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Structural studies on the light-harvesting membrane 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 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 explore the putative Ca^{2+}-binding sites in *Tch. tepidum* LH1 complex by using both native and biosynthetically Sr-substituted LH1-RC complexes. Preliminary results on crystallization of the native LH1-RC complex are reported towards future X-ray crystal structure determination.

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 $CaCl_2$, 16% w/v PEG3000).

Sr-substituted LH1-RC complex was obtained by cultivating the *Tch. tepidum* cells in a medium containing Sr^{2+} instead of Ca^{2+}, followed by the purification procedure described previously[1]. Spectroscopic and thermodynamic characterizations were carried out on the Sr-LH1-RC complex.

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 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.

The LH1-RC complex purified from Sr-substituted cells exhibited a Q_y transition at 888 nm, 27 nm shorter than that of native LH1-RC. Removal of Sr^{2+} resulted in a spectral change of the LH1 Q_y transition to around 870 nm[3]. Reconstitutions of Sr^{2+} and Ca^{2+} into the Sr-depleted complex resulted in a red-shift of the Q_y peak back to 888 nm and 908 nm, respectively, although the latter was distinctively different from the native Q_y peak position. Thermal stability of the Sr-LH1-RC was slightly lower than that of its native counterpart as revealed by differential scanning calorimetry measurement[3]. These results indicate that the Ca^{2+} ions in the native LH1-RC complex were replaced with the Sr^{2+} ions at the same or similar binding sites although the coordination states are somewhat different between each other. The differences are reflected on the absorption spectra and thermodynamic property. This study reveals evidence for the LH1 complex to have ability of adaptation to the environmental changes *in vivo* by utilizing the exogenous metal cations, and provides an alternative means to identify the metal-binding sites in this complex.

References

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