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Precursors for isoprenoid synthesis are essential in all organisms. These compounds are synthesized by one of two known routes: the well characterized mevalonate pathway [1] or a recently discovered non-mevalonate route which is used in many bacteria and human pathogens [2]. Since the second pathway is both vital and unlike any found in humans, enzymes catalysing reactions along this synthetic route are possible drug targets. The structure of one such enzyme from the thermophilic bacterium *Thermus thermophilus* has been solved to high resolution in the presence of substrate and with a substrate analogue. Enzyme co-crystallized with substrate shows only one product, cytosine monophosphate (CMP), in the active site. At the high resolution of the refinement (1.6 Å) the positions and coordination of the magnesium ions in the active site are clearly seen.

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Keywords: isoprenoid synthesis, non-mevalonate route, drug targets

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Crystal Structure of the β-subunit of Acetyl-CoA Carboxylase in *C. glutamicum*

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Acetyl-CoA carboxylases (ACCs) catalyze the first committed step of fatty acid biosynthesis. Although ACC is an essential enzyme (complex) in every organism, the structure-function relationship of ACC remains to be unclear. As the first step for elucidating the structure-function relationship of ACC, we started the crystallographic analysis of DtsR1. DtsR1 is the β-subunit of ACC multisubunit complex in *Corynebacterium glutamicum*, which catalyzes the transcarboxylation between biotin and acetyl-CoA.

DtsR1 was over-expressed in *E. coli*, purified, and crystallized by the sitting-drop vapor diffusion method using PEG 6000 as a precipitant. The approximate dimensions of the obtained crystals were 0.07x0.07x0.03mm^3. Diffraction data of the crystals were collected at NW12 of the Photon Factory (Tsukuba), revealing that the crystals belong to the space group *R*32. The crystal structure of DtsR1 was solved at 3.2Å resolution by the molecular replacement method using single-subunit coordinates of the 12S transcarboxylase (PDB ID: 1ON3) as a search model. The obtained structure suggests that the biological unit of DtsR1 is a ring-shaped hexamer with the *32*-point group symmetry. Crystallographic refinement of DtsR1 is in progress at 2.7Å resolution.

Keywords: carboxylases, fatty acid biosynthesis, structure-function enzymes

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Structural Studies of FlaA1, a UDP-GlcNAc 4,6-dehydratase

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FlaA1 is a UDP-GlcNAc 4,6-dehydratase believed to be involved in the protein glycosylation process of *Helicobacter pylori*. The crystal structures of FlaA1 in five different ternary complexes with various substrates were determined at resolutions between 1.9 and 2.8 Å. This represents the first structure of a 4,6-dehydratase that can catalyze a UDP-saccharide. Among 4,6-dehydratases, FlaA1 possesses several unique structural features including a novel C-terminal fold and a hexameric oligomerization state in the crystal. The catalytically productive conformation observed in the FlaA1•NADPH•UDP-GlcNAc ternary complex suggests that FlaA1 employs a different mechanism for the water elimination step from that proposed for other 4,6-dehydratases. Normally, an Asp and Glu residues are the two catalytic residues that effect dehydratase activity through a concerted mechanism. In FlaA1, the corresponding residues are Asp-132 and Lys-133, precluding an analogous mechanism. Computational analysis suggests that for the water elimination step in FlaA1, Lys-133 sequentially functions as catalytic acid and base while Asp-132 closely interacts with the leaving water group.

Keywords: dehydratase, catalytic mechanism, pKa calculation

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The Crystal Structure of Murine 11β-hydroxysteroid Dehydrogenase 1: an Important Therapeutic Target for Diabetes

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11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) catalyzes the conversion of 11-dehydrocorticosterone to its active form corticosterone in rodents (or cortisone to cortisol in humans). An excess of active glucocorticoids has been shown to play a key role in metabolic disorders such as diabetes and obesity. Therefore, 11β-HSD1 represents an important therapeutic target for the treatment of these diseases. To facilitate the iterative design of inhibitors, we have crystallized and determined the three-dimensional structures of a binary complex of murine 11β-HSD1 with NADP(H) to a resolution of 2.3 Å, and a ternary complex with corticosterone and NADP(H) to a resolution of 3.0 Å by X-ray crystallography. The enzyme forms a homodimer in the crystal. The structure shows a novel folding feature at the C-terminus of the enzyme. The C-terminal helix insertions provide additional dimer contacts, exert an influence on the conformations of the substrate binding loops, and present hydrophobic regions for potential membrane attachment. The structure also reveals how the 11β-HSD1 achieves its selectivity for its substrate.

Keywords: 11β-HSD1, SDR, corticosterone

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Crystal Structure of P-protein of the Glycine Cleavage System

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The glycine cleavage system (GCS) is a multienzyme complex composed of four different components (P-, H-, T- and L-proteins). In almost all organisms, the GCS plays a crucial role in the degradation of glycine, and it has been studied extensively. Three-dimensional structures of H-, T- and L-proteins from many species have been published, but only the structure of the P-protein has not yet been reported. We have determined the crystal structure of the P-protein from *Thermus thermophilus* HB8, which reveals that P-proteins do not involve the α_2-type active dimer universally observed in the evolutionarily related pyridoxal 5'-phosphate (PLP)-dependent enzymes. Instead, novel αβ-type dimers associate to form an $\alpha_2\beta_2$ tetramer, where the α- and β-subunits are structurally similar and appear to have arisen by gene duplication and subsequent divergence with a loss of one active site. The binding of PLP to the apoenzyme induces large open–closed conformational changes. The structure of the complex formed by the holoenzyme bound to an inhibitor, (aminooxy)acetate, suggests residues that may be responsible for substrate recognition. The molecular surface around the lipoamide-

P4

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 $α_2β_2$ 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 $α_2β_2$ complex. The crystal structures of the tryptophan synthase $α_2β_2$ complex from *S. typhimurium* ($Stα_2β_2$) 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 $Stα_2β_2$ (*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 $Stα_2β_2$ 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.

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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