phosphatase which is distributed among eubacteria, archia and eukaryotes. In *E. coli*, it is necessary for survival during the stationary phase, but the physiological role of SurE is not clear. Crystal structures of SurE from *Thermotoga maritima* and *Pyrobaculum aerophilum* have been determined, which revealed that SurE forms a dimer that assembles into a tetramer.

We report here the crystal structures of SurE from *Thermus thermolhilus* HB8 ($SurE_{ther}$) in a few different space groups. The $SurE_{ther}$ structure consists of a globular Rossmann fold domain and a protruded domain that mediates tetramerization as so far-reported structures. However, the angle between the protruding domain and the Rossmann fold domain of $SurE_{ther}$ is different. As a result, the dimeric- and tetrameric-structures of $SurE_{ther}$ were quite different from the known SurE structures. We studied the self-associative properties of $SurE_{ther}$ in solution using the sedimentation equilibrium analytical ultracentrifugation. Phosphatase activity assays of $SurE_{ther}$ is in progress, and its substrate specificity seems to be strict compared to SurE from other species. The relationship between enzymatic property and the variant oligomeric structure of $SurE_{ther}$ will be discussed.

Keywords: protein crystallography structures, phosphatases, protein assembly

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Crystal Structure of *Mortierella vinacea* α-galactosidase I

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α-Galactosidase (E.C. 3.2.1.22) is an exoglycosidase that hydrolyzes an α-1,6-linked galactosyl residue from galacto-oligosaccharides and polymeric galacto-(gluco)mannans. α-Galactosidase I from *Mortierella vinacea* (α-Gal I) consists of 397 amino acid residues and it shows high activity to increase the yield of sucrose by eliminating raffinose, which prevents normal crystallization of beet sugar, and is sometimes referred to as raffinase. α-Gal I is considered to be a glycoprotein, resulting from the presence of its sugar chain. In addition, the gel filtration data shows that α-Gal I might exist as a tetramer in solution.

In order to understand the catalytic mechanism, we conducted structure analysis of this enzyme. Crystals of α-Gal I were obtained by the hanging drop vapour diffusion method using the polyethylene glycol 400 as a precipitant. Diffraction experiments were conducted at the Photon Factory, and the data up to 1.6 Å resolution were collected. Structure was determined by the molecular replacement method and the final model gave a crystallographic *R*-factor of 0.133 and an R_{free}-factor of 0.157. Owing to the high resolution X-ray data, four carbohydrate chains were observed in one α-Gal I molecule and their structures were identified to be high mannose type. α-Gal I seemed to form a tetramer around the crystallographic four-fold axis.

Keywords: α-galactosidase, glycoprotein, *Mortierella vinacea*

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Structural basis for Ca^{2+}-induced Activation of Human PAD4

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Peptidylarginine deiminase 4 (PAD4) is a Ca^{2+}-dependent enzyme that catalyzes the conversion of protein arginine residues to citrulline residues. PAD4 is expressed mainly in bloodstream granulocytes and present in the cell nucleus. The recent experimental evidence that PAD4 targets multiple arginine sites in histone H3 and H4, including those sites methylated by CARM1 (H3/Arg17) and PRMT1 (H4/Arg3), has attracted considerable attention to characterize the role of histone modifications in regulating gene transcription [1, 2].

On the other hand, a recent single-nucleotide polymorphism (SNP) analysis of the PAD4 (*PADI4*) gene has identified a specific haplotype linked to an increased susceptibility for rheumatoid arthritis in Japanese people [3].

Here we present the crystal structures of Ca^{2+}-free, Ca^{2+}-bound, and Ca^{2+}-substrates bound PAD4 [4]. PAD4 has five non-EF-hand Ca^{2+} binding sites and adopts an elongated shape, consist of N- and C-terminal domain. These structural data indicate that Ca^{2+} binding in C-terminal domain induces conformational changes that generate the active site cleft. Our findings identify a novel mechanism for enzyme activation by Ca^{2+} ions.

[1] Cuthbert G. L., *et al*., *Cell.*, 2004, **118**, 545. [2] Wang Y., *et al.*, *Science*, 2004, **306**, 279. [3] SuzukiA., *et al.*, *Nat. Genet.*, 2003, **34**, 395. [4] Arita K., *et al.*, *Nat Struct Mol Biol.*, 2004, **11**, 777

Keywords: histone modification, citrullination, calcium binding

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The Crystal Structure of a Novel Subtilisin-like Alkaline Serine Protease, KP-43

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The crystal structure of an oxidatively stable subtilisin-like alkaline serine protease, KP-43 from *Bacillus* sp. KSM-KP43, with a C-terminal extension domain, was determined at 1.30-Å resolution. KP-43 consists of two domains, a subtilisin-like α/β domain and a C-terminal jelly roll β-barrel domain. The topological architecture of the molecule is similar to that of kexin and furin, which belong to the subtilisin-like proprotein convertases (SPCs), whereas the amino acid sequence and the binding orientation of the C-terminal β-barrel domain both differ in each case. Since the C-terminal domains of SPCs are essential for folding themselves, the domain of KP-43 is also thought to play such a role.

KP-43 is known to be an oxidation-resistant protease among the general subtilisin-like proteases. The structure analysis of oxidized form and the biochemical experiments have indicated that the oxidation of the methionine adjacent to the catalytic serine is not a dominant modification, but might alter the substrate specificities.

[1] Nonaka T., Fujihashi M., Kita A., Saeki K., Ito S., Horikoshi K., Miki K., *J. Biol. Chem.*, 2004, **279**, 47344. [2] Nonaka T., Fujihashi M., Kita A., Saeki K., Ito S., Miki K., *Acta Cryst.*, 2001, **D57**. 717.

Keywords: alkaline serine protease, subtilisin, oxidative stability

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Crystal Structure of Atypical Cytoplasmic ABC-ATPase SufC

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Biosynthesis of Fe-S clusters requires several specific proteins. The *suf* operon has been recently identified as a third system for the assembly of Fe-S clusters. The *suf* operon of *Eschrichia coli* comprises *suf*A, *suf*B, *suf*C, *suf*D, *suf*S, and *suf*E. Most of *suf* genes are conserved in various organisms, but the *suf* operon is seldom found in complete form. The most conserved *suf* genes are SufC and SufB. SufC is a cytoplasmic ABC-ATPase, probably the most essential Suf protein. SufC interacts with SufB and SufD in a stable complex, and the SufBCD complex interacts with other Suf proteins in the Fe-S cluster assembly.

We have determined the crystal structure of SufC from *Thermus thermophilus* HB8 in a nucleotide-free state and an ADP-Mg bound state at 1.7Å and 1.9Å resolution, respectively. The structure of SufC consists of two domains: the ABC αβ domain, which is structurally similar to the typical core fold of ABC-ATPase, and the α helical domain. In the ABC αβ domain, three residues following the end of

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