the Walker B motif form a novel 3_{10} helix, which is not observed in other ABC ATPases. This results in an unorthodox conformation of a conserved glutamate residue involved in ATP hydrolysis. Compared to other ABC-ATPase structures, significant displacement occurs at a linker region between the ABC αβ and α helical domains, leading to an atypical surface structure around the Q loop. This surface feature suggests that SufC interacts with SufB and SufD in a different manner from that observed in the structure of ABC transporters.

Keywords: ABC-ATPase, structural biology, Suf protein

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Comparisons of the Structures of Isolated Proteolytic Domains of Lon Proteases

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Crystal structure of the proteolytic domain of *A. fulgidus* B-type Lon protease (*Af*LonB) revealed significant differences in the conformation of the active site compared to two other known Lon P-domains, from *E. coli* (*Ec*LonA) and *M. jannaschii* (*Mj*LonB) [1], despite the similarity of the overall fold. The differences in the interactions of the catalytic residues in the active sites of *Af*LonB and the other two Lon proteases are primarily connected to the variable conformational state of the segment that precedes catalytic Ser509. It appears that in isolated P-domains of single chain Lon proteases this segment does not have a stable conformation that could maintain proper structure of the active site. Other ATP-dependent proteases with known structures, such as HslUV or ClpAP, are two-chain enzymes, and in their independently-folded proteolytic subunits the catalytic residues are in appropriate positions. We suggest that the interactions with other domains (the ATPase domain in particular), as well as ligand binding, might lead to rearrangements in Lon P-domain active sites. Full-length *Af*LonB is proteolytically active in an ATP-dependent manner, whereas all individually purified wild-type and mutant P-domains are inactive. These results suggest that the structure of the active site in the P-domain of *Af*LonB represents an inactive state of enzyme. This raises the possibility that the surprising differences between the catalytic mechanisms of A and B type Lon proteases [1] might be artifacts, since the structure of the P-domain of *Mj*LonB could similarly represent an inactive state of that enzyme.

[1] ImY. J., et al., *J. Biol. Chem.*, 2004, **279**, 53451.

Keywords: Lon proteases, active site, structure comparison

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Structures and Function Studies of Microbial P-loop-containing Phosphatases

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The P-loop-containing phosphatases are composed of a conserved sequence of DX(30)HCXXGXXR(T/S). It is well-known that tyrosine phosphatases (PTP), dual specificity phosphatases (DSP) and inositol polyphosphate (IPP) phosphatase are important regulators in signal transduction of cell cycle and IPP signaling molecules. Up to date, we have solved two the P-loop-containing phosphatases which are the inositol hexakisphosphate phosphatase (phytase) from *Selenomonas ruminantium* and DSP in *Sulfolobus Solfataricus*. We have solved two crystal forms of the complex structure of the phytase with an inhibitor, *myo*-inositol hexasulfate. In the "standby" and the "inhibited" crystal forms, the inhibitor is bound, respectively, in a pocket slightly away from Cys241 and at the substrate-binding site where the to-be-hydrolyzed phosphate group is held close to the –SH group of Cys241.

P-loop-containing phosphatase from *S. Solfataricus* was also solved. Comparison of the structures of *S. Solfataricus* and other phosphatases have revealed a extensive substrate binding surface which implying the possibility of low specificity. Overall, these investigations help us to evaluate the evolution of tyrosine phosphatase in microbial and the role it plays in the signal transduction among Achaea.

Keywords: dual specificity phosphatase, phytase, *myo*-inositol polyphosphates

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Structure of a Two-domain Chitinase from *Streptomyces griseus*

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Many glycoside hydrolases consist of multiple domains involved in catalysis and carbohydrate binding, which are connected by interdomain linkers. The widespread occurrence of these linkers suggests their importance in the binding and catalytic functions. However, the number of available structures of full-length glycoside hydrolases has been limited. We first revealed the whole structure of a two-domain chitinase, namely chitinase C from *Streptomyces griseus* HUT6037 (ChiC), classified into glycoside hydrolase family 19. ChiC is composed of an N-terminal chitin-binding domain, a C-terminal catalytic domain, and a linker peptide. Although the cubic crystals of full-length ChiC contain two molecules in an asymmetric unit, the electron densities are assigned to three out of the four domains because of incomplete densities. While the electron densities for the two catalytic domains are clearly defined, that corresponding to the single chitin-binding domain is obscured. This absence and obscurity of electron densities is presumably caused by conformational flexibilities of the linker peptides. The two discrete domains, chitin-binding and catalytic domains, connected by the linker peptide are distantly located without any interactions with each other in the crystal. Great flexibility of the linker must allow the two separated domains to be close to each other in solution, and hence cooperation between the domains is likely to be important for the full activity.

Keywords: chitinase, whole structure, conformational flexibility

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Structure-based Functional Analysis of Prenyltransferases: *Trans*-type OPPs from *T. maritima* and *Cis*-type UPPs from *E. Coli*

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Isoprenoids are an extensive group of natural products with diverse structures consisting of various numbers of five-carbon isopentenyl pyrophosphate (IPP) units. The enzymes responsible for the synthesis of linear isoprenyl pyrophosphates can be classified as *cis*- and *trans*-isoprenyl pyrophosphate synthase (IPPs) according to the stereochemical outcome of their products. The C_{40} product of octaprenyl pyrophosphate synthase (OPPs) constitutes the side chain of ubiquinone in *Thermotoga maritima*. Among the *cis*-polyprenyl pyrophosphates, the C_{55} product of the bacterial undecaprenyl pyrophosphate synthase (UPPs) serves as a lipid carrier in cell wall peptidoglycan biosynthesis.

OPPs is composed entirely of α-helices joined by connecting loops and is arranged with 9 core helices around a large central cavity. An elongated hydrophobic tunnel between D and F α-helices contains two DDxxD motifs on the top for substrate binding and is occupied at the bottom with one large residue F132. From the biochemical studies, F132 is the key residue for determining the product chain length.

The structures of *Escherichia coli* UPPs were determined previously as an apo-enzyme, in complex with Mg^{2+}/sulfate/Triton, and with bound FPP. In further search of its catalytic mechanism, the

wild-type UPPs and the Asp26Ala mutant are crystallized in a new trigonal unit cell with Mg^{2+}/IPP/FsPP (farnesyl thiopyrophosphate, an FPP analogue) bound to the active site. Our results here improve the understanding of the prenyltransferases reaction significantly.

Keywords: prenyltransferase, crystal structure, metal ion

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Crystal Structure of Human HMG-CoA lyase

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3-Hydroxyl-3-methylglutaryl-CoA (HMG-CoA) lyase (EC 4.1.3.4) catalyzes the divalent cation dependent cleavage of HMG-CoA to form acetyl-CoA and acetoacetate. This reaction is a key step in ketogenesis and the final step in leucine catabolism. Human HMG-CoA lyase has been previously cloned and overexpressed in *Escherichia coli.* Crystals of the lyase containing a competitive inhibitor have been obtained with PEG 8K using sitting-drop vapor diffusion method. The crystals belong to the monoclinic space group C2, with unit cell parameters a=197.0Å, b=116.1Å, c=86.8Å, and β=122.5˚. The native data set has been collected which is 99.8 % complete to 2.1 Å. The calculated Vm is 2.34 $Å^3$ Da^{-1}, with a solvent content of 43.2%, which corresponds to three dimers of the enzyme in the asymmetric unit (each dimer molecular weight, 65 kDa). One heavy atom derivative (Hg) has been obtained and the search for additional derivatives is in progress.

Keywords: HMG-CoA lyase structure, fatty acid metabolism, ketogenesis

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Structural Analysis of Thr342 Mutants of Soybean β-Amylase: The Role of Conformational Changes of Two Loops in the Catalytic Mechanism

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Soybean β-amylase has two mobile loops in the active site, a flexible loop (residue 96-103) and an inner loop (residues 340-346). The flexible loop moves about 10 Å from "open" to "closed" form to make interactions with substrate. Though the movement is relatively small (about 3 Å), two different conformations of the inner loop have been found in the enzyme/substrate complexes. In the "product form", the Thr 342 residue creates hydrogen bonds with the Glu 186 (catalytic acid) and with the glucose residues at subsites -1 and +1, whereas most of those interactions are lost in the "apo form". To elucidate the relationship between the structural states of inner loop and the catalytic mechanism, Thr 342 was mutated to Val, Ser, and Ala, respectively, and their crystal structures complexed with maltose were determined together with that of the apo enzyme at 1.27-1.64 Å resolutions. The k_{cat} values of the T342V, T342S, and T342A mutants decreased by 13-, 360- and 1700-fold, respectively, compared to that of the wild-type enzyme. Whereas the inner loops in the wild-type/matose and T342V/maltose complexes adopted the product form, those of the T342S/maltose and T342A/maltose complexes showed the apo form. Structural analyses suggested that the side-chain of Thr 342 in product form plays an important role in distorting the sugar ring at subsite -1, stabilizing the deprotonated form of Glu 186, and grasping the glucose residue of the remaining substrate at subsite +1.

Keywords: amylases, mutations, enzymatic reaction mechanism

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Crystal Structure of the Haloalkane Dehalogenase DbjA

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Haloalkane dehalogenases are key enzymes that catalyze hydrolytic conversion of halogenated aliphatic compounds to alcohol and hydrogen halide. The enzymes have good potential for bioremediation, biosensing and biocatalysis. Recently, the haloalkane dehalogenase DbjA from *Bradyrhizobium japonicum* USDA110 was revealed to have a sufficient enantioselectivity for industry scale synthesis of optically active alcohols. This is the first example of enantioselective catalysis among the haloalkane dehalogenases. To reveal the enantioselectivity mechanism of DbjA on the basis of the structure, we determined the crystal structure of DbjA at 1.47 Å resolution by the molecular replacement method. DbjA has an alpha/beta hydrolase fold. The architecture of specificity-determining cap domain is, however, different from three dehalogenases with known crystal structure. The results of structure-function analysis will be presented.

Keywords: enantioselectivity, alpha/beta hydrolase, haloalkane dehalogenase

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Structure-Function Analysis of a Novel Mitochondrial Antioxidant Protein

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Bovine mitochondrial SP-22 is a member of the peroxiredoxin family which is a novel antioxidant enzymes. It belongs to the typical 2-Cys Prxs containing three cysteines at positions 47, 66, and 168. Its co-elution with E3 of PDC indicate possible interaction between them. SP-22 can be reduced by DTT and oxidized by H_2O_2. By electronic microscopy study, SP-22 forms a stable decameric toroid consisting of five basic dimeric units. By GFC study, it is found that SP-22 stay as a decamer under different conditions (salt concentration, pH, redox statue). Preliminary crystallography study of SP-22 has been done. 3.3A data of C168S mutation SP-22 were collected.

Oxidized SP-22 needs to be reduced by thioredoxin, thioredoxin reductase system. To finish this pathway and have a better understanding of the function of SP-22, human mitochondrial thioredoxin and thioredoixn reductase were cloned, overexpressed and purified. Due to human mitochondrial thioredoxin reductase is a SeCys protein, fully active protein was under investment.

Keywords: peroxiredoxin, mitochondrial, oligomer

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Structural Studies on Novel Streptococcal Virulence Factors

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Streptococcus pyogenes (*S. pyogenes*) is responsible for a variety of illnesses ranging from mild sore throat to life-threatening toxic shock syndrome. The strain most strongly associated with highly invasive infections is *S. pyogenes* M protein serotype 1 (M1).

We have identified a number of putative genes from the *S. pyogenes* M1 genome which possess various sequence motifs that are often present in bacterial toxins. Three of the proteins encoded by these genes have been expressed, purified and crystallized with the aim of determining their structures by X-ray crystallography.

The protein encoded by gene *spy1492*, Spy1492, contains the GDSL-like lipase motif which is commonly found in lipolytic enzymes including some bacterial toxins such as hemolysin. Its lipolytic activity was detected in biochemical assays. Multiwavelength anomalous diffraction (MAD) data to 3.0 Å resolution have been