02. STRUCTURAL MOLECULAR BIOLOGY


Fab NQ10, derived from a murine monoclonal anti-phenylcyclazone antibody crystallizes in the space group D2 with a = 113.5, b = 76.3, c = 85.0 A, β = 137.5°. The Vh gene encoding most of the Vh domain of this antibody is closely related to the corresponding gene of the anti-lysozyme Dl.3. However, the light polypeptide chain of NQ10 is unrelated to that of Dl.3.

Native Fab NQ10 X-ray intensities were measured on a four circle diffractometer to 4.5 A resolution. Two heavy atom derivatives were obtained using DNP-acetate at different concentrations. An electron density map at 6 A resolution clearly revealed the molecular boundaries. Using a rigid body refinement program and the three-dimensional structure of Fab Dl.3, a model was fitted to the electron density map showing the traces of the light and heavy chains. The model was refined to 4.5 A resolution. Measurement of intensities to higher resolution and further refinement of the model are currently under way. The latest progress in the structure determination will be reviewed.

02.1-5 PRONOUNCED EFFECT OF THE SOLVENT OF CRYSTALLIZATION ON THE STRUCTURE OF A MULTI-DOMAIN PROTEIN. C.-H. Chang, W. C. Carperos, C. F. Ainsworth, K. W. Glesser, and W. Schiffer Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL; The Chemistry Department, Loyola University, Chicago, IL; U. S. A.

Radically different variable domain interactions were observed in antibody light-chain dimer crystals solubilized from either distilled water or from 1.5 M ammonium sulfate. Light chains are characterized by an amino-terminal variable domain and a carboxyl-terminal constant domain linked by an extended peptide. We have previously determined the structure of light-chain dimer Loc in a crystal form obtained from ammonium sulfate (C.-Chang, et al., 1985, Biochemistry 24, 4890). The two variable domains form a very unusual antigen binding site consisting of a protrusion with a groove on each side. We now have determined the crystal structure of Loc as crystallized from distilled water. At present, the R = 35% for 3.5 A data. In this structure, the association of the two variable domains forms a cavity that resembles the "conventional" antigen binding site that was observed in the Hcgp protein. The association of the constant domains is very similar in the two crystal forms. Two independent hinge-bending motions are required to convert the structure observed in one crystal form to that observed in the other form. Work supported by the U. S. Department of Energy under contract no. W-31-109-ENG-38.

02.1-6 CRYSTALLIZATION AND CRYSTALLOGRAPHIC STUDY OF PIG LIVER NADH-CYTOCHROME b5 REDUCTASE. By X. Kiki, S. Raia, H. Kamai, T. Iyama, K. Kubayashi, and K. Hayashi. 1Department of Applied Chemistry, Faculty of Engineering, Osaka University, Suita, Osaka 565, 2Institute of Basic Medical Sciences, University of Tsukuba, Niihari, Ibaraki, 350, and 3Institute of Scientific and Industrial Research, Osaka University, Ibaraki, Osaka 567, JAPAN

NADH-cytochrome b5 reductase (EC 1.6.2.2) catalyzes to reduce cytochrome b5 in an electron-transport chain from NADH to a terminal oxidase desaturase in the endoplasmic reticulum. The enzyme is an amphipathic membrane-bound flavoprotein with a single subunit containing one molecule of FAD. The protein molecule consists of a large, hydrophilic catalytic domain and a smaller, hydrophobic membrane-binding domain. We have crystallized the following two forms of this enzyme from pig liver microsomes: the intact molecule (35kDa) solubilized by a detergent (Triton N-101) and the catalytic domain (30kDa) solubilized by a protease (cattapasin D). Crystals have been grown from polyethylene glycol solutions by the vapour diffusion method. The crystals of the intact enzyme are needle-shaped but still too small for X-ray studies (their diameters less than 0.05mm). The crystals of the catalytic domain are large enough to be visualized by the X-ray technique.

02.1-7 CRYSTALLIZATION OF A COPPER CONTAINING NITRITE REDUCTASE by E.T. Adman, S. Turley, L.C. Sieker, Dept. of Biological Structure SMSU, Univ. of Washington, Seattle, WA 98195, USA and Jean Lekail, Dept. of Biochemistry, The Univ. of Georgia, Athens, GA, 30602


We have obtained small crystals of the cupredoxin from "A. cycloclastes" and data on its partner, NIR. NIR is a grey-green protein, a dimer of molecular weight 69K (monomer 36.8K) and three Cu per 69K. It is crystallized from 325 saturated ammonium sulfate, pH=5.0, at 30°C in 0.1M phosphate buffer at 4°C. The space group, determined from precession photographs, is P2_1, a=b=98.4 A. The ratio of crystal volume to molecular weight is 2.16 with one 36.8 K unit per asymmetric unit (42 solvent).

Preliminary oscillation photographs were taken by Dr. Samar Hasnain and Dr. Yoshinori Satow at the Photon Factory U-14 facility using a crystal, 0.25x0.25x0.3 mm and highly monochromated radiation at λ = 1.260Å. 0.1°-0.3° second polaroid exposures show clearly resolved reflections to a d-spacing of at least 1.7A. Diffraction photographs of crystals soaked in uranyl nitrate or platinum thiocyanate show intensity changes indicative of good derivatives.

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