rossmann-like domain

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Crystallographic studies of ferredoxin-NAD(P)+ reductase from *Chlorobium tepidum*

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Ferredoxin-NAD(P)$^+$ reductase (FNR) is a key enzyme that catalyzes the photoreduction of NAD(P)$^+$ to generate NAD(P)H during the final step of the photosynthetic electron-transport chain. FNR from the green sulfur bacterium *Chlorobium tepidum* is a homodimeric enzyme with a molecular weight of 90 kDa; it shares a high level of amino-acid sequence identity to thioredoxin reductase rather than to conventional plant-type FNRs. In order to understand the structural basis of the ferredoxin-dependency of this unique photosynthetic FNR, *C. tepidum* FNR has been heterologously expressed, purified and crystallized. The *C. tepidum* FNR was crystallized in two forms, I and II, from screening conditions consisting of 20% PEG 3350 containing 200 mM ammonium sulfate or diammonium tartrate as precipitant. Form I crystals belong to the orthorhombic space group $C222_1$, with unit cell parameters a = 100.5, b = 128.0, c = 128.4 Å. Assuming the presence of one dimer in the asymmetric unit, the Matthews coefficient (V_M) is 2.6 Å^3/Da corresponding to a solvent content of 53.2%. Form II crystals belong to the tetragonal space group $P4_122$ or $P4_322$, with unit-cell parameters $a = b$ = 82.0, c = 162.7 Å. The V_M value of 3.5 Å^3/Da indicates that the form II crystal contains one protein molecule per asymmetric unit. Diffraction data were collected from a form I crystal to 2.4 Å resolution on the synchrotron-radiation beamline NW12 at the Photon Factory.

Keywords: FNR, ferredoxin, thioredoxin reductase

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The crystal structure of the staphylococcal amidase AmiE reveals the active site of a metalloenzyme

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Staphylococci are gram-positive bacteria that mainly colonise human skin and the upper respiratory tract. While most staphylococcal infections are averted by a working immune system, they can be life threatening in immunocompromised hosts. Among the most common diseases are endocarditis, pneumonia and the toxic shock syndrome. The ability of staphylococci to form biofilms upon attachment to polystyrene surfaces is another pathogenic factor and especially relevant in transplantation of medical prostheses. Peptidoglycan (PGN) hydrolases such as the major autolysin AtlE from Staphylococcus epidermidis play an important role in cell wall turnover, which renders them appealing targets for drug design. During cell division AtlE splits the equatorial septum, thus allowing the formation of daughter cells. Deletion mutants exhibit a severely disordered division pattern and are biofilm-negative. We have determined the crystal structure of the catalytic domain AmiE, a N-acetylmuramyl-L-alanine amidase at 1.7Å resolution. The protein adopts a globular fold, with several α-helices surrounding a central β-sheet. Alignments with homologous proteins revealed a conserved surface cleft, which is capable of incorporating a PGN-like ligand. A divalent cation is bound in the active site and likely participates in catalysis. Analysis of the architecture of the binding site and the location of key residues allow us to postulate a mechanism of function, which is likely to be that of a metalloenzyme. Mutations of amino acids directly involved in catalysis resulted in severe changes of adjacent loops and a loss of activity. The high-resolution structure of AmiE advances our understanding in terms of ligand binding and enzymatic function, thus providing an excellent base for future drug design.

Keywords: AmiE, amidase, peptidoglycan

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Structure of the inactive mutant of arabinanase complexed with oligosaccharides

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The three-dimensional structure of the thermostable endo-α-1,5-L-arabinanase, ABN-TS, from a strain of *Bacillus thermodinitrificans* TS-3, has been determined at 1.9 Å resolution. We have started the structure analysis of the inactive mutant of ABN-TS complexed with oligosaccharides to elucidate the substrate recognition and reaction mechanism of the emzyme. ABN-TS and the inactive mutant D27A were expressed in *E.coli* as His-tag fusion proteins at their C-termini. They were purified by Ni-affinity, anion-exchange, and size-exclusion chromatographic techniques. The activity of the purified WT (ABN-TS with His-tag) was assayed using debranched arabinan as a substrate by the Somogyi-Nelson method. The WT showed the same catalytic activity as the native ABN-TS, while the mutant D27A showed only very weak activity. The crystals of the inactive mutant complexed with oligosaccharides were prepared by co-crystallization and soaking methods using PEG8000 as a precipitant. Data collection and structure analysis are now in progress.

Keywords: arabinanase, glycoside hydrolase, crystal structure analysis

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Structure of endo-1,5- α -L-arabinanase from *Penicillium chrysogenum*

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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