

Microsymposium

MS70.O04

Two dimensional crystallization of calcium transport regulatory complexes

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Phospholamban and sarcolipin physically interact with the sarcoplasmic reticulum calcium pump (SERCA) and differentially regulate contractility in smooth, skeletal, and cardiac muscle. While mutagenesis and cross-linking studies have provided insight into the mechanism of interaction, we lack a molecular understanding of these regulatory complexes. We have compared two crystal forms of SERCA in the absence and presence of phospholamban by electron cryo-microscopy. Our previous studies with phospholamban utilized both small helical crystals [1] and large two-dimensional crystals [2], where the fundamental units of both crystal forms were found to be anti-parallel dimer ribbons of SERCA molecules. The SERCA dimer ribbons have been known for decades as a rigid assembly of calcium-free SERCA molecules induced by the addition of decavanadate. While the lattice formed by the SERCA dimer ribbons is different in the helical (p2) and two-dimensional crystals (p22121), we now show that a phospholamban oligomer interacts with SERCA in a similar manner in both crystal types. With this information, we next undertook a structural investigation of SERCA and sarcolipin in the large two-dimensional crystals. Both wild-type and a gain-of-function mutant (Asn4-to-Ala) mutant of sarcolipin were utilized. Projection maps were determined for SERCA in the presence of sarcolipin to a resolution of 8.5 Å and were most consistent with a pentameric state for sarcolipin. While both phospholamban and sarcolipin interacted with transmembrane segment M3 of SERCA, the interaction of the sarcolipin pentamer was mediated by an additional density consistent with a sarcolipin monomer.

[1] H.S. Young, L.R. Jones, D.L. Stokes. *Biophysical Journal* (2001) 81:884-94., [2] J.P. Glaves, C.A. Trieber, D.K. Ceholski et al. *Journal of Molecular Biology* (2011) 405:707-23.

Keywords: electron crystallography, sarcoplasmic reticulum, calcium transport