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Inhibition of the sarcoplasmic reticulum Ca^{2+}-ATPase by thapsigargin analogues

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The sacroplasmic reticulum Ca^{2+}-ATPase is the most studied member of the P-type ATPase family of membrane pumps. Members of this family are primary transporters that mediate the movement of ions against their concentration gradients across biological membranes by the use of energy derived from the hydrolysis of ATP. Large Ca^{2+} gradients maintained by the sarcoplasmic reticulum Ca^{2+}-ATPase are involved in the relaxation of muscle contraction and in Ca^{2+}-dependent signal transduction. Several high resolution X-ray structures of the Ca^{2+}-ATPase are available [1-3] that describe the pump in different conformational states of its functional cycle. The overall structure of the Ca^{2+}-ATPase consists of three cytoplasmic domains and a membrane domain with ten membrane-spanning helices that contain ion-binding sites situated between helices M4, M5, M6 and M8.

One of the most powerful inhibitors of the sarcoplasmic reticulum Ca^{2+}-ATPase is thapsigargin, a three-ring sesquiterpene lactone derived from a Mediterranean plant *Thapsia garganica.* Thapsigargin inhibits the Ca^{2+}-ATPase by irreversibly trapping the pump in one of its conformational states by preventing further binding of calcium ions. Derivates of thapsigargin are able to selectively target cancer cells and show promise in the treatment of slowly growing prostate cancer cells that are resistant to other cancer therapies.

We have confirmed the binding and inhibitory effect of several newly synthesized thapsigargin analogues using biochemical assays. To further investigate the connection between the chemical structure of these inhibitors and their effect on the Ca^{2+}-ATPase activity, we have crystallized the pump with several thapsigargin derivatives. We obtained a high resolution structure of a complex of the pump with one of the inhibitory compounds bound in the thapsigargin-binding pocket situated at the cytoplasmic site of the pump between three transmembrane helices denoted M3, M5 and M7, and identified several amino-acid residues important for the binding properties of the inhibitor.

[1] C. Toyoshima, M. Nakasako, H. Ogawa, *Nature* **2000**, *405*, 647-655. [2] C. Olesen, T.L.-M. Sørensen, R.C. Nielsen, J.V. Møller, P. Nissen, *Science* **2004**, *306*, 2251-2255. [3] C. Olesen, M. Picard, A.-M.L. Winther, C. Gyrup, J.P. Morth, C. Oxvig, J.V. Møller, P. Nissen, *Nature* **2007**, *450*, 1036-1042

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Hydrogen-bond networks and channels revealed in the 1.9 Å structure of PSII

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Photosystem II (PSII) is a multi-subunit membrane protein complex consisting of 38 protein subunits and 114 co-factors with a total molecular weight of 700 kDa as a dimer. PSII performs a series of light-induced electron transfer reactions leading to the splitting of water and generation of molecular oxygen, the latter of which is essential for almost all life on the earth. The catalytic center for water-splitting and oxygen evolution is a Mn_4CaO_5-cluster, whose detailed structure has been successfully resolved in the recent 1.9 Å structure of PSII from *Thermosynechococcus vulcanus* [1]. This high resolution structure also revealed the presence of nearly 1,400 water molecules in a PSII monomer, some of which are directly associated with the Mn_4CaO_5-cluster and thus may serve as the substrate water molecules for the oxygen-evolving reaction. Most of the water molecules, however, are not associated with the metal cluster directly and distributed over the stromal and lumenal sides of the thylakoid membrane. A number of water molecules, in combination with some hydrophilic amino acid residues, were found to form extended hydrogen-bond networks starting from the Mn_4CaO_5-cluster toward the exterior surface of the protein complex in the lumenal side. These hydrogen-bond networks may therefore function either as proton exit channels or substrate water inlet pathways. Among these channels, a well defined hydrogen-bond network was found starting from the water molecules bound to the Mn_4CaO_5-cluster through D1-Tyr161, the so-called Y_Z, to the exterior surface of the lumenal side; this channel may therefore function as a proton exit pathway required for the proposed proton-coupled electron transfer mediated by Y_Z. Another well defined hydrogen-bond network was found to start from the Mn4 (the isolated Mn atom) side of the Mn_4CaO_5-cluster through the Cl-1 binding site toward the exterior of the protein complex, which may also serve as a proton exit channel. A similar hydrogen-bond network was found in the opposite side of the cluster and involves the Cl-2 binding site; however, this network was interrupted by a polypeptide backbone and may need a movement of this backbone in order to transfer the proton through this site. In addition, there are several other hydrogen-bond networks that may function as either proton exit or water inlet channels.

In addition to the hydrogen-bond networks, there are some continuous channels calculated based on the 1.9 Å structure of PSII. These channels may function as water inlet pathways to supply for the substrates of the oxygen-evolving reaction. We will discuss the structure and functions of these hydrogen-bond networks and channels revealed in the 1.9 Å structure of PSII.

[1] Y. Umena, K. Kawakami, J.R. Shen, N. Kamiya, *Nature* **2011**, in press, DOI: 10.1038/nature09913.

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Structural Studies on cora magnesium transporter from methanococcus jannaschii

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Divalent cations are essential elements in most cellular processes and the supply of these ions to cells and organelles at appropriate levels is critical for life. As a result, organisms have developed transporter systems for maintaining adequate concentrations of intracellular divalent cations, e.g. Mg^{2+}, while preventing over-accumulation of these metal ions. CorA is a divalent cation transporter and traditionally it is known to transport mainly Mg^{2+}. The structure of CorA from *T.maritima* determined earlier [1] did not fully answered how the transport occurs. Here we report the progress made on the elucidation of three-dimensional structure of CorA from *M.jannaschii*. This CorA represents the intermediate subclass between two distinct

CorA families [2], which show functional [3] and apparently structural differences.

Both full-length protein and its truncated version, comprising the soluble domain, were successfully overexpressed in *E.coli* and purified using Immobilized Metal Ion Affinity (IMAC) chromatography. Crystallization trials with several commercially available screens (JSCG+, Morpheus, PGA) gave a number of hits, optimization of which, yielded crystals suitable for data collection at synchrotron.

Several datasets with varying resolution from 3.5Å to 5 Å were collected at National Synchrotron Radiation Research Center (Taiwan), Diamond Synchrotron (UK) and Australian Synchrotron (Australia).

Initial analysis of collected data as well as future steps of structure determination will be presented.

[1] S. Eshaghi, D. Niegowski, A. Kohl, D. Martinez Molina, S.A. Lesley, P. Nordlund. *Science* **2006**, *313*, 354-357.[2] D. Niegowski, S. Eshaghi, *Cellular and Molecular Life Sciences* **2007**, *64*, 2564-2574. [3] Y. Xia, A.K. Lundback, N. Sahaf, G. Nordlund, P. Brzezinski, S. Eshaghi *Journal of Biological Chemistry* **2011,** DOI: 10.1074/jbc.M111.222166

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The catalytic architecture of leukotriene C_4 synthase with two arginine residues

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Leukotriene (LT) C_4 and its metabolites, LTD_4 and LTE_4, are the lipid mediators involving in inflammatory and immunoresponses. These lipid mediators are collectively called cysteinyl leukotrienes (Cys-LTs). The therapeutic effects of the intervention with the Cys-LT biosynthesis or the antagonist of the specific receptor of Cys-LT suggests that Cys-LT plays an important role as a pathogenic factor of bronchial asthma.

LTC_4 synthase (LTC_4S) is the nuclear membrane-embedded enzyme responsible for LTC_4 biosynthesis, catalyzing the key reaction to conjugate glutathione (GSH) and LTA_4 at the first step of Cys-LTs metabolism as a branch in the arachidonic acid cascade. A previous crystal structure revealed important details of GSH binding, and implied a GSH activating function for Arg104. In addition, Arg31 was also proposed to participate in the catalysis based on the putative LTA_4 binding model [1].

To elucidate structural and functional relationship of LTC4S, we constructed mutants at several arginine residues including Arg104 and Arg31, and the catalytic architecture of LTC4S was thoroughly analyzed by enzyme kinetics and X-ray crystallography of these mutants [2]. Both the arginine mutants showed decreased catalytic activities, indicating concerted catalysis with Arg104 and Arg31 described as follows. Arg104 as the base catalyst binds to thiol group of GSH, and generates thiolate anion. The activated thiolate attacks the carbon atom of the epoxide group of LTA4 to form the covalent bond between GSH and LTA4. Synchronously, guanidino group of Arg31 as the acid catalyst donates a proton to the oxygen of the epoxide group, and its positive charge accelerates the propagating reaction by the neutralization of the emerging negative charge of oxyanion generated from epoxide ring-opening.

[1] H. Ago, Y. Kanaoka, D. Irikura, B.K. Lam, T. Shimamura, K.F. Austen, M. Miyano, *Nature* **2007**, *448,* 609-12. [2] H. Saino, Y. Ukita, H. Ago, D. Irikura, A. Nisawa, G. Ueno, M. Yamamoto, Y. Kanaoka, B.K. Lam, K.F. Austen, M. Miyano, *J Biol Chem* **2011**, in press.

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Crystal structures of photosystem II complexed with electron-transfer inhibitors

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Oxygen-evolving Photosystem II (PSII) is a multi-subunit membrane protein complex performing light-induced electron transfer and water-splitting reactions, leading to the formation of molecular oxygen. After initial light excitation and charge separation in PSII, an electron is transferred to a bound plastoquinone molecule Q_A, and subsequently to an exchangeable plastoquinone Q_B. Q_B is the final electron acceptor of PSII, and leaves PSII at the stromal side to cytochrome b_6f as plastoquinol (PQH_2) after uptake of two protons and two electrons. A non-heme iron is located between Q_A and Q_B, and is coordinated by four histidine residues and one bicarbonate ion. Based on XAS experiment and DFT calculation, it was suggested recently that the coordination number of the non-heme iron changes from six to five in the electron transfer from Q_A to Q_B [1]. A class of herbicides is well-known to inhibit the electron transfer beyond Q_A by binding to the Q_B site of PSII . These herbicides have been classified into several types, namely, phenolic, triazine, uracil, and urea types. The corresponding representatives of the four types of herbicides are bromoxynil, terbutryn, bromacil, and DCMU, respectively. PSII crystals were soaked into each of 1 mM solutions of the four herbicides to prepare the electron transfer inhibitor complexes, and X-ray diffraction data were collected at BL44XU, SPring-8, Japan. The four crystal structures were solved independently with the molecular replacement technique, using the 1.9 Å resolution structure (PDB code: 3ARC) determined recently by our group [2] as a search model, and refined at resolutions of 2.3 Å-1.9 Å. All of the four herbicides were modeled into the Q_B site. The crystal structure of PSII-terbutryn complex has been reported at a resolution of 3.2Å with PDB codes 3PRQ and 3PRR [3], in which no water molecules were assigned. In contrast, our structure of PSII-terbutryn complex, determined at a resolution of 2.0 Å, consisted of many water molecules, and some of them were hydrogen-bonded to terbutryn. Furthermore, we found that the bicarbonate ion was coordinated to the non-heme iron as a monodentate ligand, resulting in a coordination number of five for the non-heme iron. This is apparently different from the coordination number of six in native PSII. Thus, the coordination number of the iron may be different in different herbicide complex of PSII. This is the first crystallographic result showing that the bicarbonate ion is able to change its coordination pattern for the non-heme iron.

[1] P. Chernev, *et al., J. Biol. Chem.* **2010**, *286*, 5368-5374. [2] Y. Umena, K. Kawakami, J.-R Shen, N.Kamiya, *Nature* **2011**, in press, DOI: 10.1038/nature09913. [3] M. Broser, *et al.*, *J. Biol. Chem.* **2011,** DOI: 10.1074/jbc.M110.215970.

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