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

P04.02.92

Acta Cryst. (2008). A**64**, C259

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

P04.02.93

Acta Cryst. (2008). A**64**, C259

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

P04.02.94

Acta Cryst. (2008). A**64**, C259–260

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