P04.16.385

Acta Cryst. (2008). A**64**, C351

Structural studies of BxlE, sugar binding protein from *S. thermoviolaceus* OPC-520

Koji Tomoo[1], Hideaki Morioka[1], Yasuhiro Miki[1], Kiho Seike[1], Toshimasa Ishida[1], Tomokazu Hasegawa[2], Akihito Yamano[2], Sadao Ikenishi[1], Katsushiro Miyamoto[1], Hiroshi Tsujibo[1]
[1]Osaka University of Pharmaceutical Sciences, Physical Chemistry, 4-20-1 Nasahara, Takatsuki, Osaka, 569-1094, Japan, [2]PharmAxess Inc., Biohills 308, 7-7-18 Saitoasagi, Ibaraki, Osaka 567-0085, Japan, E-mail：tomoo@gly.oups.ac.jp

BxlE isolated from *Streptomyces thermoviolaceus* OPC-520, together with the integral membrane proteins BxlF and BxlG, form an ATP-binding cassette (ABC) transport system that mediates the uptake of xylane. To clarify the structural basis for sugar binding by BxlE at the atomic level, recombinant BxlE was crystallized by the hanging-drop vapor-diffusion method at 290 K. The crystals belonged to monoclinic space group $P2_1$, with unit-cell parameters a=44.63, b=63.27, c=66.40 Å, β=103.05°, and contained one 48 kDa molecule per asymmetric unit (V_M=1.96 Å^3/Da). Diffraction data collected to a resolution of 1.65 Å using a rotating anode X-ray source gave a data set with an overall *R*merge of 2.6% and a completeness of 91.3%. A data set for a platinum derivative is being used for phasing by the SAD method. We have determined the crystal structure of BxlE at 2.1 Å resolution. The structure of BxlE is comprised of two domain. The two domains are linked by three hinge segments. These hinge segments would allow the opening and closing of two domains for binding of sugar.

Keywords: BxlE, sugar binding protein, domain structure

P04.16.386

Acta Cryst. (2008). A**64**, C351

The structure of AMIGO - A leucine rich repeat protein important for neuronal growth regulation

Tommi A Kajander[1,2], Juha Kuja-Panula[2], Heikki Rauvala[2], Adrian Goldman[1,2]
[1]University of Helsinki, Institute of Biotechnology, PO BOX 65, Helsinki, --, 00014, Finland, [2]University of Helsinki, Neuroscience Center, PO Box 56, FIN, 00014 Helsinki, Finland, E-mail：tommi.kajander@helsinki.fi

AMIGO is a presentative member of a novel neuronal family of membrane bound LRR proteins induced by HMGB1/Amphoterin interaction with the multifunctional receptor RAGE, and associated with neuronal growth regulation. We have determined the structure of AMIGO both by X-ray crystallography and characterization by solution scattering (SAXS). Crystal data exist for both glycosylated and deglycosylated protein from insect and mammalian systems. The structure solution was complicated by pseudo-C-centering in monoclinic crystals of the glycosylated protein. As indicated by sequence data the structure consists of 6 LRR-repeats and and Ig-domain, with N- and C-terminal caps on the LRR domain, characteristic for extracellular LRR domains, including several disulphide bonds stabilizing these regions. Both crystal and solution studies confirm the protein as a dimer linked via the LRR-domains. It appear thats dimerization and the LRR region are vital for the protein function. Details of structure solution, dimer formation and interface structure and functional implications of the structure will be discussed.

Keywords: protein-protein interactions, structural motifs, neural processes

P04.16.387

Acta Cryst. (2008). A**64**, C351

Structure of laccase from *Streptomyces coelicolor*

Tereza Skalova[1], Jan Dohnalek[1], Lars H Ostergaard[2], Peter R Ostergaard[2], Petr Kolenko[1], Jarmila Duskova[1]
[1]Institute of Macromolecular Chemistry AS CR, Heyrovskeho nam. 2, Praha 6, Czech Republic, 162 06, Czech Republic, [2]Novozymes A/S, Brudelysvej 26, DK-2880 Bagsvaerd, Denmark, E-mail：skalova@imc.cas.cz

Laccases (EC 1.10.3.2) are multicopper oxidases catalyzing the reduction of molecular oxygen to water accompanied by oxidation of a substrate, with broad substrate specificity (polyphenols, methoxy-substituted phenols, aromatic diamines). Structurally known laccases consists of three domains. The laccase from Streptomyces coelicolor reported here is the first two-domain laccase structure of which was solved. The laccase was crystallized using hanging drop vapor diffusion method with reservoir containing 0.1 M NaCl, 0.1 M glycine, pH 9.0, and 39% (v/v) PEG monomethyl ether 550 at temperature of 298 K (Skalova et al, Acta Cryst. F 63, 1077-1079, 2008). The laccase crystallizes in space group $P4_32_12$ with unit cell parameters $a = b$ = 180.9 Å, c = 177.2 Å. The phase problem was solved using MAD (multiple anomalous dispersion) on natively present copper ions at ESRF in Grenoble, BM 14. Data for structure refinement were processed up to 2.65 Å and the structure was refined in REFMAC with final R-factors R = 0.172, Rfree = 0.194. The oligomeric state of two-domain laccases (dimeric versus trimeric) has been discussed in literature during recent years. This structure confirms that the laccase from Streptomyces coelicolor forms trimers. The trimeric packing is tight and necessary for the catalytic function. The quaternion of copper ions which form the active unit lies in domain 1 and domain 2 of two neighbor monomers. The structure was deposited in The Protein Data Bank under accession code 3CG8.
Acknowledgement: This work was supported by GA AV CR, project IAA500500701, by GA CR, project 305/07/1073, and by the European Commission, integrated project SPINE2-Complexes, no. LSHG-CT-2006-031220.

Keywords: laccase, oxidoreductase, multicopper blue protein

P04.16.388

Acta Cryst. (2008). A**64**, C351–352

Crystallography of bacterial RNA polymerase complexed with transcription factors

Shunsuke Tagami[1,2], Shun-ichi Sekine[1,2], Thirumananseri Kumarevel[3], Masaki Yamamoto[3], Shigeyuki Yokoyama[1,2]
[1]the University of Tokyo, Dept. of Biophysics and Biochemistry, Graduate School of Science, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-0033, Japan, [2]Genomic Sciences Center, Yokohama Institute, RIKEN, 1-7-22 Suehiro-cho, Tsurumi, Yokohama, 230-0045, Japan, [3]SPring-8/RIKEN, Kouto, Sayo-cho, Sayo-gun, Hyogo 679-5148, Japan, E-mail：tagami@biochem.s.u-tokyo.ac.jp

Transcription is the initial, prerequisite step for gene expression. Since the gene expression is controlled principally at the transcriptional level, studies on mechanistic details of transcriptional regulation are of general significance. RNA polymerase (RNAP) is a huge, multi-subunit enzyme that plays a pivotal role in transcription. During the transcription cycle, various transcription factors associate with the RNAP, and supports or controls the RNAP action. Therefore, it is quite significant to analyze structures of the RNAP complexed with specific transcription factors. The Gre factors are known as

the bacterial transcription elongation factors, which modulate the enzymatic activity of RNAP. GreA is a transcript cleavage factor, which binds to a stalled elongation complex caused by an RNAP back tracking. It rescues the RNAP by invoking the inherent RNAP activity to cleave the extruded RNA 3' end. On the other hand, Gfh1, a close paralog of GreA, inhibits the enzymatic activity. To elucidate details of how these factors modulate the RNAP activity, we crystallized Thermus thermophilus RNAP complexed with them. Crystallization, data collection and preliminary crystallographic analysis will be presented.

Keywords: RNA polymerase, transcription regulation, macromolecular X-ray crystallography

P04.16.389

Acta Cryst. (2008). A**64**, C352

Crystal structure of the full-length Hsp110 molecular chaperone in the nucleotide-free state

Yasuhito Shomura[1], Andreas Bracher[2], F Ulrich Hartl[2], Yoshiki Higuchi[1]

[1]Graduate School of Life Science, University of Hyogo, Department of Life Science, Koto 3-2-1, Kamigori-cho, Ako-gun, Hyogo, 678-1297, Japan, [2]Department of Cellular Biochemistry, Max Planck Institute of Biochemistry, Am Klopferspitz 18, D-82152 Martinsried, Germany, E-mail：shomura@sci.u-hyogo.ac.jp

The 70kDa heat shock proteins (Hsp70) play essential roles in diverse cellular processes as molecular chaperones by preventing the substrate proteins from unfavorable aggregation. The 110 kDa heat shock protein (Hsp110) is a distal member of Hsp70 superfamily found in eukaryotes. In spite of the existence of two domains both characteristic of Hsp70, Hsp110 shows some significant different properties than canonical members of Hsp70; most importantly, Hsp110 lacks ATPase activity that is necessary for the Hsp70 function. Recently, the function of Hsp110 as a nucleotide exchange factor for canonical Hsp70s has become clear. The crystal structure of the *Saccharomyces cerevisiae* ortholog of Hsp110 in the ATP-bound state has been more recently reported, although the detailed mechanism is still poorly understood. We report the crystallization and X-ray crystallographic analysis of Hsp110 from *Schizosaccharomyces pombe*. The protein was crystallized in the absence of nucleotides and includes the C-terminal portion that was truncated in the previous study. The biochemical data suggests that the C-terminal helix bundle is critical for the nucleotide exchange activity. By comparing the two structures, possible roles of the ATP binding and the C-terminal helix bundle will be discussed.

Keywords: X-ray structure determination, protein crystallography, protein chaperone

P04.16.390

Acta Cryst. (2008). A**64**, C352

Structural insights into asymmetric cell division in drosophila

Mohammad Yousef[1,2], Hironari Kamikubo[3], Mikio Kataoka[3], Ryuichi Kato[1], Soichi Wakatsuki[1]

[1]KEK (High Energy Accelerator Research Organization), 1-1 Oho, Tsukuba, Ibaraki, Tsukuba, Ibaraki, 305-0801, Japan, [2]Biophysics Department, Faculty of Science, Cairo University,Egypt, [3]Graduate School of Materials Science. Nara Institute of Technology, Ikome, Nara630-0192, JAPAN, E-mail：yousef@post.kek.jp

Miranda is a multidomain adaptor protein involved in nueroblast asymmetric cell division in drosophila. The central domain of miranda is necessary for cargo binding of the neural transcription factor prospero. We solved the first solution structure of miranda central cargo-binding domain using small angle x-ray scattering (yousef et al, 2008). Additionally,the complex structure of prospero homeodomain bound to its dna target has been previously solved by x-ray crystallography(yousef and matthews, 2005). We have also expressed different segments of prospero and miranda in soluble forms for further structural analyses. The structural information available thus far has shed light on important mechanisms involved in asymmetric cell division.

Yousef MS, Kamikubo H, Kataoka M, Kato R and Wakatsuki S. Protein Science (2008). 17:908-917
Yousef MS and Matthews BW. Strcuture (2005). 13:601-607

Keywords: asymmetric cell division, small angle x-ray scattering, X-ray crystallography

P04.17.391

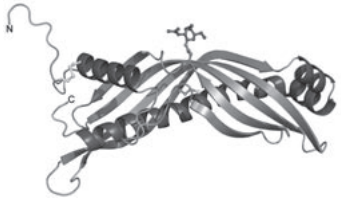
Acta Cryst. (2008). A**64**, C352

Ancestral lipid-binding fold of insect juvenile hormone binding protein

Mariusz Jaskolski[1,2], Robert Kolodziejczyk[1], Grzegorz Bujacz[2,3], Marian Kochman[4]

[1]Faculty of Chemistry, A. Mickiewicz University, Department of Crystallography, Grunwaldzka 6, Poznan, Poznan, 60-780, Poland, [2]Center for Biocrystallographic Research, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland, [3]Institute of Technical Biochemistry, Lodz University of Technology, Lodz, Poland, [4]Department of Biochemistry, Faculty of Chemistry, Wroclaw University of Technology, Wroclaw, Poland, E-mail：mariuszj@amu.edu.pl

As specific carriers of Juvenile Hormone (JH), Juvenile Hormone Binding Proteins (JHBPs) have a profound effect on insect development. The crystal sructure of G. mellonella JHBP has a remarkable fold, consisting of a long helix wrapped almost completely in a highly curved beta-sheet. JHBP resembles the folding pattern of some human lipid binding proteins, with similar organization of one cavity and an S-S bond between the helix and the beta wrap. The human molecules, however, have a tandem of JHBP-like domains, probably as a result of gene duplication. Thus, JHBP reveals an ancestral fold used for hydrophobic ligand binding. Unexpectedly, the JHBP molecule possesses two hydrophobic pockets, one at each pole. Although it was not possible to crystallize a JHBP-JH complex, several experiments indicate that JHBP binds JH in only one cavity. The fact that JHBP crystals disintegrate on contact with JH confirms a report of a conformational change on hormone binding. The nature of the second ligand is unknown but it is intriguing that with its two binding pockets the JHBP molecule has a similar binding potential as the human molecules, in which each domain has only one hydrophobic pocket.



Keywords: insect development, juvenile hormone, hemolymph