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 molluscs. 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=b=117.2 ± 0.6 Å, c = 285.86 ± 0.9 Å with $\alpha=\beta=90.0°$ and $\gamma=120.0°$. 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. Bruenger, 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 coppers in the μ-η^2:η^2 configuration, that is both oxygen atoms are equidistant from both copper atoms. There is no evidence of an endogenous bridging ligand present between the two copper atoms.

MS–03.04.02 STRUCTURE AND FUNCTION OF FERREDOXINS Keiichi 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 I. 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 phthalate dioxygenase reductase. The topology is also found in ubiquitin 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; *Peptococcus aerogenes* Fd (2[4Fe-4S], 55 residues), *Bacillus thermoproteolyticus* Fd (one [4Fe-4S], 81 residues), *Azotobacter vinelandii* Fd ([4Fe-4S] [3Fe-4S], 106 residues), and *Desulfovibrio gigas* Fd (one [3Fe-4S], 58 residues). Yet 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 *Peptococcus* 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.

MS–03.04.03 THE UNUSUAL METAL CLUSTERS OF NITROGENASE: AN ANALYSIS OF THE STRUCTURE OF MoFe–protein (CP1) AT 2.2A resolution J.T.Bolin*, N.Campobasso, S.W.Muchmore and W.Minor, Dept. of Biological Sciences, Purdue University, W.Lafayette, IN 47907, USA

Mo–dependent nitrogenases comprise two separately purifiable metalloproteins called MoFe protein and Fe protein. MoFe–protein, the component which contains the site of substrate reduction, is an $\alpha_2\beta_2$ tetramer (Mr=220,000) which binds 2 Mo and 30 Fe atoms in the form of two unusual types of metal–surfur clusters known as FeMo–cofactors and P–clusters.
We have determined the crystal structures of the MoFe protein from *Clostridium pasteurianum* (Cp1) at a resolution of 2.2A. 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 25 and 2.2A resolution. Throughout the analysis we used anomalous diffraction 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*,360,553–560).

MS–03.04.04 CRYSTAL STRUCTURE STUDIES OF TWO COMPLEXES INVOLVING AMICYANIN AND ITS ELECTRON TRANSFER PARTNERS. By L. Chen*, R. C. E. Durley 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 methylotrophic bacteria. It is the electron acceptor for methylamine dehydrogenase (MADH), a quinoprotein containing tryptophan tryptophylquinone (TTQ). *In vitro*, cytochrome c_{551i} serves as an efficient electron acceptor for amicyanin in the presence of

