# Crystal Structure of the Calcium Pump with a Bound ATP Analogue

Nature uses ion gradients across cell membranes very efficiently. When cell membrane excites, ions come into cytoplasm rapidly following the ion gradients. To restore the original resting state, the ions must be pumped back. P-type ATPase is a family of ion transporting ATPases that are responsible for establishing such ion gradients. They include $Na^+K^+$-ATPase, $Ca^{2+}$-ATPase and gastric $H^+K^+$-ATPase among others. The transport is thought to be achieved by changing the binding sites from high affinity and facing cytoplasm (E1 form) to low affinity and facing the extracellular side (E2 form). One important feature of the pumping process is that, before being released, bound cations are occluded; that is, become inaccessible from either side of the membrane. Binding in itself does not lock the cytoplasmic gate, and bound cations can be exchanged with those in the cytoplasm.

Of the P-type ATPase superfamily, the $Ca^{2+}$-ATPase of fast skeletal muscle sarcoplasmic reticulum (SR) is structurally and functionally the best studied member. It is an integral membrane protein of 110 kD, consisting of 3 (A (actuator), N (nucleotide binding) and P (phosphorylation)) cytoplasmic domains and 10 (M1-M10) transmembrane helices (Fig. 1). We already published the structures in a $Ca^{2+}$-bound state (E1·2$Ca^{2+}$; PDB accession code 1SU4) [1] and a $Ca^{2+}$-unbound state (E2(TG)) [2], stabilized by a potent inhibitor, thapsigargin (PDB accession code 1IWO), and have now succeeded in determining the structure of the ATPase with a non-hydrolysable analogue, AMPPCP, a $Mg^{2+}$ and two $Ca^{2+}$ ions bound at their respective binding sites (PDB accession code 1VFP) [3]. This structure (abbreviated as E1·AMPPCP) explains how the γ-phosphate and $Mg^{2+}$ binding to the P-domain results in the occlusion of the bound $Ca^{2+}$ at some 50 Å away.

The binding of AMPPCP and a $Mg^{2+}$ ion to the P-domain causes large and global changes in the structure (Fig. 1). The most prominent difference is that the 3 cytoplasmic domains, widely separated in E1·2$Ca^{2+}$, now form a compact headpiece, with the N- and P-domains cross-linked by AMPPCP. This causes a nearly 90° inclination of the N-domain, which now makes contacts with the A-domain. To achieve this, the γ-phosphate bends the P-domain to gain an extra ~30° of inclination. At the same time, ATP induces the binding of $Mg^{2+}$, which bends the P-domain in nearly orthogonal direction so that the M2 side is brought higher up. As a combined result of

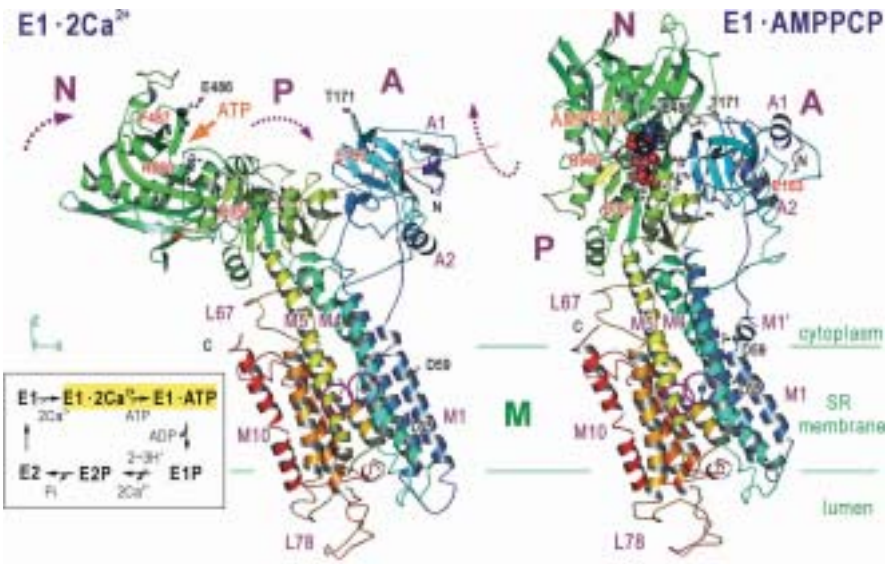


Fig. 1. Structures of $Ca^{2+}$-ATPase of sarcoplasmic reticulum in the presence of $Ca^{2+}$ with (E1·AMPPCP) and without (E1·2$Ca^{2+}$) AMPPCP, a non-hydrolysable ATP analogue. Color changes gradually from the N terminus (blue) to the C terminus (red). Two purple spheres (circled) in the membrane domain represent bound $Ca^{2+}$ ions. AMPPCP is shown in space fill. Large arrows in E1·2$Ca^{2+}$ indicate the directions of movements of the cytoplasmic domains (A, N and P) in E1·2$Ca^{2+}$ →E1·AMPPCP; the axis of tilting of the A-domain is also specified (thin red line). Inset is a simplified reaction scheme; the two states compared here are shown with a yellow background.

these, the A-domain tilts by ~30° to pull up the M1-M2 helices (Fig. 1) and to strain the loop connecting to M3. This strain appears to be the driving force for another A-domain rotation to open the lumenal gate.

The M1 helix is pulled up and largely bent at the membrane surface. The functional meaning of this movement is evident. In E1·2$Ca^{2+}$, which represents the state after the binding of both $Ca^{2+}$, Glu309, the gating residue, caps the $Ca^{2+}$ in site II (Fig. 2(a)). But a large empty space around it will allow Glu309 to adopt other side chain conformations and the site II $Ca^{2+}$ to escape. In E1·AMPPCP, this space is occupied by M1 and Leu65 makes van der Waals contacts with the Glu309 side chain (Fig. 2(b)). As a result, the conformation of Glu309 is locked and bound $Ca^{2+}$ cannot escape.

Thus, $Ca^{2+}$-ATPase changes the orientation of the A-domain to regulate the cytoplasmic gate of $Ca^{2+}$-binding pathway by moving, primarily, the M1 helix. This means that the interfaces between the A-domain and two other cytoplasmic domains are critically important and adjusted during the reaction cycle. ATP works as the principal modifier of the interfaces together with $Mg^{2+}$.

The data were obtained at beamline **BL41XU.**

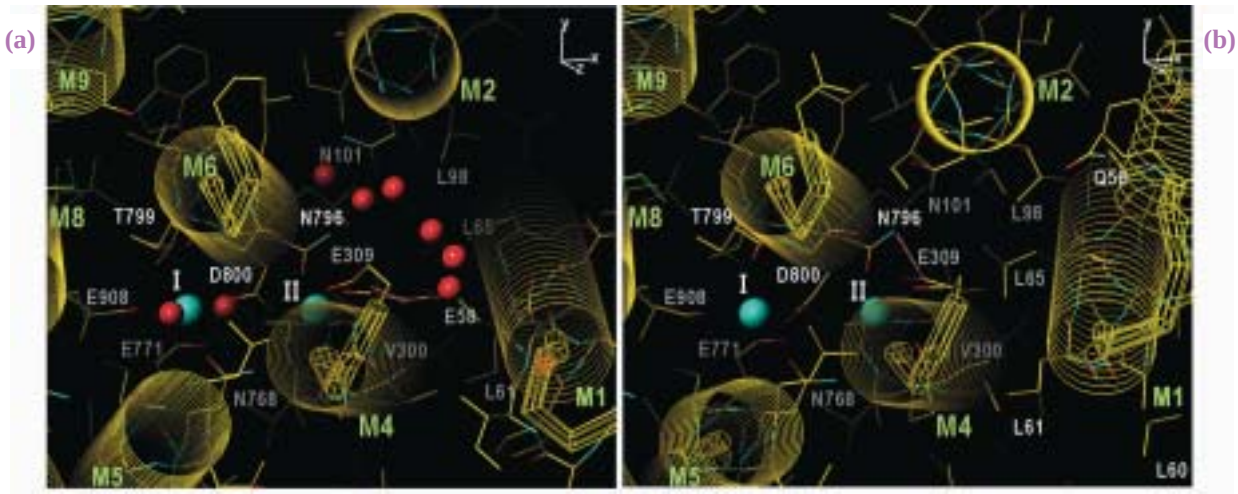


Fig. 2. Transmembrane $Ca^{2+}$-binding sites (I and II) in E1·2$Ca^{2+}$ **(a)** and E1·AMPPCP **(b)**, viewed from the cytoplasmic side. Cyan spheres represent bound $Ca^{2+}$; red spheres in **(a)** indicate water molecules in the crystals.

Chikashi Toyoshima* and Tatsuaki Mizutani

Institute of Molecular and Cellular Biosciences,
The University of Tokyo

*E-mail: ct@iam.u-tokyo.ac.jp

**References**

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