PS04.01.33 THE STRUCTURE OF HUMAN CATHEPSIN-L AT 2Å RESOLUTION. Richard Pauptit, Alec Tucker*, Simon Weston & Bob Gordon^. Zeneca Pharmaceuticals, Alderley Park, Macclesfield UK. *Pfizer Central Research, Sandwich, Kent, UK ^Jannsen, Antwerp, Belgium.

Cathepsin L is a thiol protease which plays an important role in protein degradation within the lysozymes and in the extracellular matrix, and is a leading target in the search for inhibitors of bone resorption. Human cathepsin L has been cloned, expressed in E.coli, purified, crystallised, and its three-dimensional structure has been determined by molecular replacement methods using papain as a trial model. Purification was problematic, but once 0.1 mm crystals were obtained structure solution progressed rapidly. The crystals froze readily since they were grown in 30% MPD. They are $P2_1$ with a=46.23, b=49.38, c=49.25 Å and beta=113.45 deg. The overall structure is very similar to other thiol proteases; in particular, the active site is similar to papain suggesting equivalent mechanism of action. However, there are differences in detail of chain tracing. Close to the active site in the region where inhibitors bind there are differences in the arrangement of hydrohobic sidechains, suggesting the design of specific inhibitors is feasible. The active site thiol group is oxidised, and the active site cleft contains an unidentified peptide-like fragment. While cathepsin L is biosynthesised as a pre-pro form of 333 residues and loses a signal sequence to give a 316 residue pro-form which is cleaved at low pH to yield a 219 residue form, the crystals contain a further-cleaved 2-chain form of 175 and 42 residues which are covalently linked through a disulfide bond. The solvent contains some MPD molecules, over 150 water molecules, and, apparently, cacodylate ions. The structure was refined to R=18.4% at 2Å resolution.

PS04.01.34 CRYSTAL STRUCTURE OF HUMAN FACTOR XIII BOUND TO YTTERBIUM: PROBING CALCIUM-BINDING. Brian A. Fox, Paul D. Bishop, Ronald E. Stenkamp, David C. Teller,and Vivien C. Yee, Departments of Biochemistry and Biological Structure, University of Washington, Seattle, WA, 98195 and ZymoGenetics Inc, Seattle, WA, 98102

We have determined the structure of the recombinant human blood coagulation protein, factor XIII, with ytterbium bound in its calcium binding site. In the final step of blood clotting, activated factor XIIIa crosslinks polymerized fibrin and renders it mechanically and proteolytically stable. The factor XIII active site bears a striking resemblance to that of the cysteine proteases. This structural similarity supports a transglutaminase mechanism based on the reverse of the proteolysis carried out by the papain family of cysteine proteases.

Factor XIII is activated by thrombin cleavage in the presence of physiological concentrations of calcium. In the absence of proteolytic cleavage, factor XIII can be activated by high, non-physiological levels of calcium, in the upper millimolar range. In efforts to identify the protein ligands necessary for calcium binding, and to understand the role of calcium in the activation of the enzyme, we have determined the structure of factor XIII bound to the calcium analog ytterbium.

A crystal of factor XIII zymogen soaked in 2mM $YbCl_3$ for 48 hours was used for data collection. The unit cell dimensions for this $P2_1$ crystal were a=101.06 Å , b=72.39 Å, c=135.99 Å, and beta=106.09 deg. The structure is being refined against diffraction data from 10.0-2.5 Å resolution. This work has been funded by NIH grant HL-50355.

PS04.01.35 THREE DIMENSIONAL STRUCTURE OF A PLANT CARDOON ASPARTIC PROTEINASE. C. Frazco, I. Bento, R. Coelho, J. Costa, ITQB, Apt. 127, 2780-Oeiras, Portugal, C. Faro, P. Vermssimo, E. Pires, Dep. Bioqummica, Fac. Cijncias e Tecnologia, Univ. Coimbra, 3000 Coimbra, Portugal, J. Cooper, Lab. Molecular Biology, Dept. Crystallography, Birkbeck College, Malet Street, London WC1E 7HX, UK, Z. Dauter, K. Wilson, EMBL, c/o DESY, Notkestrasse 85, 22603 Hamburg, Germany, and M. A. Carrondo, ITQB and IST, Apt. 127, 2780-Oeiras, Portugal

Although aspartic proteinases are widely dispersed in the vegetal kingdom, their structures and biological functions are less well characterized than those of animal, microbial and viral aspartic proteinases. A native, glycosylated aspartic proteinase, cardosine A, isolated from the cardoon flower (1), which is traditionally used in Portugal for cheese making, was crystallized and its structure solved by the molecular replacement method, using the structure of human cathepsin D (2) as a model. Cardosine A is composed of two polypeptide chains and is expressed with the ca. 100 residues insertion, also found in other vegetal aspartic proteinases. However, its mature native form does not show the insertion anymore.

Crystals were obtained in space goup C2, a=119.0, b=88.1, c=82.3 Å, β=104.4 , with two molecules in the asymmetric unit. Syncrotron diffraction data to 2.9 E resolution were collected at the EMBL outstation, Hamburg. Refinement is in progress, with an actual of R=29.4% and Rfree=35.0%.

(1) P. Vermssimo et al. (1996) Eur. J. Biochem., in press.
(2) E.T. Baldwin et al. (1993) Proc. Nat. Acad. Sci. USA 90, 6796-6800.

PS04.01.36 THE 2.5Å CRYSTAL STRUCTURE OF PENICILLIN G ACYLASE FROM A MUTANT FORM OF *P. RETTGERI*. Michael A. McDonough[1], Herbert E. Klei[2], Gayle K. Schulte[3], and Judith A. Kelly[1], [1]Department of Molecular and Cell Biology, and Institute of Materials Science, University of Connecticut, Storrs, CT 06269-3125, [2]Bristol-Myers Squibb, Princeton, NJ 08543-4000, [3]Pfizer Central Research, Groton, CT 06340

Penicillin G acylase (EC 3.5.1.11) catalyzes the hydrolysis of the inexpensive antibiotic penicillin G into phenylacetic acid (PAA) and 6-aminopenicillanic acid (6-APA). The 6-APA is used commercially in the synthesis of new penicillins with broader inhibitory profiles. A mutant form of the acylase from *P. rettgeri* with an altered substrate profile was crystallized in space group $P6_122$ (a=b=140.5Å, c=200.2Å) from 50mM potassium phosphate, pH 7.5, 45 percent ammonium sulfate and 10 to 15 percent v/v glycerol (Klei, H. E. *et al.*, 1995, *Protein Science* **4**, 433-441). Native and heavy atom data have been collected to 2.5Å. The native data are 96 percent complete, and the Rsym is 11 percent on I. Data were collected at 100K. The structure has been solved by molecular replacement using AMoRe with the *E. coli* penicillin acylase as a model(Duggleby, H. J. *et al.*, 1995, *Nature* **373**, 264-268). The *P. rettgeri* enzyme is a heterodimer with a 23.7kDa alpha subunit, and a 62.2 kDa beta subunit with the reactive serine residue being the first residue of the beta chain. All but ten residues at the carboxyl terminus of the alpha chain have been modeled, and the native structure is being refined using X-PLOR. Currently, the R factor is 0.24, and the rms deviations in bond distances and angles are 0.011Å and 1.6° respectively. Addition of solvent molecules to the model is in progress.

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