s1.m8.p8 **A six- or sevenfold coordinated divalent cation in the active site of human PSP makes the difference.**

C. De Ranter[a], Y. Peeraer[a], A. Rabijnsa, J.-F. Collet[b], E. Van Schaftingen[b]. [a] *Laboratory for Analytical Chemistry and Medicinal Physicochemistry, Faculty of Pharmaceutical Sciences, K.U.Leuven, B-3000 Leuven, Belgium;* [b] *Laboratory of Physiological Chemistry, Christian de Duve Institute of Cellular Pathology, Université Catholique de Louvain, B-1200 Brussels, Belgium. E-mail: camiel.deranter@pharm.kuleuven.ac.be*

Keywords: Phosphoserine phosphatase; Magnesium-dependent enzymes; Calcium:HPSP complex

Human phosphoserine phosphatase (HPSP), a member of the haloacid dehalogenase (HAD) superfamily, catalyses the last and irreversible step of the *de novo* biosynthesis of L-serine i.e. the hydrolysis of phosphoserine with the formation of L-serine and inorganic phosphate (Pi). The HAD superfamily members, except for 2-haloacid dehalogenases [1, 2], utilise Mg^{2+} as a cofactor during catalysis. The effects of various metal cations on the activity of PSP were described [3], but key features of their metal binding characteristics remained undetermined. Maximum activity of the enzyme, measured by the rate of Pi release from phosphoserine, is obtained with Mg^{2+}. In the absence of added divalent cations, the activity of PSP is only 9 to 15% of the maximal activity observed in the presence of Mg^{2+}. Of particular interest was our observation that the replacement of Mg^{2+} by Ca^{2+} in an activity test caused complete loss of activity of PSP. Furthermore Ca^{2+} inhibited the activity measured in the presence of Mg^{2+}. The expression, purification, crystallisation and elucidation of the 1.53 Å resolution, Ca^{2+} containing crystal structure have been described elsewhere (PDB ID code 1NNL) [4]. The sixfold coordinated Mg^{2+} ion present in the active site of HPSP under normal physiological conditions and in previously reported structures of the PSP family, was replaced by a Ca^{2+} ion. The plausible reason for the presence of the Ca^{2+} instead of a Mg^{2+} ion, is that we used a crystallisation condition with high concentration of $CaCl_2$ (0.7 M). The resulting HPSP structure now shows a sevenfold coordinated Ca^{2+} ion in the active site what might explain the inhibitory effect of Ca^{2+} on the enzyme. Indeed, the Ca^{2+} ion in the active site captures both side-chain oxygen atoms of the catalytic Asp20 as a ligand, while a Mg^{2+} ion ligates only one oxygen atom of this Asp residue. The bidentate character of Asp20 towards Ca^{2+} hampers the nucleophilic attack of one of the Asp20 side chain oxygen atoms on the phosphorus atom of the substrate phosphoserine [5].

[1] Hisano, T., Hata, Y., Fujii, T., Liu, J.Q., Kurihara, T., Esaki, N. & Soda, K. (1996) *J. Biol. Chem*. **27**1, 20322-20330.
[2] Ridder, I.S. & Dijkstra, B.W. (1999) *Biochem. J.* 339, 223-226.
[3] Neuhaus, F.C. & Byrne, W.L. (1959) *J. Biol. Chem*. **234**, 113-121.
[4] Peeraer, Y., Rabijns, A., Verboven, C., Collet, J.F., Van Schaftingen, E. & De Ranter, C. (2002) *Acta Cryst.* D**58**, 133-134.
[5] Peeraer, Y., Rabijns, A., Collet, J.F., Van Schaftingen, E. & De Ranter C. (2004) *European J. of Biochemistry*, in press.

s1.m8.p9 **Structural studies of FXa-Trypsin mutants complexed by dianhydrosugar-based benzamidine inhibitors.**

Armida Di Fenza,[a] Andreas Heine[a], Marko Vogler[b], Milton T. Stubbs[c], Daniel Rauh[c], Anastasia Tziridis[c], Ulrich Koert[b] and Gerhard Klebe[a], [a]*Institute for Pharmaceutical Chemistry, Philipps University, Marburg, Germany;* [b]*Department of Chemistry, Philipps University, Marburg, Germany;* [c]*Institute for Biotechnology, Martin-Luther University, Halle-Wittenberg, Germany. E-mail: difenza@staff.uni-marburg.de*

Keywords: Serine-proteinases; Protein-ligand recognition; Dianhydrohexitoles

Trypsin and factor Xa are enzymes belonging to the family of serine-proteinases, which play a crucial role in many important biological and pathological processes. Their regulation represents an attractive target for the development of new therapies. Both enzymes share high similarity in the binding pocket and catalyze the same mechanism; nevertheless they can selectively discriminate among specific substrates and inhibitors [1]. For these reasons they constitute an ideal system to study the features that differentiate selectivity. We constructed trypsin mutants in which the binding pocket is substituted partially or completely by the factor Xa binding pocket and analyzed the effect of the mutations on the binding of dianhydrosugar-based benzamidine inhibitors by measuring their corresponding Ki values. The series of ligands studied (**1-4**), is characterized by a common rigid scaffold, the dianhydrohexitoles isosorbide moiety (Fig.1).

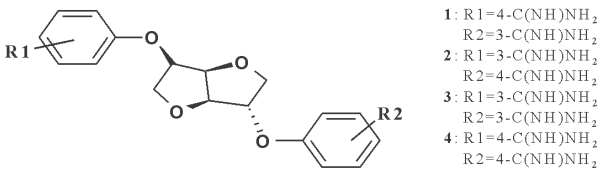

Fig.1 Dianhydrosugar-based benzamidines 1-4.

We also determined the crystal structures of some of the mutant-inhibitor complexes to investigate the adopted binding mode. The first structure was obtained in the presence of ligand **1**, with a resolution of 1.65 Å and allows the detailed analysis of the protein-ligand interactions. Despite a high degree of symmetry, the X-ray structure shows that the ligand binds in a preferred orientation. In particular, **1** interacts with the specific primarily hydrophobic S1 pocket of the enzyme, by means of its R2 substituent. This feature is particularly interesting and guided us to choose others ligands (**2-4**) to investigate the property of binding of these compounds with the Trypsin mutant. Successively, to analyse how the binding is influenced by mutations in the binding pocket, the series of ligands will be examined in complex with others trypsin mutants. This will contribute to understand the difference in the binding mode between Trypsin and Factor Xa. Our results will provide a new approach to the structure-activity relationships in particular with respect to protein family members. Infact, the binding is analyzed by varing both, the structure of the ligand and of the enzyme binding site. This information is an important prerequisite to design new drugs possessing both high affinity and selectivity.

[1] Johnson VA and Smith RL. (1976). *Arch Biochem Biophys*. **175**(1), 190-5.