03-Crystallography of Biological Macromolecules

03.04 - Metalloproteins

MS-03.04.01 The Structure of Limulus polyphemus Subunit II Hemocyanin in an Oxygenated Form. Karen A. Magnus* and Hoa Ton-That. Case Western Reserve University, Cleveland, Ohio 44106, USA.

We have determined the structure of hemocyanin subunit II from Limulus polyphemus, the American horseshoe crab. Hemocyanins are oxygen transport proteins found in some arthropods and mollusks. These extra-cellular proteins are named for the blue color they exhibit when in the oxygenated form. Hemocyanins bind one oxygen molecule using two copper active sites. The native Limulus hemocyanin is composed of 48 subunits of eight immunologically distinct types. All subunits are of molecular weight approximately 75,000. Limulus hemocyanin, like all arthropod hemocyanins, bind reversibly to one molecule of oxygen per subunit. Crystals are of the space group R32 with unit cell constants in the hexagonal setting of a=b=117.2 \pm 0.6 Å, c = 285.86 \pm 0.9 Å with α=β= 90.0° and γ= 120.1°. There is one hemocyanin subunit containing one copper site in each asymmetric unit. The phase problem was solved by molecular replacement using the Panulirus interruptus hemocyanin monomer structure (A. Volbeda and W.G. Hol, J. Mol. Biol., 1989, 209, 249-279), a deoxygenated form of the protein, as the test molecule. Refinement was performed using the program package X-PLOR (A.T. Brünger, J. Mol. Biol., 1988, 203, 80, 3-816). The structure of the Limulus subunit II was refined to 1.9 Å resolution and a crystallographic R-value of 18.6%. The two copper atoms in the active site are spaced 3.6 ± 0.2 Å apart and are liganded by six histidine residues in the protein. The hemocyanin subunit is in an oxygenated form since density for a well-ordered oxygen molecule is clearly visible in difference Fourier maps. The oxygen atoms are bound between the two copper ions in the μ-η²-η² configuration, that is both oxygen atoms are equidistant from both copper ions. There is no evidence of an endogenous bridging ligand present between the two copper ions.

MS-03.04.02 STRUCTURE AND FUNCTION OF FERREDOXINS. Keiji Fukuyama*, Kazuhiko Saeki, and Hiroshi Matsubara, Department of Biology, Faculty of Science, Osaka University, Toyonaka, Osaka 560, JAPAN

Among iron-sulfur proteins ferredoxins (Fds) have most extensively been studied by the biochemical, crystallographic, spectroscopic, and genetic methods. Fds are distributed in a wide range of living organisms and function as electron carriers in diverse metabolic pathways. Chloroplast-type Fds are distributed in higher plants and algae, and act as the electron acceptor from the photosystem 1. They have a [2Fe-2S] cluster and a unique folding of the polypeptide chain of about 95 amino acid residues, which has recently been found in the C-terminal domain of phytoheme oxygenase reductase. The topology is also found in ubiquinone and immunoglobulin binding domain of protein G. Most bacteria have Fds distinct from the chloroplast type Fds in terms of the Fe-S cluster type and sequences. Bacterial Fds have [4Fe-4S] and/or [3Fe-4S] clusters, and are diverse in the length and primary structure motif. Crystal structures of the 4 distinct types of bacterial Fds have been determined so far, Pseudomonas aeroginis Fd (2Fe-4S, 55 residues), Bacillus thuringiensis protein (1Fe-4S, 81 residues), Azotobacter vinelandii Fd (1Fe-4S), 3Fe-4S, 106 residues), and Desulfovibrio gigas Fd (2Fe-4S, 58 residues). Ye contain a common folding of the polypeptide chain, suggesting that most bacterial Fds evolved from a common ancestor. A number of bacteria possess 2 or more Fds; Rhodobacter capsulatus, a purple non-sulfur photosynthetic bacterium, has Pseudomonas type and azotobacter type Fds. By exploring the physiological roles of these Fds, in particular the relation to iron nutrition, by genetic method. We also overexpressed and isolated wild-type mutant proteins. Relation between the physicochemical properties and the structure will be presented.

MS-03.04.03 THE UNUSUAL METAL CLUSTERS OF NITROGENASE: AN ANALYSIS OF THE STRUCTURE OF MoFe-protein (CPI) AT 2.2A resolution. J.T. Bolton*, N. Camillia, S.W. Muchmore and W. Minor, Dept. of Biological Sciences, Purdue University, W.Lafayette, IN 47907, USA

Mo-dependent nitrogenases comprise two separable purifiable metalloproteins called MoFe protein and Fe protein. MoFe-protein, the component which contains the site of substrate reduction, is an αβγδ tetramer (Mr=220,000) which binds 2 Mo and 30 Fe atoms in the form of two unusual types of metal-sulfur clusters known as FeMo-cofactors and Pe-clusters. We have determined the crystal structures of the MoFe protein from Clostridium pasteurianum (CpI) at a resolution of 2.2Å. Initial phases were obtained by combination of MAD and MIR phase distributions and were improved and extended by solvent flattening and twofold electron density averaging. The structure was refined using the TNT package (Tronrud, D.E. et al. 1987 Acta Cryst. A43, 593-612) to an R-factor of 17% based on all measured data between 20 and 2.2Å resolution. Throughout the analysis we used anomalous diffraction methods to prove and define the structure of the metal-sulfur groups. Selected features of the refined structure will be described and related to biochemical and biophysical data pertaining to the structure and function of the enzyme. The stereochemistry of the metal-sulfur clusters as well as their interactions with protein groups and bound water molecules will be considered in detail. Experiments designed to test the reliability of the structures of the clusters will be reported, as well as comparisons to models published by Kim and Rees (cf. Kim, J. & Rees, D.C. 1992 Nature,360,553-560).

MS-03.04.04 CRYSTAL STRUCTURE STUDIES OF TWO COMPLEXES INVOLVING AMICYANIN AND ITS ELECTRON TRANSFER PARTNERS. By L. Chan*, R. C. E. Dutley and F. S. Mathews, Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110, U.S.A.

Amicyanin is a blue copper protein found in Paracoccus denitrificans and several other methylocrotyc bacteria. It is the electron acceptor for methylamine dehydrogenase (MADH), a quinoprotein containing tryptophan tryptophylquinone (TTQ). In vitro, cytochrome c serves as an efficient electron acceptor for amicyanin in the presence of