03-Crystallography of Biological Macromolecules

03.04.01 The Structure of Limulus polyphemus Subunit II Hemocyanin in an Oxygenated Form. Karen A. Magnus* and Hoa Ton-Thi. 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 a two copper active site. The native Limulus hemocyanin is composed of 48 subunits of eight immunologically distinct types. All subunits are of molecular weight approximately 75,000. Limulus hemocyanins, 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=117.2 ± 0.6 Å, c = 285.86 ± 0.9 Å with α=β= 90.0° and γ = 120.0°. There is one hemocyanin subunit containing one two 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.J. 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. Brunger, J. Mol. Biol., 1986, 203, 80–381). The structure of the Limulus subunit II was refined to 1.9 Å resolution and a crystallographic R-value of 19.6%.

03.04.02 STRUCTURE AND FUNCTION OF FERREDOXINS. Ketsiki Fukushima*, Toshiihiko Sasaki, 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 I. They have [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 phthalate dioxygenase reductase. The topology is also found in ubiquinol 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 proximity structure motif. Crystal structures of the 4 distinct types of bacterial Fds have been determined so far: Pseudomonas aeruginosa Fd (2Fe-4S), 55 residues, Bacteriodes bacteroides Fd (1Fe-4S), 81 residues, Azotobacter vinelandii Fd (1Fe-4S) [3Fe-4S], 106 residues, and Desulfovibrio gigas Fd (1Fe-4S), 58 residues. Ye they contain a common folding of the polypeptide chain, suggesting that most bacterial Fds evolved from a common ancestor. A number of bacteria possess two or more Fds; Rhodobacter capsulatus, a purple non-sulfur photosynthetic bacterium, has Pseudomonas type and azotobacter type Fds. We explored the physiological roles of these Fds, in particular relation to nitrogen fixation, by genetic method. We also overexpressed and isolated some mutant proteins. Relation between the physicochemical properties and the structure will be presented.

03.04.03 THE UNUSUAL METAL CLUSTERS OF NITROGENASE: AN ANALYSIS OF THE STRUCTURE OF MoFe-protein (CpI) AT 2.2Å RESOLUTION. J.T. Boll*; C.K. Camacho, 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 Fe3S clusters.

We have determined the crystal structures of the MoFe protein from Clostridium pasteurianum (CpI) at a resolution of 2.2A. Initial phases were obtained by combination of MAD and MIR phase dissections and were improved and extended by solvent flattening and twofold electron density averaging. The structure was refined using the TNT (Tuc, D.E. et al. 1987 Acta Cryst. A43, S1-62) to an R-factor of 17% based on all measured data between 20 and 2.2A resolution. Throughout the analysis we used anomalous dispersion methods to probe and define the structures 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 will comparisons to models published by Kim and Rees (cf. Kim, J. & Rees, D.C,1992 Nature,369,553-560).

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 methylo trophic 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
MADn. The structure of a binary complex between MADn and amiciyamin based on an x-ray sequence for MADn was reported previously by Chen et al., 1992, Biochemistry 31, 4959-4964).

The binary complex has now been refined at 2.5 Å resolution using the DNA-derived amino acid sequence. The current model, which includes 637 solvent molecules, has residual 0.143, with rms bond length deviations of 0.014 Å. The two proteins are oriented such that the cobalt binding region of amiciyamin is adjacent to the TFG-containing portion of MADn. The closest distance between TFG and cobalt is 9.35 Å. Most of the interactions between amiciyamin and MADn are hydrophobic. In addition, two water molecules mediate the interaction between backbone and side chain atoms.

The structure of a ternary complex composed of MADn, amiciyamin and ironcobalt complex has now been solved at 2.4 Å resolution. The location of the MADn portion was determined by molecular replacement allowing the amiciyamin molecule to be located in a single Fourier. After refinement of the MADn-amiciyamin partial structure, 147 residues of the 155 residue cytochrome could be traced in the electron density. The R-factor of the current model with 129 solvent molecules is 18.0%.

The interaction between amiciyamin and MADn is very similar in both the binary and the ternary complexes. The interface between amiciyamin and the cytochrome is much more polar, involving approximately 5 charged groups on amiciyamin and 4 charged groups on the cytochrome, including one of the heme propionate. The cytochrome is of the highly acidic s class found in methylotrophic bacteria. Its folding pattern resembles those of other bacterial c-type cytochromes, but it has a 45 residue extension at the N-terminal end and a 20-30 residue extension at the C-terminal end of the polypeptide chain. The distribution of charges over the cytochrome surface is asymmetrical, leaving the area closest to the heme relatively hydrophobic. The copper and iron atoms are approximately 24 Å apart. This is the first complex structure ever solved with three sequential protein components of an electron transfer chain. Several hypothetical electron transfer pathways will be discussed. This work has been supported by NSF grant no. MCB-8119789.

MS-03.04.05  THE IRON CENTER IN RIBONUCLEOTIDE REDUCTASE REDUCTASE by Per Nordlund, Anders Aberg, Ulla Uhlin & Hans Ekblund*; Department of Molecular Biology, Swedish University of Agricultural Sciences, Biomedical Center, S-751 24 Uppsala, Sweden

Proteins containing binuclear nonheme iron centers perform several functions which can also be made by heme containing proteins. Hemerythrin (mebemerythrin) reversibly binds oxygen, i.e. perform the same function as hemoglobin (myoglobin), monooxygenase chemistry can be performed by the non-heme methylene monooxygenase as well as the heme-protein cytochrome P450. Tyrosyl radical containing proteins also exist in both groups: ribonucleotide reductase protein R2 and prostaglandin H synthetase respectively. The crystal structure of the free radical protein R2 of ribonucleotide reductase has been determined by multiple isomorphous replacement and single-wavelength averaging. The structure has been refined to 2.24 resolution R = 0.175. The subunit structure of the R2 protein has a fold where the basic motif is a bundle of eight long helices.

The R2 dimer has two equivalent binuclear iron centers. The iron centers are well buried in the subunits. Each iron center contains two ferric ions which are coordinated by Asp44, Glu115, His118, Glu204, Gln218 and His241. The coordination is octahedral for one of the ferric ions as distorted octahedral for the other. The tyrosine heme ligand is conserved in all R2 proteins.

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