and $f(\bar{P}_i, \bar{h}_j)$ is a formula, describing reflections; d_1 and d_2 are uadratic, or robust, or entropy metrics. At the same time $\bar{h}_j$ * , computed while minimizing R_j, index jth reflections. V and N are volumes of a phase and total number of reflections at tried parameter vectors.

The minimization of (1) with respect to $\bar{P}_i$ is carried out by trying parameter values from a assumed region, and refining them by analytical fitting, which, in case of convergence, gives the best estimate, or, if initial guesses are good enough, by analytical fitting.

Reference: Zlokazov V.B. Comp.Phys.Comm.,1995,v.85,p.415-422.

PS02.06.21 PHASING THE CHOLERA TOXIN ELECTRON DIFFRACTION DATA USING THE MAXIMUM ENTROPY-LIKELIHOOD METHOD WITH NON-CRYSTALLOGRAPHIC SYMMETRY IMPOSED IN THE MICE COMPUTER PROGRAM. W.N. Nicholson and C.J. Gilmore, Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, Scotland U.K.

We have already reported our experience with applying the maximum entropy-likelihood method to phasing the two-dimensional projection data for cholera toxin (Gilmore & Nicholson (1995). Transactions American Crystallographic Association, 31 In press.). We have been working with a 2-d data set for which 56 unique image phases are available at 8.8Å resolution, and for which a further 1417 diffraction intensities extend to 4Å. The problem has been:

1. To phase the 4Å data from the 56 known phases.
2. To impose 5-fold non-crystallographic symmetry on the projection.
3. To impose envelope and solvent flattening constraints.

The maximum entropy-likelihood program (MICE) has been modified to carry out these steps. Using it we have shown that the likelihood criterion to is an accurate and reliable predictor of:

4. The effective number of atoms in the unit cell.
5. The centroid coordinates for the 5-fold non-crystallographic axes.
3. The envelope radius.

We are now extending the procedure to phase data derived from a series of electron diffraction patterns derived from a limited set of specimen tilts. The extension of two-dimension phase information into three is non-trivial especially when so little a priori phase information is available, and will be discussed in detail.

Research funded by a grant from the Human Frontier Science Program.

Metalloproteins X-Ray & EXAFS Studies

MS02.07.01 EXAFS STUDIES OF NITROGENASE AND RELATED SYSTEMS: AN OVERVIEW. Keith O. Hodgson, Department of Chemistry Stanford University, Stanford, CA 94305

X-ray absorption edge and extended fine structure (EXAFS) studies contributed significantly to the early definition of particularly the Mo- Fe-S containing cofactor (FeMoco) of the MoFe protein of the nitrogenase system both within and outside the protein (in its isolated form). The x- ray crystal stucture results have now defined the overall protein structure and the relationships and structures of the metal-containing prosthetic groups in the resting state. However, much remains to be learned about how the electronic structure of the FeMoco mediates electron transfer and dinitrogen reduction and XAS spectroscopy is now being used to probe this question. Edge studies provide information on electronic structure including covaleny and electronic distribution as a function of oxidation state. EXAFS results incorporating multiple scattering analyses provide a specific probe of the metrical details (including longer range distances) and in some cases geometry of the FeMoco. This talk will review some of these recent advances as they provide insights into the structure/function of the nitrogenase system.

MS02.07.02 EXAFS AND X-RAY STUDIES OF B_{12} MODEL COMPOUNDS. Christoph Kratky, Institut für physikalische Chemie, A-8010 Graz - Austria.

The chemical and structural complexity of the B_{12} coenzymes (5'-desoxyadenosyl cobalamin and methyl cobalamin) and their biologically relevant reactivities (Co-C bond homolysis and Co-C bond heterolysis, respectively) has been a puzzle ever since the elucidation of their structures by Dorothy Hodgkin's group more than three decades ago. For many years, the focus of structural and chemical research lay on the isolated cofactor or cofactor analogue, with special emphasis on the cobalt center and its coordination environment.

Along these lines, we have determined crystal structures of a number of cobalamins with different α- and β-substituents. Using synchrotron radiation in combination with imaging plate detectors for some of these analyses, we were able to collect very accurate and comprehensive data sets, which permitted structure refinement to a level of precision comparable to a well-determined small-molecule crystal structure. From the combined structural data of about 20 cobalamin crystal structures, correlations between several characteristic intra-molecular deformation parameters (upward-folding of the corrin ring, axial Co-N distance, orientation of the dimethylbenzimidazole base) can be established and used to estimate the relative "stiffness" of each of these deformation modes.

In recent years, the B_{12} field has advanced dramatically as a result of the elucidation of the first crystal structures of proteins binding a B_{12} cofactor (B_{12} binding domain of methionine synthase; methylmalonyl CoA mutase). In both proteins, a protein-derived histidine-imidazole occupies the α-axial coodination of the cobalt center, replacing the dimethylbenzimidazole base occupying this position in solutions of the isolated cofactor under physiological conditions. Thus, it appears that some of the above structural correlations refer to a biologically irrelevant cofactor constitution.

For a number of representative compounds, we have also collected X-ray absorption spectra as a basis for the interpretation of EXAFS spectra of cobalamins in „non-crystalline" environments (e.g. cobalamins in solution and bound to a protein). Thus, by a comparison of the spectra of Aquocobalamin perchlorate in solution and in crystalline form, we could show that there is no detect-

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 B_{12} 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 B_{12}. Methylmalonyl-CoA mutase is a member of a class of enzymes that bind the cobalt-containing 5´-deoxyadenosyl-cobalamin cofactor (coenzyme B_{12}) 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 B_{12}, 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 microrganisms. 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 B_{12}, 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 B_{12} 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 B_{12}.

MS02.07.04 EXAFS ANALYSES OF MANGANESE ENZYMES James E. Penner-Hahn[1], Timothy L. Stemmler[1], Pamela J. Riggs-Gelasco[1], and Charles F. Yocum[2], Departments of Chemistry[1] and Biology[2], 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, hydrophillic reductants such as NH_2OH 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 enyzmes, 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 plastocyanintype 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 fucntional aspects will be discussed.

[1] Zaitseva, I., Zaitsev, V., Card, G., Moshkov, K., Bax, B., Ralph, A. & Lindley, P. *J. Biol. Inorg. Chem.*, 1, (1996), 1-9.
[2] Messerschmidt, A., Ladenstein, R., Huber, R., Bolognesi, M., Avigliano, L., Petruzelli, R., Rossi, A. & Finazzi-Agró, A. *J. Mol. Biol.*, 224, (1992), 179-205.