Structure of the Cytochrome b_6f Complex of Oxygenic Photosynthesis

The photosynthetic unit of oxygenic photosynthesis is organized as two large multimolecular membrane complexes, photosystem II (PSII) that extracts low-energy electrons from water and photosystem I (PSI) that raises the energy level of such electrons using light energy to produce a strong reductant, NADPH. The two photosystems operate in a series linked by a third multiprotein complex called the cytochrome b_6f complex (Fig.1). The cytochrome b_6f complex is a membrane-spanning protein complex embedded in the thylakoid membrane of photosynthetic organisms. The molecular weight of the complex is 220,000 as a dimer with 26 transmembrane helices. The b_6f complex controls the electron transfer between the plastoquinol reduced by PSII and the electron carrier protein plastocyanin that associate with PSI. Coupled with the electron transfer, the b_6f complex also generates a transmembrane proton gradient for ATP synthesis. The crystal structures of the cytochrome b_6f complex [1,2] complete the description of the architecture of the oxygenic photosynthetic electron transport chain, since three-dimensional structures have been provided for PSI and PSII [3-5].

The structure of the b_6f complex from cyanobacterium *M. laminosus* was determined by the isomorphous replacement method using Pb and Pt derivatives and multiwavelength anomalous diffraction from native iron atoms. X-ray diffraction data from native crystals and complex crystals with the quinone-analogue inhibitor DBMIB were collected at the Osaka University beamline **BL44XU** of SPring-8. The highest resolution data of 3.0 Å from the complex crystal with another analogue inhibitor, TDS, was collected at the SBC beamline 19ID, APS. The initial model was developed into a 3.4 Å map of the native complex. Final refinement was carried out with a dataset from a co-crystal with TDS (Figs. 2, 3).

Viewed along the membrane normal, the b_6f complex is 90 Å × 55 Å within the membrane side, and 120 Å × 75 Å on the lumen (*p*) side (Fig. 2). A prominent feature of this structure is an extended quinone exchange cavity between the monomers, which exchanges lipophilic plastoquinone in the bilayer center, and also mediates the electron and proton transfer across the complex. The heme-binding 4 transmembrane helices core of the b_6f complex is almost identical to that of the analogous bc_1 complex in the respiration chain of the mitochondrial membrane. However, there are three prosthetic groups recently found in the b_6f complex that are not present in the bc_1 complex: a high spin heme *x* covalently bound to the cyt b_6 polypeptide by one thioether bond, and the pigment molecules, chlorophyll *a* and β-carotene. Heme *x* occupies the binding site of the *n*-side bound quinone in the bc_1 complex. The presence of heme *x* in contact with heme b_n and a plastoquinone in the cavity suggests the mechanism of ferredoxin-mediated cyclic electron transfer (dotted line in Fig. 1) that uses classical elements of the Q-cycle mechanism [1,2].

The quinone-mediated redox connection between the (*p*) and (*n*) sides of the complex can be visualized through (a) a plastoquinone molecule close to heme *x* on the *n*-side, and (b) a quinone analogue inhibitor, TDS, on the *p*-side of the other monomer that surrounds the cavity (Fig. 3). The position of TDS in the b_6f complex is similar to that of the *p*-side inhibitor myxothiazol in the bc_1 complex. Another *p*-side inhibitor, DBMIB, is bound near Glu in the conserved sequence in the *p*-side peripheral loop. Both of these inhibitors are >10 Å from the closest histidine ligand of the [2Fe-2S] cluster of Rieske ISP,

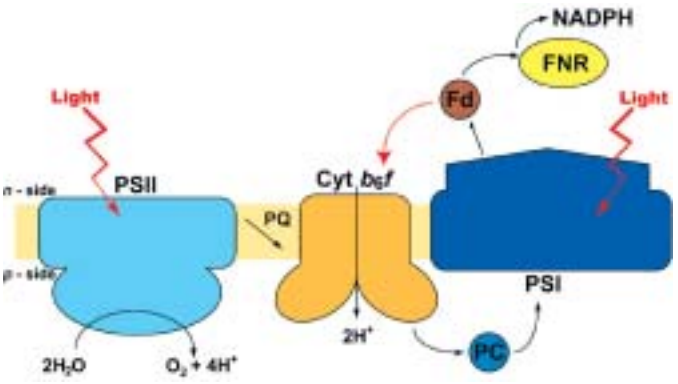

Fig. 1. Integral membrane protein complexes and electron carrier proteins responsible for electron transport and proton translocation in oxygenic photosynthesis.

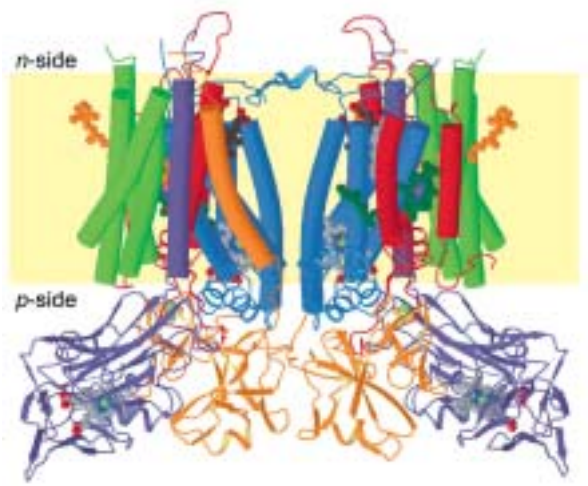

Fig. 2. Side view of eight-subunit dimeric cytochrome b_6f complex. Hemes *bn*, *bp* and *f* (grey), heme *x* (dark brown), chlorophyll a (dark green), β-carotene (orange), cyt b_6 (blue), subunit IV (red), cyt *f* (purple), iron-sulfur protein (orange), and small subunits (light green).

and cannot form an H-bond with the histidine ligand as does stigmatelline in the bc_1 complex.

The [2Fe-2S] cluster is 29 Å from the heme Fe of its electron acceptor, cyt *f*. In the case of the bc_1 complex, the positional change of the [2Fe-2S] cluster to a more c_1-proximal position in different crystal forms suggests that Rieske ISP mediates the electron transfer between the membrane-bound quinol and cyt c_1 by shuttling between the membrane-proximal and c_1-proximal states. However, only a single membrane-proximal position for the Rieske ISP has been observed in the b_6f complex. Together with the different positions of cytochrome *f* and its heme relative to cyt c_1, this implies the difference in trajectory between Rieske ISP and cyt *f*.

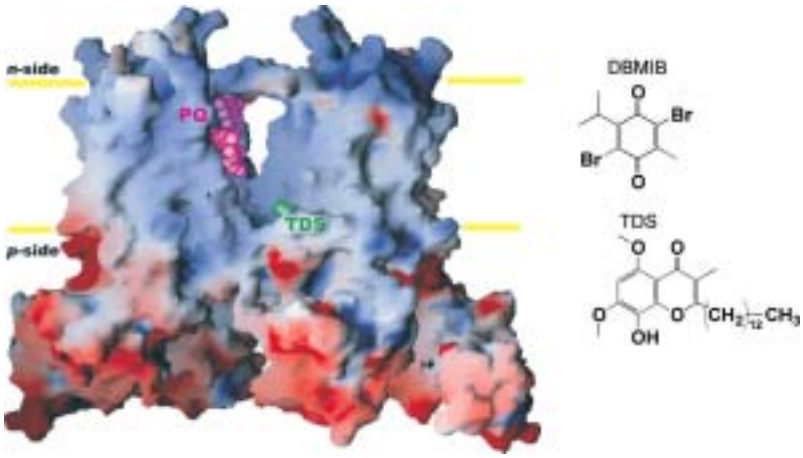

Fig. 3. Molecular surface of the complex mapping the electrostatic potential. Plastoquinone (PQ) and Q-analogue inhibitor (TDS) are drawn in the cavity as a cpk model, and the chemical formulas of inhibitors are shown on the right.

Genji Kurisu*

Institute for Protein Research, Osaka University

E-mail: GKurisu@bio.c.u-tokyo.ac.jp

**Present address* : Department of Life Sciences, The University of Tokyo

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