PS04.02.16 THE CRYSTALLOGRAPHIC STRUCTURE OF DEHYDROQUINATE SYNTHASE FROM *ASPERGILLUS NIDULANS*. E. P. Carpenter[1], A. R. Hawkins2, J. D. Moore[2], J. W. Frost[3], G. G. Dodson[1], K. A. Brown[4], [1]National Institute For Medical Research, Mill Hill, London, UK, [2]Dept of Biochemistry and Genetics, University of Newcastle, UK, [3]Dept of Chemistry, Michigan State University, East Lansing, MI, [4]Dept of Biochemistry, Imperial College, London, UK

Dehydroquinate synthase (DHQS) is the second enzyme in the prechorismate (shikimate) pathway and is a potential target for tuberculosis drugs. The enzyme requires zinc or similar metals for activity. In *Aspergillus nidulans* DHQS is the N-terminal enzyme of the pentafunctional AROM complex.

Crystals of recombinant DHQS complexed with NAD and carbaphosphonate were obtained from jeffamine and isopropanol, using the sitting drop method. Two crystal forms are observed growing in the same drop. 2.3Å data has been collected from crystals with a unit cell of 67.5Å, 80.7Å, 143.3Å using a crystal frozen at 100K in 30% sucrose. 2.8 Å data were collected with a capillary mounted crystal with a cell of 65.8Å, 71.3Å, 200.0Å. All the crystals tested so far are space group $P2_12_12_1$. An unfrozen crystal soaked in $HgCl_2$ for two hours gave data to 3.5Å. This derivative has sites for eight mercury atoms with a two fold axis relating four of the mercuries to the other four. The final Cullis R for this derivative was 60%. Two fold averaging using this axis has given an interpretable map in which a dimer is clearly visible. The monomer consists of two domains and a zinc binding site has been tentatively located. Details of the structure will be discussed.

Moore, J. D., Coggins, J. R., Virden, R. and Hawkins, A. R., (1994), *Biochemical Journal*, **301**, 297-304.

PS04.02.17 ACTIVE-SITE BINDING OF METAL IONS IN D-XYLOSE ISOMERASE. H. L. Carrell, Carol E. Afshar, Liat Shimoni-Livny, Jenny P. Glusker, The Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111, USA

The active site of the enzyme D-xylose isomerase contains two divalent metal ions, usually Mg^{2+}, Mn^{2+}, or Co^{2+}.[1,2] We have investigated the structure and characteristics of the metal-free enzyme and the enzyme containing the normally encountered metal ions (Mg^{2+}, Mn^{2+}, Co^{2+}), as well as the effects on the structure of adding divalent cations such as Ni^{2+}, Zn^{2+}, Pb^{2+}, and Ca^{2+}. The variation in enzyme activity as a function of added metal ion, and the changes that these metal ions cause in the geometry of the active site of the enzyme will be described. X-ray structural studies have all been carried out at 1.9-1.6 Å resolution. A detailed description of different metal ion environments will be provided. The extent of competition between metal ions for the two sites is revealed by this type of study. The results of our analysis of metal binding will be compared with results from previous NMR studies on some of these systems.[3]

Supported by grant CA-10925 from National Institutes of Health.

1. Carrell, Rubin, Hurley, and Glusker J. *Biol. Chem.* **1984**, *259*, 3230.
2. Carrell, Glusker, Burger, Manfre, Tritsch, and Biellmann *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 4440.
3. Sudfeldt, Schäffer, Kägi, Bogumil, Schulz, Wulff, Witzel *Eur. J. Biochem.* **1990**, *193*, 863.

PS04.02.18 CRYSTAL STRUCTURES OF STROMELYSIN: CONFORMATIONAL CHANGES INDUCED BY INHIBITOR BINDING. Longyin Chen, Timothy J. Rydel, C. Michelle Dunaway, Larry Strickland, The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, OH 45253-8707

Stromelysin, a zinc-dependent proteinase, is a member of the matrix metalloproteinases (MMP). MMPs are believed to play an important role in pathological conditions such as osteoarthritis, rheumatoid arthritis and tumor invasion. The crystal structures of native truncated stromelysin (the catalytic domain) and its inhibitor-bound complexes have been solved. The native structure has been refined to 2.0 Å resolution (R-factor 0.21, R-free 0.29). The complexes were formed both from diffusion soaking and co-crystallization, and have been refined to 2.3 Å resolution (R-factor and R-free 0.19 and 0.31 for the soaked crystals; 0.19 and 0.33 for the co-crystals, respectively). The native and the inhibitor-soaked crystals are in space group $P2_12_12_1$, with two molecules (A and B) in the asymmetric unit, associating as a dimer. The inhibitor-bound co-crystals, also existing as a dimer, are in space group C2, one molecule per asymmetric unit. There are three α-helices and one twisted, five-strand β-sheet in each molecule with one catalytic Zn^{2+}, one structural Zn^{2+} and three Ca^{2+} ions. The catalytic zinc is ligated to three histidines (H201, H205 and H211), with distances around 2.1-2.2 Å. The fourth and possibly the fifth ligands may come from substrate, inhibitor, another stromelysin molecule or solvent.

The inhibitor binding obtained by soaking or co-crystallization is basically identical. The Pl' portion of the inhibitor binds to a highly hydrophobic pocket. Most residues between the native and the inhibitor-bound complex structures match very well except the inhibitor-binding loop (residues 222-231). The major difference occurs at Y223. In native structure, the side chain of Y223 occupies the position that is occupied by the P1' portion of an inhibitor in the complex. The inhibitor has to push away the Y223 in order to fit into the binding site. Moreover, the inhibitor binding and Y223 movement induce movement of the entire loop. This makes the loop completely different in the native and in the complex. Some residues (Cα) differ by as much as 5 Å. In native stromelysin, a symmetry-related molecule B has its C-terminal (251-255) interacting with the active site of molecule A of the central unit. The last residue, T255, has its carboxylate group ligated to the catalytic zinc, forming two ligand bonds, in a similar manner as an inhibitor. This symmetrical C-terminal-active site interaction occurs for one molecule only. In molecule A, the last four C-terminal residues are disordered as a flexible tail. The situation in the inhibitor-bound complexes is more complicated. In molecule B, only the inhibitor binds to the active site. But in molecule A, inhibitor competes with the C-terminal of a symmetry-related molecule B.