s1.m8.p22 **Binary complex of calf spleen purine nucleoside phosphorylase with a potent multisubstrate analogue inhibitor.** Marija Luic,[a] Gertraud Koellner,[b] Tsutomu Yokomatsu,[c] Shiroshi Shibuya[c] and Agnieszka Bzowska[d], *[a]Rudjer Boskovic Institute, P.O.Box 180, 10002 Zagreb, Croatia, [b]Institut für Chemie-Kristalography, Freie Universität Berlin, Takustr. 6, D-14195 Berlin, Germany, [c]School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan, and [d]Department of Biophysics, Institute of Experimental Physics, University of Warsaw, Zwirki i Wigury 93, 02 089 Warsaw, Poland. E-mail: marija.luic@irb.hr*

Keywords: Purine nucleoside phosphorylase; Crystal structure; Multisubstrate inhibitor

The ubiquitous enzyme purine nucleoside phosphorylase (PNP, E.C. 2.4.2.1.) is the key enzyme of the purine salvage pathway [1]. In mammals homotrimeric PNPs catalyse the reversible phosphorolytic cleavage of the glycosidic bond of 6-oxopurine nucleosides (ribo- and 2'-deoxyribo) and some analogues, as follows: purine nucleoside + orthophosphate - base + pentose-1-phosphate. PNP deficiency in humans leads to inhibition of T-cell response. Potent inhibitors of this enzyme are therefore considered as potential immunosuppressive agents. In the 28 years since the genetic deficiency of PNP has been discovered [2], great efforts have been made to design inhibitors of PNPs with potential medical applications [1]. However, only the transition state analogue of human PNP, immucillin-H (trade name BCX-1777), has reached phase I/II clinical trials against human T-cell leukemia [3]. The important class of potent ground-state analogue inhibitors of trimeric PNPs (K_i in nM range) is composed of so called multisubstrate analogue inhibitors. These are compounds consisting of three structural parts linked together: (1) purine base, (2) acyclic chain or cyclic moiety, and (3) phosphonate or phosphate or other electronegative group [1]. These three parts mimic two substrates of PNP, namely, purine nucleoside (part 1 and 2) and phosphate (part 3) in the phosphorolytic direction or purine base and pentose-1-phosphate in the synthetic direction, hence they are expected to bind to purine, pentose and phosphate binding sites in a binary complex with the enzyme. In the Protein Data Bank there are no reported structures of the complexes of trimeric PNPs with multisubstrate analogue inhibitors. We describe here for the first time the high resolution X-ray structure of trimeric calf spleen PNP, highly homologous to the human PNP, with the potent (K_i^{app} = 16 and 18 nM at pH 7.4 with calf and human enzymes, respectively [4]) multisubstrate analogue inhibitor 9-(5,5-difluoro-5-phosphonopentyl)guanine.

[1] Bzowska, A., Kulikowska, E. & Shugar, D. (2000). *Pharmacology and Therapeutics* **88**, 349-425.
[2] Gilbert, E.R., Ammann, A.J., Wara, D.W., Sandman, R. & Diamond, L.K. (1975). *Lancet* **1** , 1010-1013
[3] Schramm, V.L. (2001). *Biochim. Biophys. Acta*. **1587**, 107-117.
[4] Halazy, S., Ehrhard, A., Eggenspiller, A., Berges-Gross, V. & Danzin, C. (1995). *Tetrahedron* **52**, 177-184.

s1.m8.p23 **Crystal Structure of Plant Threonine Synthase Complexed with S-Adenosylmethionine Reveals Allosteric Activation Mechanism**. Mas Corine [a], Biou Valérie [b] and Dumas Renaud [a], *[a] Laboratoire de Physiologie Cellulaire Végétale DRDC/CEA-Grenoble, France, [b] Laboratoire d'Enzymologie et de Biochimie Structurales, UPR 9063, CNRS, Gif sur Yvette, France. Email : mas@dsvsud.cea.fr*

Keywords: Allosteric activation; S-adenosylmethionine; Pyridoxal phosphate

Threonine synthase (TS) is a pyridoxal phosphate (PLP)-dependent enzyme that catalyses, in plants and micro-organisms, the synthesis of threonine from O-phospho-L-Homoserine (OPH). In plant, TS and Cystathionine-γ-synthase, the first enzyme in the methionine pathway shares the same substrate OPH. At this branch point, TS was activated by S-adenosylmethionine (SAM), a key metabolic downstream product of methionine synthesis. This positive allosteric TS activation triggered by SAM is specific to plant. With the aim to determine the molecular basis of this allosteric activation, biochemical and crystallographic experiments were carried out. The first structure of *Arabidopsis thaliana* TS without SAM was solved in our group [1]. In this structure, the PLP was not bound in the active site and the local conformation did not allow PLP binding in the position observed in non allosteric TS such as the *Thermus thermophilus* TS [2]. Indeed, several aminoacids that are involved in the interaction with the PLP are in a different orientation, and a variable loop that comes on top of the PLP site is disordered in the structure of the plant TS. We have now solved the structure of *A. thaliana* TS complexed with SAM at 2.8Å resolution. The asymmetric unit contains one asymmetric dimer. Two SAM molecules bind at the dimer interface and induce conformational changes and loops stabilisation. It is clear that the presence of the activator triggers conformational changes, that allows the PLP to bind. However, only one active site shows a PLP bound in the same orientation as in *Thermus thermophilus* TS, while the other site is empty. These first conformational changes explain the role of SAM in the binding of one PLP leading to substrate recognition in one monomer. Biochemical studies [3] as well as crystal structure of *Thermus thermophilus* TS in the absence and in the presence of a substrate analogue [2] indicate that binding of the substrate would induce further conformational changes leading to PLP binding in the second monomer. These results allowed us to propose a new mechanism for the allosteric activation of plant TS by SAM.

[1] Thomazeau, K., Curien, G., Dumas, R., and Biou, V. (2001) *Protein Science* **10**, 638-648.
[3] Omi, R., Goto, M., Miyahara, I., Mizuguchi, H., Hayashi, H., Kagamiyama, H., and Hirotsu, K. (2003) *J Biol Chem* **278**, 46035-45.
[3] Curien, G., Job, D., Douce, R. and Dumas, R. (1998) *Biochemistry* **37**,13212-13221