establish structure-function relationships for the enolase reaction. The crystal structures of the activated enolase (carboxylated on Lys 173) and enolase liganded with Mg and 2,3-diketohexane, a stable alternate substrate, were determined. The stereochemical course of the reaction catalyzed by the enolase was determined using stereospecifically deuterated samples of an alternate substrate. On the basis of these experiments we conclude that the enolase, the functionally divergent member of the RuBisCO superfamily uses the same structural strategy as RuBisCO for stabilizing the enolate anion intermediate , but the proton abstraction is catalyzed by a different general base.

MS03 P07

Structural Study of Selenocysteine Lyase Rie Omi[a], Suguru Kurokawa[a], Hisaaki Mihara[a], Tatsuo Kurihara[a], Nobuyoshi Esaki[a], Ken Hirotsu,[b] Ikuko Miyahara[b], [a]*Institute of Chemical Research, Kyoto University, Physics,* [b]*Graduate School of Science, Osaka City University, Japan.*
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Keywords: enzyme activity mechanism, vitamin B6, substrate binding

Selenocysteine lyase (SCL), which is a pyridoxal 5'-phosphate (PLP) dependent enzyme, catalyzes the β-elimination of L-selenocysteine to yield L-alanine and selenium. SCL is specific for L-selenocysteine and has no activity for L-cystein, therefore it is known as the key enzyme in the specific selenium-delivery pathway for selenoprotein synthesis. In order to elucidate the strict discrimination between selenium and sulfur by SCL, we have determined the three-dimensional structures of native SCL and L-cysteine complexat 1.8 Å and 1.9 Å, respectivery.
Overall and active site structure of SCL is similar to those of cysteine desulfurase which catalyze the same type of reaction as SCL but act on both cysteine and selenocysteine. Why SCL does not show activity on cysteine? In the case of SCL/L-cysteine complex structure, not the amino group of L-cysteine but the thiol group is located close to the C4A of PLP in the active site. This indicates that L-cysteine is incorporated into the active site but can not make an external aldimine with PLP. The mechanism for discrimination between selenium and sulfur will be presented on the basis of the structural comparison between SCL and cysteine desulfulase;

MS03 P08

Crystal Structure Of A Dead-End Complex With Two Isopentenyl Diphosphate Molecules Sheds Light On Substrate Recognition By Human FPPS Jean-Michel Rondeau, Emmanuelle Bourgier, Sylvie Lehmann and Wolfgang Jahnke, *Discovery Technologies, Novartis Institutes for Biomedical Research, CH-4002, Basel, Switzerland.* E-mail: jeanmichel.rondeau@novartis.com

Keywords: structural enzymology, drug mechanism, activity and mechanism of enzymes

Farnesyl diphosphate synthase (FPPS) catalyses the "head-to-tail" condensation between a homoallylic diphosphate, isopentenyl diphosphate (IPP), and an allylic diphosphate, dimethylallyl diphosphate (DMAPP) or geranyl diphosphate (GPP). Early biochemical studies have shown the existence of distinct allylic and homoallylic binding sites, and the dependence of allylic substrate binding on divalent metal ions [1]. Furthermore, the FPPS reaction has been shown to follow an ordered mechanism, with the allylic substrate binding first to the enzyme [2]. However, it is not clear how the enzyme is able to differentiate, at the molecular level, between its two, structurally highly similar, substrates, IPP and DMAPP. In the absence of DMAPP, binding of up to two IPP molecules per FPPS active site has been observed. Moreover, substrate inhibition by high IPP concentrations has been reported [1], also indicating that the allylic binding site does not fully discriminate between DMAPP and IPP. We have crystallized the FPPS ternary complex with two IPP molecules and determined its X-ray structure at 1.70Å resolution. Our data reveal that IPP adopts an energetically less favorable conformation in the allylic site, with the C(1)-C(2)-C(3)-C(4) dihedral angle taking an intermediate value (22°) between that expected for DMAPP (0°) and that observed for IPP in the homoallylic binding site (51°). Hence, discrimination between the C5-isoprenoid substrates appears to be mainly achieved through steric fit and shape recognition.

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MS03 P09

PglD – an acetyl transferase from *Campylobacter jejuni*
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Keywords: Crystallography, Acetyl transferase, CoA

PglD is a protein which is part of an N-linked protein glycosylation system in *Campylobacter jejuni*. The glycan produced by this system was identified to be GalNAc-α1,4-GalNAc-α1,4-[Glcβ1,3-]GalNAc-α1,4-GalNAc-α1,4-GalNAc-α1,3-Bac-β1,*N*-Asn-Xaa, where Bac is bacillosamine, 2,4-diacetamido-2,4,6-trideoxyglucopyranose [1]. PglD has been predicted to be an acetyltransferase which is involved in the biosynthesis of bacillosamine.
Through protein crystallography the atomic structure of PglD reveals that it is a trimer composed of three identical subunits that are related by a crystallographic three-fold rotation axis. Each subunit is composed of 2 domains: an N-terminal domain which is formed by residues 2-70 and the C-terminal domain that stretches from residues 71 to 195. The N-terminal domain consists of two α helices and three parallel β sheets. What is most striking about the C-terminal is that it folds into a left-handed parallel β helix (LβH). The chain in this domain is composed of 6 coils that are wound, in a left handed sense, around the surface of an equilateral prism. Enzymes that contain this fold are placed into a superfamily termed hexapeptide acyltransferases.
PglD adds the acetyl group from acetyl CoA to UDP-4-amino-4,6-dideoxy-α-DGlcNac to make the product bacillosamine, the first reagent required in the glycosylation system [2]. PglD was crystallised with the presence of CoA in the active site which is located along a crevice that is present between the interface of two adjacent monomers. The interactions of CoA with PglD will be discussed further on the poster.

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MS03 P10

Hyperthermophilic phosphotriesterase: insights into stability and catalytic mechanism Mikael Elias[a], Jérôme Dupuy[b], Luigia Merone[c], Sébastien Moniot[a], Claude Lecomte[a], Mosè Rossi[c], Patrick Masson[d], Guiseppe Manco[c] and Eric Chabriere[a], [a]*LCM3B, CNRS-Université Henri Poincaré, Nancy, France.* [b]*LCCP, Institut de Biologie Structurale, Grenoble, France,* [c]*IBP, Consiglio Nazionale delle Ricerche, Napoli, Italy,* [d]*CRSSA, Grenoble, France*
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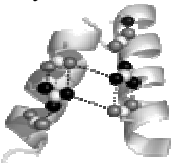
Keywords: hyperthermophile, catalytic mechanism, anomalous scattering methods.

Organophosphates (OPs) constitute the largest class of insecticides used worldwide and certain of them are potent nerve agents. Consequently, organophosphates-degrading enzymes are of paramount interest, as they could be used as bioscavengers and biodecontaminants [1]. The most efficient OP-degrading enzymes are phosphotriesterases (PTEs), whose natural substrate(s) still remains unknown. Here we report the first three-dimensional structure at 2.6 Å resolution of a hyperthermophilic PTE (*Sso*Pox), isolated from the archeon *Sulfolobus solfataricus*. This enzyme is of exceptional thermostability, as catalytic activity is still increasing over 95°C [2]. Structural analysis suggests that the increased stability of this protein is achieved by a number of fine structural differences from the less thermostable homologs. Possible determinants of thermostability in *Sso*Pox PTE are the deletion and the stabilization of flexible regions; the dimer of *Sso*Pox is more compact; an increased number of ion pairs, and their network organization. In addition, the active site region reveals some interesting features. First, the presence of an unexpected hydrophobic channel connected to the active site may represent an important clue in the search of the natural substrate of this enzyme. Second, we investigated the chemical properties of the active site constituted by a bimetallic center. Using crystallography and anomalous scattering properties, we characterized in a non ambiguous way that the active site is constituted of a heterobinuclear cobalt / iron center. Finally, many evidences provided from the structure, mutagenesis experiments, and from previous works on mesophilic PTEs, allowed us to refine the catalytic mechanism of these enzymes.

Global structure of SsoPox



One of the ion pairs networks present in SsoPox structure

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MS03 P11

Structural Details in the β-elimination Mechanism of Tyrosine Phenol-lyase Dalibor Milić,[a] Dubravka Matković-Čalogović,[a] Tatyana T. Demidkina,[b] Alfred A. Antson,[c] [a]*Department of Chemistry, Faculty of Science, University of Zagreb, Croatia.* [b]*Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia.* [c]*Structural Biology Laboratory, University of York, York, UK*
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Keywords: tyrosine phenol-lyase, protein-ligand complexes, enzyme mechanisms

Tyrosine phenol-lyase (TPL; EC 4.1.99.2) is a homotetrameric pyridoxal-5'-phosphate (PLP)-dependent enzyme that catalyses the β-elimination of L-tyrosine (the reversible hydrolytic cleavage of L-tyrosine to phenol and ammonium pyruvate [1]). The β-elimination proceeds via several intermediate steps, including the cleavage of the C_β–C_γ bond. In order to reveal details in the enzymatic reaction and understand structural events during the catalysis, we determined the X-ray structures of several different forms of TPL from *Citrobacter freundii*. All of them are the structures of non-covalent complexes which resemble the quinonoid or the aminoacrylate reaction intermediate. As previously shown [2], the TPL active site can possess two different conformations: open and closed. Our study showed that the proposed closure of the active site during the enzymatic reaction "forces" the quinonoid intermediate into the "strained" conformation, which resembles the transition structure, and thus makes the cleavage of the C_β–C_γ bond easier. The "strained" conformation of the quinonoid intermediate is stabilised by hydrogen bonding and van der Waals interactions with the active site residues.

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MS03 P12

Crystal structures of conjugates of soman with *Tc*AChE Sanson Benoît[a], Nachon Florian[b], Colletier Jacques-Philippe[c], Silman Israel[d], Sussman Joel L.[e], Masson Patrick[b], Weik Martin[a], [a]*Laboratoire de Biophysique Moléculaire, Institut de Biologie Structurale CEA-CNRS-UJF, Grenoble,France ,* [b]*Unité d'Enzymologie, Département de Toxicologie, Centre de Recherches du Service de Santé des Armées, La Tronche, France,* [c]*Molecular Biology Institute, University of California, Los Angeles, USA, Departments of* [d]*Neurobiology and* [e]*Structural Biology, Weizmann Institute of Science, Rehovot, Israel.* E-mail: benoit.sanson@ibs.fr

Keywords: organophosphorus poisons, protein crystallography, acetylcholinesterase

Acetylcholinesterase (AChE) is one of the fastest enzymes in Nature. It hydrolyses the neurotransmitter acetylcholine at ~10000 times per second, thus achieving its role in terminating neurotransmission at cholinergic synapses. AChE is the target of organophosphorus (OP) nerve