Arabinanase hydrolyzes the α-1,5-L-arabinofuranoside linkage of arabinan distributed in hemicelluloses, which comprise a large fraction of plant cell walls. AbnS1 from *P.chrysogenum* 31B and ABN-TS from *Bacillus thermodenitrificans* TS-3, which hydrolyze arabinan through an endo mechanism, show optimal activity at 333 and 343 K, respectively. The X-ray crystallographic analysis has revealed that the thermostable ABN-TS has a unique motif consisting of a five-bladed β-propeller fold. Since AbnS1 have 32% homology with ABN-TS, X-ray analysis of the mesophilic AbnS1 should provide information towards clarify the structural features that cause the difference in the optimum temperature. The recombinant AbnS1 was overexpressed in *E. coli* as a C-terminal His-Tagged protein (AbnS1-His). The first purification step was a Ni-affinitycolumn. Further purification steps were anion-exchange and gel filtration columns. The tag was not removed for the subsequent crystallization experiments, because AbnS1-His showed the same catalytic activity and optimum temperature as the native AbnS1. Crystals were obtained using PEG4000 as a precipitant. Data collection and structure analysis are under way.

Keywords: arabinanase, glycoside hydrolase, crystal structure

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Crystal structure of GMP synthetase (GuaA) from *T. thermophilus*

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GMP synthetase (GuaA) catalyzes the last step to synthesize GMP in purine nucleotide biosynthetic pathway. This enzyme catalyzes a reaction in which xanthine 5 ´ -monophosphate (XMP) is converted into GMP in the presence of ATP. The crystal structure of GuaA from *E. coli* (PDB ID 1GPM) has been determined with AMP and PPi, and the protein consists of three domains, class I glutamine amidotransferase domain, ATP pyrophosphatase domain, and dimerization domain. Here we determined crystal structures of GuaA from *Thermus thermophilus* HB8 in apo form (2YWB, space group *C*2, max res. = 2.1 Å, *R* = 23.3%, free *R* = 27.2%) and in complex with XMP (2YWC, space group *C*2, max res. = 2.2 Å, *R* = 23.6%, free *R* = 27.8%). The structure of GuaA in complex with XMP and without ATP is reported in the first time. Diffraction data for these crystals were collected at BL26B1 and BL41XU in SPring-8, respectively. Collected data were processed by using HKL2000 and, for the apo form, phase were determined by the MAD method using SOLVE/RESOLVE. Overall folding is almost same with that of *E.coli* GuaA. For both species, electron density was missing at the ATP binding site. Comparing XMP complex with apo form *T. thermophilus* GuaA, the loop near the XMP binding site moved toward XMP. Now we are analyzing the structure of the complex with ATP and the reaction mechanism of GuaA will be discussed based on their structures. MK present affiliation: RIKEN Advanced Science Institute

Keywords: GMP synthetase, amidotransferase, nucleoside metablism

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Crystal structures of the oxygenase component of an aromatic monooxygenase in ligand-bound forms

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The 4-hydroxyphenylacetate (4-HPA) 3-monooxygenase is involved in the initial step of the 4-HPA degradation pathway and catalyzes hydroxylation of 4-HPA, generating 3,4-dihydroxyphenylacetate. This enzyme consists of two components: an oxygenase (HpaB) and a reductase (HpaC). HpaB hydroxylates 4-HPA using reduced flavin and molecular oxygen, and HpaC reduces oxidized flavin using NADH, providing reduced one to HpaB. During the catalytic reaction of HpaB, an unstable intermediate of C4a-hydroperoxyflavin is formed and stabilized. To understand the structural basis of the catalytic mechanism of HpaB, crystal structures of HpaB from Thermus thermophilus HB8 were determined in three states: a ligand-free form, a binary complex with FAD, and a ternary complex with FAD and 4-HPA. HpaB is a tetrameric molecule and its monomer (481 aa) consists of three domains with an additional helix tail. The overall structure of the monomer is similar to that of medium-chain acyl-CoA dehydrogenase from pig liver without any sequence homology. Structural analysis revealed that the binding and dissociation of flavin are accompanied by conformational changes of HpaB in the loop between strands 5 and 6 and the loop between strands 8 and 9, leading to preformation of part of the substrate-binding site (Ser197 and Thr198). The latter loop further changes its conformation upon binding of 4-HPA, and obstructs the active site from the bulk solvent. These features are new to this type of protein fold, and even not observed for the Acinetobacter type of 4-HPA monooxygenase. Arg100 is located adjacent to the putative oxygen-binding site, and may play a key role in the formation and stabilization of the C4a-hydroperoxyflavin intermediate.

Keywords: enzyme mechanisms, hydroperoxyflavin, structural change

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Functional insights from structures of coactivator-associated arginine methyltransferase 1 domains

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Post-translational methylation of arginine is a widespread epigenetic modification found in eukaryotes that is catalyzed by the protein arginine methyltransferases (PRMTs). PRMTs have been implicated in a variety of biological processes, such as regulation of transcription, translation and DNA repair. Coactivator-associated arginine methyltransferase 1 (CARM1, also known as PRMT4) was identified as an enhancer of the transcriptional activation by several nuclear hormone receptors. As a transcriptional coactivator, CARM1

is a key player in the formation of large complexes on gene promoters leading to chromatin remodelling and gene activation. CARM1 has now been shown to methylate a large variety of proteins which are all vital to gene expression. CARM1 contains 608 amino acids in human and its architecture has been schematically divided into three domains. CARM1 is built around a catalytic core domain that is well conserved in sequence (and therefore in structure) among all PRMTs members. CARM1 possesses two unique additional domains attached, respectively, at the NH_2-terminal and at the COOH-terminal end of the PRMT active site. Both NH_2-terminal domain and COOH-terminal domain have been shown to be required for the coactivator function of human CARM1. We have solved six crystal structures corresponding to three isolated modules of CARM1. Five crystal structures of the CARM1 catalytic module, two free, two cofactor and one inhibitor bound forms have revealed large structural modifications and shown that the NH_2-terminal and the COOH-terminal end of CARM1 catalytic module contain molecular switches that may inspire how CARM1 regulates its biological activities by protein-protein interactions. Full detailed analysis of the structures will be presented.

Keywords: epigenetic, protein ariginine methyltransferase, CARM1

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Crystal structure of Histo-aspartic protease from *Plasmodium falciparum*

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Histo-aspartic protease (HAP) from *Plasmodium falciparum* is a promising new target for the development of anti-malarial drugs. The sequence of HAP reveals an overall similarity to aspartic proteases, but crucial replacement by histidine of one of the two catalytic aspartates, Asp32, combined with several more changes of the catalytically important residues in the active site area, indicated a possible novel mechanism of action. The structures of the recombinant HAP, as apoenzyme and a complex with pepstatin A, were solved at 2.5 and 3.3 Å resolution, respectively. In the apoenzyme crystals HAP forms a unique dimer, which has not been observed in any known aspartic proteases. The interactions between the monomers in a very tight dimer of HAP affect the conformations of two flexible loops, the functionally important “flap” (residues 77-88) and its structural equivalent in the C-terminal domain (237-249), as well as the orientation of the helix 225-235. The flap in the apoenzyme was found in an open conformation. Unexpectedly, the active site contains a zinc ion tightly bound to His38 and Asp218 from one monomer, and to Glu282 from the other monomer, with the coordination resembling its counterparts in metalloproteases. Although the mode of binding of pepstatin A in the active site of HAP is different than in other pepsin-like aspartic proteases, the presence of the inhibitor questions the previously proposed hypothesis that HAP is a serine protease. The flap is closed in the structure of the complex and Lys82, present at the tip of the flap, interacts with the inhibitor. The novel features of the active site of HAP should allow designing specific inhibitors that could be developed into antimalarial drugs.

Keywords: aspartic proteases, malaria, enzyme mechanism

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Structural and mutational studies of anthocyanin malonyltransferases

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The BAHD family is a class of acyl-CoA-dependent acyltransferases that are involved in plant secondary metabolism and show a diverse range of specificities for acyl acceptors. Anthocyanin acyltransferases make up an important class of the BAHD family and catalyze the acylation of anthocyanins that are responsible for most of the red-to-blue colors of flowers. Here, we describe crystallographic and mutational studies of three similar anthocyanin malonyltransferases from red chrysanthemum petals: anthocyanidin 3-O-glucoside-6’’-O-malonyltransferase (Dm3MaT1), anthocyanidin 3-O-glucoside-3’’, 6’’-O-dimalonyltransferase (Dm3MaT2), and a homolog (Dm3MaT3). Mutational analyses revealed that seven amino acid residues in the N- and C-terminal regions are important for the differential acyl-acceptor specificity between Dm3MaT1 and Dm3MaT2. Crystallographic studies of Dm3MaT3 provided the first structure of a BAHD member, complexed with acyl-CoA, showing the detailed interactions between the enzyme and acyl-CoA molecules. The structure, combined with the results of mutational analyses, allowed us to identify the acyl-acceptor binding site of anthocyanin malonyltransferases, which is structurally different from the corresponding portion of vinorine synthase, another BAHD member, thus permitting the diversity of the acyl-acceptor specificity of BAHD family to be understood.

Keywords: BAHD family, acyltransferase, crystal structure

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Reaction pathway of ADP-ribose pyrophosphatase, revealed by time-resolved X-ray crystallography

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ADP-ribose pyrophosphatase (ADPRase) is a member of nudix family proteins that metabolize many kinds of nucleotide diphosphates. ADPRase catalyzes the divalent metal ion-dependent hydrolysis of ADP-ribose (ADPR) to AMP and ribose 5’ -phosphate. Crystal structures of ADPRases from three organisms including *Thermus thermophilus* (*Tt*) HB8 have already been reported. The structures are very similar with each other, but two kinds of different reaction mechanisms are proposed based on 3D structures of ternary complexes with metal ions and ADPR or ADPR analogue. In order to reveal the real mechanism, we traced the reaction pathway of *Tt*-ADPRase by time-resolved X-ray crystallography. ADPRase crystals were soaked first into ADPR solution at pH 4.6 for one