02.1-27 A COMPARISON OF TWO CRYSTAL FORMS OF BOVINE β-LACTOGLOBULIN. By S.G. Hambling+, S. Yewdell*, A.C.T. North* & L. Sawyer+ +Biochemistry Department, The University, Edinburgh, EH8 9XD, UK and Astbury Biophysics Department, The University, Leeds, LS2 9JT, UK

Bovine β-lactoglobulin (BLG) has recently been shown to bear a close structural similarity to plasma retinol-binding protein. This has suggested a possible role for BLG in vitamin transport which in turn has led to the discovery of specific receptors in calf intestine (Papiz et al., Nature 324, 354; 1986). BLG is remarkably acid stable but on titration to alkaline pH undergoes at least two conformational changes which render it unstable by pH9. One of these changes occurs at physiological pH and may be functionally significant. Crystal forms of BLG have been grown at pHs on either side of this transition and we are now able to compare their structures in some detail. Lattice X (triclinic: a=38.1, b=49.7, c=56.6A, α=122.7 β=97.5, γ=104.1°; pH 6.5) and lattice Y (orthorhombic: a=55.7, b=67.2, c=81.7A; $B22_12$; pH 7.8) structures being refined by restrained least squares with data to 2.0 and 1.8A respectively. The current state of the comparison of the two structures will be reported together with some explanation of the behaviour of the proteins over the pH range.

02.1-28 THE 1.75Å RESOLUTION STRUCTURE OF CHLORAMPHENICOL ACETYLTRANSFERASE. A.G.W. Leslie and W.V. Shaw*, Imperial College of Science and Technology, London SW7 2BZ, and *University of Leicester, Leicester LE1 7RH, UK.

Bacterial resistance to the antibiotic chloramphenicol is conferred by the enzyme chloramphenicol acetyltransferase (CAT). This enzyme, a trimer of three identical subunits (MW 3x25000) catalyses the transfer of an acetyl group from acetyl-CoA to the primary hydroxyl of chloramphenicol. The modified drug no longer binds to the bacterial ribosome (its normal site of action) and is ineffective as an antibiotic. A number of variants of CAT have been isolated and characterised from both gram positive and gram negative bacteria (Shaw, W.V. CRC Crit. Rev. Biochem. 1983, 14, 1-46), and the nucleotide sequences of eight naturally occuring variants have been determined.

The structure of the Type III enzyme from E. coli has been determined from a 2.7Å resolution m.i.r. electron density map based on six derivatives and refined at 1.75Å resolution. The present model, which includes bound chloramphenicol and 200 solvent molecules has excellent stereochemistry and an R-factor of 18.9% for all data between 10Å and 1.7Å. The structure of the monomer is dominated by a six-stranded antiparallel β-sheet flanked by five α-helices in an "open sandwich" arrangement. There is no obvious homology to any published protein structure. A structurally isolated strand in the monomer forms hydrogen bonds with the final strand of the β-sheet of a three-fold related subunit, forming a seven-stranded sheet which extends across subunit boundaries. This feature, in conjunction with the hydrophic nature of the subunit interface, helps to explain the stability of the trimer.

Chloramphenicol binds in a narrow, predominantly hydrophobic pocket at the subunit interface, and the acetyl acceptor is within 2.8Å of the essential active site histidine. Model building suggests a possible binding site for acetyl-CoA. The enzyme has been cloned, and several mutants produced by site-directed mutagenesis. Crystallographic study of some of these mutants is planned.

02.1-29 CRYSTALLOGRAPHIC STUDIES ON BOVINE HEART CYTOCHROME C OXIDASE. By S. Yoshikawa*#, T. Tera*, Y. Takahashi*, T.Tsukihara° and W.S.Caughey#, *Department of Biology, Konan University, Kobe, Japan, °Faculty of Engineering, Tottori University, Tottori, Japan and # Department of Biochemistry, Colorado State University, Fort Collins, CO, USA

Cytochrome c oxidase, a Key enzyme in energy production in aerobic organisms, recieves four electrons from cytochrome c and four protons from the medium to reduce one O_2 molecule to two H_2O molecules.This enzyme spans the inner mitochondrial membrane and, as isolated, consists of more than ten protein subunits as well as two hemes, 2.5 coppers, one zinc and one magnesium per minimal catalytic unit. Inter-relationships between tertiary structure, reaction mechanism and catalytic function are of great interest. However, since it is a membrane protein, previous attempts to prepare a crystal that gave an adequate X-ray diffraction pattern have not been successful.

The enzyme, purified from bovine heart as described earlier(1), was solvilized by Brij-35 (a non-ionic detergent). After a search for an optimal condition, crystals were obtained from a solution of highly concentrated protein in 0.5 mM sodium phosphate buffer, pH7.4, at 0°C. Small crystals were hexagonal bipyramid; upon becoming larger, a clear shape was lost. The crystals are very fragile, and easily deteriorate with a change in pH or a rise in temperature. The diffraction experiment was carried out at 5 to 10°C by Cu-Kα radiation generated at 40KV-20mA by Rigaku rotating anode RU-300 equipped with fine focus cathode(0.1 x 1mm^2). X-ray beam was optically focused in radius of 0.1mm at a film position with two nickel mirrors. A crystal (0.3x0.5x0.7mm^3) gave diffraction as high as 8 Å resolution; deterioration was observed after exposure for 30 hrs. One precession and 28 oscillation photographs at different settings were obtained, from which space group of $P6_2$ or $P6_4$, and cell dimensions of a= b=175 Å, c=282Å, α =β=90° and γ=120°, were determined.

The apparent molecular weight of the enzyme was 200,000 including the detergent. If an asymmetric unit consists of two molecules of the enzyme, a reasonable value of 3.1 for Vm was obtained. Dimers of the enzyme estimated as 80x80x100Å^3 from the electron microscopic evidence (2) packed well in the crystal lattice as depicted in the figure.

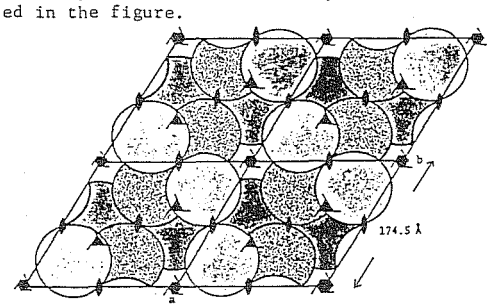

Figure. Packing of cytochrome c oxidase dimers in the crystal. Each circle indicates the dimer.

References (1) Yoshikawa,S., Choc,M.G., O'Toole,M.C. and Caughey,W.S.(1977) J.Biol.Chem. 252, 5498-5508
(2) Fuller, S.D., Capaldi,R.A. and Henderson, R.(1979) J.Mol.Biol. 134, 305-327