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Opening of the Safety-belt Loop of Human Aldose Reductase

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Aldose reductase (ALR2; EC 1.1.1.21) is a member of the aldo-keto reductase superfamily and it catalyzes the NADPH-dependent reduction of aldeydes to their corresponding alcohols. It is implicated in the polyol pathway and in diabetic complication.

The crystal structure of native aldose reductase has been determined to a resolution of 0.82 Å with a final R = 9.50 and R_{free} = 10.90 and a mean coordinate error for the fully occupied sites of the protein of 0.011 Å (from fully matrix inversion). The structure contains a large number of multiple conformations: 78 out of 316 residues were modeled in two conformations.

The overall structure folds into an eight-stranded α/β barrel with the active site located at the C-terminal end of the barrel and the $NADP^+$-binding site near the hydrophobic binding pocket [1]. The cofactor is held in place by the so-called 'safety-belt' (a loop between residue 216 and 227 of the canonical α/β barrel) [2].

The active site of the structure contains a citrate molecule in two conformations. One of the conformations stabilizes the closed position of the safety-belt, whereby the other permits the safety-belt to open. Due to the high resolution, the partially opened conformation of the safety-belt can be observed in the electron density.

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Keywords: high-resolution refinement, active-site structure, loop modeling

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Crystal Structure of *Caenorhabditis elegans* Spermidine Synthase: in Preparation

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Caenorhabditis elegans (C.elegans) is a free living worm and a well established model organism to study general biological processes like development.

Consistent with reports on other organisms, growth of *C. Elegans* depends on polyamines. Polyamines such as putrescine, spermidine and spermineare aliphatic polycations, essential for regulation of cell proliferation and differentiation. Spermidine synthase isoneofthekey enzymes in the polyamine biosynthetic pathway.

This enzyme catalyzes the transfer of the aminopropyl group from decarboxylated S-adenosylmethionine to putrescine in the biosynthesis of spermidine.

Spermidine synthase from *C. Elegans* has been over-expressed in Escherichiacoli, purified by affinity chromatography and co-crystallized with putrescine, which is the substrate. The crystals diffract to 2.5Å and belong to the monoclinic P21 space group with unit cell dimensions, a=59.99, b=99.23, c=67.85Å and β=107.2°. The asymmetric unit contains two molecules. Model building and refinement are ongoing.

Keywords: *Caenorhabditis elegans*, polyamines, spermidine synthase

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Structure of *Ralstonia solanacearum* Fucose Binding Lectin at 0.94Å Resolution

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Ralstonia solanacearum is a soil-born bacterium belonging to the group of beta-proteobacteria. It is responsible for bacterial wilts in over 200 plants including potato, tomato and banana, and is capable of living for prolonged periods in soil, infecting hosts via the roots.

A 9.9 kDa fucose-binding lectin (RSL) has been found in *R. Solanacearum* extract. Ultra-high resolution diffraction data to 0.94Å data were collected from crystals of the recombinant RSL: α-methyl-fucose complex at ESRF, Grenoble. Superb phasing was obtained using the RSL:seleno-methyl fucoside complex, showing the crystals to contain three monomers, each of two 4-stranded β-sheets, with two sugar sites per monomer. The three monomers associate to form a 6-bladed β-propeller; the first time such an arrangement has been observed. ITC microcalorimetry and surface plasmon resonance studies are underway to define the fine specificity to fucosylated oligosaccharides present in plant cell walls, that may be the target for the lectin in soil.

Keywords: lectin crystallography, atomic resolution crystallography, synchrotron radiation crystallography

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Crystal Structures of Ribosomal Protein L10 in Complex with L7/12 N-Terminal Domains

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The L7/12 stalk of the large ribosomal subunit comprises protein L10 and multiple copies of L7/12. It is involved in binding of translation factors and stimulation of factor-dependent GTP hydrolysis. The stalk is disordered in available crystal structures of ribosomes or 50S subunits. We have determined crystal structures of *Thermotoga maritima* L10 in complex with three L7/12 N-terminal domain (NTD) dimers. The structures are in agreement with a multitude of biochemical data. A globular NTD of L10 encompasses the binding region for 23S rRNA. A long C-terminal helix (α8) of L10 shows a modular design with consecutive binding sites for L7/12 dimers. L10 helix α8 assumes different positions with respect to the NTD in different crystal forms and thus constitutes a mobile platform for the attached L7/12 molecules. The number of L7/12 dimers varies with the length of L10 helix α8 in different species. The structure of the L7/12 NTD dimers agrees with one mode of dimerization observed in isolated L7/12. The hinge region of L7/12 can bind in α-helical form to the NTD in isolation but is displaced by L10 upon complex formation and becomes disordered. The organization of the complex supports its function in factor recruitment and GTPase activation.

Keywords: L10-L7/12 complex, L7/12 stalk, ribosome structure

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Comparative Study of Thrombin Binding of Potassium vs. Sodium

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Thrombin, a critical serine protease responsible for blood coagulation, is an allosteric enzyme that binds an alkali metal cation near the substrate-binding site. We have obtained a crystal structure to a resolution of 1.9 Å for the potassium-bound form of thrombin and compared it to the existing structure of sodium-bound thrombin.[1]

The crystal packing of K+-bound thrombin is quite different from that of Na+-bound thrombin. Crystal contacts in K+-bound thrombin distort the cation-binding site of one of the two molecules of the asymmetric unit such that the residues that normally coordinate the alkali metal are disordered. However, the cation-binding site of the other molecule is intact and can be compared with the sodium-binding site of Na+-bound thrombin.

Potassium in K+-bound thrombin is 7-coordinate with three-backbone carbonyl oxygen atoms and four water molecules as ligands. The key water molecule that communicates with the substrate binding site is the water molecule that bridges the cation and the side chain of Asp189. The distance between this water and the cation is about 0.5 Å longer in the K+-bound form than in the Na+-bound form.

[1] Pineda A. O., Carrell C. J., Bush L. A., Prasad S., Caccia S., Chen Z., Mathews F. S., di Cera E., *J. Biol Chem.* 2004, **279**, 31842.

Keywords: proteases, metal binding, packing

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Crystal Structure of Hyperthermostable Thioredoxin Peroxidase from *Aeropyrum pernix* K1

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Thioredoxin peroxidase from an aerobic hyperthermophilic archaeon, *Aeropyrum pernix* K1, (ApTPx) participates in the thioredoxin system, which is an antioxidant system to reduce hydrogen peroxide. ApTPx belongs to the peroxiredoxin family. We determined the crystal structure of ApTPx at 2.0 Å resolution. The overall structure is a decameric ring consisting of five homodimers with outer and inner diameters of approximately 130 and 50 Å, respectively. The monomer structure can be divided into two domains, a main domain and an arm domain. The arm domain is characteristic to ApTPx among peroxiredoxins. The redox active resolving cysteine is located on the arm domain and occupies the characteristic position when compared with mesophilic peroxiredoxins. A dimer interface is created by interaction between main domains. The dimerization results in formation of an intersubunit β-sheet. The arm domains stick out of the main body of the dimer. Assembly of homodimers to form a decameric ring is contributed by two types of interactions, one is by main domains and the other is by main and arm domains, latter of which is solely observed in ApTPx. Higher proportion of the monomer surface is buried in the decameric ring of ApTPx compared with mesophilic peroxiredoxins, suggesting that the high affinity in the protein complex contributes to the hyperthermostability of ApTPx.

Keywords: peroxiredoxin, *Aeropyrum pernix*, thioredoxin system

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Thymidine Kinase of Mycoplasmic Origin – an Enzyme with Lasso

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Thymidine kinase, TK1, is a well-known enzyme of importance in nucleotide metabolism as well as an activator of antiviral and anticancer drugs as AZT. TK1 has narrower substrate specificity than the other deoxynucleoside kinases and phosphorylates only deoxythymidine and deoxyuridine. TK1-like sequences are found in a broad variety of organisms. Recently, thymidine kinase from *Ureaplasma urealyticum (Uu*-TK) was characterized.

U.urealyticum is a human pathogen colonizing the urogenital tract. Interestingly, no genes for the *de novo* synthesis of deoxyribonucleotides have been found in the *U. urealyticum* genome. Therefore, this bacterium has to rely solely on salvage for synthesis of DNA precursors making *Uu*-TK a potential target for antibacterial drugs blocking the bacterial but not the human TK1.

Here the Xray-structure of *Uu*-TK in complex with the feedback inhibitor deoxythymidine triphosphate (dTTP) is presented, [1]. The enzyme has a tetrameric structure where each subunit contains an α/β-domain and a unique lasso-type domain. The domains are connected via a structural zinc. The active site is buried between these two domains and the thymidine of dTTP is hydrogen bonded to main-chain atoms predominantly coming from the lasso loop.

[1] Welin M., Kosinska U., Mikkelsen N.E., Carnrot C., Zhu C., Wang L., Eriksson S., Munch-Petersen B., Eklund H., *Proc. Natl. Acad. Sci. USA*, 2004, **101**, 17970.

Keywords: thymidine kinase, prodrug activation, mycoplasma

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Structure of Heterotetrameric Sarcosine Oxidase (TSOX) at 1.85 Å Resolution

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Heterotetrameric Sarcosine Oxidase is a bacterial flavoenzyme isolated from *Pseudomonas maltophilia*. It contains three coenzymes (FAD, FMN and NAD^+ and comprises 4 different subunits (α, 103 kDa; β, 44 kDa; γ, 22 kDa; δ, 11 kDa; total MW 180 kDa). TSOX catalyzes the oxidation of sarcosine (N-methylglycine) to yield hydrogen peroxide and formaldehyde. In the presence of tetrahydrofolate (THF), the oxidation is coupled to the formation of 5,10-methylenetetrahydrofolate (5,10-CH_2-THF). Sequence analysis suggests that NAD^+ as well as the 5,10-CH_2-THF synthase site are located in the α subunit whereas the covalent FMN site and the noncovalent FAD site, where sarcosine oxidation and peroxide formation take place, are located in the β-subunit.

The structure of selenomethionine-substituted TSOX was determined at 2.0 Å resolution by MAD phasing at three energies from data collected at the Biocars beamline 14ID of the APS. Location of 28 selenium sites with SOLVE and phasing with SHARP allowed automatic fitting of the solvent leveled map using Arp/Warp. Native TSOX was then solved at 1.85 Å resolution using MOLREP.

As predicted, the NAD^+ and putative folate binding sites are located in the α-subunit and the FAD binding site is in the β-subunit. The FMN is bound between the α and β subunits. Unexpectedly, a zinc ion was discovered bound to the δ-subunit and coordinated by 3 cysteine and 1 histidine side chain.

Keywords: flavoenzymes, channelling, MAD phasing

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Structure of Plant ATG12, a Ubiquitin-like Modifier Essential for Autophagy

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Atg12 is a post-translational modifier that is activated and conjugated to its single target, Atg5, by a ubiquitin-like conjugation system [1]. The Atg12-Atg5 conjugate is essential for autophagy, a starvation-induced response that mediates the bulk degradation of cytoplasmic components in lysosomes/vacuoles. In autophagy, a double-membrane structure called an autophagosome sequesters cytoplasm and fuses with the lysosome/vacuole to deliver its contents into the organelle lumen. The Atg12-Atg5 conjugate plays a critical role for autophagosome formation [1], but its mechanism remains to be elucidated. In order to clarify the role of Atg12 in autophagy, we determined the crystal structure of *Arabidopsis thaliana* (At) ATG12 at 1.8 Å resolution by MIRAS phasing.

In spite of no-detectable sequence homology with ubiquitin, the structure of AtATG12 shows a ubiquitin fold, strikingly similar to those of mammalian Atg8 homologs such as LC3 [2]. Two types of