as problem to be solved. We determined a crystal structure of a complex of ArgRS from *Pyrococcus horikoshii*, $tRNA^{Arg}_{CCU}$ and ATP analog with R_{factor} = 0.215 (R_{free} = 0.259) at 2.0 Å resolution and could give one solution for this problem using newly obtained structural information about position of ATP. The experimental results show that the ArgRS protein lacking the additional N-terminal domain characteristic for ArgRS possesses sufficient catalytic activity in the aminoacylation reaction for tRNA. Modeling of relative positions of amino acid, Ade76 of tRNA and ATP on ArgRS was made to find the suitable position to tRNA-assisted formation mechanism of Arg-AMP. It was found that formation of hydrogen bond between 2'OH of Ade76 of tRNA and O2 of carboxy group -C-O2H=O1 of arginine can be achieved in one conformation by rotation around C α-C of carboxy group. In ATP-PPi exchange reaction at low pH, reversible conversion between C=O1 and C-O1-Pα is controlled by the formation of this hydrogen bond. On the other hand, at pH8.0, experimental results in the deacylation reaction of Arg-tRNA is also understood by mechanism that NH^+ of guanidium group $-N^+H=C-(NH_2)_2$ of Arg-tRNA donates a proton to C=O2 of ester bond of Arg-tRNA, resulting in carbonium C^+-O2H. We discuss in detail these mechanisms.

Keywords: aminoacyl-tRNA synthetases, tRNA, catalytic mechanisms

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Crystal structures of α -amino- ε -caprolactam racemase from *Achromobacter obae*

Seiji Okazaki[1], Atsuo Suzuki[2], Tsunehiro Mizushima[3], Hidenobu Komeda[4], Yasuhisa Asano[5], Takashi Yamane[6]
[1]Nagoya University, Department of Biotechnology, School of Engineering, Fur-cho, Chikusa, Nagoya, Aichi Prefecture, 464-8603, Japan, [2]Nagoya University, Fur-cho, Chikusa, Nagoya, Aichi Prefecture, 464-8603, Japan, [3]Nagoya University, Fur-cho, Chikusa, Nagoya, Aichi Prefecture, 464-8603, Japan, [4]Toyama Prefectural University, Kurokawa 5180, Imizu, Toyama Prefecture, 939-0398, Japan, [5]Toyama Prefectural University, Kurokawa 5180, Imizu, Toyama Prefecture, 939-0398, Japan, [6]Nagoya University, Fur-cho, Chikusa, Nagoya, Aichi Prefecture, 464-8603, Japan, E-mail：okazaki.seiji@d.mbox.nagoya-u.ac.jp

α-Amino-ε-caprolactam (ACL) racemase (EC 5.1.1.15) is a 51 kDa enzyme that catalyzes the interconversion of L- and D-ACL. Recently, amino acid amide racemizing activity was found in ACL racemase [1], and the combined method of ACL racemase with D-stereospecific amino acid amidase has been developed for industrial D-amino acid production from D,L-amino acid amide [2]. To clarify the structute-function relationships of ACL racemase, the crystal structures of the native and ε-caprolactam complex of ACL racemase were determined at 2.3 and 2.4 Å resolutions, respectively. ACL racemase belongs to the fold-type 1 group of PLP-dependent enzyme [3]. The crystal structures of serine racemase which belongs to fold-type 2 (PDB ID 1V71) and alanine racemase which belongs to fold-type 3(1SFT) were already determined. However, the crystal structure of fold-type 1 racemase has not been determined yet. If the structure of ACL racemase can be determined, we can understand the catalytic mechanism of racemase which belongs to fold-type 1 for the first time. The structure of ACL racemase is composed of three segments; (1) an N-terminal segment, (2) a large, pyridoxal phosphate (PLP) binding domain and (3) a C-terminal domain. The C4· in PLP covalently bonded to the ε-amino group of Lys267 forming the internal aldimine. From structural comparison with racemase belongs to fold-type 2 and 3, Lys267 and Tyr137 of ACL racemase may be candidates for two bases which are essential for proceeding racemization. The functional significance of ACL racemase will be discussed.

Reference
[1] Asano, Y. & Yamaguchi, S., *J. Mol. Catal. B. Enzym.* **36**, 22-29.
[2] Asano, Y. & Yamaguchi, S., *J. Am. Chem. Soc.* **127**, 7696-7697.
[3] Grishin *et al*., *Protein Sci.* **4**, 1291-1304.

Keywords: enzyme structure, racemases, X-ray biocrystallography

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FBPase allosteric transition: Crystal structures of liver and muscle isoforms from rodents and human

Armin Ruf, Markus Rudolph, Catherine Joseph, Jörg Benz, Brigitte Schott, Tim Tetaz, Bruno Fol
F. Hoffmann-La Roche Ltd, Pharma Discovery Research, Bldg 65 / 36 , Grenzacherstrasse 124, Basel, Basel, 4070, Switzerland, E-mail：armin.ruf@roche.com

FBPase (Fructose-1,6-bis-phosphatase) is a key enzyme in gluconeogenesis. Together with its counterpart enzyme in glycolysis, Phosphofructokinase, FBPase regulates glucose metabolism. FBPase forms a tetramer that is allosterically inhibited by AMP. The allosteric transition from the active R-state to the inactive T-state was intensely investigated over the last 20 years both biochemically and structurally and now serves as a textbook example for allosteric regulation in proteins. However, structural knowledge on FBPase stems almost exclusively from pig kidney/liver FBPase and thus may be biased toward this organism. Muscle FBPase is reported to be 100-fold more sensitive to AMP inhibition than liver FBPase while rodent liver FBPases are roughly 10-fold less sensitive to allosteric inhibition than their human or pig counterparts. Do the textbooks convey the correct picture of the allosteric transition? Or do they purport a biased and, thus, misleading view of the pig special case? To clarify this issue we determined several crystal structures of FBPase liver and muscle isoforms from rat, mouse, and human.

Organism Tissue	Human Muscle	Human Muscle	Rat Muscle	Human Liver	Rat Liver	Mouse Liver	Mouse Liver
Inhibitor	F26P (comp.)	AMP	AMP	-	-	-	AMP-analog
Conformation	T	T	T	R	R	R	T
Space group	C222	C222	P1	P41212 or P43212	I222	P21212	C2
Cell	219Å, 227Å, 79Å	219Å, 234Å, 72Å	56Å, 87Å, 145Å, 101° 92° 92°	122Å, 122Å, 317Å	73Å, 78Å, 130Å	108Å, 81Å, 79Å	165Å, 144Å, 102Å, 123°
Molecules / ASU	4	4	8	6	1	2	6
Resolution	2.3 Å	2.3 Å	2.7 Å	2.2 Å	1.9 Å	1.8 Å	3.0 Å

Keywords: allosteric regulation, enzyme, fructose-1,6-bisphosphate

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Catalytic promiscuity and mechanistic determinants of ODCase - A high-resolution investigation

Masahiro Fujihashi[1], Shingo Kuroda[1], Lakshmi P Kotra[2,3,4], Emil F Pai[5,6], Kunio Miki[1]
[1]Kyoto University, Department of Chemistry, Graduate School of Science, Sakyo-ku,, Kyoto, Kyoto, 6068502, Japan, [2]Toronto General Institute, University Health Network, Toronto, Ontario M5G 1L7, Canada, [3]Departments of Pharmaceutical Sciences and Chemistry, University of

Toronto, Toronto, Ontario, Canada, [4]Department of Chemistry and Biochemistry, The University of North Carolina, Greensboro, NC 27412, USA, [5]Ontario Cancer Institute, University Heatlh Network, MaRS Centre, 101 College Street, Toronto, ON, M5G 1L7, Canada, [6]Department of Biochemistry, Medical Biophysics, Molecular Genetics, University of Toronto, Toronto, ON, M5S 1A8, Canada, E-mail：mfuji@kuchem.kyoto-u.ac.jp

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

Rika Obata[1], Kenji Miyamoto[1], Hiromichi Ohta[1], Masayoshi Nakasako[1,2]
[1]Faculty of Science and Technology, Keio University, Department of Physics, Hiiyoshi 3-14-1, Kohoku-ku, Yokohama, Kanagawa, 223-8522, Japan, [2]The RIKEN Harima Institute/SPring-8, Kouto 1-1-1, Sayo-cho, Sayo-gun, Hyogo, 679-5148, Japan, E-mail：obata@phys.keio.ac.jp

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

Shun-ichi Tanaka, Hiroyoshi Matsumura, Yuichi Koga, Kazufumi Takano, Shigenori Kanaya
Osaka university, Material and Life Science, Graduate School of Engineering, 2-1 Yamadaoka, Suita, Osaka, 565-0871, Japan, E-mail：tanaka@bio.mls.eng.osaka-u.ac.jp

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

Hirohito Ishikawa[1], Noriko Nakagawa[1,2], Seiki Kuramitsu[1,2], Ryoji Masui[1,2]
[1]Osaka University, Biological Sciences, 1-1 Machikaneyamacho, Toyonaka, Osaka, 560-0043, Japan, [2]RIKEN Harima Institute, 1-1-1 Kouto, Sayo-cho, Sayo-gun, Hyogo, 679-5148, Japan, E-mail：daijin@bio.sci.osaka-u.ac.jp