SERCA1a and Phospholamban Cocrystallisation

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The Sarco(Endo)plasmic Reticulum Ca^{2+}-ATPase (SERCA) is a membrane Ca^{2+}-pump with a crucial role in the relaxation/contraction mechanism of the muscular cells.

SERCA1a has been purified from Sarcoplasmic Reticulum vesicles, isolated from rabbit fast twitch muscles. Ca^{2+}-ATPase concentration was increased within SR vesicles using different techniques: high ionic strength was employed to eliminate myosin and many membrane proteins and vesicles were treated with EDTA with the same purpose. Furthermore SR membranes have been purified by an extraction with low concentration of deoxycholate. Purified membranes were solubilised using a non-ionic detergent, $C_{12}E_8$, at 1.8 mg/ml final concentration. The supernatant was directly used for crystallization. Crystals of E1 SERCA1a grew in few days at 19°C with the hanging drop technique, using a precipitant solution containing: 15% (w/v) PEG 6000, 4% (v/v) tert-butanol, 15% (v/v) glycerol, 5 mM β-mercaptoethanol, 200 mM sodium acetate [1].

Synthesized PLB was solubilised in a solution containing chloroform/methanol with a ratio of 1/2 to a 31 mg/ml final concentration. SERCA1a and PLB were mixed to a 1:5 final molar ratio. Cocrystals grew in approximately a week, using the same precipitant utilized in SERCA1a crystallization.

[1] Sorensen T.L., Moller J.V., Nissen P., *Science*, 2004, **304**, 1672.

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