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

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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

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

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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

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Structure of laccase from *Streptomyces coelicolor*

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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.
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Keywords: laccase, oxidoreductase, multicopper blue protein

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Crystallography of bacterial RNA polymerase complexed with transcription factors

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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