01.6—1   STRUCTURE OF BOVINE PANCREATIC TRYPSIN INHIBITOR: JOINT REFINEMENT WITH HIGH RESOLUTION X-RAY AND NEUTRON DATA By Jochen Walter and Robert Huber, Max Planck Institut für Biochemie, Martinsried, F.R.G., and Lennart Sjölin, Dept. of Inorganic Chemistry, Chalmers Polytechnic, Göteborg, Sweden, and <u>Alexander Wlodawer</u>, National Bureau of Standards, Washington, DC 20234, and National Institutes of Health, Bethesda, Maryland 20205, U. S. A.

The structure of BPTI has been investigated by a combination of X-ray and neutron diffraction methods. High resolution (0.94 Å X-ray and 1.8 Å neutron) data were measured from crystals of form II (space group $P2_12_12_1$, a=74.1 Å, b=23.4 Å, c=28.9 Å). The structure was initially refined with the X-ray data only, using the refined model of the crystal form I as a starting point. This refinement was terminated when the R factor reached 0.196 for a highly restrained model with anisotropic temperature factors. This model was later used as a starting point for joint neutron/X-ray refinement (Wlodawer and Hendrickson, Acta Cryst. A38, 239, 1982). Joint refinement led to a model with R factors of 0.200 (X-ray) and 0.197 (neutron) with isotropic temperature factors. Most hydrogens whose positions are determined by stereochemistry and also many other hydrogens became clearly visible in the neutron maps. This led to a number of changes, including the reorientation of the side chain of Gln31, and to the reinterpretation of the structure of bound solvent. Positions of the parts of the chain on the C- and N- termini of the molecule were established. Refinement of the neutron data led to the elucidation of the pattern of amide hydrogen exchange (Wlodawer, this volume).
Supported in part by a NATO grant 623/83.

01.6—2   JOINT NEUTRON AND X-RAY STRUCTURAL REFINEMENT OF PORCINE INSULIN By <u>Hugh Savage</u> and Alexander Wlodawer, National Bureau of Standards, Washington, DC 20234, and National Institutes of Health, Bethesda, Maryland 20205, U. S. A.

Neutron diffraction data for porcine insulin were measured using the flat-cone diffractometer equipped with a 1 m long linear detector and situated at the National Bureau of Standard's Reactor. An insulin crystal of ~5mm³ in volume was provided by Dr. Liang Dong-Cai, Academia Sinica, Peking, People's Republic of China. Data extending to 2.2A resolution were measured. The R factor for the scaled data was 5.3% (F's) and in the final data set, 60% of the reflections in the 2.2 to 10.0A shell had significant intensities $(I>1.5\sigma(I))$.
A joint neutron/X-ray restrained least squares refinement (Wlodawer and Hendrickson, Acta Cryst. A38, 238, 1982) has been performed using the 2.2A neutron data and the 1.5A X-ray data obtained from Dr. G. Dodson, University of York, England. At the present stage the R-factor agreement between the model — which includes 1616 protein positions (some disordered) and ~320 solvent sites — and the neutron and X-ray data are 0.216 and 0.177 respectively. Most of the strongly bound solvent appears to occupy similar positions in the neutron and X-ray $2F_o-F_c$ maps, but there are large differences in the more disordered regions away from the protein surface. Further refinement is under way to resolve some of the disordered side chains, the solvent structure and to ascertain the occupancies of the exchangeable hydrogen sites on the molecule.

01.6—3   H/D EXCHANGE AND STRUCTURE OF LYSOZYME BY NEUTRON DIFFRACTION. By <u>S.A. Mason</u>, G.J. McIntyre and G.A. Bentley, Institut Laue-Langevin, 156X, 38042 Grenoble Cédex, France.

Combining the restrained least-squares technique with extensive use of difference maps has substantially improved our 1.4 Å model of deuterated triclinic hen egg-white lysozyme. The degree of exchange has been determined reliably for all but five backbone amide hydrogen atoms, and for certain side-chain hydrogens. Comparison with the degree of exchange calculated by interpolation from [1]H n.m.r. data on lysozyme in solution under similar conditions (pH 4.2, 23 C) suggests that both the average molecular structure and fluctuations about it are similar in the two states. The exchange data will be analysed in terms of geometrical factors, including solvent accessibility and hydrogen bonding. Analysis of the ordered solvent using diffraction data from both deuterated and non-deuterated crystals has improved the clarity of the solvent density maps and allowed us to distinguish several nitrate ions from water molecules.

Modelling of the disorder of Trp62 as several discrete components will be described, as will experience with anisotropic temperature factors.

The good agreement between observed and calculated structure amplitudes (conventional residual = 0.13) even without explicit treatment of disordered solvent led us to incorporate a new target model for the protein backbone into the refinement. The improved geometry, which mostly concerns H/D atoms, was derived from a weighted average of the current lysozyme model. Although it takes no account of thermal motion, such an approach should be of general interest as more protein structures are determined to near atomic resolution.

01.6—4   DIFFRACTION STUDIES OF MODEL AND BIOLOGICAL MEMBRANES AT A PULSED NEUTRON SOURCE. By A.M. <u>Balagurov</u> and V.I. Gordeliy, Laboratory of Neutron Physics, Joint Institute for Nuclear Research, Dubna, USSR.

A new Dubna high flux pulsed reactor IBR-2 has been operated for over 1.5 years (Frank, Pacher, Physika (1983) 120B, 37). A series of experiments with model and biological membranes have been carried out recently at the TOF-diffractometer DN-2 with a position sensitive detector. Membrane samples were prepared as thin multilayers spread on glass slides 2.5x2.5 cm. Typically, a sample containing 15 mg of lipid has disorientation of layers not above 5°. Diffraction spectra were measured at the average scattering angle of 10° or 20° within the limits of 10° and within the wavelength range 2-20 Å. In the experiments the structure factors of the first ten Bragg reflections were determined at 20C, several values of relative humidity and for different ratios of H_2O/D_2O. An hour is enough to acquire 1% statistic accuracy for structure factors of strong reflections ($\pm$ 0.5 Å for spacing d). The effects of saturating multilayers with water and of isotope exchange on the variation of intensity and position of the first three orders were investigated. The time of measuring of the whole spectrum was 1 min. The acquired data allowed the determination of phases of structure factors and the calculation of the bilayer profiles at 6Å resolution.