s8a.m1.p11 **The structure of SAICAR synthase-substrate complexes at 1Å resolution.** S.V. Antonyuk[1,2*], V.M. Levdikov[1], V.V. Barynin[1,3], A.I. Grebenko[1], D.V. Urusova[1], A.N. Popov[1,4,] W.R. Melik-Adamyan[1] and V.S. Lamzin[4], *[1]Institute of Crystallography, Russian Academy of Sciences, Leninsky pr. 59, Moscow 117333, Russia; [2]CLRC Daresbury Laboratory, Warrington, Cheshire WA4 4AD, England, U.K.; [3]Krebs Institute for Biomolecular Research, Department of Molecular Biology and Biotechnology, University of Sheffield, P.O. Box 594 Sheffield, S10 2TN, U.K.; 4European Molecular Biology Laboratory (EMBL), c/o DESY, Notkestrasse 85, 22603 Hamburg, Germany.*
Keywords: enzyme catalysis, protein engineering.

SAICAR synthase (EC 6.3.2.6) catalyses the composition of SAICAR from CAIR and aspartic acid in the presence of ATP and Mg ions at the seventh step (out of ten) in the biosynthesis of purine nucleotides. SAICAR is the key intermediate in the purine biosinthesis, The reaction products are SAICAR, ADP and phosphate. Preliminary identification of the ATP binding site was made from the enzyme-ATP complex of SAICAR synthase from *Saccharomyces cerevisiae* [1]. Now we present the structures of SAICAR synthase complexed with ASP-acid, ATP and the substrate analogue AICAR. Diffraction data for all four complexes were measured at cryo-temperatures at the EMBL Hamburg Outstation.

Crystals of complexes with AICAR + ATP and AICAR were obtained by soaking native crystals in the mother liquid containing substrates. Data from these two complexes collected to 1.4 and 1.55 Å resolution and the structures were refined to R-factors of 13.4 and 16.4%, respectively. The difference electron density maps cleally reveal the location of ATP and a Mg ion near the phosphate groups of the ATP and phosphate with sugar groups of the AICAR molecule in the active site of the AICAR+ATP complex. A second binding site of AICAR opposite to the active side cleft was found in both complexes.

Crystals of SAICAR synthase complexed with ASP-acid and with AICAR+ATP+succinate+Mg^{2+} were grown by co-crystallisation using the vapour diffusion at 20°C. Data were collected to 1.05 and 1.0 Å resolution. The structure of the ASP-acid complex (R-factor 14.5%) unambiguously identified the location of the aspartic acid at the active site. The structure of the AICAR+ATP+succinate+Mg^{2+} complex was solved by molecular replacement and refined to an R-factor of 12.0%. The difference electron density map showed the AICAR, ADP and a Mg^{2+} ion near the ADP in the active site of the enzyme. The binding site for the phosphate group decomposed from ATP was found. The active cleft of the native protein is constricted when ATP and AICAR are present.

The mechanism of the SAICAR biosynthesis is proposed on the basis of these structural data.

[1] Levdikov, V.M., Barynin, V.V., Grenenko, A.I., Melik-Adamyan, W.R., Lamzin, V.S., Wilson, K.S. Structure (1998) 6: 363-376.

s8a.m1.p12 **Threonine synthase from *Arabidopsis thaliana* : 2.2 Å resolution structure and reaction proposal.** K. Thomazeau ‡, R. Dumas §, G. Curien §, V. Biou ‡, *‡ Institut de Biologie Structurale Jean-Pierre Ebel (UMR 5045) CNRS/CEA/Université Joseph Fourier, 41 rue Jules Horowitz, F-38027 Grenoble Cedex, France. § Unité Mixte CNRS/Aventis (UMR 1932),Aventis CropScience, 14-20 rue Pierre Baizet, F-69263 Lyon cedex 09, France. Correspondence e-mail : biou@ibs.fr*
Keywords: aminoacid synthesis, plant, allostery.

Threonine synthase is a pyridoxal-phosphate-dependent dimeric enzyme, which catalyses the last reaction to yield threonine from aspartate. The catalysed reaction is an elimination of a phosphate group along with the hydration of Cß to yield threonine.

In plants, the methionine pathway shares the same substract, O-phospho-L-homoserine and threonine synthase is activated by a down-product of methionine synthesis (1)]. The activator is S-adenosyl-methionine.

Free threonine synthase was crystallised from PEG and Lithium Chloride solutions (Thomazeau et al., in preparation). The structure was solved on those triclinic crystals using MAD data from the selenomethionated protein. The monomer structure reveals a four-domain protein with a two-stranded beta-sheet arm swapped from one monomer onto the other. This may be a lever through which the allosteric effect is transmitted. Regions which are potential sites for activator binding are disordered in its absence.

The structure will be presented and the substrate specificity of this pyridoxal phosphate enzyme will be discussed. In addition, aspects of the MAD structure solution using oxidised and reduced selenomethionine will be reported.

[1] Curien, G., Job, D., Douce, R., Dumas, R. Allosteric activation of Arabidopsis threonine synthase by S-adenosylmethionine., 1998. Biochemistry. 37 (38):13212-21 0006-2960.