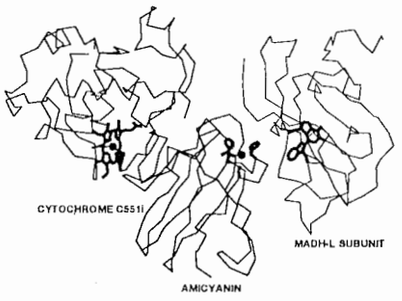
MADH. The structure of a binary complex between MADH and amicyanin based on an x-ray sequence for MADH was reported previously (L. Chen *et. al.*, 1992, *Biochemistry* 31, 4959-4964)

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.