02. STRUCTURAL MOLECULAR BIOLOGY

02.1-27 A COMPARISON OF TWO CRYSTAL FORMS OF BOVINE α-LACTOGLOBULIN. By G.C. Hambly*, S. Yeudell*, A.C.T. North* & L. Sawyer†, Biochemistry Department, The University, Edinburgh, EH3 9XD, UK and Aston Biophysics Department, The University, Leeds, LS2 9JT, UK.

Bovine α-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 (Pepin 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 pH 9.

The structure of the monomer is depicted in the figure. There are two dimers in the asymmetric unit. Each dimer consists of two related subunits, forming a seven-stranded sheet which extends across subunit boundaries. This feature, in conjunction with the hydrophobic 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 2.8 Å 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.

02.1-28 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 LEI 7RH, UK.

Bacterial resistance to the antibiotic chloramphenicol is conferred by the enzyme chloramphenicol acetyltransferase (CAT). This enzyme, a trimer of 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

This enzyme spans the inner mitochondrial membrane and, as isolated, consists of more than ten protein subunits as well as two hemes, 2.5 copper, 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 solubilized 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 M sodium phosphate buffer, pH 4.4, at 0°C. Small crystals were hexagonal bipyramids; 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 50KV-20mA by Rigaku rotating anode HU-300 equipped with fine focus cathode(0.1×1mm) 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) 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_3 or P6_4, and cell dimensions of a=173Å, c=282Å, α=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 from the electron microscopic evidence (2) packed well in the crystal lattice as depicted in the figure.