02.1-24 FUNCTIONAL IMPLICATIONS OF CARBOXYPEPTIDASE A STRUCTURES IN NATIVE AND LIGANDED FORMS. By D. C. Rees, M. Lewis, and W. N. Lipscomb, Gibbs Chemical Laboratory, Harvard University, Cambridge, MA 02138 USA

The structure of the metalloenzyme carboxypeptidase A (CPA) has been refined at 1.5A by the restrained least-squares algorithm of Hendrickson-Konnert to a crystallographic R-factor of 0.175. The structures of complexes of CPA with dipeptides, substrate analogues, inhibitors and a small protein inhibitor of CPA have also been determined at resolutions between 2.0 and 2.8A. Comparison of these structures has resulted in the following observations of particular relevance for the catalytic mechanism: (a) the coordination number for the zinc is five in the native enzyme, and five or six in the complexes, (b) binding modes of compounds exhibiting a wide range of kinetic behavior towards CPA are described, (c) solvent structure in the native enzyme has been characterized, as well as solvation changes accompanying ligand binding. The relationship of these results to the catalytic mechanism, and to inhibitory and productive binding modes to CPA will be discussed.

02.1-25 TOWARD AN ASPARTIC PROTEASE MECHANISM OF ACTION: INHIBITOR BINDING TO THE ASPARTIC PROTEASE FROM RHIZOPUS CHINENSIS AT 2.5 Å. By R. Bott, E. Subramanian and D. R. Davies, LMB, NIAMDD, NIH, Bethesda, MD

The aspartic proteases represent a large class of enzymes that includes the human enzymes: renin, pepsin and cathepsin D. The mechanism of action and the structural basis of substrate specificity for these enzymes has yet to be determined. The aspartic protease from Rhizopus chinensis crystallizes in the space group $P2_12_12_1$, with a=60.33, b=60.66 and c=107.0 Å. The three-dimensional crystal structure has been refined at 2.5 Å using restrained least squares to an agreement factor R=26.2%. The data is now being extended to 1.8 Å. This structure is remarkably similar to that of the three other aspartic proteases; porcine pepsin and the fungal proteases from Pencillium janthinellum and Endothia parasitica. This structural similarity along with the biochemical similarities: two active aspartic acids, specificity for peptide bonds bracketed by large hydrophobic residues, and the existence of a group of universal aspartic protease inhibitors, suggests a highly conserved class of enzymes.

All aspartic proteases are inhibited by pepstatin, a hexapeptide isolated from streptomyces. Pepstatin has an unusually high binding constant (K_i=1x10^{-10}M for pepsin) and this has led to speculation that by virtue of an unusual residue, statine (3-hydroxy-4-amino-6-methyl-heptanoic acid), pepstatin may approach a transition state analog. We have prepared crystals of the protease with pepstatin bound, both by soaking and co-crystallization, with the same result; pepstatin was bound at the active site with the 3-hydroxyl of statine within hydrogen bonding distance and straddling the two active aspartate carboxyls. In addition, the hydrophobic side chains of pepstatin interact at several apparent substrate binding sites. The pepstatin binding suggested a mode of substrate binding which has been tested by model building. These studies have led us to propose a mechanism of action, based on general acid-base catalysis with no covalent intermediates.

02.1-26 HIGH RESOLUTION X-RAY STUDIES OF THE EVOLUTION, SPECIFICITY AND CATALYTIC MECHANISM OF ACID PROTEINASES. By L. H. Pearl, H. B. Jones, G. L. Taylor and T. L. Blundell, Department of Crystallography, Birkbeck College, Malet Street, London WC1E 7HX, UK.

The acid or aspartate proteinases are so called because they have a pH optimum for catalytic activity in the range 1.5 - 5.0 and two aspartates in the active site. They include digestive enzymes such as chymosin and pepsin, some of which are of commercial interest as rennet in the dairy industry, and renin and cathepsin-D which are implicated in hypertension and inflammation respectively.

X-ray studies in several laboratories have confirmed the existence of homologous bilobal structures with extended active site clefts for both microbial and mammalian enzymes. The structure of endothia pepsin refined at 2.5Å using electron density modification and restrained least-squares techniques is shown in the Figure. Medium resolution X-ray studies of mucor chymosin indicate a similar structure and difference Fouriers of crystals of a lactyl pepstatin define the binding site for this inhibitor. Refinement of these structures is proceeding to high resolution to further detail the environment of Asp 32 in the active site which gives rise to its unusual pK and to describe any conformational differences on inhibitor binding.

The microbial enzymes will be compared with pepsin and chymosin for which X-ray studies using synchrotron radiation, molecular replacement and computer graphics are carried out in our laboratory.