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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Keywords: prestin, STAS domain, SulP/SLC26 anion transporters

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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-protectans, 2.8 Å resolution X-ray crystal structure was obtained. Purification, crystallization and structural analysis of MexB will be presented.

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Keywords: membrane, protein, crystallography

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Isolation and crystallization studies of selected proteins from plant photosystem II

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Abreviation: 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

optimized. It was shown that oxygen-evolving complex is possible to wash out from thylakoid membrane by contain of different salts. According preliminary crystallization experiments it was shown that success of crystallization trials is dependent on purification steps of this complex. It was shown that degradation of subunits is caused by presence of Na^+, K^+, Ca^{2+} and Mg^{2+} ions, but Ca^{2+} are sufficient additive for crystallization. Our results showed that development of reproducible purification protocol is a crucial step toward reproducible crystallization experiments.

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Keywords: plant photosystem II, oxygen-evolving complex

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ProSMART - procrustes structural matching alignment and restraints tool

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ProSMART (Procrustes Structural Matching Alignment and Restraints Tool) is a tool to aid in the comparative analysis and refinement of protein structures, intended to be complementary to existing resources. Primarily, it is used for conformational invariant/independent pairwise structural alignment, allowing identification of local similarities. The tool provides residue-based dissimilarity scores for assessment of local similarity, identifies rigid substructures, and outputs sets of superposed coordinates. Utilising information from external structures, atomic distance restraints may be generated for subsequent use during crystallographic refinement.

Pairwise structural alignment is achieved by performing an all-on-all comparison of n-residue structural fragments between two chains. Individual structural fragments are compared using Procrustes analysis, quickly achieving local backbone root mean square deviation. A fragment alignment is achieved using a dynamic programming algorithm, which is then further refined. In order to maintain conformation-invariance, the alignment is filtered to enforce global rigidity of neither chains nor domains. This feature makes the tool suited to the analysis of domain movement and other conformational changes, as well as for the identification of structural units that are conserved between seemingly different structures.

Following identification of the alignment, the structures are searched for conserved rigid substructures. For each identified substructure, superposed coordinates are output. ProSMART has a variety of features, useful in different applications. One such feature is the ability to colour (superposed) structures according to various residue-based dissimilarity scores, to be viewed in PyMOL. ProSMART also allows dissimilarity scoring of side chain conformation, relative to local coordinate frame. This feature is of particular use when comparing the side chains of two near-identical structures in different conformations and/or bound states, due to the ability to detect subtle changes.

Given an alignment, ProSMART can be used to generate external restraints on the distances between relatively close, non-bonded, atoms. Using one or more similar structures, the software generates restraints that are intended to help the target protein adopt a conformation that is more reasonable for structures in the class in which it belongs, whilst allowing global flexibility. The assertion is that the target structure's local atomic distances should be reasonably similar to those from similar structures. External restraints from ProSMART can be applied during crystallographic refinement by REFMAC5. These restraints have been found to stabilise refinement in some cases, especially at low resolution (3-6Å) where experimental data alone may not be sufficient. Tests are promising, suggesting that external restraints might be used to improve reliability in future.

ProSMART may be used with data from various methods, including structures from crystallography and electron microscopy, and ensembles from nuclear magnetic resonance and molecular dynamics simulations. A pre-release version of ProSMART is available by contacting the author.

Keywords: alignment, comparison, restraints

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Towards the complete structure of the S-layer protein SbsC

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Monomolecular paracrystalline surface layers (S-layers) are composed of a single (glyco)protein and are the most commonly observed cell surface structures of bacteria and archaea. Because of their diverse properties S-layers have various potential applications in nanobiotechnology [1]. However, detailed structural information on S-layer proteins is very scarce. In order to determine the structure-function relationship of SbsC, the S-layer protein from *Geobacillus stearothermophilus*, deletion mutants were produced. It was shown that the N-terminal part is responsible for binding to the secondary cell wall polymer (SCWP) and that the C-terminal part is essential for self-assembly [2]. Recently, the crystal structure of the C-terminally truncated form $rSbsC_{(31-443)}$ was solved to 2.4 Å [3].

We continued the work with different N-terminal truncations and crystals of 3 different protein constructs were obtained. The structure of one construct was solved by producing different heavy atom derivatives. The structure consists of 3 Ig-like domains connected with the short linker. The refinement of the crystals from two other constructs is in progress.

Small angle X-ray scattering measurement of all constructs was performed. All constructs consist of domains similar in size and shape. We can conclude that the full length SbsC protein consists of 9 domains. The first coiled-coil domain followed by 8 Ig-like domains.

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A study of how different ligands and ph may influence insulin crystallisation by using powder diffraction

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