s8a.m1.p31 Channeling of ammonia in glucosamine-6P synthase. A. Teplyakov, G. Obmolova, M.-A. Badet-Denisot, and B. Badet, *Center for Advanced Research in Biotechnology, Rockville, Maryland 20850, U.S.A. and Institut de Chimie des Substances Naturelles, CNRS, Gif-sur-Yvette, France.*
Keywords: amidotransferase, glucosamine-6P synthase, intramolecular ammonia channel.

Glutamine dependent amidotransferases are responsible for utilisation of the amide nitrogen of glutamine in a variety of biosynthetic reactions. Glucosamine 6-phosphate synthase (GlmS) catalyses the first step in hexosamine metabolism, converting fructose-6P into glucosamine-6P in the presence of glutamine. The end-product of the pathway, N-acetylglucosamine, is an essential building block of bacterial and fungal cell walls. The mammalian enzyme plays a key role in desensitising the insulin-responsive glucose transport system. GlmS functions as a homodimer. Each subunit is composed of two structurally and functionally distinct domains. The N-terminal 30-kDa glutaminase domain catalyses hydrolysis of glutamine to glutamate and ammonia. The C-terminal 40-kDa isomerase domain utilises this ammonia for fructose-6P to glucosamine-6P conversion.

The three-dimensional structure of *E. coli* GlmS as well as those of the glutaminase and isomerase domains complexed with substrate analogues and reaction products have been determined by X-ray crystallography. The structures revealed the mechanism of glutamine hydrolysis as that of the N-terminal nucleophile type and suggested a mechanism of glutamine dependent fructose-6P isomerisation through an enol intermediate (rather than by a hydride shift). Dimerization of the enzyme provides an essential catalytic histidine to the sugar phosphate site for initiating the isomerisation by opening a sugar ring.

An internal channel connects two active sites and serves for devivering of ammonia from the glutaminase domain to the sugar phosphate site. Channelling of ammonia seems to be a common feature of amidotransferases. This enzyme family includes carbamoyl phosphate synthetase, phosphoribosyl pyrophosphate amidotransferase, GMP synthetase, and Asn synthetase for which the 3-D information is available. In spite of differences in both the acceptor and the glutamiase domains, these enzymes share similarities in the overall design of the chanel. On the other hand, GlmS is unique in a way that it cannot use exogenous ammonia as a nitrogen donor. The C-terminal fragment of the protein with the GlmS fingerprint sequence plays a key role in substrate binding, catalysis and communication between domains. It may also be a critical element of the mechanism of fructose-6P induced activation of glutamine hydrolysis.

s8a.m1.p32 Structure-function relationship in serralysins, probed by site-directed mutagenesis and X-ray crystallography. T. Hege, U. Baumann, *Department of Chemistry and Biochemistry, University of Berne, Berne, Switzerland*.
Keywords: site-directed mutagenesis, metallo-protease, *Erwinia chrysanthemi*.

Met-226 of the 50 kDa metallo-endo-protease from *Erwinia chrysanthemi* (Protease C) is the name giving and hence highly conserved residue in the family of the metzincins. The reason for this conservation is not clear, yet. The turn including the Met-226 contains one of the ligands of the active zinc-ion (Tyr-228), but the Met-226 itself doesn't have the right distance and orientation - the sulfur is pointing away from the active site - to be part of the proposed catalytic mechanism for serralysins[1].

In order to elucidate why Met-226 is so highly conserved, we have changed this residue by site-directed mutagenesis into Ala, Leu, Ile, Cys, Asn and His. The catalytic activity of these mutants lies between the one from the wild type (M226L) and none at all (M226C).

The crystal structures of the mutants M226L and M226C have been determined and refined to a crystallographic *R*-factor of 0.191 and 0.196 at 2.0 Å and 2.4 Å resolution, respectively, building on the wild type structure.

To get more information about the active site, some of the amino acids directly involved in the catalytic mechanism have been changed. These mutants have been crystallized, too.

[1] Mock W. L. "Kinetic Characterisation of the Serryalysins: A Divergent Catalytic Mechanism Pertaining to Astacin-Type Metalloproteases", Biochemistry, (1997), 36: 4949 - 4958.