02.8-06 DIFFERENCE FOURIER REFINEMENT OF CRYSTAL STRUCTURE OF INSULIN. By Liang Dong-cai, Wang Da-cheng, Dai Jin-bi and Chang Wen-rui, Institute of Biophysics, Academia Sinica, Beijing, China.

We obtained MIR maps of rhombohedral crystals of pig insulin at 2.5 Å and 1.8 Å resolution and determined the three-dimensional structure of this hormone protein in 1971 and 1973. Recently, the structure was refined at 1.8 Å resolution by the difference-Fourier technique. A set of programs with automatic analysis of the shifts has been set up and used in the refinement. After initial refinement, the measured coordinates of the insulin model gave an R-value of 0.388. At the beginning of the refinement, only the protein atoms with overall thermal parameters were put in. After 5 cycles of the refinement, the R factor decreased to 0.275. Then the individual isotropic thermal parameters replaced B. Two more cycles made the R factor decrease to 0.250. From this time on we began to determine the water molecules from the difference Fourier maps and to put them in the refinement. At the 11th cycle of the refinement, the R factor descended to 0.210. During the refinement, the bond distances and angles were continuously computed in order to check the deviations from standard stereochemistry and adjustments were made to maintain reasonable stereochemistry of the molecule.

After the refinement, the specified structure of the protein was improved and a number of water molecules in the crystal were determined. Water molecules are shown clearly in the refined maps. The structure and probable actions of water molecules in the crystal of insulin will be discussed.

02.8-07 WATER STRUCTURE IN RHOMBOHEDRAL 2 ZINC INSULIN CRYSTAL AT 1.1Å RESOLUTION. By K. Sakabe, K. Sasaki and N. Sakabe, Faculty of Science, Nagoya University, Chikusa, Nagoya, 464 Japan.

Water molecules are essential in maintaining the structure of biologically active proteins. Rhombohedral 2 zinc insulin crystals contain about 30% of solvent, about 280 molecules per asymmetric unit. The structure of this crystal was refined by a block-diagonal least-squares method with 1.1Å resolution data (to be presented in this meeting). The water molecules are packed mainly both in the large channels penetrating the whole length of the insulin hexamer along the three-fold axis and in the regions surrounded by the hexamers. The flexibility of these water molecules was estimated on the basis of the r.m.s. displacement related to the Bs. The r.m.s. displacements of the main-chain atoms are between 0.3Å and 0.5Å but those of water molecules distribute in the range from 0.4Å to 1.1Å. Water molecules within 3.2Å from insulin oxygen or nitrogen atoms have always a bit higher B value than those atoms to which they are hydrogen-bonded. There are water molecules as zinc ligands. These zinc ions on the 3-fold axis make distorted octahedral coordination with three imidazole groups of B10 His and three water molecules. There are about 150 water sites within 3.5Å from insulin, and about 60 water molecules of them are hydrogen-bonded to peptide backbone NH or CO. These water molecules have rather less flexibility and these hydrogen-bonded water molecules were divided into 6 groups. Generally the direction of water to NH group is more restricted than that to CO group, and there are suitable conformations of peptide to make water bridge between main chain atoms (The definition of the geometry has been published in the book: Water and metal cations in biological systems, 117, (1979), Japan Scientific Societies Press). Most of polar groups in side-chains are hydrogen-bonded to water molecules. We have found some features about the geometry and the flexibility of these waters.

02.8-08 STRUCTURE OF BOUND WATER IN CRYSTALS OF METMYOGLOBIN FROM NEUTRON DIFFRACTION DATA. Raghavan N. V. and Benno P. Schoenborn, Biology Department, Brookhaven National Laboratory, Upton, NY 11973.

Neutron diffraction is a suitable technique for the determination of bound water in protein crystals since the neutron scattering from D_2O is of the same magnitude as the scattering from the atomic constituents of the protein. The location of both the oxygen and deuterium atoms can be obtained leading to a more detailed interpretation of hydrogen bond geometry. The refinement of metmyoglobin data and the determination of bound water will be discussed. Important features of the water stucture will be described and compared to the solvent structure determined by x-ray diffraction.

(Supported by the U.S. Department of Energy)

02.8-09 INTERCALATION OF WATER MOLECULES BETWEEN NUCLEIC ACID BASES: CRYSTAL AND MOLECULAR STRUCTURE OF 6-AZATHYMINE HEMIHYDRATE.* T. Srikrishnan and R. Parthasarathy, Center for Crystallographic Research, Roswell Park Memorial Institute, Buffalo, New York 14263 U.S.A.

The crystal structure of 6-azathymine hemihydrate was studied as part of an ongoing project[1,2] on hydrated bases and nucleosides in which a water molecule is "sandwiched" between nucleic acid bases. Crystals of the title compound, obtained from methanol/water are monoclinic, space group $P2_1/n$ with unit cell parameters: a = 8.861(1), b = 13.177(3), c = 20.662(2)Å, β = 93.35 (1)°, D_x = 1.50 g.cm^{-3}, D_m = 1.51 g.cm^{-3} and Z = 16. From diffractometer data ($2\theta \leq 165°$ for CuKα) comprising 5127 reflections, the structure was solved by a combination of multisolution technique and refined by full-matrix least squares method to a final R value of 0.064 for 2982 reflections ($I \geq 2\sigma$).

Of the four molecules A, B, C and D, A and D have similar bond distances, angles and hydrogen bonding pattern. Likewise B and C form a pair and have bond distances and angles slightly different from A and D. For example, molecules B and C have d(C4-O4)≥d(C2-O2), similar to the anhydrous form[3], whereas molecules A and D have d(C2-O2)≥d(C4-O4), similar to thymine. Molecules A and D are each donors of two hydrogen bonds N_3-H···O4 and N_1-H···O2 to C and B, respectively, and each receive from C and B, respectively, one hydrogen bond N3-H···O2. The most remarkable feature of this structure is the intercalation of water molecules between nucleic acid bases; OW1 between molecules A and C, and OW2 between B and D, thereby forming two independent "sandwiched" water molecules. In either sandwich, there are no hydrogen bonds from the water oxygen to the bases at its top or bottom; but the water molecule in one sandwich is hydrogen bonded to bases in the adjacent sandwich, adding stability to the packing of the sandwiches.