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CRYSTALLOGRAPHY OF ENZYMES

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Knowledge of three-dimensional structure of enzymes plays an important role in our understanding their function and is required for design and modification of enzymatic properties and stability. In recent years we have worked with several enzymatic systems where structural studies provided very important insights into the enzyme behaviour, its classification, and helped discovery of previously not known activity or uncovered structural changes upon ligand binding.

In structural studies of -galactosidase from *Arthrobacter* sp. C2-2 a unique way of arrangement of the molecules in functional hexamers was discovered. The functional form of the enzyme revealed by its crystal structure has consequences for the actual substrate and product logistics. The sphere-like hexamers form a large cavity inside the cluster with three types of channels connecting it with exterior. The six active sites of the hexamer are open into the internal cavity and are not accessible from the outside. Thus any substrate or ligand must pass through the channels of the hexamer to reach the catalytic site. In further studies it was confirmed that ligands can indeed access the active sites inside the cavity via the existing channels. Also presence of the so called shallow binding mode of this glycosyl hydrolase was confirmed by observation of inhibitor binding in this 660 kDa structure [1].

Structural arrangement and stabilization features uncovered by crystal structure of the small laccase from *Streptomyces coelicolor* for the first time proved existence of the trimerization-dependent laccase, in which quaternary organization in an interesting way makes the second domain of laccase redundant and ensures extreme stability [2]. Here, an exceptionally high solvent content value of 83% was observed. The high solvent content was interesting from the point of view of methodology of structure solution as it enabled very effective application of solvent flattening to the initial phases acquired from a MAD experiment on copper ions. Further studies of ligand binding led to variation of enzyme arrangement in the crystal and improved quality of data connected with ferrocyanide binding (acting as an electron donor). The structure solution revealed also presence of a central channel which can serve as a route for access to the trinuclear copper cluster. Its role still remains unexplained.

Structure of the plant nuclease TBN1 with confirmed anticancerogenic properties opened up many topics regarding non-specificity of this enzyme capable of degradation of ss and ds DNA and RNA. Structural similarity to another enzyme led to confirmation of phospholipase C-like activity and initiated other investigations [3]. Especially mapping of the molecular surface electrostatics and differences between single strand and double strand processing nucleases of this type suggest some amino acid residues being responsible for such specificity. Structural studies also brought insights into aggregation/dimer formation of this enzyme, which for the first time raises questions about specific peptide binding in a nuclease active site.

Structural study of a bacterial organophosphorous acid anhydrolase provided for the first time a complete view of the enzyme and proved that the enzyme classes of prolidase and OPAA are basically identical [4]. Access to the active site differs in the human and bacterial enzymes although dependence on manganese ions remains conserved. The solved structures showed details important for enzyme dimerization, which is required for function. Covalent modification of the peptide chain of the bacterial OPAA was observed, which was identified as nickel forming covalent bonds to the main chain nitrogen atoms.

X-diffraction analysis of enzymes brings essential information directly related to function of the studied systems. The most outstanding are information about oligomerization and its exact mechanism, positioning and exact function of the catalytic amino acids, ligand and metal binding, and mechanisms of structural stabilization. Solid results of structural analysis pave route to further modifications of the enzymatic systems and utilization in biotechnological and medical applications.

Support is acknowledged from the Czech Science Foundation (no. P302/11/0855), MEYS (LG14009, EE2.3.30. 0029) and BIOCEV CZ.1.05/1.1.00/02.0109 from the ERDF.

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SL5

NEW FAMILY OF BACTERIAL LECTINS WITH SEVEN-BLADED -PROPELLER FOLD

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Burkholderia pseudomallei and *Chromobacterium violaceum* are bacteria of tropical and subtropical soil and water that occasionally can cause fatal infections in human and animals [1-3]. Lectins of these bacteria, BP39L and CV39L, were discovered and characterized. Lectins are proteins, which are capable of specific and reversible binding to carbohydrate moieties. Carbohydrate-mediated recognition plays an important role in the ability of pathogenic bacteria to adhere to the surface of the host cell in the first step of their invasion and infectivity [4].

The crystal structures of BP39L and CV39L were determined based on a new class of lanthanide complexes for heavy atom derivatization (SAD experiment). The BP39L lectin crystallized in I222 space group (a = 46.08 Å, b = 87.55 Å, and c = 159.10 Å) and CV39L in $P2_12_12_1$ (a = 65.71 Å, b = 123.78 Å and c = 180.93 Å). The structures of both lectins revealed seven-bladed -propeller fold with possibly seven binding sites per monomer. The BP39L lectin was observed as a monomer and CV39L as a dimer in solution, which was confirmed also in the crystal structure. The knowledge about the structure together with sequence and structure predictions indicates that the proteins belong to an undescribed new family of lectins. The binding sites of BP39L are highly conserved. On the other hand, CV39L has not equivalent binding sites but the architecture is very similar. In both lectin binding sites, highly conserved Trp residues were observed, which play a key role in saccharide stabilization via stacking and polar (hydrogen bond) interactions. Isothermal titration calorimetry experiments conducted on a series of saccharides confirmed the preference of BP39L for d-mannose and -1,3(-1,6)-d-mannotriose, and CV39L for l-fucose and l-galactose. As the architecture of the binding sites of both lectins has a shape of wider shallow pocket, it indicates that the lectins can recognize more complex saccharides as just monosaccharides. This structural feature was also indicated by the molecular docking simulations based on the orientation of the saccharide ring of d-mannose (BP39L), l-fucose and l-galactose (CV39L).

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We thank the synchrotron facility PETRA-III (DESY, Hamburg, Germany) for access to their macromolecular beam line P13 and the allocation of synchrotron beam time. This work was supported by the Czech Science Foundation (P207/11/P185 and GA13-25401S).

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CRYSTALLIZATION AND PRELIMINARY X-RAY DIFFRACTION ANALYSIS OF GLYCERALDEHYDE DEHYDROGENASE FROM *THERMOPLASMA ACIDOPHILUM*

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Synthetic Cascade Biomanufacturing is a cell free enzyme cascade developed for environmentally gentle chemicals production [1]. The glyceraldehyde dehydrogenase from *Thermoplasma acidophilum* (TaAlDH) is a part of such cell free system for production of isobutanol and ethanol from glucose. A mutant of TaAlDH carrying mutations F34M+Y399C+S405N was constructed in order to improve enzyme's properties essential for its functioning within the cascade: substrate selectivity, activity, product tolerance and acceptance of NAD^+ as a cofactor [2]. The aim of our research is solving the 3D structure of TaAlDH (F34M+Y399C+S405N) in order to explore the mutations influence on the enzyme's function.

For this purpose protein crystallization with further X-ray diffraction analysis of obtained crystals has been used.

TaAlDH (F34M+Y399C+S405N) was successfully crystallized by means of different crystallization screens using the Gryphon crystallization robot (Art Robbins Instruments, USA). Two different forms of crystals appeared at room temperature: form A in condition G2 of Morpheus screen (Molecular Dimensions Ltd., UK) and form B in A1 of PEGs Suite (QIAGEN, Netherlands). Crystals from PEGs Suite were suitable for X-ray diffraction analysis without optimization of crystallization conditions (crystal form B). Crystals from Morpheus screen grew quite big (200 300 m) but plate and multilayer. Therefore, the optimization of crystallization conditions by variation of protein and precipitant concentrations was provided. Certain improvement of crystals quality was observed after the optimization and the final size of crystals was about 100 x 100 500 m (crystal form A). Full diffraction data sets were collected at the BESSY II electron-storage ring operated by the Joint Berlin MX-Laboratory (Berlin-Adlershof, Germany) up to the resolutions of 2.14 and 2.2 Å for crystal form A and B, respectively.

Further work on determination of TaAlDH mutant structure by molecular replacement is currently in progress.

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