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

The structures of the two independent molecules of CHYMOTRYPSIN were refined using Hendrickson's PROLSQ program. Intensity data at 1.65 Å resolution were measured using only one crystal at low X-ray power and calculated with the program O. After 23 cycles of refinement, the R-factor of 0.282 but after 5 cycles 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 (2Fo - Fc) and (Fo - Fc) maps were examined and the structure was adjusted with the aid of non-crystallographic symmetry methods. After 2 cycles, 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 resolution data 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 average structure (Fo - Fc) 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 r.m.s. asymmetry is 0.4 Å for main chain atoms and 1.0 Å for side chains with total rms shifts of 0.47 Å and 0.2 Å, respectively, from the trial structure. Deviations from the idealized structure are small (bond lengths = 0.06, plane = 0.01, vdW = 0.3 Å). The 2.8 Å resolution data were examined with SIR2559. The structures of both these forms from chicken heart mitochondria have been determined to 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.


The structure of an inhibited derivative of the sulphhydryl protease papain, 2-hydroxetylthiopapain, has been solved and refined by a restrained least-squares technique to a conventional R-factor of 0.094 and an r.m.s. deviation of 0.1 Å for bond lengths and bond angles, respectively. 172 protein 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 488 sequence homology, demonstrates amazing conservation of tertiary structure with a r.m.s. deviation of 0.5 Å for 94% of the corresponding Cα atoms in the two structures. 57% of the corresponding α and β 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.