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Crystallization and characterization of the metal-substituted light-harvesting-reaction center core complexes

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1 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 (a and b, 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 915 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].

The crystal structure of LH1-RC from *Tch*. *tepidum* has been determined [3], in which the Ca-binding sites have been identified. On the other hand, the Ca^{2+} ions in LH1 can be replaced by other divalent metal ions (e.g., Sr^{2+} and Ba^{2+}), resulting in a substituted LH1-RC with the LH1 Q_y in a range of 880 ~ 890 nm [1, 2]. Here, we report crystallization and characterization of the Sr- and Ba-substituted LH1-RCs.

2 Experiment

In the last step of purification, the LH1-RC fractions were eluted by a linear gradient of $SrCl_2$ or $BaCl_2$ from 10 mM to 50 mM. Crystallization of the Sr- and Ba-LH1-RCs and post-crystallization treatment were carried out following the same method as described previously [3].

3 Results and Discussion

Figure 1 shows the absorption spectra of Sr- and Ba-substituted LH1-RC crystals. The LH1 Q_y bands were observed at 888 nm for both Sr- and Ba-LH1-RCs. A notable feature is that the substitution with Sr^{2+} is much slower compared to the Ba^{2+} although the final LH1 Q_y transitions become the same. Both Sr- and Ba-substituted LH1-RC complexes show a Q_y peak at the same position as that obtained from the biosynthetically Sr^{2+}-substituted LH1-RC. This indicates that the substitution has been completed. Both the absorption spectra of Sr- and Ba-LH1-RCs are similar to that of the native LH1-RC of *Allochromatium vinosum*, a mesophilic counterpart and close relative of the *Tch*. *tepidum*.

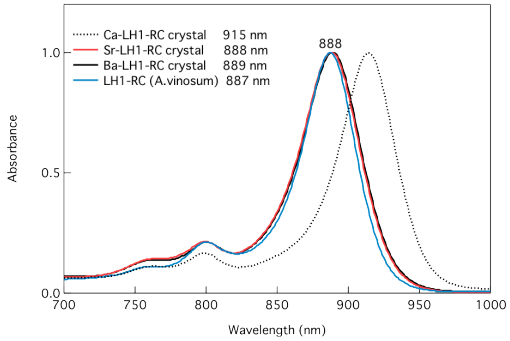

Fig. 1: Absorption spectra of the metal-substituted LH1-RC complexes.

Figure 2 shows the crystals of the Sr- and Ba-substituted LH1-RC complexes. They have the similar sizes to that of the native Ca-LH1-RC complex. Now, the diffraction measurements and structure analysis are in progress.

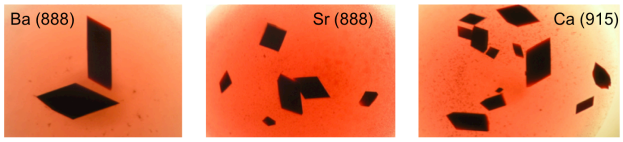

Fig. 2: Crystals of the metal-substituted and native LH1-RC complexes.

References

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