms positioned between two cysteine residues at the active site near the FAD prosthetic group. The result strongly suggests that flavocytochrome c is involved in the sulfide oxidation *in vivo*. Detailed discussion is given in a published paper [3] on the relationships between the crystal structures and the spectroscopic properties observed for these proteins.

References

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