C-16 01. DETERMINATION OF MACROMOLECULAR STRUCTURES

01.4-12 REFINEMENT OF THE MONOCLINIC HEN EGG WHITE LYSOZYME AT 2Å RESOLUTION. By S. T. Rao, J. Hogle and M. Sundaralingam, Department of Biochemistry, College of Agricultural & Life Sciences, University of Wisconsin-Madison, Madison, WI 53706

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Monoclinic crystals of hen egg white lysozyme grown at pH 4.4 contain two independent molecules in the asymmetric unit with cell dimensions a = 28.0, b = 62.9, c = 60.5Å; β = 90.0°; space group P2₁. The backbone chain was traced from a 4Å MIR map phased with four derivatives (Acta Cryst. B37 (1981) in press). The tetragonal model fitted to this chain trace provided the starting model for the two molecules. The phases were extended to 2.5Å using Mayer's graphics system at Texas A&M and a model was rebuilt into a density calculated with 2Fo-Fc as coefficients. Segments of the polypeptide chain being fitted were excluded from the phasing calculations. This model, comprising of two molecules, was subjected to 20 cycles of difference Fourier refinement followed by idealisation of stereochemistry (Dodson, Isaacs & Rollett, Acta Cryst. A32, 311 (1976)). This model had a few short contacts between side chain atoms in both molecules. These contacts have been relieved and the model coordinates have been subsequently improved by two complete rounds of 2Fo-Fc maps using the Vector-General graphics in our laboratory, R = 0.29 for 6535 reflections. We are now carrying out reciprocal space refinement at 2Å (13,000 reflections) using the Konnert-Hendrickson procedure.

Supported in part by NSF grant PCM76-23268.

01.6-01 CONTRAST VARIATION STUDIES IN LOW RESOLUTION NEUTRON CRYSTALLOGRAPHY: AN APPLICATION TO THE NUCLEOSOME CORE PARTICLE. G.A. Bentley, J.T. Finch, A. Lewis-Bentley and N. Rost, x I.L.L., Grenoble, France + M.R.C. Cambridge, U.K.

The method of H/D contrast variation, which has been successful in small angle neutron scattering to distinguish protein and nucleic acid in complex macromolecules (e.g. chromatin, viruses), is being applied to the nucleosome core particle to distinguish protein and DNA in low-resolution crystal structure analysis.

The results show that the nucleosome core particle consists of a central protein core surrounded by approximately 1.8 superhelical turns of DNA.

01.6-02 A SINGLE-CRYSTAL NEUTRON DIFFRACTION STUDY OF SPERM- WHALE CYTOGLOBIN. By S.E.V. Phillips, MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, England, and Department of Biology, Brookhaven National Laboratory, Upton, NY 11973 USA.

Neutron diffraction data have been collected to approximately 1.7 Å resolution on large crystals of oxymyoglobin in D₂O buffer, using the protein crystallography station at the High Flux Beam Reactor, Brookhaven National Laboratory. The diffractometer was equipped with a 2-dimensional multwire detector, and a cooling device to maintain a temperature of -15°C at the crystal to retard oxidation of the heme iron. Two crystals were used to collect 14,611 independent reflections, the merging R between crystals being 14.1% on intensities.

An F = F map, phased from the refined X-ray co-ordinates of C, O, S, Fe atoms, showed peaks for half the H and D atoms. Adding all observed H and D atoms, and those whose positions were known from stereochemistry, to the model gave R=33% for 10,151 reflections with I>1.5σ(I). After 10 cycles of Jack-Levitt refinement (R=18.8%) a difference map showed a strong peak for D bonded to N of His E7, indicating a good H-bond from the proximal imidazole to the oxygen ligand. The function of this histidine in myoglobin and haemoglobin is to stabilize the heme-oxygen complex.

The pattern of H/D exchange is clear in the maps, and will be discussed, together with a description of hydrogen bonding and water structure.