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.

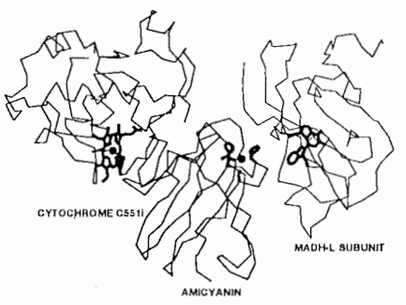
Amicyanin is a blue copper protein found in *Paracoccus denitrificans* and several other methylotrophic bacteria. It is the electron acceptor for methylamine dehydrogenase (MADH), a quinoprotein containing tryptophan tryptophylquinone (TTQ). *In vitro*, cytochrome c_{551i} serves as an efficient electron acceptor for amicyanin in the presence of

MADH. The structure of a binary complex between MADH and amicyanin based on an x-ray sequence for MADH was reported previously (L. 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 copper-binding region of amicyanin is adjacent to the TTQ-containing portion of MADH. The closest distance between TTQ and copper is 9.35 Å. Most of the interactions between amicyanin and MADH are hydrophobic. In addition, two water molecules mediate the interaction between backbone and side chain atoms.

The structure of a ternary complex composed of MADH, amicyanin and cytochrome c_{551i} (L. Chen *et. al.*, 1993, *Protein Science* 2,147-154), has now been solved at 2.4 Å resolution. The location of the MADH portion was determined by molecular replacement allowing the amicyanin molecule to be located in a difference Fourier. After refinement of the MADH-amicyanin 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 amicyanin and MADH is very similar in both the binary and the ternary complexes. The interface between amicyanin and the cytochrome is much more polar, involving approximately 5 charged groups on amicyanin and 4 charged groups on the cytochrome, including one of the heme propionates. The cytochrome is of the highly acidic c_L 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-9119789.



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

Proteins containing binuclear non-heme iron centers perform several functions which can also be made by heme containing proteins.Hemerythrin (myohemerythrin) reversibly binds oxygen, i.e. perform the same function as hemoglobin (myoglobin), monooxygenase chemistry can be performed by the non-heme methane 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 two-fold molecular averaging. The structure has been refined at 2.2A resolution to 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 canters. The iron centers are well buried in the subunits. Each iron center contains two ferric ions which are coordinated by Asp84, Glu115, His118, Glu204, Glu238 and His241. The coordination is octahedral for one of the ferric ions an distorted octahedral for the other. The tyrosine harboring the stable free radical is buried in the protein with its η-oxygen 5.1A away from the closest iron ion.
The R2 protein without iron, apoR2, is a precursor of active R2 and folds into a stable protein which is transformed into active R2 by ferrous ions and molecular oxygen. Diffraction data on apoR2 crystals was collected to 2.5A and the structure has been refined to a crystallographic R-value of 18.7%. A comparison with the iron containing protein shows no large global differences. Differences found are local and mainly restricted to the former metal sites and their environment.
The removal of iron results in a clustering of four carboxylate side chains in the interior of the subunit. The distances between Asp84, Glu115, Glu204 and Glu238 are short, Suggesting that some of them are uncharged. In the case of protein R2 the energy cost of the clustering of carboxylate side chains in the interior of the protein must have been accounted for by other means. Hydrogen bonding to polar side chains in the vicinity of the carboxyl residues party reduces the effect of the charges. Most importantly, the folded state of the subunit is stabilized by extensive van der Waals interactions and hydrogen bonds between the unusually long helices of R2.
ApoR2 has a very strong affinity for four stable Mn^{2+} ions. The manganese containing form of R2, named Mn-R2 has been studied by X-ray crystallography. It contains binuclear manganese clusters in which the two manganese ions occupy the natural irons sites and are only bridged by carboxylates from glutamates 115 and 238. Mn-R2 could provide a model for the active diferrous form of protein R2.
Guided by the three-dimensional structure of the R2 protein of E.coli ribonucleotide reductase, we have aligned the sequences of two different methane monooxygenases (MMO) with the sequences of the iron coordinating four helix bundle in R2.The model confirmed that the central four helix bundle of R2 should be present also in MMO. The iron coordination is similar in MMO and R2 with two histidine ligands and four carboxyl acid ligands in both cases. The terminal carboxyl ligands appear to have less restrictions than the other ligands and may be Asp or Glu. In R2 only His241 is hydrogen bonded by an Asp residues. This may allow high transient oxidation states of the binuclear iron center in MMO are significantly smaller in MMO than in R2 allowing binding site is lined by residues Cys151, Thr213, Ile217 and Ile(Val)239.
This binding site in R2 can be involved in other reactions than producing the radical species of the protein as shown by the mutation of Phe208 to Tyr. this Tyr is transformed into a catechol in the oxygen reaction and a blue protein with ferric dopa interaction is created. The structure of this mutant has been determined at 2.5A resolution. The coordination geometry is changed significantly and the dopa 208 and Glu238 both become bidental.