

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.<sup>1</sup>

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.

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Twenty-four apoferritin subunits (each 54 x 27 x 27 Å<sup>3</sup>) 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  $\alpha$ -helix is distorted, possibly to 3.0<sub>10</sub> helix, for a short length. Again in this region, Tb(III) and UO<sub>2</sub>(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.

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The structures of the binuclear iron complexes in methydroxo- and metazido-hemerythrin, a non-heme iron, oxygen transport protein will be presented. Crystallographic refinement of these structures at 2.0 Å and 2.2 Å 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 metazido-hemerythrin, 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:  $a = 117.24$ ,  $c = 286.94$  Å. Each asymmetric unit includes one subunit in its oxygenated form. The three-dimensional structure of the subunit was determined to 5.5 Å 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 Å. 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  $\beta$ -barrel. The copper atoms appear to be toward the outside of the molecule and near one end.

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