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Oxygen-evolving photosystem II (PSII) is the site of light-induced water-splitting and provides us with molecular oxygen indispensible for life on the earth. The components of PSII include 17 membrane-spanning subunits, 3 hydrophilic, peripheral subunits, and over 70 cofactors, including chlorophylls, carotenoids, Mn, Ca, Fe, Cl, and plastoquinones, which give rise to a total molecular mass of 350 kDa for a monomer. Chloride ion (Cl⁻) is an essential cofactor for oxygen evolution of PSII, and is closely associated with the Mn_4CaO_5 -cluster. Two Cl-binding sites have been identified in the vicinity of the Mn_4CaO_5 -cluster by substitution of Cl⁻ with bromide ion (Br) or iodide ion (I⁻). Substitution of Cl⁻ with I⁻ completely inhibited oxygen evolution of PSII, whereas substitution of Cl⁻ with Br did not inhibit oxygen evolution [1].

Recently, we succeeded in solving the crystal structure of PSII at 1.9 Å resolution, and confirmed the two chloride-binding sites in native PSII [2]. However, the cause for the inhibition of oxygen evolution by I has not been clarified. In order to elucidate the inhibition mechanism of oxygen evolution by I⁻, we crystallized Br and I⁻ substituted PSII crystals, and analyzed these crystal structures at resolutions of 2.06 and 2.03 Å, respectively. The diffraction data of PSII crystals was collected at beamline BL41XU of SPring-8 in JAPAN and processed with MOSFLM. Each initial phase angles of the reflections were determined by the molecular replacement method with MOLREP in the CCP4 program suit using the previously determined 1.9 Å resolution structure of PSII (PDB code: 3ARC) as a search model. Model improvement and structural refinement were carried out using COOT and REFMAC5, respectively. The results showed that two Iions (I-1, I-2) indeed replaced the two Cl⁻-binding sites in the vicinity of the Mn₄CaO₅-cluster; however, the positions of the two I⁻binding sites were slightly different from those of the two Cl-binding sites, whereas two Br ions bound to the same positions of Cl-. Moreover, fine structural changes were found in the residues surrounding the I-2 site, especially in the C-terminal residue of D1 subunit, Ala344, the carboxylate group of which is directly coordinated to Ca and Mn2 of the Mn₄CaO₅-cluster. These results suggest that the inhibition of oxygen evolution by I⁻ can be ascribed to the structural changes caused by substitution of Cl⁻ with I⁻.

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Production of membrane histidine kinases from mycobacterium tuberculosis

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Bacterial pathogens frequently use Two-Components Systems (TCS) to recognize and respond to the changing environmental conditions within the host, and they normally do this by means of a phosphotransfer reaction between a membrane-localizated histidine kinase sensor protein (HK) and a cytoplasmic response regulator (RR), usually a transcription factor. Mycobacterium tuberculosis contains few

TCS compared to many other bacteria [1], even so, these TCS appear to play important roles in early intracellular survival of the pathogen as well as in aspects of virulence [2], what makes them potential targets for the development of new chemotherapeutic agents.

We have selected all the integral membrane HKs present in the Mycobacteria tuberculosis genome for its production to initiate structural studies. Here we describe our medium-throughput strategy including ligation independent cloning in two T7 based expression vectors, auto-induction protein expression in Escherichia coli, and a membrane solubilization screening with the most successful detergents used in structural analysis.

After optimization of protein expression and detergent-mediated solubilization, we achieved large-scale purification of 70% of the membrane HK cloned, yielding enough quantities for biochemical, biophysical and structural analysis.

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Exploring the Na⁺/K⁺-ATPase : src kinase complex

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The Na⁺/K⁺-ATPase is a plasma membrane protein of fundamental physiological significance. During one reaction cycle, 3 Na⁺ ions are exported and 2 K⁺ are imported under the consumption of one ATP molecule. The ion gradients being formed are important for maintenance of resting potentials and regulation of cell volume, and are exploited by ion channels as well as secondary transporters that facilitate the transport of e.g. ions, nutrients or neurotransmitters across biological membranes.

Research over the last few years suggests that the Na⁺/K⁺-ATPase is implicated in interactions with several other proteins including the Src kinase, where it serves as a signal transducer [1]. The Src kinase is inhibited while it is bound to the Na⁺/K⁺-ATPase. Upon binding of cardiotonic steroids (e.g. ouabain) to the extracellular side of the Na⁺/K⁺-ATPase, the kinase domain of Src is supposedly released and activated. Subsequent phosphorylation of downstream proteins by Src activates for instance MAPK signal cascades and production of mitochondrial reactive oxygen species.

To expand the availability of Na⁺/K⁺-ATPase from different animal sources, the purification of the Na⁺/K⁺-ATPase from bovine kidneys was established based on the purification of pig Na⁺/K⁺-ATPase [2]. The individual domains of the Src kinase are produced recombinantly in *E. coli*. Interactions between the Src kinase domains and the Na⁺/K⁺-ATPase are analyzed using sucrose cushion assays and microscale thermophoresis [3]. The complexes are furthermore applied in screenings for crystallization conditions.

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