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Proton transfer inhibition by molecular anion substitutions in Photosystem II

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Photosystem II (PSII) performs a series of light-induced electron transfer leading to the splitting of water and generation of molecular oxygen. PSII is a membrane protein complex consisting of 40 subunit-proteins in a dimer and a total of 114 co-factors with an overall molecular weight of 700 kDa. Chloride ion (Cl-) is known as one of the most essential co-factors, and it have been reported that other monovalent anionic ions (Br-, I-, NO3-, CH3COO- and N3-) compete for the Cl--binding site and therefore depress the oxygen evolution activity [1]. Previously, we have identified two Cl--binding sites near the catalytic core of a Mn4CaO5-cluster by analyzing Br- or I- substituted PSII crystal structures [2], and these results were confirmed by the crystal structure analysis of native PSII at 1.9 Å resolution from a thermophilic cyanobacterium [3]. Both Cl- binding sites (CL-1 and CL-2) were surrounded by water molecules similarly, but differences were found in their immediate protein environment. CL-1 was surrounded by polar residues (D1-Asp61 and D2-Lys317), whereas CL-2 was surrounded by backbone nitrogen atoms. The roles of these Cl--binding sites have not been fully understood yet.

In order to understand the roles of each Cl--binding site in the water-splitting reaction of PSII, we crystallized cyanobacterial PSII with the Cl- substituted by inhibitive anions, azide ion (N3-) or nitrate ion (NO3-). The replacement of Cl- by these anions was performed with a co-crystallization technique. We succeeded to solve the N3- and NO3- substituted PSII crystals at 2.0 and 2.1 Å resolutions respectively, and confirmed the replacement of Cl- by these anions by anomalous different Foureir map collected at the wavelength of 1.7 or 1.9 Å. We found that the structure around CL-2 was not influenced by the N3- or NO3- substitutions; however, the structure around CL-1 was changed in which a salt-bridge between D2-Lys317 and D1-Asp61 was formed in place of the hydrogen-bond interaction between Cl- and D2-Lys317. Interestingly, this conformation change at CL-1 was similar to the simulation model of Cl- depletion in PSII [4]. Therefore, we concluded that the role of CL-1 is maintaining the separation of the negative charge on D1-Asp61 from D2-Lys317, which is important for the proton transfer through D1-Asp61. Formation of the salf-bridge between D1-Asp61 and D2-Lys317 by the substitutions of Cl- by other anions will inhibit the proton transfer, thereby suppressing the oxygen evolution activity of PSII.

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