02.1-30 STRUCTURE OF TRICLINIC LYSOZYME AND ITS Cu^{2+} COMPLEX AT 2Å RESOLUTION. By M. Ramanadham, Neutron Physics Division, Bhabha Atomic Research Centre, Trombay, Bombay 400085, India, L.C. Sieker and L.H. Jensen, Depts. Biological Structure and Biochemistry, University of Washington, Seattle, WA 98195, USA and B.J. Birknes, Dept. of Chemistry, University of Tromso, N-9001 Tromso, Norway.

The structure of triclinic hen-egg white lysozyme is refined at 2Å resolution to an R-value of 0.169. In addition to 1001 protein atoms, the present model consists of 239 solvent molecules ranging in occupancies from 1.0 to 0.5. Individual isotropic B-values are refined for all the atoms. B-values are found to be generally smaller than those obtained in most of the other protein structures. Average B-values for the main-chain atoms, side-chain atoms and for the entire protein are 8.2, 9.6 and 8.9 $Å^2$ respectively. However, the last 30 residues, especially in the vicinity of residue 103 and the terminal carboxyl group, have comparatively larger B-values. Distribution of the B-values seems to be generally consistent with the secondary structure of the protein molecule and the inter-molecular interactions involving individual atoms. The crystal structure is extensively hydrogen bonded involving well-ordered solvent molecules and side chains. A qualitative comparison of the average B-values is carried out with the corresponding B-values from tetragonal lysozyme (Sternberg et al. J. Mol. Biol. (1979) 130, 231-253). On an average, B-values in tetragonal lysozyme seem to be comparatively larger by a factor of 2. In addition, certain residues seem to have widely different B-values in the two structures. Barring these differences, the general patterns of the main-chain B-values in these two structures of lysozyme do not seem to be very much different.

Three dimensional data consisting of more than 6900 independent observations at 2Å resolution, recorded from a single crystal of Cu^{2+}-lysozyme complex, prepared by soaking the native crystal in the mother liquor containing $CuSO_4$ is used in the study of Cu^{2+} binding to triclinic lysozyme. Two binding sites are located in a remarkably clean (F_D-F_P) map. The entire model consisting of the protein atoms, solvent atoms and the two Cu^{2+} positions is refined by the method of restrained least-squares. One of the two Cu^{2+} ions is bound in the active site of the enzyme in the vicinity of GLU-35. Distances of OE1, OE2 atoms of GLU-35 and OD1, OD2 of ASP-52 from this site are 2.1, 3.0, 5.6 and 4.7A respectively. The second Cu^{2+} ion is bound in the neighbourhood of HIS-15, ASP-87 and THR-89 residues. The closest protein atoms are NE2 of HIS-15 and OG1 of THR-89 at distances of 2.1 and 2.4Å respectively. In addition, these two Cu^{2+} sites are surrounded by a number of solvent molecules. An extensive comparison of these results is made with a number of other crystallographic (for example, Cu^{2+} binding to tetragonal lysozyme; Teichberg et al. J. Mol. Biol. (1974) 87, 357-368) and spectroscopic studies of metal-ion binding to lysozyme.

02.1-31 X-RAY STUDIES ON THE BINDING OF BROMOPHENOL RED AND BROMOPHENOL BLUE TO LYSOZYME. By H.M. Krishna Murthy and M. Vijayan, Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560012, India; and S. Gurnani, Biochemistry and Food Technology Division, Bhabha Atomic Research Centre, Bombay 400085, India.

Solution studies have indicated the presence of a binding site for phenolsulfophthalein dyes like bromophenol red (BPR) and bromophenol blue (BPB) in lysozyme (Krishnamoorthy, Prabhananda & Gurnani (1979), Biopolymers 18, 1937-1963; Krishnamoorthy & Prabhananda, personal communication). The dye-bound enzyme is active against the hexasaccharide but not against the bacterial cell wall suggesting thereby that the binding site of the dyes is important in the action of lysozyme against its natural substrate. X-ray data upto a resolution of 5.5 Å were collected from the native tetragonal crystals grown at pH 4.6, crystals soaked in tris buffer at pH 8, those soaked in a BPR solution in tris buffer at pH 8 and those soaked in a BPB solution in acetate buffer at pH 4.6. Appropriate difference Fourier maps were computed using the known phase angles of the structure factors of the native crystals, made available by Prof. Sir D.C. Phillips. A preliminary examination of the maps, especially that involving the data from crystals soaked in the BPB solution, appears to indicate that the dye binding site is far removed from the cleft region. A detailed examination of the maps is in progress.

02.1-32 THE STRUCTURE OF TAKA-AMYLASE A AT 3.0 Å RESOLUTION AND THE D-FOURIER STUDIES OF SUBSTRATE BINDING SITES. M. Kusunoki, W. Harada, N. Tanaka and M. Kakudo, Institute for Protein Research, Osaka University, Suita, Osaka, 565, Japan.

Taka-Amylase A is an α-amylase [EC.3.2.1.1] with molecular weight of about 50,000 produced by *Aspergillus oryzae*. The three-dimensional structure has been determined at 3.0 A resolution and two main substrate binding sites were identified. The space group is P21 with cell dimensions of a=91.9, b=133.3, c=94.3 A and β=102.7°. The asymmetric unit contains three molecules which are related by non-crystallographic three-fold screw symmetry along [101]. Protein phases were determined with derivatives of HgCl2, UO2(NO3)2 and KAu(CN)2. About 45,000 independent reflections up to 3.0 A resolution were measured with a 4-circle diffractometer (40kV,200mA). <m> is 0.73. Although the noise level of the e.d. maps of the three molecules is not low, averaging of the e.d. over the three molecules gave a very clear map. The molecular model was built using this map in the Richards' box. Although the primary structure has not been completed yet considerable part of the sequence has been determined. The dimensions of the molecule are 35×40×80 A. The molecule is composed roughly of the two domains, main and C-terminal ones which are connected by only one peptide chain. The main domain has a β-barrel structure composed of parallel eight strands around which nine α-helices surround approximately in parallel to the axis of the β-barrel. These α-helices and β-strands alternate along the peptide chain. Stereo drawings of the main chain viewed from the direction of the β-barrel are shown in the figure. This super-secondary structure is similar to those of triose phosphate isomerase and A-domain of pyruvate kinase. At present the protein phases are being refined by the molecular replacement method in real space.

In order to clarify the catalytic mechanism, the D-Fourier maps have been calculated for complex crystals of