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.

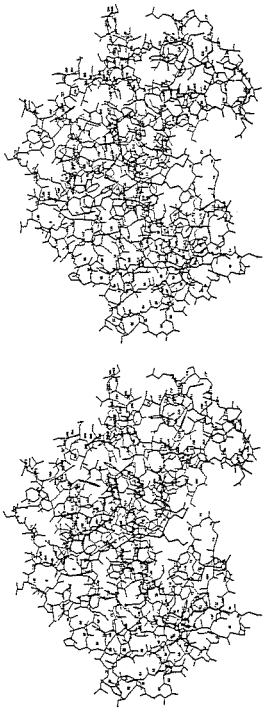
All aspartic proteases are inhibited by pepstatin, a hexapeptide isolated from streptomyces. Pepstatin has an unusually high binding constant (K_i=1x10^{-10}M for pepsin) and this has led to speculation that by virtue of an unusual residue, statine (3-hydroxy-4-amino-6-methyl-heptanoic acid), pepstatin 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.



The structure of endothia pepsin

02.1-27 CRYSTAL STRUCTURE OF PORCINE PEPSINOGEN. By S.N. Rao, S.N. Koszelak and J.A. Hartsuck, Oklahoma Medical Research Foundation, Oklahoma, OK 73104

Porcine pepsinogen, molecular weight 39,630, crystallizes from lithium sulfate solutions of pH 6.0 in space group C2 with a=104.8, b=43.1, c=88.4 Å and β=91.4°. Diffraction data have been collected by diffractometry to 5.4 Å resolution for the native crystal and for a K_2PtCl_4 derivative. Molecular replacement techniques have placed a model in the pepsinogen crystal space. The model was derived from the penicillopepsin atomic coordinates and corresponds to 64% of the pepsinogen molecule. Translation parameters were determined by a Tl translation function and were confirmed by packing analysis. For the platinum derivative, five sites were identified by direct methods on normalized structure factor differences. A difference Fourier map phased by molecular replacement yielded the same five platinum locations. Phasing of the x-ray data was achieved by a combination of the isomorphous replacement and molecular replacement information.

Pepsinogen differs from pepsin and the other active acid proteases in that it has 44 more residues at the amino terminal end of the protease chain. This activation peptide is spontaneously removed when pepsinogen is exposed to low pH. Peaks in the pepsinogen map which are not due to the model can be attributed to the activation peptide. Our crystal structure shows that the peptide wraps around the pepsin molecule and contains little or no secondary structure. It blocks access to the pepsin active site even though it is not located in the active site cleft. A conformation change to place the activation peptide in contact with the pepsin active site must occur as activation takes place.

Photographic data at 2.5 Å resolution are being collected and results of those experiments will be discussed.

02.1-28 A STRUCTURAL STUDY OF THE ELASTASE-ELASTATINAL COMPLEX AT 2.5Å RESOLUTION. L. Presta, G. Cole, and E. Meyer, Biographics Laboratory, Department of Biochem. and Biophys., Texas A&M University, College Station, Texas 77843 U.S.A.

Elastase is a serine protease possessing an extended substrate binding site. An understanding of the energetics of recognition and binding may be sought only after resolution of the binding interaction into discrete components (H-bonds, hydrophobic associations, etc.). A structural study of elastase + substrate would help illuminate the several structural aspects of these interactions.

Elastatinal is a naturally occurring, highly specific inhibitor of porcine and human elastase, resembling a tetrapeptide. The hemiacetal complex formed with Ser 195 at the active site gives a geometry which should closely resemble the tetrahedral intermediate involved in normal substrate hydrolysis. Thus, the structure of the elastase + elastatinal complex may indicate the conformations and interations involved in extended substrate binding, which could be useful to those who seek to design inhibitors of disease-related elastases.

A set of 11000 diffractometer data was collected from a crystal soaked first in elastatinal (pH5), then in His buffer(pH7). Initial phases were derived from our 1.7A elastase + methanol structure. Refinement using EREF (Jack-Levitt-Deisenhofer) is in progress. Results will be presented in pictorial form.

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02.1-29 CRYSTAL STRUCTURE OF STREPTOMYCES ERYTHRAEUS LYSOZYME. By R. Sarma, S. Harada, T. Ikenaka and S. Hara, Department of Biochemistry, State University of New York, Stony Brook, N.Y. 11794, U.S.A. and Department of Chemistry, Osaka University, Toyonaka, Osaka 560, Japan.

Streptomyces erythraeus lysozyme found in its culture broth differs from other mammalian lysozymes in its molecular weight, amino acid composition and specificity. The enzyme has about 185 residues in its primary structure and has a molecular weight of about 20,000 daltons.

The three-dimensional structure of the bacterial lysozyme has been determined using x-ray crystallographic data to 2.9A resolution and refined using 2.5A resolution. The tertiary structure is different from that of hen egg white lysozyme or the T4-phage lysozyme.

The primary structure of the bacterial lysozyme is only partially determined, it resembles the primary structure of lysozyme from the fungus chalaropsis. This similarity has been used to speculate on the structure of the active site of the enzyme.