P04.01.16

Acta Cryst. (2008). A**64**, C235

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

P04.01.17

Acta Cryst. (2008). A**64**, C235

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

P04.01.18

Acta Cryst. (2008). A**64**, C235

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

P04.01.19

Acta Cryst. (2008). A**64**, C235-236

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

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S100A13 is a member of the S100 family of EF-hand-containing calcium-binding proteins. S100A13 plays an important role in the secretion of fibroblast growth factor-1 and interleukin-1α, two pro-angiogenic factors released by the non-classical endoplasmic reticulum-Golgi-independent secretory pathway. To gain insight into the structural basis of the interactions with these proteins, we determined the crystal structure of human S100A13 at pH 7.5 at 1.8-Å resolution. Human S100A13 was heterologously expressed in *Escherichia coli* purified and crystallized by the hanging-drop vapor diffusion method using PEG 3350 as the precipitant and at pH 7.5. The crystals diffracted X-rays from a synchrotron-radiation source beyond 1.8-Å resolution. The crystal space group was assigned as primitive orthorhombic $P2_12_12_1$ with unit-cell parameters a= 39.7Å, b= 59.2Å, c = 77.6Å. The structure was solved by molecular replacement and has been refined to a final R factor of 19.0% and a free R of 22.7%. The structure revealed that human S100A13 exists as a homodimer with two calcium ions bound to each protomer. The protomer is composed of 4 α-helices (α1-α4) forming a pair of EF-hand motifs. Dimerization occurs by hydrophobic interactions between helices α1 and α4 and by intermolecular hydrogen bonds between residues from helix α1 and the residues between α2 and α3 of both chains. Comparison between the crystal structures of human S100A13 at pH 7.5 (this study) and pH 6.0 (Li *et al.*, 2007) exhibited recognizable differences in the relative orientation (ca. 2.5°) between the protomers within the dimer and also remarkable differences in the side-chain conformations of several amino-acid residues.

Keywords: calcium-binding proteins, EF-hand proteins, X-ray crystallography of proteins

P04.01.20

Acta Cryst. (2008). A**64**, C236

X-ray analysis of FliJ, a cytoplasmic component of the flagellar type III protein export apparatus

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The flagellum is a motile organelle composed of the basal body rings and the tubular axial structure. The axial proteins synthesized in the cytoplasm are transferred into the central channel of the flagellum by the flagellar protein-export apparatus, which is classified into the type III protein export system. The apparatus is composed of six transmembrane proteins (FlhA, FlhB, FliO, FliP, FliQ, FliR) and three soluble components (FliH, FliI, FliJ). FliJ is an essential component for protein export. Although FliJ is thought to be a general chaperone, its function is still unclear. Here we report purification, crystallization and X-ray analysis of FliJ. Native FliJ was difficult to handle because of its strong tendency to form insoluble aggregates. Recently, we found that FliJ with extra three residues attached to the N-terminus as a remainder of His-tag is highly soluble. We obtained hexagonal bi-pyramid crystals from this FliJ variant, and determined the structure at 2.2 angstrom resolution using anomalous data from a mercury derivative crystal collected at SPring-8 BL41XU. We will discuss details of the structure and possible function of FliJ.

Keywords: Flagellum, FliJ, type III protein export apparatus

P04.01.21

Acta Cryst. (2008). A**64**, C236

Crystallographic study of zinc finger domain of Eco1 involved in sister chromatid cohesion

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The sister chromatid cohesion apparatus mediates physical pairing of duplicated chromosomes. This pairing is essential for appropriate distribution of chromosomes into the daughter cells upon cell division. The cohesion apparatus is also involved in gene expression and development. For instance, the Roberts syndrome in humans is caused by mutations in Eco1 homologous protein ESCO2, which is a component of the cohesion apparatus. Eco1 is originally found in budding yeast. Eco1 proteins are composed of variable N-terminal region and conserved C-terminal region composed of PCNA interacting protein box, zinc finger and acetyltransferase domains. Eco1 isn't required for binding of cohesin to chromosomes, but is needed to establish cohesion during S phase in *S. cerevisiae*. Furthermore, it has been reported that Eco1 interacts with PCNA, suggesting that it has a critical role during DNA replication. Although Eco1 has acetyltransferase activity in vitro, its inherent substrates in vivo are still unknown. Recent study shows that acetyltransferase domain of Eco1 is dispensable for S phase cohesion, but required for DSB-induced cohesion in G2/M. The zinc finger domain is alternatively required for chromosome segregation. Here we report crystallographic study of zinc finger domain of Eco1. Zinc finger domain of Eco1 was overexpressed as a GST-fused Protein in *E. coli*. The recombinant protein is purified by an affinity resin, removal of affinity tag by protease, gel-filtration chromatography. The purified protein was successfully crystallized using hanging drop vapor diffusion method. X-ray diffraction studies reveal that the crystal belongs to trigonal/hexagonal system with the cell dimensions of a = b = 73, c = 81Å, γ = 120°.

Keywords: sister chromatid cohesion, crystallization, zinc finger

P04.01.22

Acta Cryst. (2008). A**64**, C236–237

Crystallization of carbohydrate oxidase from *Microdochium nivale*

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Carbohydrate oxidase from *Microdochium nivale* is a flavoenzyme containing 475 amino acids with covalently linked flavin. The