Location and Function of Chloride Ions in Oxygen-Evolving Photosystem II Revealed by X-Ray Crystallography

Most of the oxygen on the earth is produced by photosystem II (PSII), a membrane protein complex found in thylakoid membranes from prokaryotic cyanobacteria to eukaryotic higher plants. PSII performs a series of light-induced electron transfer reactions, coupled with this is the water-splitting reaction leading to the evolution of molecular oxygen, which is indispensable for oxygenic life on the earth. PSII from cyanobacteria contains 17 membrane-spanning subunits and 3 peripheral, membrane-extrinsic proteins, and over 70 cofactors including chlorophylls, carotenoids, plastoquinones, heme-irons and a non-heme iron, Mn, Ca, Cl, with a total molecular mass of around 350 kDa for a monomer. The structure of cyanobacterial PSII has been reported at resolutions of 3.8-2.9 Å [1-4], which provided much information on the overall arrangement of protein subunits and most of the cofactors, although the current resolution is not high enough to elucidate the detailed structure of PSII required for the full understanding of the light-induced water oxidation reaction.

The water-oxidizing complex of PSII is composed of 4 Mn atoms and 1 Ca atom, most of which are coordinated by D1, one of the reaction subunits of PSII, with only one residue provided by CP43, a chlorophyll-binding protein. It has been long known that in order for the Mn_4Ca-cluster to operate properly, chloride ions (Cl^-) are required. In the absence of Cl^-, the water-splitting reaction cannot proceed beyond some steps, and oxygen cannot be formed. However, the exact number and binding site(s) of Cl^- in PSII have not been identified due to the limited resolution of the current structure available, as well as the difficulty to detect Cl^- by X-ray diffraction analysis.

In order to identify the binding sites for Cl^- within PSII, we substituted Cl^- with either bromide ions (Br^-) or iodide ions (I^-), and crystallized, solved the crystal

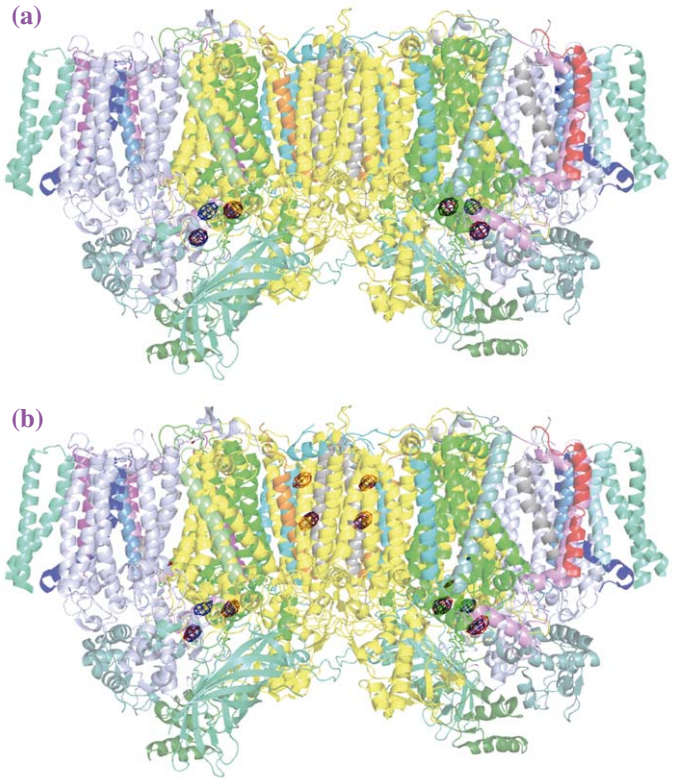

Fig. 1. Difference-Fourier maps and anomalous Fourier maps of Br^- or I^--substituted PSII, overlapped with the structure of PSII dimer. **(a)** Difference-Fourier map of Br^--substituted PSII *minus* Cl^--PSII, and anomalous Fourier map of Br^--substituted PSII collected at 0.9 Å wavelength, which were superimposed with the PSII dimer structure at a view perpendicular to the normal of the membrane plane. Red: Difference-Fourier map; Blue: Anomalous Fourier map. **(b)** Difference-Fourier map of I^--substituted PSII *minus* Cl^--PSII, and anomalous Fourier map of I^--substituted PSII collected at 1.0 Å wavelength superimposed with the PSII dimer structure. Red: Difference-Fourier map; Blue: Anomalous Fourier map.

structure of Br^--substituted and I^--substituted PSII respectively [5]. Since both Br^- and I^- have significant effects of anomalous dispersion around 1.0 Å, their detection was greatly facilitated by their anomalous scattering effects. Fig. 1 shows the difference Fourier maps between native-PSII (Cl^--PSII) and Br^- or I^- substituted PSII, as well as the anomalous Fourier maps of Br^- and I^- substituted PSII, respectively, which were overlapped with the overall structure of PSII dimer. It was found that two binding sites for Br^- are existed around the Mn_4Ca-cluster in both the difference Fourier map and anomalous Fourier map of Br^--substituted PSII, and there were no additional binding sites for Br^- in the other area of PSII. The same two sites were also found in the difference Fourier map and anomalous Fourier map of I^--substituted PSII, indicating that both Br^- and I^- bind to the same two sites around the Mn_4Ca-cluster. In the I^--substituted PSII, however, three additional sites were found which are located distantly from the Mn_4Ca-cluster, these three sites are thus concluded not involved in the oxygen-evolving reaction [5].

Since I^--substitution for Cl^- completely inhibited oxygen evolution, and this inhibition was completely reversed upon re-substitution of I^- with either Br^- or Cl^-, we concluded that both Br^- and I^- bind to the Cl^--binding sites in PSII; in other words, the above determined two sites surrounding the Mn_4Ca-cluster represent the Cl^--binding sites in native PSII [5]. The structure around these binding sites is depicted in Fig. 2. It was found that these two sites are coordinated to two residues that provided direct ligands to the Mn_4Ca-cluster, namely, Glu333 of D1 subunit and Glu354 of CP43 subunit [5], suggesting that Cl^- ions may be required for maintaining the correct structure of these residues, thereby maintaining the structure of the Mn_4Ca-cluster required for the water-splitting reaction to proceed. In addition, one of the two Cl^--binding sites was located in the exit of a proposed proton channel, suggesting that this site may be required to maintain the structure of the channel [5]. X-ray diffraction experiments were performed at beamline **BL41XU**.

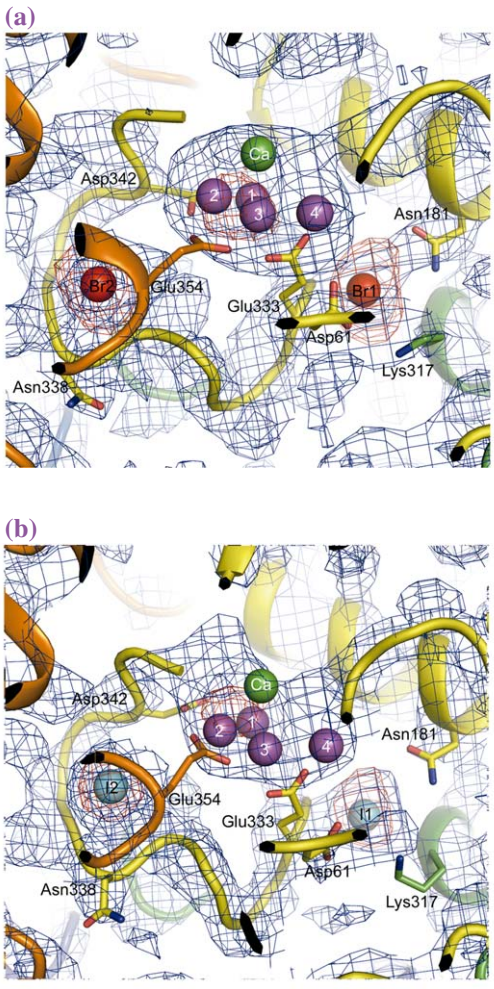

Fig. 2. Location of the two anion binding sites in PSII. **(a)** Composite omit Fo-Fc map (blue) and anomalous map (red) of Br^--substituted PSII (blue), respectively, superimposed with the structure of the Mn_4Ca-cluster and its surrounding regions. Color codes for the residues are as follows: Yellow, D1; Green, D2; Orange, CP43. **(b)** Composite omit Fo-Fc map (blue) and anomalous map (red) of I^--substituted PSII (red), respectively, superimposed with the structure of the Mn_4Ca-cluster and its surrounding regions. The color codes for the residues are the same with panel **(a)**.

Jian-Ren Shen

Graduate School of Natural Science and Technology, Okayama University

E-mail: shen@cc.okayama-u.ac.jp

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