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The plasma membrane calcium pump. Structure, function, regulation

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The plasma membrane Ca-pump is the largest of all P-type ion pumps. It forms a phosphorylated intermediate in the catalytic cycle (an aspartyl-phosphate) and is inhibited by vanadate. It interacts with Ca with high affinity, but has a low total Ca transporting capacity. First discovered in erythrocytes, it has been subsequently found in many other cells, and is now assumed to be an obligatory component of eukaryotic plasma membranes. It is stimulated by calmodulin and, in its absence, by a number of alternative treatments like the exposure to acidic phospholipids (including, and especially, the phosphorylated derivatives of phosphatidylinositol), a controlled treatment with proteinases, e.g., trypsin or calpain, phosphorylation reactions by protein kinases A or C and self-association to form dimers or, more likely, oligomers.

Recent cloning work [1,2] has shown that the pump is a single polypeptide chain of molecular mass about 134 kDa. The pump is the product of a multigene family: four genes have been recognized in humans, and three have been assigned to chromosomes 1, 3 and 12. Additional isoforms originate through alternative mRNA splicing. The general architecture of the pump (Fig. 1) follows the general pattern of the P-type pump family, i.e., most of the pump mass protrudes into the cytoplasmic medium, very short loops connecting the putative transmembrane domain on the external side. Ten putative transmembrane domains have been identified, concentrated in the N- and C-terminal portions of the pump. The transmembrane organization of the pump is predicted from hydropathy plots and is thus still tentative. However, some of it has been directly validated by work with monoclonal antibodies and with other chemical labelling techniques. Three main units protrude into the cytosol, the first between transmembrane domains 2 and 3, the second between transmembrane domains 4 and 5, and the third from transmembrane domain 10. The first protruding unit has been proposed, based on the analogy with other P-type pumps, to permit the coupling of ATP hydrolysis to the transport of Ca (transducing unit). The protruding portions of the pump (Fig. 1) consist of antiparallel β-sheets, α-helices and parallel β-sheets. The C-terminal portion of the second protruding domain which contains the active site(s) of the pump (i.e., the site of aspartyl-phosphate formation and the binding site for ATP), is assumed to be connected to the membrane by a flexible 'hinge' which permits the catalytic aspartic acid to approach the bound ATP during the reaction cycle.

The calmodulin binding domain of the pump has been identified with the help of bifunctional crosslinkers coupled to calmodulin [3], and located next to the C-terminus of the pump. The domain is a positively charged segment of about 25 residues, which has a strong α-helix propensity in its N- and C-terminal portions (sub-domains A and B). Work with trypsin has shown that the pump is sequentially degraded to fragments of electrophoretic molecular masses 90, 85, 81, 76 kDa. The 90 kDa fragment has low basal activity and is stimulated by both calmodulin and acidic phospholipids. During the formation of the 85 and 81 kDa fragments, the pump gradually loses its sensitivity to calmodulin, but retains that to acidic phospholipids, whereas the formation of the 76 kDa fragment is accompanied by the loss of phospholipid sensitivity as well. The removal of the calmodulin binding domain and of the pump portion C-terminal to it during the formation of these fragments leads to the stimulation of the basal activity of the pump, suggesting that the domain could act as an autoinhibitor of the pump. In the formation of the 90 to 76 kDa fragments, trypsin also cuts the pump between putative transmembrane domains 2 and 3, but it is doubtful whether the portion

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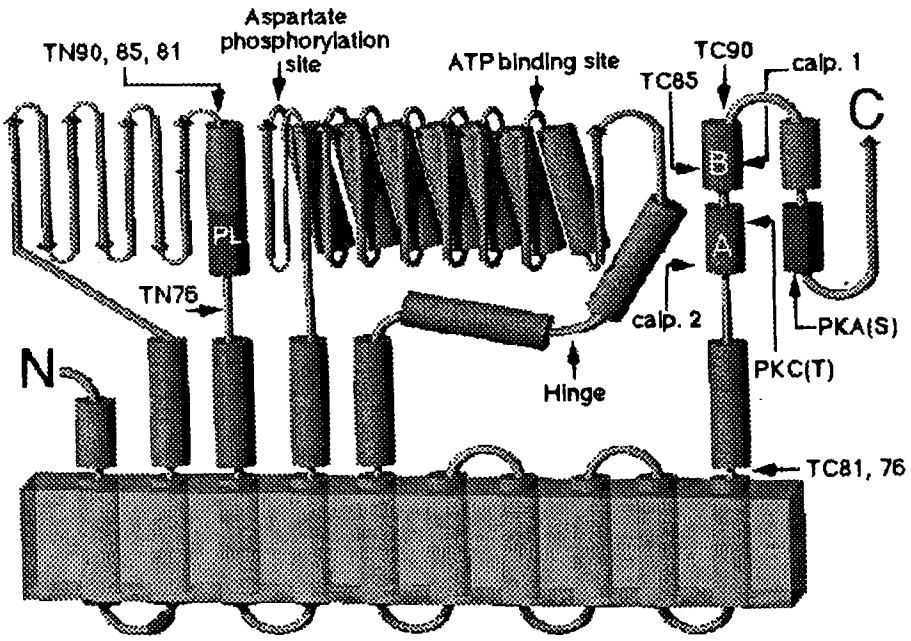

Fig. 1. Architecture of the pump. Cylinders indicate predicted α-helices, arrows β-sheets. The two calmodulin binding sub-domains are indicated by A and B. Calp 1 and calp 2 are the two sequential sites of calpain cleavage. PKA (S) and PKC (T) are sites of serine and threonine kinase phosphorylation. TC is the notation for the C-terminal cleavage by trypsin, TN that of the N-terminal cleavage. PL is the domain responsive to acidic phospholipids.

of the pump N-terminal to the cut is actually removed.

The Ca-dependent proteinase calpain also activates the pump, and does so by removing in two steps its calmodulin binding domain and the portion C-terminal to it. The resulting 124 kDa fragment has been used to test the suggestion of the auto-inhibitory function of the calmodulin binding domain. The latter, suitably derivatized with a photoactivatable crosslinker [4] has been found to label two domains of the pump, one located between the sites of aspartyl-phosphate formation and of ATP-binding, the other in the first protruding unit of the pump. The finding that the 'receptor' sites for the calmodulin binding domain are located in close proximity to the active site(s) of the pump conveniently rationalizes the 'inhibitory' action of the domain.

On the N-terminal side, trypsin cleaves the pump between transmembrane domains two and three. The N-termini of the fragments of 90, 85, 81 kDa differ from that of the 76 kDa fragment by a heavily charged stretch of about 50 residues. Work with the synthetic C-terminal portion of the stretch, which is very rich in basic amino acids, (labelled PL in Fig. 1) has shown [5] it to be one of the sites of the pump responsible for the response to acidic phospholipids (the other site appears to be the calmodulin binding domain).

None of the isoforms of the pump shows variations in the domains which are preserved throughout the family of P-type ion-motive ATPases, e.g., the domains surrounding the active site(s). Most of the diversity concerns the regulatory domains and leads to differences in regulatory properties, e.g., alternative mRNA splicing can eliminate the domain phosphorylated by protein kinase A (PKA(s) in Fig. 1). A particularly interesting mRNA splicing process leads to inserts of increasing length between calmodulin binding subdomains A and B. The newly inserted domain(s) duplicate somewhat the original calmodulin binding domain, except that the positively charged amino acids are histidines instead of arginines and lysines. This confers to the newly inserted domain(s) pH sensitivity in the binding of calmodulin, and may thus endow the pump with additional modulation possibilities.

One domain of the pump which is still unknown is that containing the catalytic Ca binding site. By analogy with suggestions on the Ca pump of sarcoplasmic reticulum, this Ca binding site could be located within some of the transmembrane domains. Another Ca binding site, possibly regulatory, has recently been found immediately C-terminal to the calmodulin binding domain.

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