02.1-58 THE DETERMINATION OF THE THREE-DIMENSIONAL STRUCTURE OF TRICHOSANTHIN AT 4 Å RESOLUTION. Trichosanthin Structure Research Group, Chinese Academy of Sciences, organized by Shanghai Institute of Organic Chemistry (Xia Zongxiang, Tian Gengyuan), Fujian Institute of Research on the Structure of Matter (Pan Kezhen, Zhang Yongmao), and Institute of Biophysics (Dong Yicheng, Chen Shizhi).

Trichosanthin, a Chinese herb medicine, is a plant protein extracted from Trichosanthes kirilowii Maxim, Cucurbitacecae. After batch-wise precipitation from acetone, the purified product has a molecular weight of 24,000 and contains 217 amino acid residues. Single crystals of trichosanthin suitable for diffraction work have been grown successfully by using the method of equilibrium dialysis. The crystals are monoclinic, belong to the space group C2 and have the following cell dimensions $a = 75.64$, $b = 75.52$, $c = 88.85$ Å, $\beta = 99.51^\circ$ with 8 trichosanthin molecules in the unit cell. Each asymmetric unit contains two molecules. Two heavy-atom derivatives UO_2Ac_2 and $K_2Pt(NO_2)_4$ were obtained by soaking. Diffraction data at 4 Å resolution were collected with the native crystal as well as with the two derivatives so obtained. The positions of the heavy atoms were then determined by using Patterson functions and refined by least-squares method. On the basis of the joint probability of the isormorphous replacement and the anomalous dispersion for these two heavy-atom derivatives, it has been possible to calculate the phases of the native protein. We also have been able to calculate an electron density map at 4 Å resolution. It is believed that there is only one polypeptide chain present, and its trace in the unit cell, the molecular dimensions, as well as the relative orientation between molecules, can be ascertained. Moreover, rotation functions have been calculated, and thus it is possible to find out the idealized non-crystallographic symmetry of the two molecules in an asymmetric unit. Further work at higher resolution is still in progress.

02.2-01 COMPARISON OF THE INDEPENDENT STRUCTURES OF α-CHYMOTRYPSIN DIMER AT 1.8 Å RESOLUTION. By M.A. Frentrup and A. Tulinsky, Department of Chemistry, Michigan State University, East Lansing, Michigan 48824, U.S.A.

α-Chymotrypsin (α-CHT) crystallizes as a dimer at pH 3.5 with 2 molecules/asymmetric unit which show differences and asymmetry in tertiary structure at 2.8 Å resolution. These reside on the surface and in the interface regions of the dimer. A 1.8 Å resolution map of α-CHT has been calculated with MIR 2.8 Å resolution electron density-modified, 1.8 Å resolution extended and refined phase angles (Raghavan, N.V. and Tulinsky, A., Acta Cryst., B35, 1776 (1979)). Thus, the high resolution electron density is unbiased with respect to assumed structure. The Kendrew model of one α-CHT molecule based on a 2.8 Å resolution map required considerable but generally minor adjustment to fit the higher resolution map. The coordinates of the readjusted model were measured with the aid of a surveyor's transit and a cathetometer. The measured coordinates of about 1,800 atoms fit idealized coordinates with a rms of 0.17 Å and an average deviation of 0.15 Å. The latter were then subjected to several cycles of real-space refinement and re-idealization in preparation to further refinement.

The 2nd molecule of α-CHT was fitted to the density by examining the fit of the 1st molecule to the local 2-fold related density of the 2nd molecule, upon which the difference density between the two molecules was additionally superimposed. In regions of insignificant difference density, local 2-fold related coordinates of the 1st molecule were assigned to the 2nd molecule. Otherwise, the model was refitted and new coordinates measured.

The most significant differences between the main chains of the two molecules are in the β-bends, which are located near the surface, and in the dimer interface region, where the molecules cannot exhibit two-fold symmetry for steric reasons because of close contacts about the symmetry axis. The active site region and the specificity binding site, which are in the dimer interface, also show such asymmetry. Side chain conformation is variable not only on the surface but to a lesser extent in the interior, where the hydrophobic side chains seem not to have been "insulated" from surface perturbations but rather, to have responded by a network of minor inter-related side chain adjustments. The main chain in the interior is basically the same in both molecules. We are now in the process of a detailed and exhaustive comparison of the two molecules aimed at uncovering generalities of the asymmetry and principles applicable to other oligomeric systems. We also plan to carry out structural comparisons of the independent molecules with that of a highly refined structure of γ-CHT (G.H. Cohen, E.W. Silverton and D.R. Davies, personal communication).

We are grateful to NIH (GM 21225) for supporting this work.