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

hydrophobic patches are present on the surface of AtATG12: one is conserved in both Atg12 and Atg8, while the other is unique to Atg12. Considering that they share Atg7 as an E1-like enzyme, we suggest that the first hydrophobic patch is responsible for the conjugation reaction, while the latter is involved in Atg12-specific functions.

[1] Mizushima N., Noda T., Yoshimori T., Tanaka Y., Ishii T., George M.D., Klionsky D.J., Ohsumi M., Ohsumi Y., *Nature*, 1998, **395**, 395. [2] Sugawara K., Suzuki N.N., Fujioka Y., Mizushima N., Ohsumi Y., Inagaki F., *Genes Cells*, 2004, **9**, 611.

Keywords: protein crystallography, ubiquitin system, autophagy

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Crystal Structure of Novel Cyan-emitting Fluorescent Protein from *Acropara* Stony Coral

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Variants of green fluorescent protein (GFP) existing in different spectral features with blue, cyan and yellow-green emissions were originally generated from the bioluminescent jellyfish *Aequorea Victoria*. We have solved the crystal structure of a novel cyan-emitting fluorescent protein (CFP) from *Acropara* coral to a resolution of 2.0Å. The protein possesses a tyrosine residue in the chromophore, while enhanced CFP, one of mutants of *Aequorea* GFP, has tryptophan residue at this position. In our crystal structure, two protomers pack closely together to form a dimer. The protein fold is in the shape of a cylinder, comprising 11 strands of the β-barrel threaded by an α-helix running up the axis of the cylinder and short helical segments on the ends of the cylinder. The chromophore is attached inside the cylinders, and it is consistent with the formation of aromatic systems made up of Tyr70 with reduction of its C^{α} - C_{β} coupled with cyclization of the neighboring glutamine (Gln69) and glycine (Gly71) residues. The number of polar groups and structured water molecules are buried adjacent to the chromophore. Also, the structural identification of the dimer contacts may allow mutagenic control of the state of assembly of the protein.

Keywords: structural biology, fluorescence, structure and properties

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Structural Study of Atg5 and Atg16 Essential for Autophagy

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Atg5 is a 34 kDa protein which is covalently modified by a ubiquitin-like protein, Atg12 by a ubiquitin-like conjugation system [1]. The Atg12-Atg5 conjugate then forms a multimeric complex with Atg16 [2]. Atg12-Atg5/Atg16 complex is essential for autophagy, the main pathway for the degradation of cytoplasmic components such as proteins and organelles in all eukaryotic cells. In autophagy, cytoplasmic components are enclosed by double-membrane structures termed autophagosomes, which subsequently fuse with the vacuole / lysosome. Atg12-Atg5/Atg16 complex is localized to autophagosome precursors (isolation membrane). The localization implies that the complex plays major roles in the development of autophagic isolation membranes into autophagosomes.

We determined the crystal structure of Atg5 in complex with the N-terminal region of Atg16 using methods of multiple isomorphous replacement with anomalous scattering (MIRAS) and multiwavelength anomalous dispersion (MAD). Atg5 consists of two ubiquitin-like domains and a helical domain. The N-terminal region of Atg16 has a long helical structure, which binds to the helical domain of Atg5 via salt bridges and hydrophobic interactions. Biological analysises of Atg5/Atg16 complex are now in progress.

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Keywords: membrane trafficking, autophagy, ubiquitin system

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Crystal Structure of Novel Orange-emitting Fluorescent Protein from Stony Coral

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Green fluorescent protein (GFP) from *Aequorea* and GFP-like proteins are now ubiquitously used as biological markers. The chromophore is formed in an autocatalytic cyclization of encoded tripeptide segment. The fluorescent properties of the proteins should connect to the environment around the chromophore, in which the chromophore interacts with amino acids forming hydrogen-bonds, π-π stacking and so on. Thus, to understanding better the physicochemistry of GFP and GFP-like proteins, it is of importance to have 3D-structural information, especially regarding their chromophores.

We have crystallized a novel orange-emitting fluorescent protein from a stony coral, which shows emission peak maxima at 548nm, and subsequently, succeed in determination of the structure to 1.7Å resolution using molecular replacement method. The protein shares β can fold which is specific to the fluorescent proteins. Comparison of the environment around the chromophore with that of the other structural-known GFP and GFP-like proteins have emerged that a cation-πinteraction between the chromophore and a charged amino acid affects orange-emitting fluorescent property of the protein.

Keywords: fluorescent proteins, structural biochemistry, structure-properties relationships

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Proline Isomerization in Stefin B: a Crucial Step Towards Amyloid Fibril Formation

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For prion proteins as well as cystatins it has been suggested that formation of the 3-dimensional domain swapped dimers is the crucial step in fibril formation process, whereas higher order oligomers have not been characterized so far. One of the mutants of stefin B, the P79S, exhibited a higher stability and a prolonged lag phase of fibrillation. It forms tetrameric oligomers, which we were able to crystallize and determine their structure at 1.4 Å resolution (a=120Å, b=31Å, c=51Å, $\alpha=\gamma=90^{o}$, $\beta=96^{o}$, space group C2). The tetramer structure is built from a pair of domain-swapped dimers related by a crystallographic 2-fold axis. The structure comparison with the native stefin B structure revealed that the flip of the Ser72-Leu80 loop is associated with the trans to cis isomerization of the peptide bond of Pro74, which is the only absolutely conserved proline residue in the cystatin family of the cysteine protease inhibitors. The crucial role of the proline peptide bond trans-cis isomerization is further supported by the activation energy needed for stefin B P79S mutant to undergo tetramerization,, which corresponds to the energy of proline isomerization. These data suggest that the proline isomerization may be the crucial step governing the kinetics of stefin fibril growth.

Keywords: protein crystallography, amyloidogenesis, protein structure and folding