01. DETERMINATION OF MACROMOLECULAR STRUCTURES

01.5-8 RESTRAINED LEAST SQUARES REFINEMENT OF CHEMOTAXIN DIMER AT 1.65 Å RESOLUTION. By A. Tallarita and R.A. Blevins, Department of Chemistry, Michigan State University, E. Lansing, MI 48824.

The structures of the two independent molecules of the complex 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 (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 the R-factor of the 3.0 Å range also decreased very significantly with the higher resolution refinement (to 0.186). The trial structure is also being refined restricting the coordinates of Birktoft and Blow (calcium binding loop of trypsin) is disordered in one molecule. 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 (calculus binding loop of trypsin) is disordered in one molecule but not the other.

At this point, the rms asymmetry is 0.41 Å for main chain atoms and 1.0 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 distances < 0.03 Å, angles < 0.06, plane = 0.01 Å, 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 of side chains on the surface and the dimer interface regions (rms asymmetry = 0.77 Å and 0.84 Å, respectively) which reflects the overall flexibility of the 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 (calculus 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 GM0125.