

able difference in the cobalt environment between the two states and that the conformation observed in the crystal structure is also representative for the aqueous solution.

Very pronounced differences exist in the near-edge region between cobalt(II), cobalt(III) and alkyl (e.g. methyl) cobalamins. For several *Sporomusa Ovata* proteins carrying a p-cresolyl-cobamide cofactor, strong indications could be derived from the EXAFS and near-edge regions of the X-ray absorption spectrum that the cofactor occurs in the methylated form under oxidative ambient conditions.

MS02.07.03 HOW COENZYME B₁₂ RADICALS ARE GENERATED: METHYLMALONYL-COA MUTASE AT 2Å RESOLUTION. P.R. Evans and F. Mancia - MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK

This structure shows how the enzyme catalyses the formation of the adenosyl radical from coenzyme B₁₂. Methylmalonyl-CoA mutase is a member of a class of enzymes that bind the cobalt-containing 5'-deoxyadenosyl-cobalamin cofactor (coenzyme B₁₂) and catalyse 1,2 intramolecular rearrangements in which a hydrogen atom is exchanged with a group on an adjacent carbon. Methylmalonyl-CoA mutase catalyses the interconversion between (2R)-methylmalonyl-CoA and succinyl-CoA. Such reactions involve radical intermediates: the initial radical arises from the homolysis of the unique Co-C bond of coenzyme B₁₂, amongst the very few metal-carbon bond known in nature. A long-standing puzzle has been how the protein weakens this Co-C bond towards homolytic cleavage.

Methylmalonyl-CoA mutase is the only adenosylcobalamin-dependent enzyme present in both animals and microorganisms. In the bacterium *Propionibacterium shermanii* it is a key enzyme in the fermentation to propionate, whilst in mammalian liver it is responsible for the conversion of odd-chain fatty acids and branched-chain amino acids to succinyl-CoA for further degradation. The *P.shermanii* enzyme is an α,β heterodimer of 150kD total molecular weight with one active site per dimer. We have solved the structure of the ternary complex between the recombinant protein expressed in *E.coli*, coenzyme B₁₂, and the partial substrate desulpho-CoA (coenzyme A with the final sulphur atom replaced by a hydrogen).

Each subunit has essentially a two domain architecture. In the catalytic α chain, the B₁₂ is sandwiched between a C-terminal flavodoxin α/β domain and an N-terminal β/α TIM barrel. A conserved histidine from the flavodoxin-like domain provides axial coordination to the cobalt atom in a very similar way to that seen in methionine synthase. The histidine-cobalt distance is very long (2.5Å compared to 1.95-2.2Å in free cobalamins), suggesting that the enzyme positions the histidine in order to weaken the Co-C of the cofactor and favour the formation of the initial radical. The substrate is bound through a hole along the axis of the barrel, pointing into a deeply buried active site on the 5'-deoxyadenosyl or catalytic side of the B₁₂.

MS02.07.04 EXAFS ANALYSES OF MANGANESE ENZYMES James E. Penner-Hahn¹, Timothy L. Stemmler¹, Pamela J. Riggs-Gelasco¹, and Charles F. Yocum², Departments of Chemistry¹ and Biology², The University of Michigan, Ann Arbor, MI 48109-1055 USA

X-ray absorption spectroscopy has been used to characterize the local Mn environments of the multinuclear Mn clusters in Mn catalase and in the photosynthetic oxygen evolving complex (OEC). Manganese catalase contains a dinuclear Mn site, while the OEC contains a tetranuclear Mn cluster, together with Ca and Cl as inorganic co-factors. Although the Mn ions are magnetically interacting in reduced, Mn(II)/Mn(II), catalase, the enzyme lacks a

detectable Mn^{•••}Mn EXAFS signal. This is similar to findings for other dinuclear Mn enzymes, but distinct from the behavior of crystallographically characterized Mn models. In contrast, the OEC contains readily detectable Mn^{•••}Mn interactions at 2.7 and 3.3 Å. The dependence of the OEC structure on chemical perturbations points to the presence of two functionally distinct Mn dimers. Large, hydrophobic reductants such as p-dihydroquinone, completely disrupt the cluster structure, giving a state containing two Mn(II) ions. Small, hydrophilic reductants such as NH₂OH give a reduced enzyme with a structure that is essentially unchanged from the resting state. One of the striking differences between Mn catalase and the OEC is the oxidation state of the resting enzymes, with Mn(II) favored in the former, and Mn(III)/Mn(IV) favored in the latter. Detailed characterization of their nearest neighbor environments, and comparisons of these with crystallographically characterized Mn models suggests that the stability of lower oxidation states in the catalase is a consequence of the presence of nitrogen containing ligands as compared to alkoxide/phenoxide/or hydroxide ligands in the latter.

MS02.07.05 THE STRUCTURE OF HUMAN CERULOPLASMIN AT 3.0Å RESOLUTION: THE BEGINNING OF THE END OF AN ENIGMA. Peter Lindley, Irina Zaitseva, Vjacheslav Zaitsev, Adam Ralph, Graeme Card, CCLRC Daresbury Laboratory, Warrington WA4 4AD, UK

Ceruloplasmin is a member of the multi-copper oxidase family of proteins which includes laccase and ascorbate oxidase. Sequence homology also suggests that it is structurally related to blood clotting factors V and VIII. The protein comprises a single polypeptide chain of 1046 amino acid residues and upto four glycan chains (7 - 8% by weight) giving an overall molecular weight of some 132 kDa

The X-ray structure of human ceruloplasmin has been elucidated at a resolution of 3.0 Å [1]. The structure reveals that the molecule is comprised of six plastocyanin type domains arranged in a triangular array. There are six integral copper atoms, three of which form a trinuclear cluster sited at the interface of domains 1 and 6, and three mononuclear sites in domains 2, 4 and 6. The mononuclear copper in domain 6 and the trinuclear cluster form a four-copper oxidase centre almost identical to that found in ascorbate oxidase [2] and strongly suggesting an oxidase role for ceruloplasmin. Each of the mononuclear coppers is coordinated to a cysteine and two histidine residues and those in domains 4 and 6 also coordinate to a methionine residue to give typical "blue" type I copper centres. For the copper in domain 2 the methionine residue is substituted by a leucine and this copper is probably in the reduced state. The trinuclear cluster contains a pair of type III spin-paired coppers and a type II copper. The cluster is bound to the protein by eight histidine residues, four each from domains 1 and 6.

Ceruloplasmin has long been known as "the enigmatic blue plasma protein" since its precise functions have not been defined. The X-ray structure confirms that probability that the protein is multi-functional and suggests strong evidence for ferroxidase and anti-oxidant activity. The putative role of ceruloplasmin in iron metabolism and other functional aspects will be discussed.

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[2] Messerschmidt, A., Ladenstein, R., Huber, R., Bolognesi, M., Avigliano, L., Petruzzelli, R., Rossi, A. & Finazzi-Agró, A. *J. Mol. Biol.*, 224, (1992), 179-205.