02.X-01 THE IMPORTANCE OF REFINED STRUCTURES TO THE UNDERSTANDING OF ENZYME FUNCTION. By M.N.G. James and A.R. Sielecki, MRC Group in Protein Structure and Function, Biochemistry Department, University of Alberta, Edmonton, AB, T6G 2H7, Canada.

It is generally accepted that enzymes catalyze reactions by lowering the free energy of ("stabilizing") transition states that occur along the pathway from substrates to products. There are a number of hypotheses that attempt to provide explanations for the large catalytic rate enhancements by enzymes (e.g. strain, induced fit, etc.). Many of these proposals rely heavily on a knowledge of the stereochemistry of groups on the enzyme that are involved with the covalency changes. It has long been recognized that a full description of an enzymatic mechanism will require that the determination of atomic coordinates be better than 0.1 Å (Levitt, J. Mol. Biol. 82:393 [1974]).

X-ray crystal structure analysis offers the possibility of providing the geometry at the active site of the enzyme, but with varying degrees of accuracy. Until recently, interpretations of enzyme structure have been from MIR phased electron density maps. However, there are now several powerful algorithms that have been successful in protein structure refinement. We have used the Hendrickson-Konnert algorithm to refine the crystal structures of 4 enzymes, SGPA, SGPB, α-lytic protease and penicillopepsin. The first three are bacterial serine proteases of the α-chymotrypsin family, the fourth is an aspartyl protease.

In the case of SGPA, interpretation of difference electron density maps of the enzyme in complexation with several oligopeptide substrates, led to erroneous deductions of the binding for two major reasons: incomplete refinement (inaccurate phases) and distortion of the difference density for the complex due to displacement of bound solvent in the active site. These erroneous conclusions (Acta Cryst. A34:S56 [1978], collected abstracts) have been clarified and revised after extensive continued refinement of native SGPA (1.5 Å resolution, 17,194 reflections, R = 0.126 for a molecular model including 23 solvent sites) in addition to parallel refinements at 1.8 Å resolution of the complexes of SGPA with two tetrapeptides and a tetrapeptide aldehyde inhibitor (AcProAlaProPheCHO) (James, Sielecki, Brayer, Delbaere & Bauer, J. Mol. Biol. 144:43 [1980]). The two tetrapeptides, AcProAlaProPhe and AcProAlaProTyr bind to SGPA as product carboxylates. The refined distance of O^{γ} Ser195 to the carboxylate carbon atom is 2.6 Å; the carboxyl group is planar. A partial bond exists between O^{γ} and this carbon atom. The least-squares refinement has confirmed that the tetrapeptide aldehyde inhibits SGPA by forming a covalent tetrahedral hemiacetal bond with O^{γ} Ser195 of refined length 1.73 Å. The results of these several refinements have important consequences for the interpretation of the serine protease mechanism.

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02.X-02 CONFORMATIONAL CHANGES IN ENZYME CATALYSIS. by Carl-Ivar Brändén, Department of Chemistry and Molecular Biology, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden.

Large substrate-induced conformational changes of a similar nature have recently been established crystallographically for two enzymes, liver alcohol dehydrogenase and hexokinase, and inferred for a third enzyme, phosphoglycerate kinase. The subunits of these enzymes are all typical two-domain structures with the active site in a crevice between the domains. The conformational change consists of a rotation of one domain with respect to the other such that the crevice becomes more narrow after substrate binding and the reactive parts of the substrate is buried in the interior of the subunit.

Refined structures to medium resolution of the two conformations as well as the amino acid sequence is available for alcohol dehydrogenase. Details of the conformational differences will be described for this enzyme as well as the effect on the geometry and accessibility of the active site.

The conformational change is induced by coenzyme binding in alcohol dehydrogenase. We have studied crystallographically the binding of different coenzyme analogues and inhibitor molecules in order to understand the factors that stabilize these two different conformations. These studies will be described and discussed.

02.X-03 CONFORMATIONAL CHANGES DURING CATALYSIS BY MITOCHONDRIAL ASPARTATE AMINOTRANSFERASE. By J.N. Jansonius, G. Eichele, G.C. Ford, J.F. Kirsch, D. Picot, C. Thaller and M.G. Vincent, Biozentrum, CH-4056 Basel, Switzerland; P. Christen, H. Gehring and H. Kirsten, Biochemisches Institut, Univ. of Zürich, CH-8032 Zürich, Switzerland.

This pyridoxal-P enzyme transfers an amino group from aspartate or glutamate to oxaloacetate or α-keto glutarate. Its spatial structure is known to 2.8 Å resolution. The topography of the active site requires a rotation of the coenzyme during catalysis. Such a coenzyme reorientation is indeed observed in difference electron density maps of analogs of the covalent intermediates of catalysis. A second conformational change observed in crystallographic studies of dicarboxylic acid inhibitor derivatives consists of a shift of an entire domain (the "small" domain) over about 3 Å towards the active site.
A proton donor/acceptor function is necessary in the reaction mechanism. The only candidates in the active site are Lys 258 and Tyr 70* (other subunit). Their position is compatible with the stereo-chemistry of the transamination reaction and the coenzyme movements which we propose.