

The structure and function of the sarcoplasmic reticulum Ca^{2+} -ATPase

Poul Nissen, A.M.L. Jensen, A. Marchand, C. Olesen, P. Holm, M. Picard, B. Vilsen, C. Jaxel, P. Champeil, J.P. Andersen, M. le Maire, J.V. Møller

University of Aarhus, Department of Molecular Biology, Gustav Wieds Vej 10C, DK - 8000 Aarhus C

Abstract. P-type ATPases perform active transport coupled to ATP hydrolysis via formation and breakdown of phosphoenzyme intermediates. Crystal structure determinations of several functional states of the sarcoplasmic reticulum Ca^{2+} -ATPase from rabbit combined with biochemical and mutational studies provide the basis for a thorough analysis of the function of this cation pump. (www.actabiomedica.it)

Key words: P-type ATPase, cation pump, calcium, ATP

Introduction

P-type ATPases are of crucial importance in all eukaryotes where cation pumps of this protein family maintain the cation gradients and the membrane potential. The sarco(endo)plasmic reticulum Ca^{2+} -ATPase from skeletal muscle (SERCA1a) is responsible for transporting the Ca^{2+} released during muscle contraction back into the sarcoplasmic reticulum store. In the functional cycle of SERCA1a two Ca^{2+} ions are pumped out of the cytosol (with cytosolic Ca^{2+} ions binding to the "E1 states" of the pump) followed by the countertransport of 2-3 protons (E2 states). The energy for this up-hill transport scheme is derived from ATP hydrolysis via formation and breakdown of a phospho-enzyme intermediate. Over the last few years a number of crystal structures have been obtained which have added to our understanding of how SERCA accomplishes its task. We describe the coupling between activities at the phosphorylation site and cation binding in the membrane and how it ensures a vectorial transport.

Material and methods

Crystals were obtained of native SERCA1a (1-4) and of protein obtained from a yeast expression system (5). Functional studies were conducted on native enzyme (1-3) and on mutant forms in COS cells (6). Details at the phosphorylation site were investigated by the use of various ATP analogs (AMPPCP, AMPPNP, ADP:AlF₄⁻), and of genuine ATP bound to a mutant form. Defined ionic conditions allowed for analysis of cation binding in the membrane (1, 2), at the phosphorylation site (1-3), and at a regulatory site (6). Data sets ranging from 2.6 to 3.8 Å maximum resolution were obtained at synchrotron sources.

Outcomes

Large conformational changes are observed in response to the binding of cations in the membrane and of ligands at the phosphorylation site (1,2,3). We observe that two Mg^{2+} sites are involved in the recruitment and activation of ATP for phosphorylation (1-

3). A regulatory K^+ site occupied in the phosphorylated states has also been revealed (6).

Conclusions

We observe a strict coupling of the transition state of phosphorylation with Ca^{2+} occlusion and of dephosphorylation with H^+ occlusion. Such occluded transition states form the basis for vectorial transport (7, 8). Two Mg^{2+} sites are involved at the phosphorylation site - one for early ATP recruitment and modulation (1, 3) and one for catalysis (1, 2). We anticipate that the basic mechanisms of SERCA1a are conserved in P-type ATPases, yet crystal structures of other P-type ATPases remain to be determined. A combination of structural and functional studies of P-type ATPases will remain important in future studies of these important pumps.

References

1. Sørensen TL, Møller JV, Nissen P. Phosphoryl transfer and calcium ion occlusion in the calcium pump. *Science* 2004; 304: 1672-5.
2. Olesen C, Sørensen TL, Nielsen RC, Møller JV, Nissen P. Dephosphorylation of the calcium pump coupled to counterion occlusion. *Science* 2004; 306: 2251-2255
3. Jensen AL, Sørensen TL, Olesen C, Møller JV, Nissen P. Modulatory and catalytic modes of ATP binding by the calcium pump. *EMBO J* 2006; 25 : 2305-2314
4. Sørensen TL, Olesen C, Jensen AL, Møller JV, Nissen P. Crystals of the sarcoplasmic reticulum Ca^{2+} -ATPase. *J. Biotech* 2006; epub
5. Jidenko M, Nielsen RC, Sørensen TL et al. Crystallization of a mammalian membrane protein overexpressed in *S. cerevisiae*. *Proc. Natl. Acad. Sci USA* 2005; 102: 11687-11691
6. Sørensen TL, Clausen JD, Jensen AL et al. Localization of a K^+ -binding Site Involved in Dephosphorylation of the Sarcoplasmic Reticulum Ca^{2+} -ATPase. *J. Biol. Chem.* 2005; 279: 46355-46358.
7. Møller JV, Olesen C, Jensen AL, Nissen P. The structural basis for coupling of Ca^{2+} transport to ATP hydrolysis of the Sarcoplasmic Reticulum Ca^{2+} -ATPase. *J. Bioenerg. Biomem.* 2005; 37: 359-364.
8. Møller JV, Nissen P, Sørensen TL, Maire M. Transport mechanism of the sarcoplasmic reticulum Ca^{2+} -ATPase pump. *Curr. Op. Struct. Biol.* 2005; 15: 387-393.

Correspondence: Poul Nissen,
Department of Molecular Biology,
University of Aarhus, Aarhus, Denmark
Tel.: +45 8942 5025, Fax: +45 8612 3178,
e-mail: pn@mb.au.dk