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.

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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