PS04.02.33 CRYSTALLIZATION OF SUPEROXIDE DISMUTASE FROM A HALOPHILIC ORGANISM. Terence P. Lo, Ilona L. Canestrelli, John A. Tainer and Elizabeth D. Getzoff. Department of Molecular Biology, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, California, 92037, USA

Superoxide dismutase from the phototrophic purple sulfur bacterium Ectothiorhodospira halophila has been isolated, purified and crystallized. The importance of this enzyme lies in its ability to scavenge toxic superoxide free radicals. Although these free radicals are a normal byproduct of metabolism, they are responsible for the breakdown of biological macromolecules and thus contribute to such conditions as aging and cancer. In humans, Cu,Zn superoxide dismutase has been implicated in the neurodegenerative disease Familial Amyotrophic Lateral Sclerosis, and so has been the subject of intense scrutiny.

Ectothiorhodospira halophila is a halophilic bacterium found in sunny, hypersaline lakes, and is able to survive in solutions having a salt concentration approaching 4 M. Ectothiorhodospira halophila is also slightly thermophilic, having optimum growth at 42 degrees Celsius. The enzyme was purified directly from the bacterium by assaying for superoxide dismutase activity. While superoxide dismutases from a variety of sources have been found to be highly thermostable, the enzyme from Ectothiorhodospira halophila is extremely thermostable, retaining activity even after boiling. In addition, this superoxide dismutase is highly resistant to cleavage by proteases.

Superoxide dismutase from Ectothiorhodospira halophila has been crystallized in the orthorhombic space group $P2_12_12_1$ with unit cell dimensions a=58, b=74, c=105. The search for a molecular replacement solution is underway.

PS04.02.34 PRELIMINARY CRYSTALLOGRAPHIC DATA OF SUBUNIT III AND SUBUNIT IV OF HEMOCYANIN FROM LIMULUS POLYPHEMUS. Karen A. Magnus and Shenping Liu. Depart. Biochem., Case Western Reserve Univ. School of Medicine, Cleveland, Ohio 44106, USA.

Hemocyanins are oxygen carriers with dicopper oxygen binding sites. Their basic assembly units are hexamers. The hemocyanin from Limulus polyphemus has 8 distinct subunits which form a 48mer as the biological active molecule. These subunits have different oxygen affinities, different cooperative phenomena and different assemblely characteristics. Subunit IIIa is the only subunit in Limulus hemocyanin which can catalyze the decomposition of H_2O_2 to O_2 and H_2O. Subunit IV is the only subunit which assembles into hexamer under low ion strength in the presence of Ca^{2+} ion. We crystallized these two hemocyanin subunits under conditions favoring hexamization using hanging drop vapor diffusion methods. For subunit III, we use 8.5-9.0% PEG3350 as the precipitant in 0.3M sodium cacodylate, 1M NaCl, pH=6.2-6.4. Crystals appear within 2 weeks and grow to 0.8*0.5*0.5 mm in abouout 90 days at 4°C. At room temperature they show 6Å difrraction with our in-house X-ray generator. The space group is C222(1) with a=218.21, b=368.43, c=237.86, a=b=g=90.0 . Assuming 2 hexamers/per asymmetric unit, the Vm is 2.6Å?/Da. Subunit IV crystals appear in several days and reach their full sizes within two weeks under the following conditions: 6-9%PEG3350, 0.2M Bis-tris, $0.22MCaCl_2$, pH=4.9-5.4, at 4°C. Crystals diffract at least 3Å at room temperature. They are an orthorombic space group with a=106.84 b=127.41, c=346.37, a=b=g=90.0. With a hexamer /per assymetry unit, it gives a Vm=2.6Å?/Da. NSF MCB 9305250

PS04.02.35 DMSO REDUCTASE FROM *RHODOBACTER CAPSULATUS.* A. S. McAlpine, S. Bailey, CCLRC Daresbury Laboratories, Daresbury, Warrington, WA4 4AD, Cheshire, England

DMSO reductase from the photosynthetic bacteria *Rhodobacter capsulatus* has been crystallised in two forms which are suitable for X-ray structure determination. DMSO reductase from *Rhodobacter* is the simplest molybdenum oxotransferase known and as a result makes it an ideal model to study the structure and function of this class of enzymes.

Both crystal forms belong to the same space group, $P\ 4_12_12$ and have cell dimensions a = b = 80.81 Å c = 229.75 Å (type I) and a = b = 89.30 Å, c = 230.05 Å (type II). Data have been collected from crystal type I at both room temperature and at 100K.

Several heavy atom derivatives have been collected leading to poor MIR phases. Density modification has improved the electron density maps and interpretation of these maps is underway.

PS04.02.36 STRUCTURAL ROLES OF CRITICAL RESIDUES IN COOPERATIVITY OF SCAPHARCA DIMERIC HEMOGLOBIN. A. Pardanani, M. Bonham, W. E. Royer, Jr., Program in Molecular Medicine, University of Massachusetts Medical Center, Worcester, MA 01605

High resolution atomic structures of the cooperative dimeric hemoglobin found in Scapharca inaequivalvis have implicated several residues in the cooperative mechanism. Of these, residues Phe 97, Thr 72 and His 101 have been mutated and subjected to functional and structural analysis in order to elucidate their effects on cooperative oxygen binding. Phe 97, whose side-chain undergoes the largest ligand-linked movement, has been hypothesized to be the key regulator of oxygen affinity. Mutation to leucine increases oxygen affinity by an order of magnitude with cooperativity diminished but not eliminated. Crystallographic analysis reveals that many aspects of ligand-linked transitions observed with wild-type are still present in F97L mutant. An important aspect of the ligand-linked structural transitions of Scapharca dimeric hemoglobin is a dramatic change in a cluster of interface water molecules upon ligation. To probe these interface water molecules, we have mutated Thr72 to Val which removes a single hydrogen bond between each subunit and the water network of the deoxygenated species. This isosteric mutation increases oxygen affinity to an even greater extent than seen with the F97L mutant. Interestingly, cooperativity is completely preserved, and may even be marginally enhanced. Structural studies have defined the only change to be loss of the single hydrogen bonded water molecule, demonstrating the importance of these water molecules in the cooperative mechanism. This proximal histidine, residue 101, provides the only direct coordination of the heme group to the polypeptide chain. Following work of Barrick on sperm whale myoglobin, we have mutated this residue to glycine, and are using exogenous imidizole to funtionally replace the lost histidine side chain. This mutation will directly test the importance of the direct link of the iron with the polypeptide chain in the cooperative mechanism. These mutations are providing novel insights into the manner by which two subunits can communicate.