Dephosphorylation of the Calcium Pump Coupled to Conterion Occlusion

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We have crystallized rabbit sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA1a) in complex with aluminium fluoride (E2-AlF) and solved its structure at 3.0 Å resolution using PHASER. We find the planar aluminium fluoride group to be located between the conserved Asp351 side chain and a water molecule positioned for hydrolysis. Further, we find the cation-conducting pathway to be in an occluded and protonated state. Further supported by biochemical data we conclude that the transition state of hydrolysis of the phosphoenzyme intermediate couples with occlusion of bound H+ counter-cations[1]. The mechanism is then similar to that of phosphorylation from ATP, which couples to the occlusion of Ca2+ ions[2]. A regulatory K+ site, identified by difference Fourier analysis using crystals prepared in RbCl[3], is observed to stabilize a helix cluster which positions the conserved TGES motif to activate the hydrolysis at the phosphorylation site. Initial identification of crystallization conditions was based on a screen of 48 combinations of PEG, salt, pH and alcohol additives, which we have found to be of general value for the crystallization of Ca2+-ATPase in various functional states.

[1] Olesen C. et al., *Science*, 2004, **306**, 2251. [2] Sorensen T.L., et al., *Science*, 2004, **304**,1672. [3] Sorensen T.L., et al., *J Biol Chem*, **279(45)**, 46355-8.

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