02.1-06 CRYSTALLOGRAPHIC STUDIES OF THE 2Fe FERREDOXIN FROM *HALOBACTERIUM* OF THE DEAD SEA By: J.L. Sussman, M. Harel and A. Yonath, Department of Structural Chemistry, Weizmann Institute of Science, Rehovot, Israel.

Bacteria of the genus *Halobacterium* are obligate halophiles, i.e. they require for their growth an extracellular salt concentration higher than 2M NaCl. In fact, they pump in salt, so that their internal salt concentration is higher than that of the environment. It is surprising that halophilic proteins can function under such hostile conditions whereas most other proteins are inactive. Upon lowering the salt concentration in vitro, most of the halophilic enzymes are inactivated. A detailed three-dimensional molecular structure of a halophilic protein can provide some insight to explain this phenomenon.

Crystals of 2Fe-2S ferredoxin from *Halobacterium* of the Dead Sea have been grown in 3.8M phosphate buffer pH = 7.0 . The crystals belong to space-group $P6_322$ with cell dimensions of a=b=60.3 Å c=127.5 Å. In order to prolong the crystals' life-time under X-ray radiation the mother liquor was saturated with styrene monomer. This enabled the collection of complete data sets from single crystals.

X-ray data were collected on a CAD4 diffractometer using monochromated CuKα radiation. From two crystals, independent native data sets for the unique 1/24 of the sphere of reflection were collected, together with Friedel pairs out to 2.4 Å resolution, with a maximum intensity decay of 20%.

One heavy-atom derivative - $K_2Pt(CN)_4^=$ - data set was collected in a similar way out to 2.4 Å resolution. Data were corrected for absorption, LP factor and decay. Scaling each of the two native data sets to the derivative yielded R_F = 8.8% and 8.9% respectively. Scaling the two native data sets yielded R_F = 4.9%.

A difference Patterson map based on the averaged native and derivative data using all reflections with $F > 3\sigma$, (about two thirds of the total number of reflections) shows a single prominent heavy-atom site. A least-squares refinement of this site is in progress. Following this we plan to calculate an SIR map.

02.1-07 THE STRUCTURE OF THE RUBREDOXIN FROM DESULFOVIBRIO GIGAS. By M. FREY, G. PEPE and L.C. SIEKER, M. BRUSCHI, J. LE GALL, CRMC2, Campus de Luminy, case 913, 13288 Marseille cedex 9, France – Laboratoire de Chimie Bactérienne, 13274 Marseille cedex 9, France.

Rubredoxins are small proteins (M.W. 6000 DLTS.) which work in electron transfer systems of micro-organisms such as sulfate reducing bacteria. The active center is a single iron atom bonded tetrahedrally to a "cluster" of four cysteine residues.

A 2 Å resolution E.D. map of the rubredoxin from Desulfovibrio Gigas (RBDG), has been derived from the amplitudes of RBDG and phases of the rubredoxin from Desulfovibrio Vulgaris (RBDV) (Adman et al., J. Mol. Biol. (1977) 112, 113). The atomic model has been easily constructed thanks to the interactive graphics program *Bilder* (R. Diamond).

The three rubredoxins RBDG, RBDV and from Clostridium Pastorianum (RBCP) (Watenpaugh et al., J. Mol. Biol. (1979) 131, 509) do show substantial variations in their chemical sequences while their 3-D models resemble each other ; the structural differences concern the nature and the distribution over the surface of most of the charged residues.
The results of the first stages of the refinement and structural comparisons will be presented.

02.1-08 AN INTERIM REPORT ON THE THREE-DIMENSIONAL STRUCTURE ANALYSIS OF RABBIT PLASMA TRANSFERRIN. BY B. Gorinsky, P. F. Lindley, A. Mydin, D. Moss and J. Watson, Department of Crystallography, Birkbeck College, Malet Street, London, WC1E 7HX, UK.

Plasma transferrin is the key protein in the iron metabolism of vertebrates. The physiological significance of this glycoprotein lies in its central role in the cyclic process whereby iron derived from the catabolism of haemoglobin is conserved by its almost quantitative return to haemopoietic tissue. Transferrin also participates in the regulation of iron absorption and protects against iron toxicity.

Transferrin is a single-chain protein of molecular weight ca. 80,000, with two specific iron (III) binding sites. The metal protein complex is stabilised by the concomitant binding of one (bi)carbonate anion per iron atom. At physiological pH and oxygen tension the effective affinity constants of the metal sites are $\sim 10^{22}$ M^{-1}, precluding spontaneous dissociation of iron. Rapid iron transfer to cells is affected by reversible binding of the protein to specific membrane receptors.

Rabbit plasma transferrin crystallises in space group $P4_12_12$ (or enantiomorph) with $a = b = 127.4(3)$ and $c = 145.4(3)$ Å, and one molecule per asymmetric unit. The native crystals are grown within the temperature range 4-8 °C since at higher temperatures they are unstable. Approximately 70% of the crystal volume is occupied by solvent. Heavy-atom derivatives (mercuric-chloride, potassium chloroplatinate and uranyl acetate) have been prepared by soaking the native crystals in solutions of heavy-atom reagents in the mother liquor. X-ray diffraction intensity data for the native crystals and these three derivatives have been collected on a four-circle diffractometer. The method of isomorphous replacement

has been used to obtain an electron density map of the protein to a resolution of 6.0Å. This map reveals the bilobal nature of the molecule.[1]

Recent work has included a search for further heavy-atom derivatives to assist the determination of the X-ray phases, the determination of the hand of the molecule and the extension of the data collection to higher resolution using photographic and diffractometric techniques. This paper will report on the progress that has been achieved.

1. Evidence for the Bilobal Nature of Differric Rabbit Plasma Transferrin. Nature, 1979, 281, 157-158.

02.1-09 INTER-SUBUNIT INTERACTIONS AND METAL BINDING SITES IN HORSE SPLEEN APOFERRITIN.

By P.E.Bourne, G.A.Clegg, P.M.Harrison, J.M.A.Smith and R.F.D. Stansfield, Department of Biochemistry, The University, Sheffield S10 2TN, U.K.

Twenty-four apoferritin subunits (each 54 x 27 x 27 Å^3) are arranged in 432 symmetry to form a compact shell (130Å diameter) with a central cavity (approximately 80Å diameter) allowing storage of up to 4500 Fe(III) atoms as 'FeOOH' (Clegg et al. Prog.in Biophys. and Mol.Biol. 36, 53 (1980)). It will be shown that the molecule can be approximated as a truncated rhombic dodecahedron with two subunits lying on each face, their long axes parallel to the rhomb edges. Dissection of the molecules allows the formulation of possible assembly intermediates.

Inter-subunit interactions are also examined with the aid of the amino acid sequence (Heusterspreute et al. 16th Congr.Int.Soc.Hematology, Montreal (1980)), now tentatively fitted to our electron density maps at 2.8Å resolution. Of particular interest are the interactions between the two subunits on the rhomb faces, which overlap along most of their length. On the inside surface of the molecule a number of salt bridges are seen to be present. Interactions around the 4-fold axes are also of importance, since these define the channels allowing access of Fe atoms to the cavity.

One of the long helices on the inside surface of the shell has a sequence of five polar residues halfway along its length and near the inter-subunit diad. One of these residues, a lysine, seems to form an internal salt bridge with a glutamic acid within the same subunit. In this region the α-helix is distorted, possibly to 3.0_{10} helix, for a short length. Again in this region, Tb(III) and UO_2(II) sites are found and these may represent iron-binding sites involved in ferritin formation. Ligands in these and other metal sites are now indicated.

02.1-10 STRUCTURE OF THE IRON COMPLEX IN HEMERYTHRIN. by R.E. Stenkamp, L.C. Sieker and L.H. Jensen, Dept. of Biol. Structure, SM-20, Univ. of Washington, Seattle, WA 98195, USA.

The structures of the binuclear iron complexes in methydroxo- and metazidohemerythrin, a non-heme iron, oxygen transport protein will be presented. Crystallographic refinement of these structures at 2.0 A and 2.2 A resolution respectively have been carried out. Distortions in the model not prevented by the application of restraints will be discussed. The redundancy in the structure (four subunits in the asymmetric unit) allowed internal checking of the conformational parameters as the refinement progressed. The complex consists of two iron atoms joined by a mu-oxo bridge with carboxylate groups bound to each iron by separate oxygen atoms. In metazidohemerythrin, both iron atoms are hexa-coordinate, the remaining ligands being five histidine residues and an azide ion. The complex in the methydroxo form is rather unusual, there being no small molecule bound to the complex, making one of the iron atoms penta-coordinate. Correlations of the structural, spectroscopic and biochemical data for these molecules will also be presented.

02.1-11 THE LIMULUS II HEMOCYANIN STRUCTURE AT 5.5 Å RESOLUTION. By Karen A. Magnus* and Warner E. Love, The Johns Hopkins University, Baltimore, Maryland 21218 U.S.A.

Hemocyanins are multisubunit proteins in arthropods and mollusks. The active site of hemocyanin contains two copper atoms (there is no heme moiety) and each site reversibly binds one molecule of oxygen. In arthropods, the whole molecules can be dissociated and the subunits fractionated. All subunits are single polypeptide chains weighing about 73,000 daltons and each contains one oxygen-binding site with two coppers. Crystals of a subunit of hemocyanin from the horseshoe crab, Limulus polyphemus were grown using polyethylene glycol as a precipitation agent. The crystals have the symmetry of the trigonal space group R32 with hexagonal lattice constants: a = 117.24, c = 286.94 A. Each asymmetric unit includes one subunit in its oxygenated form. The three-dimensional structure of the subunit was determined to 5.5 A resolution using multiple heavy-atom isomorphous replacement to obtain the protein phases. Each hemocyanin subunit is roughly kidney-shaped and is about 95 x 60 x 45 A. There is a cigar-shaped region of low density running along the long axis of the subunit. The core of density surrounding the hole appears at low resolution to be a β-barrel. The copper atoms appear to be toward the outside of the molecule and near one end.

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*Present address: University of Chicago, Chicago, Illinois 60637, U.S.A.