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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

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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

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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

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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

function of the enzyme consists in catalytic oxidation of the primary alcohol in various oligosacharides with the highest activity towards D-cellobiose (Kulys et al., 2001). Crystallization experiments were performed using Hampton Research Crystal Screen and Index solutions. Crystals show variable morphology depending on crystallization conditions and feature varying stability. The measured hexagon-shaped crystal grew with Jeffamine ED2001 as precipitant. X-ray diffraction data were collected at beamline BM14, ESRF in Grenoble using the MARMosaic 225 detector. The crystal diffracted up to 2.7 Å resolution, however, a rapid intensity fall off occurred beyond 3.5 Å resolution. The space group was indicated as $P6_222$, with unit cell parameters $a = b = 55.7$ Å, $c = 610.4$ Å. The crystallographic symmetry was verified with program Pointless (Collaborative Computational Project, Number 4, 1994). Extensive molecular replacement trials with a model of 39% sequence identity (PDB code 1zr6) failed. The size of the molecule, size of the unit cell and high symmetry of the space group inadvertently resulted in significant overlaps of the 'solutions'. The real crystallographic symmetry is lower (subgroup of $P6_222$) and one or more symmetry operators arise from twinning of the crystal. Further experiments to produce well diffracting crystals without twinning are in progress.
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Collaborative Computational Project, Number 4 (1994). Acta Cryst. D50, 760-763.
Kulys, J., Tetianec, L. & Schneider, P. (2001). J. Mol. Catal. B: Enzym. 13, 95-101.

Keywords: carbohydrate oxidase, crystallization, X-ray structure analysis

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Crystal structure of actinohivin; A novel anti-human immunodeficiency virus protein

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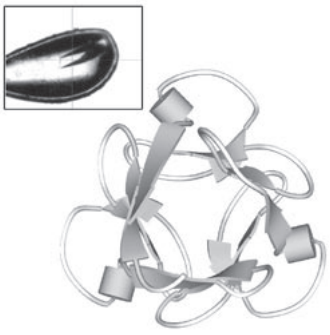
Actionohivin (AH) is a protein found in a culture filtrate of the newly discovered genus actinomycete *Longispora albida*. AH is a potent anti-human immunodeficiency virus (HIV) protein that inhibits viral entry to cells by binding high-mannose-type saccharide chains of HIV gp120. Consisting of highly conserved sequence which has three-tandem repeats, it might belong to carbohydrate binding module (CBM) family 13. To confirm mechanisms of specific binding of sugar to AH and to provide new approach for activity improvement, it was examined by crystallization and X-ray diffraction. Single crystals were obtained for several months. Crystals were soaked in a solution of platinum and were diffracted at maximum resolution of 1.1Å. Single wavelength anomalous dispersion method was used for initial phase determination. The crystal structure of AH has a pseudo threefold axis and consists of three domains which are analogous to each other. Three segments are consistent with highly conserved tandem repeats. The active site, LD and QXW motif, was positioned in central area of each segment.



Keywords: HIV drug design, high-resolution crystal structures, AIDS inhibitors

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Structural and functional analysis of an important *Pseudomonas aeruginosa* redox protein

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Pseudomonas aeruginosa is ubiquitous in soil and water and also occurs regularly on the surfaces of plants and occasionally animals. *P. aeruginosa* is an opportunistic human pathogen. It almost never infects healthy tissues, yet there is hardly a tissue that it cannot infect if defences are compromised in some manner. Therefore, *P. aeruginosa* is often responsible for nosocomial infections. To survive within a host, bacteria produce and secrete a variety of virulence factors. These are typically protein molecules that specifically influence host function and allow the bacterium to thrive. Even though each pathogenic species possesses a specific repertoire of virulence factors, a common feature of these molecules is that they are usually secreted to the surface of the cell or released into the extracellular environment to interact with host components. Often, these virulence factors contain disulfide bonds, which are incorporated to stabilize tertiary structure in a foreign environment. This involves the oxidation of two cysteine residues. It is now well established that the disulfide bond (Dsb) family of proteins catalyzes the formation of disulfide bonds in the bacterial periplasm. The key enzyme, DsbA, catalyzes disulfide bond formation by donation of its active-site disulfide to a folding protein substrate, via a mixed disulfide intermediate. The reduced DsbA produced by this reaction is then re-oxidized by the integral inner membrane protein DsbB. We have determined the 1.6 Å resolution crystal structure of the DsbA enzyme of *P. aeruginosa* (PaDsbA). Additional functional analysis of purified PaDsbA has enabled us to compare PaDsbA to previously characterized oxidoreductases. This information is vital to future studies aimed at combating this important pathogen.

Keywords: crystallographic structure determination, protein crystallography, protein biochemistry

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Crystallization of serine proteases for neutron single crystal structure determination

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