02. STRUCTURAL MOLECULAR BIOLOGY

02.1-27 A COMPARISON OF TWO CRYSTAL FORMS OF BOVINE β-LACTOglobulin. By G.C. Hepburn, 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

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 (Pepin et al., Nature 324, 356; 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 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 bipiramide; 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 60KV-20mA by Rigaku rotating anode RU-300 equipped with fine focus cathode(0.1 X 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 A 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 P63, or P61, and cell dimensions of a=173 Å, b=182 Å, a=90° and y=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.

02.1-29 CRYSTALLOGRAPHIC STUDIES ON BOVINE HEART CYTOCHROME C OXIDASE. By S. Yoshikawa*, T. Tera, Y. Falahashi*, T. Takahara* and W.D. 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, recycles four electrons from cytochrome c and four protons from the medium to reduce one O₂ molecule to two H₂O 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 copper ions, 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 bipiramide; 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 60KV-20mA by Rigaku rotating anode RU-300 equipped with fine focus cathode(0.1 X 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 A 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 P63, or P61, and cell dimensions of a=173 Å, b=182 Å, a=90° and y=120°, were determined.

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References
(1) Yoshikawa, S., Choe, W.C., O'Toole, M.C. and Caughey, W.B. (1977) J.Biol.Chem. 252, 5498-5508