s8a.m1.p13 **Structural analysis of the allosteric transition of *Pseudomonas putida* catabolic ornithine carbamoyltransferase.** B. Clantin1, G. Sainz2, C. Tricot3, V. Stalon1,3 and V. Villeret3 *1Laboratoire de Microbiologie,Université Libre de Bruxelles 1, Avenue Emile Gryson B-1070 Brussels, Belgium 2 European Synchrotron Radiation Facility, BP 220, F-38043 Grenoble Cedex, France 3 Institut de Recherches Microbiologiques Jean-Marie Wiame 1, Avenue E. Gryson B-1070 Brussels, Belgium.*

Keywords: allosteric mechanism, oligomeric enzymes, effector binding sites.

Pseudomonas putida is an arginine prototrophic organism that possesses two distinct ornithine carbamoyltransferases (OTCase, EC 2.1.3.3.). An anabolic OTCase (aOTCase) encoded by the *argF* gene is involved in the arginine biosynthesis pathway and catalyses *in vivo* the thermodynamically favoured formation of citrulline and phosphate from ornithine and carbamoylphosphate. The anabolic enzyme is a homotrimer which displays Michaelis-Menten kinetics[1]. *P. putida* possesses a second OTCase (cOTCase) encoded by the *arcB* gene, which is involved in the anaerobically inducible arginine deiminase pathway and promotes *in vivo* the phosphorolysis of citrulline yielding ornithine and carbamoylphosphate[2]. This cOTCase does not carry out the biosynthetic reaction because of a poor affinity and a marked cooperativity for the carbamoylphophate[3]. Moreover, the enzyme is heterotropically activated by nucleoside monophosphates (AMP, GMP, CMP, UMP) and inhibited by polyamines such as spermidine and putrescine[4]. The *P. putida* cOTCase displays an homododecameric quaternary structure with the 23 point group symmetry.

Sequence comparison with the *Pseudomonas aeruginosa* cOTCase shows an identity of 90%, which underlines the similarities of these two cOTCases. The crystal structure of the active R ("Relaxed") state of cOTCase from *P. aeruginosa* was recently determined at 2.5 Å resolution[5]. However, crystals of the T ("Tense") state enzyme could not be obtained so far. In order to complete our studies on the allosteric behaviour of cOTCases, we initiated structural investigations on the closely related enzyme from *P. putida*. R and T form crystals have been grown for this enzyme. T state crystals have been obtained in the presence of high concentration of spermidine (40 mM), a negative effector for the allosteric transition. They belong to the space group P21212. The refinement at 2.8 Å resolution of this crystal form structure is in progress and will be presented. A complete data set at 3.4 Å has also been measured for a R state crystal obtained in the presence of AMP (80 mM), a positive effector. The data for both crystal forms have been measured at the ESRF on the ID14 beamlines. For T and R crystals, molecular replacement solutions could be identified. Trials to obtain higher resolution data for both crystal forms are in progress.

The analysis of the T and R structures will be shown in the presentation, with their comparison to the closely related structures of the *P. aeruginosa* cOTCase previously reported[5,6]. Possible cOTCase allosteric transition mechanisms suggested by our structural analysis will be discussed.

[1] Y.Itho, L. Soldati, V. Stalon, P.Falmagne, Y. Terawaki, T. Leisinger and D. Haas (1988) J. Bacteriol. 170, 3225-3234.
[2] C. Vander Wauven, A. Pierard, M. KleyReyman and D. Haas (1984) J. Bacteriol. 160, 928-934.
[3] H. Baur, C. Tricot, V. Stalon and D. Haas (1990) J. Biol. Chem. 265, 14728-14731.
[4] C. Tricot, V.T. Nguyen and V. Stalon (1993) Eur. J. Biochem. 215, 833-839.
[5] G. Sainz, J. Vicat, R Kahn, E. Duee, C. Tricot, V. Stalon and O. Dideberg J. Mol. Biol. (submitted)
[6] V. Villeret, C. Tricot, V. Stalon and O. Dideberg (1995) Proc. Natl. Acad. Sci. USA 92, 10762-10766.

s8a.m1.p14 **High-resolution Structure of the Phosphohistidine-containing and Vanadate-inhibited Forms of *E. coli* Cofactor-dependent Phosphoglycerate Mutase.** C.S. Bond, M.F. White and W.N. Hunter. *Department of Biochemistry, University of Dundee, MSI/WTB Complex, Dow St., DUNDEE, DD1 5EH, Scotland.*

Keywords: phosphohistidine, tetravanadate, phosphoglycerate mutase.

The atomic structure of the cofactor-independent phosphoglycerate mutase from *Escherichia coli* has been elucidated by crystallographic methods to a resolution of 1.25 Å in its active, phosphohistidine-containing form. A further structure of the vanadate-inhibited protein has been solved to 1.30 Å resolution. The active conformation of the protein involves stabilisation of a catalytic phosphohistidine and the ordering of a C-terminal tail, both of which have previously eluded crystallographic investigation. The very high resolution of these structures has provided new insight into the mechanism of action of this class of enzyme.