

## High-resolution crystal structure of the LH1-RC complex from *Thermochromatium tepidum*

Photosynthesis is a process by which light energy from the sun is converted into chemical energy indispensable for the survival of living organisms on the earth. Photosynthetic bacteria can also perform this process (Fig. 1), although they do not evolve oxygen as a side product. In order to perform photosynthesis efficiently, photosynthetic organisms have developed various light-harvesting pigment-protein complexes to compensate for the low light energy density available on the surface of the earth. In purple photosynthetic bacteria, there are usually two kinds of light-harvesting (LH) antennas named LH1 and LH2, which absorb light energy first and then transfer them to the reaction center (RC) to initiate photochemical reactions. LH2 is a group of peripheral antenna with different numbers of the  $\alpha/\beta$ -subunits to form octamers or nonamers in different bacteria, whereas LH1 is the core antenna and encircles the RC to form an integral pigment-membrane protein supercomplex called LH1-RC supercomplex. RC contains 3-4 subunits (L, M, H and Cyt) and LH1 consists of 14-17 pairs of  $\alpha/\beta$ -subunits or  $\alpha/\beta/\gamma$ -subunits, giving rise to a total molecular weight over 400 kDa for the LH1-RC supercomplex. The RC complex from a purple bacterium is the first membrane protein whose structure was determined in 1985 [1], and from then on, several techniques have been employed to determine the structure of the LH1-RC supercomplex in the last decades. Recently, several structures of the LH1-RC complexes were reported by either X-ray crystallography [2] or cryo-electron microscope (cryo-EM) [3] from different species of bacteria at various resolutions. However, the high-resolution crystal structure of this supercomplex has not been determined until recently.

We purified the LH1-RC supercomplex from a thermophilic photosynthetic bacterium *Thermochromatium tepidum* isolated from Mammoth Hot Springs in Yellowstone National Park, and crystallized, determined its structure at a resolution of 1.9 Å [4]. The diffraction data was collected at SPing-8 BL41XU at a wavelength of 1.0 Å with a beam size of 35×22 μm<sup>2</sup>. The initial structure was solved by the molecular replacement method using the previous structure determined at 3.0 Å [2] as the search model with calcium ions, lipid and solvent molecules omitted. Incorporation of cofactors, lipids and detergent molecules and model modification were manually performed and the final structure was refined to 1.9 Å resolution [4].

The overall structure shows that the RC contains

Cyt, L, M and H subunits and is surrounded by 16 heterodimers of the LH1  $\alpha/\beta$ -subunits containing 32 bacteriochlorophyll (BChl) *a* and 16 spirilloxanthin molecules, forming a completely closed elliptical ring [4] (Fig. 2). Compared with the previously determined structures of isolated RC [5] and LH1-RC complex [2], relatively large deviations were found in some regions of the RC subunits and the N- and C-termini of the LH1 subunits. Owing to the high resolution achieved, a number of cofactors and structural details were identified for the first time. These include a number of lipids and additional ubiquinone molecules, a large amount of water molecules that form a potential proton transfer pathway to the secondary electron acceptor Q<sub>B</sub>, detailed coordinating environment for 16 Ca<sup>2+</sup> ions in LH1, etc.

The differences found in the RC structure between the current “intact” LH1-RC supercomplex and previously reported isolated RC-only complex include the N-terminal region and a loop region (residues 172-196) of the Cyt subunit, and a loop region of the H subunit (residues 44-58). Similar differences are found between a recently reported cryo-EM structure of LH1-RC from *Blc. viridis* [3] and those of the isolated RC-only complex [5]. These differences are considered to arise from the interactions of the RC subunits with the surrounding LH1 subunits in the intact supercomplex that are absent in the isolated RC-only complex. Thus, the RC structure represents its native state more closely.

Q<sub>A</sub> and Q<sub>B</sub> are menaquinone-8 (MQ8) and ubiquinone-8 (UQ8) molecules bound to the M, L subunits of RC respectively, and serve as the primary and secondary electron acceptors of the electron

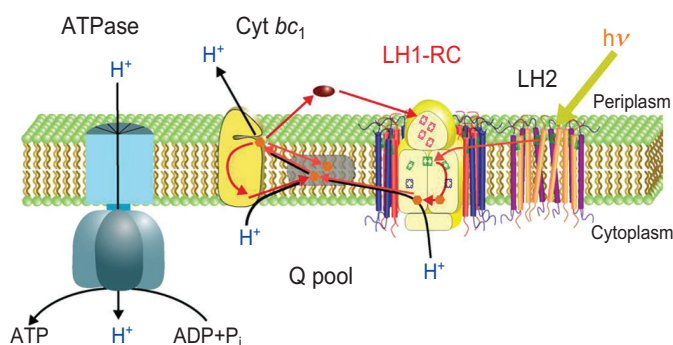
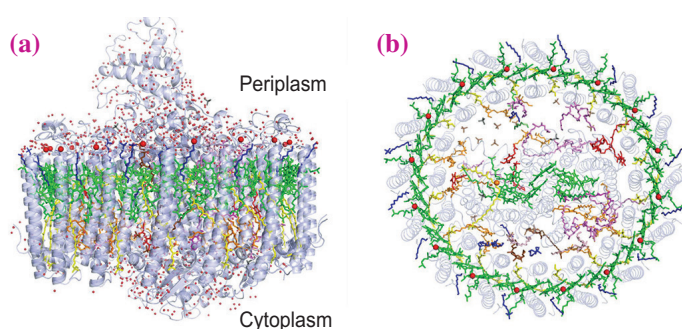


Fig. 1. Schematic illustration of a photosynthetic unit in the intracellular membrane of purple bacteria. Red arrows show the energy transfer from LH2 to LH1 and the cyclic electron-transport pathway.



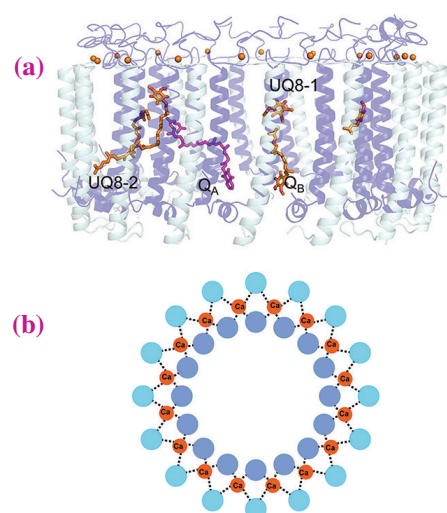
**Fig. 2.** Side view **(a)** and top view **(b)** of the LH1-RC super-complex from *Tch. tepidum*. Color codes: protein subunits, gray; BChls, green; spirilloxanthin, yellow;  $\text{Ca}^{2+}$  ions, red; water, raspberry.

transfer chain. In our structure, we identified one MQ8 and five UQ8s, among which, the MQ8 and one UQ8 are located in the  $\text{Q}_\text{A}$  and  $\text{Q}_\text{B}$ -binding sites respectively. The additional four UQ8s are located in the gap region between RC and LH1 (**Fig. 3(a)**). In particular, one of these UQ8 molecules was found to insert its isoprenoid tail into a potential channel formed by the LH1  $\alpha/\beta$ -subunits, which suggests that it is undergoing transport between the inside and outside of the ring through this possible exchange channel. This channel has been suggested in the previous studies [2], however, the present result provides the direct experimental evidence for the transport of UQ8s through this channel. Since there are 16 pairs of the LH1  $\alpha/\beta$ -subunits, there are potentially 16 such channels to facilitate the transport of the UQ8 molecules.

The *Tch. tepidum* LH1-RC supercomplex has two special features compared with other mesophilic counterparts, namely, red-shift of LH1 Qy transition and enhanced thermostability of the supercomplex. These features have been suggested to be brought about by the binding of  $\text{Ca}^{2+}$  ions. However, the detailed binding environments of the  $\text{Ca}^{2+}$  ions have not been determined unambiguously in the medium resolution due to the flexibility of the region surrounding the  $\text{Ca}^{2+}$ -binding site [2]. In the high resolution structure, all ligands including water molecules were identified; they include the side chain of Asp49, the carbonyl oxygens of  $\alpha$ -Trp46,  $\alpha$ -Ile51 and (n+1)  $\beta$ -Trp45, and two water molecules, giving rise to a six-coordination structure. A large number of interactions were found between the LH1  $\alpha/\beta$ -polypeptides that are mediated by the  $\text{Ca}^{2+}$ -binding site; this may greatly contribute to the tight connection of the neighboring LH1  $\alpha/\beta$ -polypeptides (**Fig. 3(b)**), resulting in the higher thermostability. Since the  $\alpha$ -Trp46 and (n+1)  $\beta$ -Trp45 residues coordinated

to the  $\text{Ca}^{2+}$  ions are also hydrogen-bonded with the neighboring BChl, binding of the  $\text{Ca}^{2+}$  ions may stabilize this region, resulting in the red-shift of the Qy absorption.

The novel features of LH1-RC supercomplex revealed by this high-resolution structure provide important information regarding the energy transfer between LH1 and RC, the exchange of  $\text{Q}_\text{B}$  molecule and shuttling of UQ8s through LH1 to the cytochrome  $bc_1$  complex, proton transfer pathways to  $\text{Q}_\text{B}$ , and the possible effects of  $\text{Ca}^{2+}$ -binding on the LH1 red-shift and thermostability of the LH1-RC supercomplex. This information greatly advances our understanding on the bacterial photosynthetic reactions and will also provide important clues for the development of artificial photosynthetic systems.



**Fig. 3.** **(a)** Distribution of the menaquinone (magenta) and ubiquinone (orange) molecules over the LH1-RC supercomplex. **(b)** Schematic model for the coordinating pattern of the 16  $\text{Ca}^{2+}$  ions in the LH1 complex.

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