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Acta Cryst. (2011) A67, C743**Crystallization and structure analysis of a PsbM-deletion mutant of PSII**

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Photosystem II (PSII) is a membrane protein complex located in the thylakoid membranes of oxygenic photosynthetic organisms, and performs light-induced oxygen evolution from water. PSII consists of four large trans-membrane (TM) subunits, thirteen small TM subunits and three peripheral hydrophilic subunits. PsbM is one of the small TM subunits of PSII, and is located in the monomer-monomer interface of PSII dimer. The functions of PsbM include stabilization of the PSII dimer and the plastoquinone (Q_B) binding site. In order to elucidate the structural basis for PsbM function, we purified and crystallized oxygen-evolving PSII core complex from a PsbM-deletion mutant of *Thermosynechococcus vulcanus*, and started its crystallographic analysis.

Mutant cells were grown in a phosphoric acid culture media. The mutant PSII dimer was purified by anion exchange chromatography after LDAO and β -DDM solubilization with slight modifications described in [1]. Crystallization was carried out with an oil-batch method, and the crystals obtained were soaked into a cryoprotectant solution followed by an exposure to air for dehydration before flash-cooling in a nitrogen gas stream at 100K. X-ray diffraction images were collected at BL41XU of SPring-8. The diffraction data was processed to 2.45 Å resolution by *mosflm*, and structure analysis was carried out with the molecular replacement method with the wild type PSII structure refined at 1.9 Å resolution as a search model [2]. From the results obtained, we confirmed the lack of PsbM in the $F_{\text{mutant}} - F_{\text{wild-type}}$ isomorphous difference Fourier map calculated with the phase information of the wild-type crystal, and found small but significant differences between the subunit structures of mutant and wild type PSII based on the structural model obtained, and structural changes may be expected to be visible after the structure refinement is completed at 2.45 Å resolution.

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Acta Cryst. (2011) A67, C743**A C-terminal Pathway in the calcium pump**

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The sarco(endo)plasmic reticulum calcium pump (SERCA) is an integral membrane protein of the P-type ATPase family, responsible for calcium transport from the cytoplasm into the sarco- or endoplasmic reticulum. Its activity, maintaining low (micromolar) calcium levels in the cytoplasm while refilling intracellular calcium stores, is vital for muscle function and calcium signaling processes.

We have solved a 2.2 Å crystal structure of SERCA 1a from rabbit hind leg muscle, stabilized by thapsigargin and aluminium fluoride in the calcium-free transition state of dephosphorylation. The relatively high resolution and low mosaicity data of this crystal form allow us to locate several well-defined water molecules within the membrane-embedded region of the enzyme, pinpointing a hydrated cavity close to the C-terminus of the enzyme. A systematic comparison of SERCA crystal structures in six different catalytic states reveals that this cavity is exclusive to calcium-free states. Molecular dynamics simulations confirm that the C-terminal cavity constitutes an open, fully hydrated connecting path between the ion binding sites and the cytoplasm in calcium-free SERCA. In agreement with mutational studies [1-4], and in analogy to a similar pathway recently described in the related sodium-potassium pump [5], we suggest that this C-terminal pathway plays a functional role in proton and/or calcium exchange with the cytoplasm.

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Acta Cryst. (2011) A67, C743-C744**Crystal structure of the STAS domain of the SLC26 anion transporter prestin**

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The SulP superfamily of anion transporters includes over 200 sequenced members from archaea to mammals. Among these, the members of the mammalian SLC26 family are multipurpose anion exchangers with fundamental roles in human physiology; many of them are involved in genetic diseases. As an extreme example of versatility, prestin (SLC26A5) works as a voltage-driven membrane motor rather than a transporter, and it is responsible for the outer hair cells somatic electromotility that increases hearing sensitivity and frequency selectivity in mammals [1]. In stark contrast with the wealth of information accumulated on cationic transport proteins, the structure and transport mechanisms of anion transporters remain largely unexplored, the only exception being ClC chloride channels.

SulP/SLC26 anion transporters carry a hydrophobic core with a variable membrane topology and a C-terminal cytosolic domain (around 240 amino acids long in prestin) that is essential in plasma membrane targeting and protein function. In prestin, deletions, mutations or replacement with analogous C-terminal portions of related SLC26 transporters (pendrin or PAT-1) have been reported to be lethal for function. As other SLC26 and SulP transporters, the

prestin C-terminal part is mainly composed by a STAS domain, whose name (Sulfate Transporters and Anti-Sigma factor Antagonist) is due to a remote but significant sequence similarity with bacterial spoIIAA (ASA, Anti-Sigma factor Antagonist) proteins.

We present the crystal structure at 1.57 Å resolution of the STAS domain of prestin, the first 3D structural characterization of a mammalian SLC26/Sulp STAS domain [2]. We show that it significantly deviates from those of related bacterial ASA proteins. In particular, we have found that the N-terminal region from residue 505 to residue 525 (prestin numbering), previously considered merely a generic linker region between the last transmembrane region and the STAS domain, is indeed fully part of the STAS domain from a structural point of view. This implies that the STAS domain lies just beneath the plasma membrane, most probably being able to interact with the lipid bilayer and/or with portions of transmembrane domains of the protein in a functionally relevant manner. The emerging view is that the STAS domain is a core scaffold fundamental for the proper organization of the supramolecular assembly responsible for the transport function. The structure presented here can help in guiding functional studies aimed at deciphering the transport mechanism not only of prestin but also of other SLC26 and, more generally, of SulP anion transporters.

Aberrant cellular localization and loss of function associated to mutations, deletion mutants, chimeric proteins or other manipulations involving the STAS domain of SLC26 transporters are well interpreted on the basis of this structure and, at least in the analyzed cases, they can be attributed to two different reasons: large misfolding of the domain or subtle perturbations that do not substantially alter the 3D structure but that can negatively effect its ability to properly establish intra- or intermolecular interactions. These findings could have important consequences for the planning of therapeutic-potential intervention.

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Crystal structure analysis of multidrug transporter MexB

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Pseudomonas aeruginosa is an opportunistic human pathogen that causes severe infections. It is known that *P. aeruginosa* has an ability to acquire multidrug resistance. The resistance results from mainly low permeability of the outer membrane and an over-expression of tripartite efflux pumps. One of the tripartite efflux pumps, MexAB-OprM complex, mainly participates in multidrug resistance of this bacteria. The complex consists of three components, MexA, MexB, and OprM. The inner membrane component, MexB, recognizes and binds very broad range of substrates such as antibiotics, biocides, dyes, organic solvents, and detergents. After recognition and binding of the substrates, MexB transports them to the outer membrane component, OprM, using energy of proton electrochemical gradient. The substrates transported from MexB are discharged to extracellular space by passing through OprM. MexA anchors to the inner membrane via fatty acid attached to the N-terminal cysteine residue, and it is assumed to cross-bridge the MexB and OprM.

MexB performs the multidrug recognition of substrates, so is considered a key component of MexAB-OprM. In other words, MexB structure is essential for understanding of multidrug resistance. Recently, MexB structure has been solved at 3.0 Å resolution, [1]. However, higher resolution data is required for further understanding of multidrug resistance, especially, the interaction between MexB and substrates. The aim of this research is to clarify the mechanism of multidrug resistance by high resolution X-ray crystal structure of MexB.

To obtain the high resolution X-ray crystal structure, we examined the purification and crystallization conditions. As a result of the study, high quality crystals are reproducibly obtained at the crystallization condition, 20 mM citrate pH4.0, 19 ~ 20 % PEG400. After optimization of conditions of cryo-protectants, 2.8 Å resolution X-ray crystal structure was obtained. Purification, crystallization and structural analysis of MexB will be presented.

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Isolation and crystallization studies of selected proteins from plant photosystem II

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Abbreviation: PSII – photosystem II, OEC–oxygen/- evolving complex

Photosynthesis is a process in which electromagnetic energy is converted into the chemical energy used for biosynthesis of organic cell materials. The thylakoids of green plants, algae and cyanobacteria are the places of the photosynthetic light-dependent reactions. These reactions include light-driven water oxidation and oxygen evolution, the pumping of protons across the thylakoid membranes coupled with the electron transport chain of the photosystems and cytochrome b6f complex, and ATP synthesis provides by the ATP synthase utilizing the generated proton gradient. Photosystem II (PSII) is the heart of the photosynthetic process. This multisubunit complex is embedded in the thylakoid membrane of plants, algae and cyanobacteria[1]. The function of the PSII in different organisms is identical while the composition of their subunits is different[2]. Recently the 3D X-ray structures of cyanobacterial PSII were determined to the maximum resolution of 2.9Å[3]. Cyanobacterial PSII consists of different extrinsic proteins compared with plant PSII; also the light-harvesting complex is not bound in thylakoid membrane to PSII core as it is in plant PSII. The process of photosynthesis cannot be understood without detailed knowledge of the structure of PSII and its single subunits. Our research is focused on the isolation of the plant PSII and its selected subunits to elucidate structure of this complex.

The isolation of the protein complex consisting of proteins PsbO, PsbP, PsbQ from *Pisum sativum* L. for other structural analysis was