02.1-36 CRYSTALLISATION AND HEAVY ATOM DERIVATIVES FOR CHOLESTEROL OXIDASE. By A. Vrielink, D.M. Blow, Imperial College of Science and Technology, London SW7 2AZ, U.K.

Cholesterol oxidase (3β-hydroxysteroid oxidase) is an extracellular steroid binding protein which catalyzes the oxidation of 5β hydroxyl groups in steroids with a trans A:B ring junction. The protein is used in clinical assays for the determination of serum cholesterol concentration. It has been isolated from Brevibacterium sterolicum (Uwajima, T., Yagi, H., Terada, O., Agricultural Biological Chemistry, 1974, 38, 1149) as a monomer of molecular weight 32,500 and contains one mole of FAD per mole of protein.
Orthorhombic needles of this protein have been obtained by vapour diffusion. The space group is $P2_12_12_1$ and the cell dimensions are a=56.7 Å, b=85.4 Å, c=88.1 Å. The crystals diffract to better than 2.4 Å resolution.
Data have been collected to 2.7 Å resolution using synchrotron radiation. Data collection of heavy atom derivatives is in progress. A readily interpretable difference Patterson is obtained with 1.0mM p-chloromercuric benzoate (PCMB). Other heavy atoms are known to give significant differences.

02.1-37 PAPAIN-HEPTAPEPTIDE COMPLEXES. By F. R. Ahmed, S. Hasnain, C.P. Huber, A.C. Storer, K.I. Varughese, and G. Willick, Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada K1A 0R6.

The enzyme papain is believed to bind seven consecutive residues of a protein substrate in its active site during hydrolysis. Available information about papain-substrate binding concerns two of the seven subsites. In an attempt to extend this knowledge to other subsites we have prepared complexes of papain with two heptapeptides. Both are of the type ala-ala-phe-cys-val-ala-ala; one has the natural L-cys residue and the other has D-cys. The two complexes crystallize in several different crystal forms.

Diffractometer data to 2.7 Å resolution have been measured for an orthorhombic form of the D-cys complex which is isomorphous with the D form of native papain. Refined coordinates of the latter [Priestle et al., Acta Crystallogr. A40, C17 (1984), kindly provided by Dr. Priestle] have been used to calculate difference maps of this complex. Model fitting is in progress on a PS300 graphics system, and preliminary results suggest that the heptapeptide may not be in a fully extended conformation.

CAD-4 data to 2.3 Å resolution have also been obtained for a monoclinic form of the L-cys complex which is isomorphous with the B form of native papain. Results will also be presented for this complex.

02.1-38 LYSINE/FIBRIN BINDING SITES OF KRINGLES MODELLED AFTER THE STRUCTURE OF PROTHROMBIN FRAGMENT 1. By A. Tulinsky[a], C.H. Park[a], B. Mao[c] and M. Llinas[b], Departments of Chemistry, Michigan State University, E. Lansing, MI[a] and Carnegie Mellon University, Pittsburgh, PA[b] and The Upjohn Company, Kalamazoo, MI[c].

Kringle (K) sequences are highly conserved three disulfide, triple loop entities known to occur 11 different times in 5 different proteins of blood coagulation and fibrinolysis where they appear to display recognition and impart specificity. K2 of prothrombin (PT) binds to the heavy chain of membrane bound Factor Va in the formation of the prothrombinase complex; K1 and K4 of plasminogen (Plgen) bind to fibrin, Lys and ω-amino-carboxylic acids, particularly ε-amino-caproic acid (ACA) and K5 of Plgen binds to benzamidine; K2 of tissue-Plgen activator (tPA) also binds to fibrin.

Residues Asp56 and Arg71 of K4 of Plgen have been implicated in the Lys binding site of that kringle. We have modelled this region using the 2.8A resolution three dimensional structure of K1 of PT, molecular modelling methods, published and unpublished 300 and 600 MHz proton NMR observations and energy minimization procedures with CHARMM. The K4 structure was generated from K1 of PT using positions of common atoms as guide coordinates and was then modulated to conform to NMR implications. This approximate model was improved with a mild energy refinement to optimize van der Waals and other contacts but was fairly constrained to conform to the X-ray NMR model. Nonetheless, very significant improvements in energy occurred and structures and energies, with and without ACA ligand, were compared. Binding of ACA to K1 and K4 of Plgen induced only minor conformational changes in the calculations in agreement with NMR observations.

Both K4 and K1 Lys binding sites are surrounded by a dipolar surface and the binding in the two sites appears to be different. The latter is probably related to the difference in binding constants displayed by these kringles. The modelled binding site of K4 suggests that it might be kinetically faster, especially with bulkier ligands. Lastly, K2 of tPA appears to achieve the cationic center of the binding site in a different manner from that of K1 and K4 of Plgen.

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