

01.5-6 RESTRAINED LEAST SQUARES REFINEMENT OF CHYMOTRYPSIN DIMER AT 1.65 Å RESOLUTION. By A. Tulinsky and R.A. Blevins, Department of Chemistry, Michigan State University, E. Lansing, MI 48824.

The structures of the two independent molecules of α -CHT are being refined using Hendrickson's PROLSQ program. Intensity data at 1.65 Å resolution were measured using only one crystal at low X-ray power (10% decay). Of the 50,912 reflections to this limit, 28,617 were observed (56%).

The refinement was initiated with an exact 2-fold structure using the coordinates of Birktoft and Blow (J.Mol.Biol., **68**, 187 (1972)) which were obtained from a model fitted to a 2-fold averaged electron density map. The trial structure calculated well at 3.0 Å resolution (conventional $R = 0.364$). After 17 cycles of refinement, including individual standard deviations of structure amplitudes based upon intensity statistics, R decreased to 0.227. An asymmetrical dimer structure developed rapidly during this refinement with most of the asymmetry confined to side chains. The $(2F_o - F_c)$ and $(F_o - F_c)$ maps were examined and the structure was adjusted with the aid of FRODO. The resolution was then extended to 2.5 Å; R increased to 0.320 but decreased steadily to 0.203 after 23 more cycles of refinement. The R -factor of the 3.0 Å range also decreased very significantly with the higher resolution refinement (to 0.186). The resolution was then increased to 2.0 Å; R increased to 0.282 but after 5 cycles including individual restrained isotropic B -values for the first time, R decreased to 0.250. The above maps at 2.0 Å resolution were examined with FRODO and the model was adjusted again. Comparison of the (8.0 - 2.0 Å) and (5.0 - 2.0 Å) $(F_o - F_c)$ maps located 63 highly probable solvent molecules. These were included into calculations as water; they reduced R to 0.244 and the refinement is continuing from here.

At this point, the rms asymmetry is 0.41 Å for main chain atoms and 1.02 Å for side chains with total rms shifts of 0.47 Å and 0.92 Å, respectively, from the trial structure. Deviations from the idealized structure are small (rms distance = 0.03, angle = 0.06, plane = 0.01, vdW = 0.3 Å) and the Ramachandran angles of both molecules are excellent. The side chains of 43 residues have at least one atom with an asymmetry > 1.0 Å. The fidelity of the main chain remains intact showing only 2 residues with asymmetry > 1.0 Å. Most of the asymmetry resides in the configurations and conformations of side chains on the surface and the dimer interface regions (rms asymmetry = 0.77 and 0.84 Å, respectively) which reflects the overall flexible adaptability of protein molecules. Although the catalytic sites of the independent molecules are similar, they are not exact. In addition, less asymmetry is present in the specificity site folding domain compared to that of the other folding domain. Finally, the region from 70-80 (calcium binding loop of trypsin) is disordered in one molecule but not the other.

The trial structure is also being refined restraining it to conform to non-crystallographic symmetry. Preliminary results show that with tight restraints (0.05 Å), agreement deteriorates with increased resolution while more moderate restraints (0.5 Å) at lower resolution (3.0 Å) essentially lead to a symmetry unrestrained refinement. This work is also continuing. The research was supported by grant GM21225.

01.5-7 CRYSTALLOGRAPHIC REFINEMENT OF MITOCHONDRIAL ASPARTATE AMINOTRANSFERASE. By M.G. Vincent, D. Picot, J. Priestle and J.N. Janssonius, Biozentrum, University of Basel, Switzerland and G.C. Ford, Department of Biochemistry, University of Sheffield, U.K.

Aspartate aminotransferase is a pyridoxal-phosphate dependent enzyme functioning in amino acid metabolism. It is a dimer of $M_r \sim 90000$ and catalyses the reaction aspartate + 2-oxo-glutarate \rightleftharpoons glutamate + oxaloacetate. During the catalytic cycle the enzyme shuttles between its pyridoxal and pyridoxamine forms. The structures of both these forms from chicken heart mitochondria have been determined to 2.8 Å resolution (Ford *et al.*, PNAS (1980) **77**, 2559). On binding of substrates the enzyme undergoes a conformational change which effectively locks the substrate in the active site pocket. In order to gain insight into the processes governing this conformational change, the enzyme has been cocrystallized with the competitive inhibitor maleate. The structure of this complex has recently been solved at 2.8 Å resolution using phase-combination techniques. Parallel refinements of the pyridoxal form of the enzyme (ultimately at 1.9 Å) and of its complex with maleate (ultimately at 2.3 Å) are currently underway. We will report on the progress of these refinements.

01.5-8 RESTRAINED LEAST-SQUARES REFINEMENT OF THE SULPHYDRYL PROTEASE PAPAIN TO 2.0 Å. By J.P. Priestle, G.C. Ford, M. Glor, E.L. Mehler, J.D.G. Smit, C. Thaller and J.N. Janssonius, Biozentrum, University of Basel, Switzerland.

The structure of an inhibited derivative of the sulphhydryl protease papain, 2-hydroxyethylthiopapain, has been solved and refined by a restrained least-squares technique to a conventional R -factor of 14.8% for the 13,472 reflections between 10 and 2.0 Å. The final structure has a r.m.s. deviation from ideality of 0.014 Å and 2.2° for bond lengths and bond angles, respectively. 172 solvent molecules have been located. The r.m.s. movement of atoms during refinement was 0.9 Å with several side chains on the surface of the molecule having to be rebuilt by hand. Two peptide bonds were manually flipped through 180° as well. Without manual intervention, peptide rotations of up to 60° were seen. There is one cis-proline residue. A comparison with the refined structure of actinidin, a related sulphhydryl protease with 48% sequence homology, demonstrates amazing conservation of tertiary structure with a r.m.s. deviation of 0.5 Å for 94% of the corresponding C_α atoms in the two structures. 67% of the corresponding ϕ and ψ conformation angles are within 10° of one another. The oxidized form of papain has been refined by Drenth *et al.* (I.G. Kamphuis, Thesis Groningen, 1983). A comparison with that structure will be carried out.