[1] Young, N.M., et al., *Structure of the N-linked glycan present on multiple glycoproteins in the gram-negative bacterium, Campylobacter jejuni.* Journal of Biological Chemistry, 2002. 277(45): p. 42530-42539.
[2] Olivier, N.B., et al., *In vitro biosynthesis of UDP-N,N '-diacetylbacillosamine by enzymes of the Campylobacter jejuni general protein glycosylation system.* Biochemistry, 2006. 45(45): p. 13659-13669.

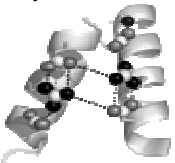
MS03 P10

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*
E-mail: mikael.elias@lcm3b.uhp-nancy.fr

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

[1] LeJeune K.E., Wild J.R., Russell A.J. (1998). Nerve agents degraded by enzymatic foams. *Nature* 395, 27-8. Merone, L., Mandrich, L., Rossi, M., and Manco, G. (2005). A thermostable phosphotriesterase from the archaeon *Sulfolobus solfataricus*: cloning, overexpression and properties. *Extremophiles* 9, 297-305.

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*
E-mail: dmilic@chem.pmf.hr

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.

[1] Phillips, R.S.; Demidkina, T.V.; Faleev N.G. *Biochim. Biophys. Acta*, 2003, 1647, 167.
[2] Milić, D.; Matković-Čalogović, D.; Demidkina, T.V.; Kulikova, V.V.; Sinitzina, N.I.; Antson, A.A. *Biochemistry*, 2006, 45**,** 7544.

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