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Minireview

Structural basis of ion pumping by Ca^{2+}-ATPase of sarcoplasmic reticulum

Chikashi Toyoshima*, Hiromi Nomura, Yuji Sugita

Institute of Molecular and Cellular Biosciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-0032, Japan

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Abstract The structures of the Ca^{2+}-ATPase (SERCA1a) have been determined for five different states by X-ray crystallography. Detailed comparison of the structures in the Ca^{2+}-bound form and unbound (but thapsigargin-bound) form reveals that very large rearrangements of the transmembrane helices take place accompanying Ca^{2+} dissociation and binding and that they are mechanically linked with equally large movements of the cytoplasmic domains. The meanings of the rearrangements of the transmembrane helices and those of the cytoplasmic domains, and the mechanistic roles of the phosphorylation are now becoming clear.

Key words: Crystal structure; Ion pump; Ca^{2+}-ATPase; SERCA1a; Ca^{2+} binding; Domain movement

1. Introduction

The Ca^{2+}-ATPase from rabbit skeletal muscle sarcoplasmic reticulum (SERCA1a) is an integral membrane protein of 110 kDa and structurally and functionally the best characterized member of the P-type ion translocating ATPase superfamily. It consists of 10 transmembrane helices (M1–M10), three cytoplasmic domains (A, actuator or anchor; N, nucleotide-binding; P, phosphorylation) and small luminal loops. SERCA1a can transport two Ca^{2+} per ATP hydrolyzed against a concentration gradient across the membrane. Active Ca^{2+} transport has been postulated to be achieved by changing the enzyme structure from E1 to E2 (Fig. 1, inset). In E1, transmembrane Ca^{2+}-binding sites have high affinity and face the cytoplasm; in E2, the binding sites have low affinity and face the lumen of the sarcoplasmic reticulum (extracellular side). Actual transfer of bound Ca^{2+} is thought to take place between E1P and E2P (where P indicates that the ATPase is phosphorylated).

We have determined the crystal structures of this enzyme in five different states, namely, Ca^{2+}-bound state (E1·Ca^{2+}; PDB accession code, 1EUL) [1], Ca^{2+}-unbound but thapsigargin (TG)-bound state (E2(TG); 1IWO) [2], Ca^{2+}-bound and AMPPCP (adenosine 5′-(β,γ-methylene) triphosphate)-bound state (E1·AMPPCP), Ca^{2+}-bound and ADP- and AlF_x-bound state (E1·AlF_x·ADP), and Ca^{2+}-unbound but MgF_4^{2-}-bound state (E2·MgF_4^{2-}). E1·AMPPCP is an analogue of E1ATP in Fig. 1, with AMPPCP as the non-hydrolyzable analogue of ATP. E2·MgF_4^{2-} is a stable analogue of E2P [3], with MgF_4^{2-} as the stable analogue of phosphate. E1·AlF_x·ADP is thought to represent a state immediately after the hydrolysis of ATP to ADP and Pi. Of these five structures, only two [1,2] have been published so far. In this minireview, we very briefly overview the mechanism of ion pumping based on the crystal structures of Ca^{2+}-ATPase.

2. Ca^{2+}-ATPase as a membrane protein

Because P-type ATPases are membrane proteins, their relationships with the lipid bilayer may have critical importance for stability and function. For example, the positions of the cation-binding sites in the direction normal to the membrane may be a critical parameter in interpreting the results of physiological measurements and obviously depend on the orientation of the enzyme with respect to the membrane plane. The orientation of the enzyme becomes even more important in comparing different forms and interpreting the meanings of the conformation changes.

Although the crystallization of SERCA1a required addition of exogenous phospholipid (phosphatidylcholine), lipid bilayers were not visualized directly within the crystal lattice. This is because conventional crystallography discards low-resolution data that contain information on the lipid bilayer. Nevertheless, crystal structures give useful information on the position of the bilayer. This is because the protein molecules are inserted in the membrane in alternating directions. This means that a symmetry axis (two-fold rotation axis) runs through the center of the bilayer. In the E1·Ca^{2+} crystals there is only one such axis, which determines the position of the M1 helix with respect to the membrane, that is, Cys70 comes to the center of the bilayer. Fortunately, one of the E2(TG) crystal forms belongs to a $P4_1$ space group and contains two monomers related by a non-crystallographic (i.e. local) two-fold symmetry in an asymmetric unit. Crystals of the E2·MgF_4^{2-} form grew with either C2 or $P2_1$ symmetry, providing other instances. Thus, we now have a series of points that will specify the center of the lipid bilayer. These positions indicate that, at least as a first approximation, the lipid bilayers run parallel to the crystallographic *ab* plane in all the crystals. The coordinates for the aligned models can be ob-

*Corresponding author. Fax: (81)-3-5841 8491.
E-mail address: ct@iam.u-tokyo.ac.jp (C. Toyoshima).

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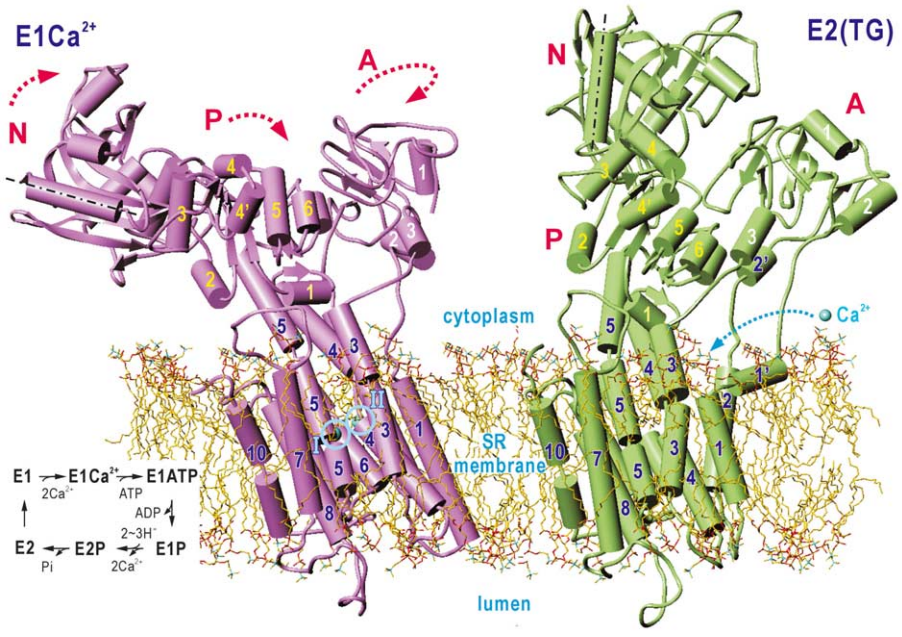

Fig. 1. E1·Ca^{2+} and E2(TG) forms of Ca^{2+}-ATPase in lipid bilayer. Inset, a simplified reaction scheme (showing only the forward direction). Cylinders represent α-helices, arrows β-strands. The lipid bilayer is generated by molecular dynamics simulation of DOPC molecules. Two cyan spheres (circled also) in the transmembrane region of E1Ca^{2+} represent bound Ca^{2+} (sites I and II). Helices in the A and P domains and those in the transmembrane domain are numbered. Red arrows in E1Ca^{2+} indicate the direction of movement of the cytoplasmic domains during the change from E1·Ca^{2+} to E2(TG). Cyan arrow in E2(TG) shows the proposed pathway [2] for Ca^{2+} to enter into the binding cavity.

tained from the authors' homepage (http://www.iam.u-tokyo.ac.jp/StrBiol/model).

In Fig. 1, E1·Ca^{2+} and E2(TG) are aligned and inserted into the bilayer of dioleoylphosphatidylcholine (DOPC), which supports full activity of Ca^{2+}-ATPase [4]. The coordinates of DOPC molecules are generated by a molecular dynamics calculation (Y. Sugita, M. Ikeguchi and C. Toyoshima, unpublished work). This figure alone shows many interesting features. For example, a short amphipathic part of M1 (M1′ in Fig. 1) is located within the interface region of the lipid bilayer. This is an expected feature that supports the orientations of the models with respect to the bilayer. The positions of the surface loops and those of interface-forming residues (Trp, Tyr, Lys and Arg [5]) are also consistent with these orientations.

Nevertheless, presumably the most important features are that the locations of the Ca^{2+}-binding sites are offset (4.4 and 7.3 Å) to the cytoplasmic side and that site II is closer to the cytoplasmic surface than site I (Fig. 1). Two Ca^{2+}-binding sites are located side by side (Fig. 2), but the binding of two Ca^{2+} is sequential, site I being the binding site for the first Ca^{2+}[6]. Site II Ca^{2+} is exchangeable without affecting the binding of site I Ca^{2+} [7]. Lee and East [8] pointed out that there is a hole lined by Glu58 and Glu309 carboxyls leading to the Ca^{2+}-binding sites. They suggested that this hole might be the entry pathway. The question is whether this hole is accessible from bulk water or sealed by lipid molecules. This question is not easy to answer. The hole is certainly located within the hydrophobic core of the membrane, but M1 and M2 helices might shield the hole from lipids. Also crystal packing might prevent the proper dispositions of lipid molecules. In the E2(TG) form, Glu309 is clearly accessible from the cytoplasm [2] through a pathway different from the one proposed by Lee and East [8] (Figs. 1 and 3a). There is an increasing number of results suggesting that Ca^{2+} ions enter into the binding cavity via Glu309 and the locking of its side chain conformation by rearrangements of transmembrane helices causes the 'occlusion' of the bound Ca^{2+} ions. However, to answer the question how Ca^{2+} reaches the binding sites still needs careful studies.

3. Rearrangement of transmembrane helices

As described previously [2], large-scale rearrangements of

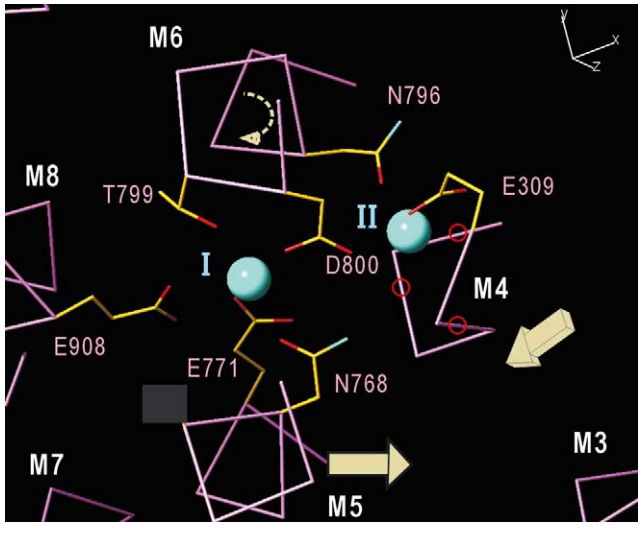

Fig. 2. Transmembrane Ca^{2+}-binding sites viewed approximately normal to the membrane from the cytoplasmic side. The arrows show the direction of movement in E1·Ca^{2+} to E2(TG) transition. Small red circles indicate approximate positions of carbonyl oxygens that contribute to the binding of Ca^{2+} in site II.

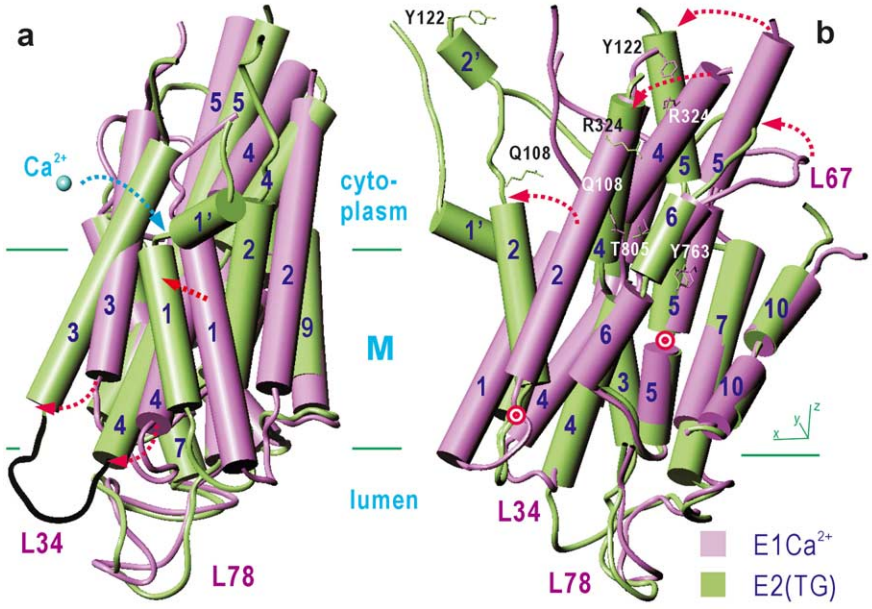

Fig. 3. Rearrangements of the transmembrane helices between E1·Ca^{2+} (violet) and E2(TG) (light green) forms. The models for E1·Ca^{2+} and E2(TG) are superimposed and viewed from the right (a) and the rear (b) of that presented in Fig. 1. M8 and M9 are removed in b, so that 'domino' movements of the M5, M4 and M2 helices can be seen clearly. Double circles show pivot positions for M2 and M5. Red arrows indicate the directions of movements in E1·Ca^{2+} → E2(TG). Cyan arrow shows the proposed pathway for the first Ca^{2+} ion [2].

transmembrane helices take place during E1·Ca^{2+} → E2(TG) transition (Fig. 1). Of the four transmembrane helices that form two Ca^{2+}-binding sites (M4–M6 and M8; Fig. 2), M8 does not move but the other three helices all move (Fig. 3). Particularly important movements in E1·Ca^{2+} → E2(TG) are (i) a shift of M4 towards the luminal (extracellular) side by one turn of an α-helix (Fig. 3), (ii) bending of the upper part of M5 towards M4 (Fig. 3), and (iii) nearly 90° rotation of the unwound part of M6 (Fig. 2). As a result the number of oxygen atoms that can coordinate to Ca^{2+} decreases. Therefore, it is clear that the Ca^{2+}-binding sites are destroyed, but these movements are perplexingly large. Homology modelling

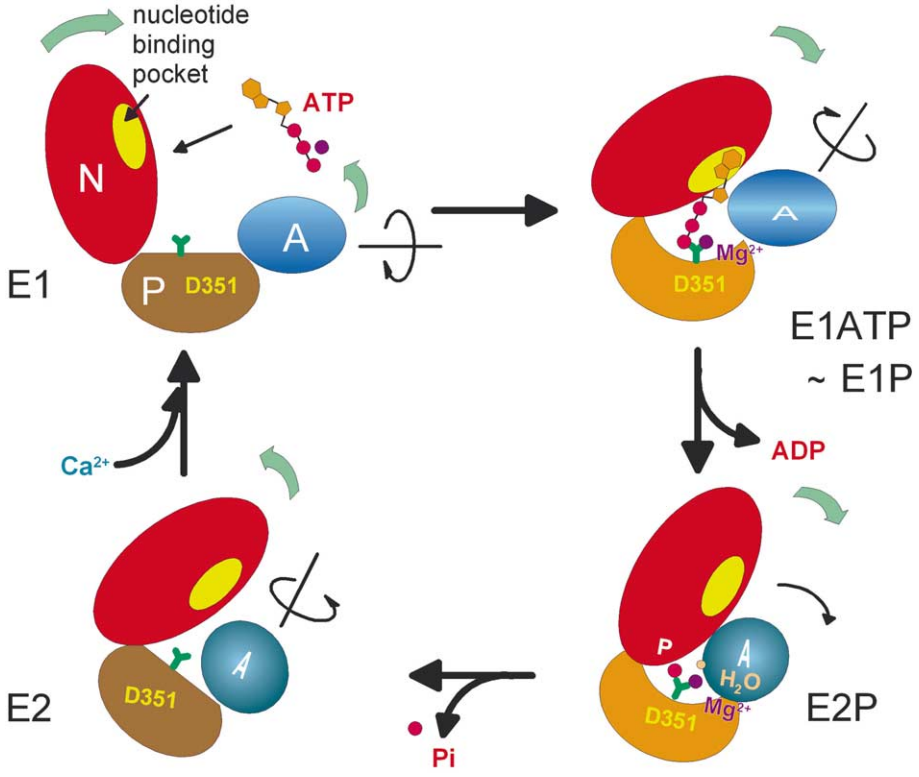

Fig. 4. Schematic models for four distinct arrangements of the three cytoplasmic domains in the reaction cycle (Fig. 1, inset) of Ca^{2+}-ATPase. D351 is the residue of phosphorylation. Transfer of 'occluded' Ca^{2+} to the luminal side is thought to take place between E1P and E2P.

of the cation binding sites of the Na^+K^+-ATPase, however, provides an explanation [9].

It was surprising to see that very regular coordination geometry could be made immediately for the K^+-binding sites of the Na^+K^+-ATPase [9], starting from the main chain conformation derived from the E2(TG) model of the Ca^{2+}-ATPase [2]. The residues involved in the cation-binding sites appear essentially the same between Ca^{2+}- and Na^+K^+-ATPases [9]. The only critical difference is that Asn796 in Ca^{2+}-ATPase (Fig. 2) is replaced by Asp811 in Na^+K^+-ATPase. Asp811 is a key residue in K^+ binding, playing a similar role to Asp800 in Ca^{2+} binding of the Ca^{2+}-ATPase and Asp815 in Na^+ binding of the Na^+K^+-ATPase, by contributing to both sites I and II (Fig. 2). The position of Asp811 (Asn796 in Ca^{2+}-ATPase) is one turn below Asp815 (Asp800 in Ca^{2+}-ATPase) towards the extracellular side. To form a cation-binding site there, M4 must move towards the extracellular side so that carbonyl oxygens (Fig. 2) can contribute to the binding. Hence, the movement of M4 ensures the release of one cation (Ca^{2+} or Na^+) and the binding of the other cation (H^+ or K^+) at the different level with respect to the membrane. This will certainly help in avoiding competition between the binding cations.

This movement of M4 can be generated by the bending of the upper part of M5 (or by the inclination of the P domain) (Fig. 3). This bending allows different residues on different faces of the M5 helix to be used for cation binding (e.g. Ser can be used instead of Glu in K^+ binding at site I). This will allow profound reorganization of the binding residues to accommodate a larger cation (K^+) with a higher affinity, and also to ensure the release of Na^+.

4. The A domain as the 'actuator' domain

M4 and M5 helices are integrated in the P domain as a part of a Rossmann fold (fig. 3 in [2]). The P domain is linked with M3 through the P1 helix and thus indirectly with the A domain. During E1–E2 transition, the A domain undergoes a 110° rotation, which appears to be another key event in active transport [10]. In fact, the A domain has different positions in all of the four states, and does so the M1 helix. This appears to be the way by which Ca^{2+}-ATPase regulates the cytoplasmic and luminal gates of the ion pathway.

In E1·Ca^{2+}, all three cytoplasmic domains are well separated. The M1 helix is deeply embedded in the lipid bilayer (Fig. 1). There is no vestibule that leads to the transmembrane Ca^{2+}-binding sites. The pathway from the luminal side to the Ca^{2+}-binding sites is sealed by hydrophobic residues just below the Ca^{2+}-binding sites. In E2(TG), several hydrogen bonds are formed between A–P and A–N domains. The M1 helix is pulled towards the cytoplasm (Fig. 1); the N-terminal amphipathic part (M1′; Figs. 1 and 3) is kinked and forms a short helix lying on the membrane. In E2·MgF_4^{2-}, the A domain is pulled towards the P domain (Fig. 4) to form a tightly sealed hydrophobic environment around the phosphorylation residue; this is to allow a particular water molecule to attack the acylphosphate. The presence of Mg^{2+} in addition to phosphate (analogue) in E2P appears critical for the positioning of the A domain. The kinked part of the M1 helix is pulled further into the cytoplasm, compared to E2(TG). This moves the luminal end of the M4 helix to open the luminal gate.

In E1·AMPPCP and E1·AlF_x·ADP, which have virtually the same structures, the A domain is pushed into an extreme position by the N domain, which is crosslinked to the P domain by ATP analogues (Fig. 4). In this position, the link between the A domain and the M3 helix is stretched whereas the M1 helix is pulled towards the cytoplasm but in an orientation different from that in E2(TG) or E2·MgF_4^{2-}. This appears to be the way for making the bound Ca^{2+} to be 'occluded' by locking the conformation of Glu309.

As the position of the A domain is affected by the N and P domains, the interfaces between them must be critical and have to be altered during the reaction cycle. Binding of Ca^{2+} and phosphorylation (or ATP binding) will be the two most important factors. Binding of Ca^{2+} places strong constraints on the bending of the M5 helix, and so on the movements of other transmembrane helices and the P domain. Phosphorylation or the binding of γ-phosphate makes the position of the hinge between the N and the P domains substantially higher and also changes the direction of the inclination of the N domain. The binding of γ-phosphate also requires the binding of Mg^{2+}, which directly alters the position of the A domain in E2P. Thus, one of the roles of phosphorylation is to change the interface between the cytoplasmic domains.

5. Roles of the closed configuration of the cytoplasmic domains in E2 state

Ca^{2+}-ATPase appears to undergo, in the absence of Ca^{2+} and Pi, large-scale thermal movements continuously involving both transmembrane and cytoplasmic domains, and the P domain is the coordinator of the movements. That must be the reason why the backward reaction (E1·Ca^{2+} → E2) is possible. If so, we would expect that the sarcoplasmic reticulum vesicles with loaded Ca^{2+} show significant leakage and that thapsigargin, a very potent inhibitor, will be able to stop it. This has indeed been demonstrated (G. Inesi, private communication). On the other hand, the closed configuration of the cytoplasmic domains will limit such movements and therefore the leakage. Another role of the closed configuration is to stop the reaction cycle, which is regulated essentially by Ca^{2+} alone. ATP can bind to the enzyme even when Ca^{2+} is absent but, without Ca^{2+}, the reaction cycle cannot proceed. Although the N domain approaches the P domain, γ-phosphate of ATP cannot reach the phosphorylation residue Asp351. It requires even deeper inclination of the N domain, or the rotation of the A domain to release the N domain. This in turn requires the binding of Ca^{2+}. Whether this requires only the first Ca^{2+} or both Ca^{2+} to bind will be an interesting problem to explore.

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