## MS5-P14 Elucidation of the structure and mechanism of CouO,a small molecule C-methyltransferase from *Streptomyces rishiriensis*

Tea Pavkov-Keller[1,2], Kerstin Steiner[1], Mario Faber[3], Martin Tengg[3], Helmut Schwab[3], Karl Gruber[2], Mandana Gruber-Khadjawi[1]

1. Austrian Centre of Industrial Biotechnology GmbH, Graz, Austria
2. University of Graz, Institute of Molecular Biosciences, Graz, Austria
3. Technical University, Graz, Austria

email: tea.pavkov@uni-graz.at

The addition of a methyl group to biologically active molecules such as hormones, proteins, lipids and nucleic acids causes a change in the physico-chemical properties of the molecules. The methyltransferase (MT) enzymes catalyze this methylation reaction.The biological functions of methylation include biosynthesis, detoxification, metabolism, signal transduction, and nucleic acid processing. Although several classes of MT enzymes are known, the great majority of methylation reactions are catalyzed by the S-adenosylmethionine-dependent MTs (SAM-MTs).

The small-molecule C-methyltransferase CouO from Streptomyces rishiriensis is involved in the biosynthesis of the antibiotic coumermycin A1 [1]. The enzyme was cloned and expressed in Escherichia coli, crystallized [2] and enzymatically characterized in our group [3]. Here we report the high-resolution crystal structure of CouO. The overall structure exhibits a core with a Rossman-like α/β-fold typical o Type I SAM-dependent methyltransferases [4]. CouO is present as a dimer in the asymmetric unit and S-adenosyl-L-homocysteine (SAH) is bound in the cofactor bindingsite. Based on the crystal structure of CouO and docking experiments, the mutagenesis studies of active site amino acids were performed. As a result, we propose a mechanism for the alkyl transfer reaction.

[1] Pacholec M, Tao J, Walsh C T. CouO and NovO: C-Methyltransferases for Tailoring the Aminocoumarin Scaffold in Coumermycin and Novobiocin Antibiotic Biosynthesis. Biochemistry 2005; 44:14969–14976.

[2 ] Lyskowski A, Tengg M, Steinkellner G, Schwab H, Gruber-Khadjawi M, Gruber K. Crystallization of the novel S -adenosyl-L-methionine-dependent C-methyltransferase CouO from Streptomyces rishiriensis and preliminary diffraction data analysis. Acta Cryst. 2012; F68:698–700.

[3] Stecher H, Tengg M, Ueberbacher BJ, Remler P, Schwab H, Griengl H, Gruber-Khadjawi M. Biocatalytic Friedel-Crafts alkylation using non-natural cofactors. Angew Chem Int Ed Engl.2009; 48(50):9546-8.

[4]Schubert H L, Blumenthal R M, Cheng X. Many paths to methyltransfer: a chronicle of convergence. Trends Biochem. Sci. 2003; 28(6):329–335.

**Keywords:** C-methyltransferase, S-adenosyl-L-homocysteine

## MS5-P15 Crystal structure of *Streptomyces rimosus* extracellular lipase

Ivana Leščić Ašler[1], Ivana Leščić Ašler[1], Zoran Štefanić[1], Marija Luić[1], Biserka Kojić-Prodić[1]

1. Ruđer Bošković Institute, Bijenička c. 54, 10000 Zagreb, Croatia

email: ilescic@irb.hr

Upton & Buckley (1995) recognized a new family of lipolytic enzymes, which they originally named GDS(L) lipolytic enzymes after the conserved motif containing the catalytic serine. A new name, SGNH hydrolases, was proposed after the four amino acids that are conserved in the active site (Mølgaard et al., 2000). This particular group comprises lipases, proteases, thioesterases, arylesterases, lysophospholipases, carbohydrate esterases and acyltransferases. Some of the SGNH hydrolases display high flexibility in the active site which enables them to accept a wide range of different types of substrates, but it is as yet unclear whether this property of promiscuity is common to all enzymes of this family.

We have recently discovered that the extracellular lipase from *Streptomyces rimosus* (SrLip) exhibits substrate and catalytic promiscuity (Leščić Ašler et al., 2010). In order to gain better insight into the structural and mechanistic basis of enzyme catalysis, we investigated the interactions of this enzyme with several inhibitors. The general mechanism-based serine protease inhibitor 3,4-dichloroisocoumarin (DCI) was shown to be the most effective and covalently bound to the active-site serine (Zehl et al., 2004).

Purified SrLip was incubated with 600-fold molar excess of DCI and crystallisation screens were set. Only one crystal grew from 0.1 M MES pH 6.5, 25% PEG 2000 MME. This crystal was used for data collection on the XRD beamline of the Elettra synchrotron, Trieste, Italy. A 98.4% complete data set was collected to a resolution of 1.7 Å at 100 K. The protein crystallized in the monoclinic space group $P2_1$, with unit-cell parameters a = 38.1, b = 78.7, c = 56.6 Å, β= 104.5°. The starting model for the molecular-replacement procedure was built using the Phyre server, based on Phospholipase A1 from *Streptomyces albidoflavus* NA297.

Crystal structure of SrLip will be discussed in detail, with special emphasis on active site shape and topology, considering this enzyme's broad substrate specificity.

Leščić Ašler, I., Ivić, N., Kovačić, F., Schell, S., Knorr, J., Krauss, U., Wilhelm, S., Kojić-Prodić, B. & Jaeger, K. E. (2010). *Chembiochem* **11**, 2158–2167.

Mølgaard, A., Kauppinen, S. & Larsen, S. (2000). *Structure* **8**, 373–383.

Upton, C. & Buckley, J. T. (1995). *Trends Biochem. Sci.* **20**, 178–179.

Zehl, M., Leščić, I., Abramić, M., Rizzi, A., Kojić-Prodić, B. & Allmaier, G. (2004). *J. Mass Spectrom.* **39**, 1474–1483.

**Keywords:** SGNH hydrolase, Streptomyces rimosus, catalytic promiscuity, 3,4-dichloroisocoumarin, crystal structure