

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-affinity column. 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

P04.02.125

Acta Cryst. (2008). A64, C270

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 C2, max res. = 2.1 Å, R = 23.3%, free R = 27.2%) and in complex with XMP (2YWC, space group C2, 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 metabolism

P04.02.126

Acta Cryst. (2008). A64, C270

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

P04.02.127

Acta Cryst. (2008). A64, C270-271

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