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Crystallization and preliminary X-ray analysis of Ca^{2+}-free primary Ca^{2+}-sensor of Na^{+}/Ca^{2+}exchanger

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The plasma membrane Na^{+}/Ca^{2+} exchanger (NCX) regulates intracellular Ca^{2+} levels in cardiac myocytes. Two Ca^{2+} binding domains (CBD1 and CBD2) exist in the large cytosolic loop of NCX. The binding of Ca^{2+} to CBD1 results in conformational changes that stimulate the exchange to exclude Ca^{2+}ions, whereas CBD2 maintains the structure, suggesting CBD1 being the primarily Ca^{2+}-sensor. To clarify the structural scaffold for the Ca^{2+}-induced conformational transition of CBD1 at the atomic level, the X-ray structural analysis of its Ca^{2+}-free form was tried: the structure of Ca^{2+}-bound form is now available. Recombinant CBD1 (NCX1 372-508) with molecular weight of 16kDa was crystallized by the sitting-drop vapor-diffusion method at 293 K. The crystals belonged to hexagonal space group $P6_222$, with unit-cell parameters a=b=56.99, c=153.86Å, β=120°, and contained one molecule per asymmetric unit (VM=2.25Å^3/Da). Diffraction data were collected to 3.00 Å using a R-AXIS IP detector and gave a data set with an overall Rmerge of 10.8 % and a completeness of 92.8 %.

Keywords: ion exchange, NCX, crystallization of proteins

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The crystal structure of villin domain 6

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Villin is an actin filament bundling, capping, severing and nucleating protein. Villin is regulated in these functions through calcium-binding, PIP2-binding and phosphorylation. Villin is related to gelsolin, in that it contains six homologous domains of the gelsolin/cofilin fold (V1-V6), but differs from gelsolin by having a seventh unrelated domain at the C-terminus. This extra domain, the villin head-piece (V-HP), binds to the side of actin-filaments and is generally thought to impart the bundling activity on villin, a property that is absent from gelsolin. To date only the structures of V1 and V-HP have been solved. Here we present the X-ray structure of V6 in an inactive buffer environment: low calcium, no PIP2 and no phosphorylation. The V6 structure contains a straight long helix which more closely resembles that of active gelsolin domain 6 (G6) rather than the inactive form in which the helix is bent. This suggests the role of calcium with respect to V6, in the context of the whole protein, is to release interdomain contacts that allow the straightening of this V6 helix, which in itself propagates further conformational changes.

Keywords: actin, villin, gelsolin

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Crystallization and X-ray structure analysis of Complex II from adult *Ascaris suum* mitochondria

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Mitochondrial complex II from adult *A. suum* consists of four subunits (Fp, Ip, Cy*b*L and Cy*b*S) and five prosthetic groups. Hydrophilic Fp and Ip subunits carry FAD and Fe-S clusters, respectively, and membrane-anchoring Cy*b*L and Cy*b*S subunits contain heme *b*. Adult *A. suum* living in a low-oxygen environment uses the anaerobic phosphoenolpyruvate carboxykinase-succinate pathway, and the complex II catalyzes the rhodoquinol oxdation in conjunction with the fumarate reduction. In this study, the *A. suum* complex II was crystallized and its crystal structures bound by malonate as well as by a specific inhibitor, flutolanil, were determined. Using sucrose monolaurate, the complex II was solubilized and purified from *A. suum* mitochondria. Crystals (space group $P2_12_12_1$, a=127.4, b=124.3, c=221.9 Å) were obtained using PEG3350 as a precipitant in the presence of a detergent mixture. X-ray diffraction data were collected at SPring-8 BL44XU and PF NW12. The structure complexed with malonate was determined by the molecular replacement method using the porcine complex II structure (1ZOY) and refined to R=0.25 (free-R=0.28) at 2.8 Å resolution. The structure complexed with flutolanil was also determined at 2.9 Å resolution (R=0.21, free-R=0.29). The structure of *A. suum* complex II is similar to those previously determined, and amino acid residues located within 4 Å from the prosthetic groups are highly conserved among complex IIs. The binding site of rhodoquinone could be identified, and flutolanil, which shows more inhibitory effect to *A. suum* complex II (IC_{50}=81 nM) than bovine complex II (16 μM), is bound to the same site as rhodoquinone. In order to study the structure-function relationship of *A. suum* complex II, the detailed examination of the structures is now in progress.

Keywords: membrane protein crystallization, parasites, membrane protein X-ray crystal structure

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The crystal structure at 1.8 Å resolution of the calcium-bound human S100A13 at pH 7.5

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