s8a.m1.p27 **New insights in the thermolysin crystallogenesis and inhibition.** J-F. Gaucher[1], M. Selkti[1], H. Chen[2], B. Roques[2], T. Prangé[1] and A. Tomas[1]. *1: LCRB, EP2075, CNRS, Faculté de Pharmacie, 4 av. de l'Observatoire 75006 Paris, France. 2: INSERM U266, CNRS UMR 8600, Faculté de Pharmacie, 4 av. de l'Observatoire 75006 Paris, France.*
Keywords: enzyme catalysis, protein engineering.

The bacterial zinc endopeptidase thermolysin (TLN, EC 3.4.24.27) provides a structural framework for some physiological peptidases such neprylisin (NEP, EC 3.4.24.11), and angiotensin converting enzyme (ACE, EC 3.4.15.1). Thus, several complexes have been solved which provide structural data about the catalytic mechanism and to design new inhibitors of these enzymes.

All the crystals structures so far obtained were in presence of important concentrations of dimethyl sulfoxide (5 to 15% (v/v)[DMSO]) which the effect has not been fully evaluated. In these studies, we have crystallized the protein in new conditions, without any organic solvent using thiocyanate as crystallizing agent. The crystal structure has been refined at 2Å resolution. A dipeptide, Val-Lys is observed in sites S1' and S2', whereas a thiocyanate molecule binds the catalytic domain. The structure of site S1 is modified, with the movement of the catalytic Tyr 157, which provides a new network of electrostatic bonding.

We describe also the binding mode of a new dual inhibitor of NEP and ACE, co-crystallized with TLN. This inhibitor fits the sub-sites S1, S1' and S2' and interact with the catalytic Zn by it's phosphonamidate function. We will discuss the binding mode of this inhibitor to NEP and ACE.

s8a.m1.p28 **Structural studies of inhibiting mechanism E.coli inorganic pyrophosphatase by calcium ions.** V.R.Samygina[1], A.N. Popov[2], V.S. Lamzin[2], N.N. Vorobeva[3], S.A. Kurilova[3], T.I. Nazarova[3], S.M. Avaeva [3]. *[1]Institute of Crystallography, Russian Academy of Sciences, Leninsky pr. 59, Moscow 117333, Russia, [2]Europen Molecular Biology Laboratory (EMBL), c/o DESY, Notkestrasse 85, 22603 Hamburg, Germany, [3]Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119899, Russia.*
Keywords: pyrophosphatase, atomic structure, inhibiting mechanism.

Inorganic pyrophosphatases (PPases) belong to the family of phosphoryl-transfer enzymes. They catalyze the hydrolysis of the phosphoanhydride bond in the pyrophosphate molecule. The presence of divalent metal cofactors, where Mg^{2+} is most effective, is necessary for PPases functioning. Ca^{2+} inhibits all PPases. Thus inactivating effect of Ca^{2+} is important for regulation of PPases activity.

We report two first atomic structures (1.1 and 1.2 Å) of E.coli PPase complexed with calcium (Ca-EPPase) and calcium-pyrophosphate (CaPPi-EPPase). They have been refined to an R-factor 11.7% and 12.8% respectively. Ca-EPPase contains two metal ions per active site while CaPPi-EPPase contains three metal ions and pyrophosphate molecule.

The positions of Ca^{2+} are similar to Mg^{2+}/Mn^{2+} positions in reported earlier PPase structures [1,2]. However metals coordination is different and the presence of a Ca^{2+} clearly affects the detailed conformation of the active site, especially loop 96-102 contains protein ligands of two metal ions. As a result the substrate is located in the active site in such a way that there is no water molecule which could be nucleopfile. So the hydrolysis is impossible.

We suggested the substrate occupies another position in the presence of Mg^{2+}/Mn^{2+} and we have generated it using CaPPi-EPPase structure. This finding confirms the proposal that nucleophile is a bridging water molecule between two metal ions[2]. It also allows to describe more accurate further substrate transformation.

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