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New crystal structures of PII-type ATPases: excitement continues

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P-type ATPases are ATP-powered ion pumps, classified into five subfamilies (PI–PV). Of these, PII-type ATPases, including Ca^{2+}-ATPase, Na^+,K^+-ATPase and gastric H^+,K^+-ATPase, among others, have been the most intensively studied. Best understood structurally and biochemically is Ca^{2+}-ATPase from sarcoplasmic reticulum of fast twitch skeletal muscle (sarco(endo)plasmic reticulum Ca^{2+}-ATPase 1a, SERCA1a). Since publication of the first crystal structure in 2000, it has continuously been a source of excitement, as crystal structures for new reaction intermediates always show large structural changes. Crystal structures now exist for most of the reaction intermediates, almost covering the entire reaction cycle. This year the crystal structure of a missing link, the E1·Mg^{2+} state, finally appeared, bringing another surprise: bound sarcolipin (SLN). The current status of two other important PII-type ATPases, Na^+,K^+-ATPase and H^+,K^+-ATPase, is also briefly described.

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Introduction

SERCA1a is the simplest of the PII-type ATPases (see [1] for a review of the P-type ATPase family), and is composed of a single polypeptide chain of 994 residues. It consists of three distinct cytoplasmic domains designated as A (actuator of transmembrane gates), N (nucleotide binding) and P (phosphorylation) domains, 10 transmembrane helices and small lumenal loops [2]. More than 50 crystal structures have been registered in the PDB from four laboratories [2–5], and together they almost cover the entire reaction cycle (Figure 1). This is partly because SERCA1a is naturally abundant and easily obtained, and can be used for crystallisation without extensive purification. We can now describe a quite detailed scenario for the active transport of Ca^{2+} by this ATPase (see [2] for a full account). The crystal structures show that very large rearrangements of the cytoplasmic domains and the transmembrane helices take place during the reaction cycle. A 110° rotation of the A-domain [2] is one of the largest domain movements ever observed as far as the authors are aware. Fluorescence resonance energy transfer has been applied to study such large domain movements [6] in addition to molecular dynamics simulations [7–13].

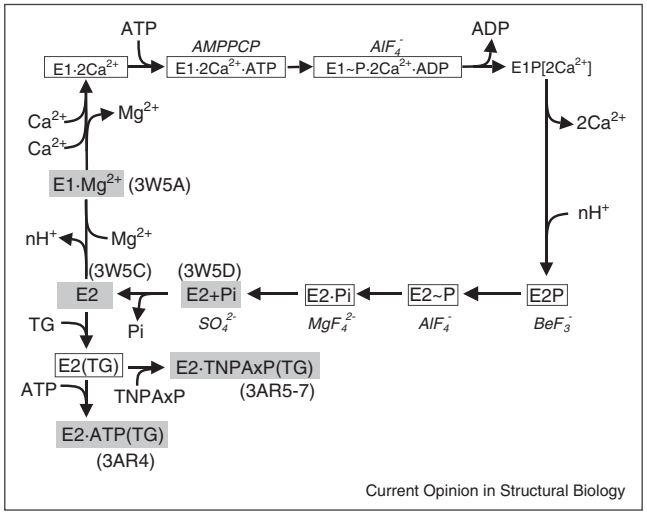
Compared to Ca^{2+}-ATPase, structural elucidation of Na^+,K^+-ATPase lags far behind. Na^+,K^+-ATPase is a substantially more complex pump than SERCA1a, consisting of α and β subunits and an auxiliary FXYD protein (e.g. [14] for a recent review). The α-subunit is the catalytic subunit, with a very similar architecture to SERCA1a. The β-subunit is considered to be the molecular chaperone of the α-subunit and is heavily glycosylated; this subunit is unique to Na^+,K^+-ATPase and H^+,K^+-ATPase. The FXYD protein is a tissue specific regulator for fine tuning of catalytic activity and stability [15]. Na^+,K^+-ATPase is the target of digitalis-like compounds (now called cardiotonic steroids (CTS)), which have been prescribed for more than two centuries for treatment of heart failure and arrhythmias. The first crystal structure of this ATPase was published in 2007 by Morth *et al.* [16]. It showed an E2·2K^+·Pi state at only 3.5 Å resolution and lacked information on most of the β-subunit. A 2.4 Å resolution crystal structure for the same state published two years later [17] provided a description of nearly the entire molecule, including details of K^+-coordination. This crystal structure gave answers to several fundamental questions, such as, why Na^+,K^+-ATPase countertransports K^+ whereas Ca^{2+}-ATPase does not, even though almost the same residues are present in the binding sites (reviewed in [14]). Simulation studies based on this structure [18,19•] demonstrate that the protonation state of the acidic residues is key to the cation specificity. Yet, although two crystal structures were published for the ouabain-bound ATPase [20,21•], we are still waiting for high-resolution crystal structures of Na^+-bound E1 states and CTS-bound forms.

H^+,K^+-ATPase is a close homologue of Na^+,K^+-ATPase, but only two-dimensional crystals for electron microscopy have been generated, and the resolution is limited to 6.5–8.0 Å [22,23,24•]. Therefore, in this review, we mainly focus on the advances in the SERCA field.

Ca^{2+}-ATPase: overview

We can now say that the mechanism of active transport by SERCA1a is roughly understood, as crystal structures of most of the intermediates in the reaction cycle have been determined (Figure 1) [2,3]. Yet, in the structural

Figure 1



Simplified reaction diagram of Ca^{2+}-ATPase. Only forward direction is shown with phosphate analogues (in italics) used for fixing the reaction intermediates. ATP can bind to SERCA1a at various steps (e.g. E2, see Figure 4a). New additions are shaded and those published previously are boxed. PDP accession codes are specified for the new entries from our laboratory described in the text.

elucidation, there are still critical gaps that leave fundamental questions unanswered. First, how do Ca^{2+} ions reach the transmembrane binding sites and induce such large conformational changes? Molecular dynamics simulations have been employed to address this question [10,11]. However, the critical problem here is that the starting structure must be E1, not E2 [10]; the structure of E1 cannot be generated by simply removing two Ca^{2+} from the atomic model of E1·2Ca^{2+} [11]. Therefore, a crystal structure of the E1 form is crucial. Second, what is the activation signal produced from the binding of the two Ca^{2+}? How is it transmitted from the Ca^{2+}-binding sites to the phosphorylation site? Conversely, how is unproductive phosphoryl transfer from ATP to the invariant Asp prevented in E1 or E1·1Ca^{2+}? To answer these questions, we need an E1 structure. Third, how do physiological regulators, phospholamban (PLN) and sarcolipin (SLN), control SERCA activity? Such regulation of SERCA is of extreme medical importance, as impaired calcium sequestration into SR is observed in failing hearts [25,26•]. Furthermore, SLN has now been shown to be critically involved in muscle based thermogenesis. We need crystals of SERCA with bound PLN/SLN to better understand the mechanism of regulation, and thereby to develop effective drugs. Fourth, how does SERCA interact with phospholipids [27•,28]? To what extent does the lipid bilayer move together with the transmembrane helices? It seems that limited flexibility of the lipid bilayer plays an important role in the mechanism of active transport by SERCA1a [2]. Structural knowledge of interactions of membrane proteins with the lipid bilayer remains rather scarce. This is partly because the lipid bilayer is not visualised in the crystal structures, although all PII-type ATPase crystals are made in the presence of phospholipids. In conventional crystallography, the low-resolution part that contains structural information on the lipid bilayer is discarded. Yet, simple inclusion of lower resolution terms in calculating electron density maps [27•] is ineffective, as the atomic model for protein does not have the potency to determine the phases of the low-resolution terms where contributions from the lipid bilayer dominate. In fact, the *R*-factor increases to >50% at 25 Å resolution with the crystals of SERCA1a when a simple bulk solvent correction is applied, as it assumes that the outside of the protein is filled with uniform solvent, ignoring the lipid bilayer. Molecular dynamics simulations have been used to investigate protein–lipid interactions ([27•] for SERCA1a), and can now be carried out for longer than 500 ns for this size of molecule [8], but the insight gained so far is rather limited. Clearly a better experimental strategy is required to answer the questions.

Thus, a breakthrough would be a crystal structure of the E1 state, which is predominant at pH 7 or higher in the absence of Ca^{2+} [29]. According to the conventional E1–E2 formalism, transmembrane cation binding sites in E1 have a high affinity for Ca^{2+} (i.e. ions transported in the forward direction) and face the cytoplasm. In E2, cation binding sites have a low affinity for Ca^{2+} but have a high affinity for H^+ (i.e. ions countertransported) and face the lumen of SR. Thus, at pH > 7, protons that stabilised the empty Ca^{2+}-binding sites have been spontaneously released into the cytoplasm, and the Ca^{2+}-binding sites are ready to accept new Ca^{2+} in the cytosol with high affinity. Only at acidic pH, SERCA1a remains in E2, with some protons bound. This situation is well described in [29].

On this point, confusion still exists in the literature, as many authors do not pay sufficient attention to pH. This problem is not just a matter of terminology. For instance, several groups (e.g. [10]) have tried MD simulations of Ca^{2+}-binding based on atomic models derived from E2 crystals with bound inhibitors. Fortunately, however, new E2 crystals free from exogenous inhibitors show virtually no difference from those with bound thapsigargin [30••].

Crystal structure of Ca^{2+}-ATPase in the E1·Mg^{2+} state

Crystallisation of SERCA1a in E1 is difficult, as the ATPase quickly denatures even within the native membrane if Ca^{2+} is removed at pH > 7. This is because there is nothing to prevent thermal movements of transmembrane helices with clusters of unneutralised negative charges. However, addition of Mg^{2+} stabilises the transmembrane region allowing crystallisation of SERCA1a in E1·Mg^{2+} at pH 7.5 as well as of inhibitor-free E2 at pH 6.5