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Orotidine-5'-monophosphate decarboxylase (ODCase) catalyzes the decarboxylation of orotidine 5'-monophosphate (OMP) to uridine 5'-monophosphate (UMP), the last step of de novo pyrimidine biosynthesis. This enzyme is known as one of the most proficient enzymes, accelerating the decarboxylation reaction by 17 orders of magnitude without using any cofactors, metal ions or delocalization effects when compared to the spontaneous reaction in aqueous solution at neutral pH. The reaction is classified as an electrophilic substitution. We found that this enzyme surprisingly catalyzes two types of novel nucleophilic reactions in addition to the physiological electrophilic reaction. The first reaction is the conversion from 6-cyano-UMP into 6-hydroxyl-UMP (BMP). The conversion proceeds very slowly ($t_{1/2}$ = about 1 day), but its product was confirmed by the high resolution crystal structure of the complex (at 1.4 Å resolution), the mass-spectroscopy, and the enzymological assay. Another novel reaction is the formation of covalent enzyme-ligand complexes with several substrate analogues. Again, the complexes were confirmed by crystallographic analysis and mass-spectroscopy. In addition, we determined numerous crystal structures of ODCase (wild-type and mutants) complexed with a variety of ligands at 1.2 - 1.8 Å resolution. Several ligands displayed a distorted conformation at C6 of the pyrimidine ring, the reactive center. These discoveries suggest that ODCase can catalyze both nucleophilic and electrophilic substitutions, with distortion on the reactant part of the substrate part of catalysis. Details are shown in the presentation.

Keywords: ODCase, crystal structure, catalytic mechanism

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Crystal structures of arylmalonate decarboxylase -Implications for enantioselective reaction

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Arylmalonate decarboxylase from *Alcaligenes bronchiseptics* (AMDase, EC. 4.1.1.76) catalyses an enantioselective decarboxylation of prochiral alpha-aryl-alpha-methylmalonate to produce optically pure alpha-arylpropionate in high enantiomeric excess in high yield. The SH group of the Cys188 residue together with Gly74 is known to play a crucial role in the reaction. By a double mutation, Gly74Cys/Cys188Ser results in an enzyme producing the totally opposite enantiomer. AMDase was crystallized into an orthorhombic crystal form, in that four molecules of the enzyme occupied a crystallographic asymmetric unit. The crystal structure was solved using the multiple isomorphous replacement method and crystallographically refined at a resolution of 2.1 Å. AMDase was composed of two compact domains of a/b-fold similar to the domain architectures in glutamate racemase and aspartate racemase. The structural details within domains and the relative orientations of the two domains were different from the racemases. The active site located in the groove between the two domains was formed by the Gly74-Thr75-Ser76 at the N-terminal face of a short helix, the side chains of Tyr80 and Tyr126, and the Cys188-Gly189-Gly190-Leu191 loop. The active-site structures were in varieties in the conformations of the residues in acompaying with the relative movement of the two domains. The findings suggest the inherent flexibility in the domain interface including the active site. One glycerol molecule, bound to the Gly74-Thr75-Ser76 region in a side-by-side contact, suggested a plausible binding site of the carboxylic groups of substrates. Based on the binding mode, a mechanism to explain the enantioselective reaction for substrates was proposed.

Keywords: arylmalonate decarboxylase, enantioselective reaction, decarboxylation

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Study on the Ca^{2+}-dependent maturation mechanism of subtilisin from a hyperthermophilic archaeon

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Tk-subtilisin from the hyperthermophilic archaeon *Thermococcus kodakaraensis* is a homologue of bacterial subtilisins and is matured from pro-Tk-subtilisin upon autoprocessing and degradation of the propeptide. This maturation process proceeds stepwisely. However, unlike bacterial subtilisins, Tk-subtilisin requires Ca^{2+} for maturation. Here we determined the crystal structures of unautoprocessed, autoprocessed, and mature forms of Tk-subtilisin. The overall structure of Tk-subtilisin is similar to those of bacterial homologues in both propeptide and mature domains, but marked differences are found in the mature domain. It is almost fully folded prior to autoprocessing and contains seven Ca^{2+} binding sites far from the active site. All the sites except Ca1 are unique for Tk-subtilisin. Four of them (Ca2-Ca5) apparently stabilize the central aba substructure of the mature domain, suggesting that these sites are required for folding of Tk-subtilisin. Ca7 is unstable in the unautoprocessed form while stable in the other two forms. Interestingly, along with the formation of this site in the unautoprocessed form, the N-terminal region of the mature domain is disordered with the scissile peptide bond contacting with the active site. This region is kept disordered and mostly truncated in the autoprocessed and mature forms, respectively, suggesting that Ca7 is required to promote the autoprocessing reaction. These crystal structure determinations provide insights into a unique Ca^{2+}-dependent maturation of Tk-subtilisin.

Keywords: Tk-subtilisin, crystal structure, Ca^{2+}-dependent maturation mechanism

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Structural and functional analysis of TTHA0252, a novel RNase of the β-CASP family

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TTHA0252 is a conserved hypothetical protein that belongs to the β-CASP family, within the metallo-β-lactamase superfamily. Archetypal metallo β-lactamases degrade β-lactam antibiotics, whereas the β-CASP family proteins degrade nucleic acids. Recently, it was reported that two β-CASP family proteins from *Bacillus subtilis* are functional homologues of *E. coli* RNase E. RNase E is a key enzyme for mRNA degradation in *E. coli* but no homolog is found in most bacteria. To reveal the biological role of this novel RNase family with a β-CASP fold in RNA metabolism, we currently address the structure and function of TTHA0252 from *Thermus thermophilus* HB8. We have determined the crystal structure of TTHA0252, which represents the first report of the tertiary structure of a β-CASP family protein (1). TTHA0252 comprises two separate domains: a metallo-β-lactamase domain and a clamp domain. The active site of the enzyme is located in a cleft between the two domains. The width of the cleft (10 Å) suggests that TTHA0252 can recognize a single-stranded region, but not a double-stranded region (diameter of 20 Å), of RNA as substrate. The active site of TTHA0252 comprises two zinc ions and seven conserved residues which are similar to those of other β-lactamases. A sulfate ion was also observed near the active site. Since the position of the sulfate ion, appears to mimic the 5'-terminal phosphate group of the substrate, we predicted TTHA0252 to have 5' to 3' exonuclease activity. TTHA0252 actually showed single-strand-specific 5' to 3' exonuclease activity to various oligonucleotides. The effects of mutations of active site residues are also discussed.

(1) Ishikawa, H., et al. (2006) *J. Biochem.* **140**(4), 535-542

Keywords: ribonuclease, protein structure, protein X-ray crystallography

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Structural basis for different substrate specificities of two ADP-ribose pyrophosphatase

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ADP-ribose (ADPR) is one of the main substrates of Nudix proteins. Among the eight Nudix proteins of *Thermus thermophilus* HB8, we previously determined the crystal structure of Ndx4, an ADPR pyrophosphatase (ADPRase)[1)]. In this study we show that Ndx2 of *T. thermophilus* also preferentially hydrolyzes ADPR and FAD, and have determined its crystal structure. We have determined the structures of Ndx2 alone, and in complex with Mg^{2+}, with Mg^{2+} and AMP, and with Mg^{2+} and a nonhydrolyzable ADPR analogue[2)]. Although Ndx2 recognizes the AMP moiety in a manner similar to other ADPRases, it recognizes the terminal ribose in a distinct manner. The residues responsible for recognition of the substrate in Ndx2 are not conserved among ADPRases. This may reflect the diversity in substrate specificity among ADPRases. Based on these results, we propose the classification of ADPRases into two types: ADPRase-I enzymes, which exhibit high specificity for ADPR; and ADPRase-II enzymes, which exhibit low specificity for ADPR. In the active site of the ternary complexes, three Mg^{2+} ions are coordinated to the side chains of conserved glutamate residues and water molecules. Substitution of Glu90 and Glu94 with glutamine suggests that these residues are essential for catalysis. These results suggest that ADPRase-I and ADPRase-II enzymes have nearly identical catalytic mechanisms[3)] but different mechanisms of substrate recognition.

[1)] Yoshiba, S. *et al.* (2004) *J. Biol. Chem.* **279**(35), 37163-37174
[2)] Wakamatsu, T. *et al.* (2008) *J. Bacteriol.* **190**(3), 1108-1117
[3)] Ooga, T. *et al.* (2005) *Biochemistry* **44**(26), 9320-9329

Keywords: hydrolase, proteins structure, protein X-ray crystallography

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Dimerization is important for the GTPase activity of chloroplast translocon components

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Arabidopsis Toc33 (atToc33) is a GTPase and a member of the Toc (translocon at the outer-envelope membrane of chloroplasts) complex that associates with precursor proteins during protein import into chloroplasts. By inference from the crystal structure of psToc34, a homologue in pea, the arginine at residue 130 (Arg130) has been implicated in formation of the atToc33 dimer and inter-molecular GTPase activation within the dimer. Here we report the crystal structure at 3.2 Å resolution of an atToc33 mutant, atToc33(R130A), in which Arg130 was mutated to alanine. Both in solution and in crystals, atToc33(R130A) was present in its monomeric form. In contrast, both wild-type atToc33 and another pea Toc GTPase homologue, pea Toc159 (psToc159), were able to form dimers in solution. Dimeric atToc33 and psToc159 had significantly higher GTPase activity than monomeric atToc33, psToc159 and atToc33(R130A). Molecular modeling using the structures of psToc34 and atToc33(R130A) suggests that, in an architectural dimer of atToc33, Arg130 from one monomer interacts with the ・-phosphate of GDP and several other amino acids of the other monomer. These results indicate that Arg130 is critical for dimer formation, which is itself important for GTPase activity. Activation of GTPase activity by dimer formation is likely to be a critical regulatory step in protein import into chloroplasts.

Keywords: atToc33, GTPase activity, psToc159

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Crystal structure of RuBisCO-like protein from *Bacillus subtilis*

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