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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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