binding channel shows conservation of positively charged residues, which are possibly involved in complex formation with the H-protein. These results provide insights into the molecular basis of nonketotic hyperglycinemia.

Keywords: multienzyme complexes, disease-related structures, vitamin B6

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Structure and Inhibition Mode of Protein I^c in Complex with Carboxypeptidase Y

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Carboxypeptidase Y (CPY) inhibitor I^c from the yeast, consisting of 204 amino acid residues, belongs to the phosphatidylethanolamine-binding protein (PEBP) family. The 2.7 Å crystal structure of the I^c-CPY complex has been solved by molecular replacement [1, 2].

The structure of I^c consists of a major β-type domain and an N-terminal helical segment. I^c has two CPY-binding sites: the N-terminal inhibitory reactive site and the secondary CPY-binding site which interact with the S1 substrate-binding site of CPY and the hydrophobic surface flanked by the active site of the enzyme, respectively. I^c also has the ligand-binding site, the putative binding site of the polar head group of phospholipid, which is conserved among PEBPs and accommodates a sulfate ion in the crystal structure.

Along with the complex structure of I^c, its mutational analyses for inhibitory activity and binding to CPY demonstrate that the N-terminal inhibitory reactive site is essential for the complex formation with CPY as well as enzyme inhibition and that the I^c binding to CPY forms a novel mode of the proteinase-protein inhibitor interaction. The unique binding mode of Ic toward CPY gives insights into not only the inhibitory mechanism of PEBPs toward serine proteinases but also the biological functions of I^c belonging to the PEBP family.

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Keywords: CPY inhibitor, I^c, PEBP family

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Structures of a Novel *N*-acetyl-L-ornithine Transcarbamylase

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N-acetyl-L-ornithine transcarbamylase, a new member of the transcarbamylase family, is an essential enzyme to synthesize arginine in a few of eubacteria. Since this enzyme is not present in other bacteria, plants, animals and human, *N*-acetyl-L-ornithine transcarbamylase could provide a potential non-toxic target for specific inhibition to control certain agriculture and human pathogens. We report here the crystal structures of the binary complexes of enzyme from *Xanthomonas campestris* with its substrate carbamoyl phosphate or *N*-acetyl-L-ornithine only and the ternary complex with carbamoyl phosphate and *N*-acetyl-L-norvaline. Comparison of the structures of the enzyme in the different substrate binding states demonstrates that the binding mechanism of this novel transcarbamylase is different from those of aspartate and ornithine transcarbamylases. The enzyme can bind carbamoyl phosphate and *N*-acetyl-L-ornithine independently, and does not require one of substrate binds first in order to bind the second substrate. The main conformational change is the ordering of the 80's loop upon binding the carbamoyl phosphate besides a small domain closure around the active site. The structures of the complexes provide insight into how the enzyme facilitates the carbamoyl group transfer, and provide a starting point for inhibitor design.

Keywords: carbamoyltransferase, acetylornithine, arginine pathway

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Crystal Structure of Spermidine Synthase from *Helicobacter pylori*

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Spermidine synthase (putrescine aminopropyltransferase, PAPT) catalyzes the transfer of the aminopropyl group from decarboxylated *S*-adenosylmethionine (dcAdoMet) to putrescine in the biosynthesis of spermidine. PAPT of *Helicobacter pylori* (HpPAPT) is encoded by the *speE* gene. HpPAPT has less than 20% of sequence identity with other PAPTs, even containing no signature sequence. The three-dimensional structure of HpPAPT has been determined by multiwavelength anomalous dispersion (MAD) in this study. HpPAPT consists of an N-terminal beta-stranded domain and a C-terminal Rossmann-like domain, with a binding pocket between two domains. The oligomerization of HpPAPT is mostly made by the N-terminal domain and sensitive to the pH values of buffer. Our structure illustrates that HpPAPT has a distinctive binding pocket with a bigger space, a unique electrostatic potential surface of less acidity, and numerous unconserved residues. Due to the lack of the gatekeeping loop, HpPAPT may need to perform a significant conformation change to accommodate the ligand binding.

Keywords: spermidine synthase, putrescine aminopropyl-transferase, *Helicobacter pylori*

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Helix-formation Due to the Binding of α- with β_2-subunit of Tryptophan Synthase

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When the tryptophan synthase α- and β_2-subunits combine to form the $\alpha_2\beta_2$ complex, the enzymatic activity of each subunit is stimulated by one to two orders of magnitude. In order to elucidate the structural basis of this mutual activation, it is necessary to determine the structures of the α- and β-subunits alone and together with the $\alpha_2\beta_2$ complex. The crystal structures of the tryptophan synthase $\alpha_2\beta_2$ complex from *S. typhimurium* (*St*$\alpha_2\beta_2$) has been reported. Therefore, we determined the crystal structure of the tryptophan synthase α-subunit alone from *E. coli* (*Ec*α) at 2.3Å resolution. The biggest difference between the structures of the *Ec*α and the α-subunit in the *St*$\alpha_2\beta_2$ (*St*α) was as follows. The helix-2' in the *St*α including an active site residue (Asp60) changed to a flexible loop in the *Ec*α. The conversion of the helix to a loop resulted in collapse of the correct active site conformation. This region is also an important part for the mutual activation in the *St*$\alpha_2\beta_2$ and interaction with the β-subunit. These results suggest that the formation of helix-2' essential for the stimulation of the enzymatic activity of the α-subunit is constructed by the induced-fit mode involved in conformational changes upon interaction between the α- and β-subunits.

[1] Nishio K., et al.. *Biochemistry*, 2005, **44**, 1184

Keywords: protein crystallography, biological structure-activity relationships, protein-protein interactions

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Structure of Statironary Phase Survival Protein SurE from *Thermus thermophilus*

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Stationary-phase survival protein SurE is a metal ion-dependent

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.

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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.

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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