01.4-12 REFINEMENT OF THE MONOCLINIC HEN EGG WHITE LYSOZYME AT 2A RESOLUTION. By S. T. Rao, J. Hogle[+] and M. Sundaralingam, Department of Bio-Chemistry, 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 = 60.5A; β = 90.8°; space group $P2_1$. The backbone chain was traced from a 4A 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 2.5A 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. The 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 the 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 2A (13,000 reflections) using the Konnert-Hendrickson procedure.

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01.6-01 CONTRAST VARIATION STUDIES IN LOW RESOLUTION NEUTRON CRYSTALLOGRAPHY : AN APPLICATION TO THE NUCLEOSOME CORE PARTICLE. G.A. Bentley[x], J.T. Finch[+], A. Lewit-Bentley[x] and M. Roth[x]
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The method of H_2O/D_2O 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 crystal structure determination at low resolution. The neutron scattering cross-sections of H_2O and D_2O are quite different and this property is used to match the scattering density of either the protein or the nucleic acid moieties in order to visualise each separately.

The effect of solvent contrast on the low resolution structure factors (d-spacing $\gtrsim$ 15 Å) may be usefully exploited to obtain relative phase information independent of any prior structural interpretation. For centrosymmetric terms, the structure factor is a linear function of the H_2O/D_2O ratio. This property may be used to scale together data sets from crystals soaked in different H_2O/D_2O buffers and to obtain the relative phase change from one contrast to the next. In the case of non-centrosymmetric structure factors the magnitude of the relative phase change may be determined but not its sign. These relationships, which depend only on the magnitudes of the observed structure factors measured in known contrasts, can be used to transfer phase information between the different H_2O/D_2O contrasts.

We have measured low-resolution 3-D data from crystals of the nucleosome core particle at contrasts of 0 % D_2O, 39 % D_2O (protein matched out) and 65 % D_2O (DNA matched out). We shall describe the use of the contrast variation method in solving the structure and its ability 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 OXYMYOGLOBIN. 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 D_2O buffer, using the protein crystallography station at the High Flux Beam Reactor, Brookhaven National Laboratory. The diffractometer was equipped with a 2-dimensional multiwire detector, and a cooling device to maintain a temperature of -5°C at the crystal to retard oxidation of the haem iron. Two crystals were used to collect 14,411 independent reflexions, the merging R between crystals being 14.1% on intensities.

An F_o-F_c map, phased from the refined X-ray co-ordinates of C,N,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 reflexions 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_ε of His E7, indicating a good H-bond from the proximal imidazole to the dioxygen ligand. The function of this histidine in myoglobin and haemoglobin is to stabilize the haem-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.