02.1-30. STRUCTURE OF TRICLINIC LYSOZYME AND ITS Cu^{2+} COMPLEX AT 2Å RESOLUTION. By <u>M. Ramanadham</u>, 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) <u>130</u>, 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 (Fp-Fp) 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) <u>87</u>, 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 <u>H.M. Krishna Murthy</u> 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.
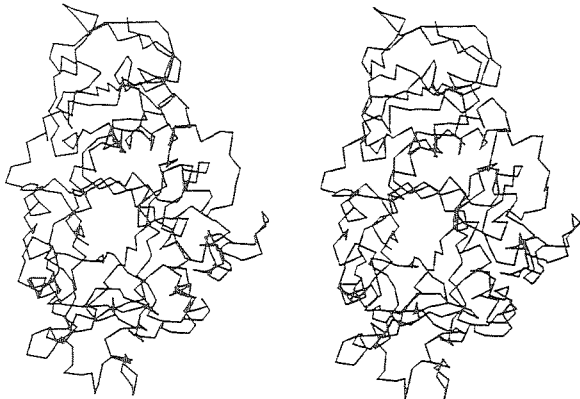
Solution studies have indicated the presence of a binding site for phenolsulfophthalein dyes like bromophenol red (BPR) and bromophenol blue (BPB) in lysozyme (Krishnamoorthy, Prabhananda & Gurnani (1979), Biopolymers <u>18</u>, 1937-1963; Krishnamoorthy & Prabhananda, personal communication). The dye-bound 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 <u>M. Kakudo</u> , 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

substrates and inhibitors, namely, maltotriose, maltose, glucose and four kinds of iodine derivatives of substrates. These D-Fourier maps showed two main binding sites I and II. The site II is at the bottom of the cleft in the main domain and also located at the C-terminal end of the β-barrel. The site I is at one of the four S-S bridges and about 30.0 A away from the site II along the molecular surface. There are not binding sites between the site I and II. The D-Fourier map for the PCMB complex crystal prepared in the presence of EDTA showed that the PCMB binds to the site close to the S-S bridge at the site I. PCMB is reported to bind to the SH-group of the enzyme which is one of chelating groups to the essential calcium ion. His and Asp are speculated to be catalytic residues judging from the pH dependence of the enzyme activity. The two residues at the edge of the site II seems to be assigned as His and Asp referring to the e.d. map, the primary structure and heavy atom binding.

– Substrate analogues bind to two different regions of the molecule : one corresponds to the active site cleft, the other is located about 30 Å from this crevice and on the surface of the molecule. The latter may function as a storage or a regulation site. In this respect, it is noteworthy that bile salts, which act as effectors of the amylase activity (O'Donnel et al., Enzymes (1975), 19, 129-139), bind only to this second site. If one will confirm that this secondary substrate-binding site has a regulatory function, its importance for the mechanism of α-amylase action must be stressed.

02.1-33   PORCINE PANCREATIC α-AMYLASE : CRYSTAL STRUCTURE AND BINDING SITES. By G. BUISSON, E. DUEE (D.R.F., C.E.N., 85 X, 38041 Grenoble cedex, France) and R. HASER, F. PAYAN, N. DARBON (CRMC², Campus de Luminy, case 913, 13288 Marseille cedex 9, France).

The crystal structure study of porcine pancreatic α-amylase I (Mr≈53000) has now been extended to 2.9 Å resolution. The electron density map is currently being interpreted and the earlier conclusions inferred from the 5 Å resolution model are already confirmed.

Among the prominent features of the molecule are the predominance of extended chain conformation and the large cleft previously identified as the active site region (Payan et al., Acta Cryst. (1980) B36, 416-421). There is no obvious division of the molecule into two identical subunits as proposed by Robyt et al (Arch. Biochem. Biophys. (1971) 144, 160-157) or into two similar structural domains as suggested by Fitzgerald et al (J. Mol. Biol. (1979) 135, 753-756). A number of additional interesting features will be discussed in the light of known properties of the enzyme, current sequence work (Pasero et al., Biochimie (1981) 63, 71-76) and previous proposals about its catalytic activity :

– The Cl⁻ ion, which is known to activate the enzyme (Levitzki and Steer, Eur. J. Biochem. (1974) 41, 171-180), binds in a pocket near the active site cleft.

– The edge of this cleft provides also the region involved in binding one calcium ion. Since this calcium ion is easily replaced by a strontium one it may not be the firmly bound and essential ion for the enzymic activity (Vallee et al., J. Biol. Chem. (1959) 234, 2901-2929 ; Levitzki and Steer (1974)). However, no other calcium ion site has been located so far.

02.1-34   MALTOHEPTAOSE BINDING TO PHOSPHORYLASE a AT 0.25 nm RESOLUTION. By E. Goldsmith, S. Sprang, R.J. Fletterick, Dept. of Biochemistry & Biophysics, University of California, San Francisco, CA 94143.

Glycogen activates glycogen phosphorylase through binding at a site distant from the catalytic site. This interaction has been modeled by crystallographic analysis at 3.0 Å of maltoheptaose, bound to phosphorylase a (Kasvinsky et al., J. Biol. Chem. (1978) 253, 1290-1296) and of maltotriose bound to phosphorylase b (Weber et al., Nature (1978) 274, 433-437). We have extended the resolution of the first study to 2.5 Å, and have included use of partially refined phases. The improved resolution has permitted the determination of the structure of maltoheptaose as it is bound to the enzyme, and has allowed analysis at the interactions with the protein and the protein conformational changes.

The maltoheptaose conformation is a left-handed helix with helical parameters close to those observed in small molecule crystal structures and estimated for amylase. The H-bond O(2)→O(3') found in related small molecules is preserved (Quigley et al., J. Amer. Chem. Soc. (1970) 92, 5834-5839).

Two molecules of maltoheptaose bind. One of these is well-localized and all 7 glycopyranosides are observed to some extent. A second molecule is bound (to site E of ref. 1) with only 2 sugars localized. The orientation of this sugar puts the reducing O(1) near the O(6) of the first ring of the tightly-bound maltoheptaose. The relative orientation suggests that phosphorylase binds α(1-6) branch points on glycogen, and further that it binds β-chains rather than loose ends.

The maltoheptaose makes numerous H-bonding interactions with the protein to ionic and polar side chains, causing local conformational changes, and changes at the active site.