S27-1 Mechanism of Ca^{2+} pump as revealed by mutations, development of stable analogs of phosphorylated intermediates, and their structural analyses

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Sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA), a representative member of P-type ion transporting ATPases, catalyzes Ca^{2+} transport coupled with ATP hydrolysis. The ATPase is activated by binding of two cytoplasmic Ca^{2+} ions at the transport sites, then forms an auto-phosphorylated intermediate (*E*P) producing the Ca^{2+} occlusion within the transport sites. Its isomerization from the ADP-sensitive form (*E*1P) to ADP-insensitive form (*E*2P) results in Ca^{2+} deocclusion and release into the lumen. The ATPase possesses three cytoplasmic domains; Nucleotide binding (N), Phosphorylation (P), and Actuator (A); and ten transmembrane helices M1~M10. The 3 domains largely move during ATP hydrolysis, and these changes are thought to be coupled with rearrangements of the transmembrane helices in which the transport sites are situated. For understanding structural mechanism of the key events of the energy coupling, the *E*P isomerization and Ca^{2+}-deocclusion/release, we have developed the stable analogs of each of *E*P intermediates; *E*1PCa_2, *E*2PCa_2, and *E*2P by using metal fluoride compounds, and also introduced a series of mutations and analyzed structural and kinetic properties of the mutants. The results gave essential information for understanding how the 3 domains moves and changes their interactions in successive structural events; *E*1PCa_2 → *E*2PCa_2 → *E*2P + $2Ca^{2+}$ to cause Ca^{2+} deocclusion and release.