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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.

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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.

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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