

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