It appears that only in the last two cases $I_R(h)$ could have been determined directly from the dispersion function $f'(\lambda) + f''(\lambda)$.

The influence of statistical and systematic errors on the results will be discussed.

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L4

Local Coordination Geometry in Fe and Ca Metalloproteins by XANES using Synchrotron Radiation

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X-ray absorption spectroscopy using synchrotron radiation is opening new perspectives in the study of fundamental biological problems of structure and function of metalloproteins because of its unique capability to give direct structural information on the metal active sites of proteins in solution. The direct structural information in X-ray absorption spectra arise because of their interpretation in terms of electron scattering by neighbour atoms of the electrons photoemitted at the metal site. EXAFS oscillations arising from single scattering processes can give distances, coordination numbers and the type of neighbour atoms!

A recent development on the X-ray absorption near edge structure: XANES [1] extending over about 50 eV energy range demonstrates that this part of the X-ray absorption spectrum also can be interpreted in terms of electron scattering by neighbour atoms. The spectra show strong multiple scattering resonances, like low energy electron spectroscopy, determined by atomic geometrical distribution of the neighbour atoms. The actual 'state of art' is such that after distance determination by EXAFS, XANES is able to distinguish between different possible geometrical structures [2].

We report XANES spectra of hemoglobin and related heme-proteins where we have found evidence of:

(1) Fe displacement relative to the porphyrin plane which cannot be determined by other methods in solution.

(2) Variation of the ligand bonding angle Fe–C–O and Fe–C–N in hemoglobin in solution where CO is tilted and CN is vertical relative to the porphyrin plane.

(3) Time resolved XANES can provide information on the dynamics of atoms in the active sites during protein function.

In calcium proteins [4, 5] troponin-C and calmodulin [6] different orientations of COO^- groups of aspartic and glutamic residues bound to Ca^{2+} in the EF-hand loop can be distinguished and different sites are identified.

The presence of the allosteric role of Mg^{2+}, and the effects of drugs on calmodulin have been found.

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L5

Iron EXAFS Studies of the Iron–Molybdenum Cofactor of Nitrogenase and the 3Fe Ferredoxin II of *Desulfovibrio Gigas*

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Chemical and physical analyses indicate that the iron–molybdenum cofactor (FeMo(co)) of nitrogenase contains 6–8 mol of iron and 4–6 mol of sulfur per mol of molybdenum. The physical properties of this cofactor suggest that it contains a novel Mo–Fe–S cluster. Extended X-ray absorption fine structure (EXAFS) data taken at the Mo edge indicate that the molybdenum has two or three iron atoms and four or five sulfur atoms as nearest neighbors. Several models are consistent with these data. More information concerning the iron environment is needed to better define the structure of the FeMo(co). In this talk, we will present our recent results [1] on the iron edge EXAFS of the FeMo(co) from *Azotobacter vinelandii* and relate structural information about the iron sites in the cluster. In a related study, we have obtained the iron K-edge EXAFS of the 3Fe ferredoxin II of *Desulfovibrio gigas* in the oxidized and reduced states [2]. For both states, interpretation of the EXAFS data suggests that the Fe–S distance is near 2.25 Å, in agreement with crystallographic studies of model compounds and proteins containing 2Fe-2S and 4Fe-4S centers, as well as with a recent crystallographic study of *Azotobacter vinelandii* ferredoxin I (D. Ghosh, W. Furey, Jr., S. O'Donnell and C. D. Stout, *J. Biol. Chem., 256,* 4185 (1981).). The Fe–Fe distance of 2.7 Å, however, agrees with similar distances observed in other Fe–S centers, but disagrees with the 3Fe cluster in the *Azotobacter vinelandii* ferredoxin I structure, for which an Fe–Fe distance of 4.2 Å was reported. We conclude that the two 3Fe ferredoxins may have substantially different core dimensions, a possibility apparently unique to 3Fe centers among known Fe-S systems in proteins. The implication of such structural variation of 3Fe centers in the nitrogenase problem will be discussed.

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L6

EXAFS Studies of Copper and Zinc in Metallothionein and Bovine Superoxide Dismutase

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We have accomplished the measurement and interpretation of the copper and zinc K-edge EXAFS for these elements in two protein environments; metallothionein, from a variety of sources, and bovine erythrocyte superoxide dismutase.

Metallothioneins are a ubiquitous class of proteins which are believed to play important roles in the control of zinc and copper metabolism and in the detoxification of metals, such as cadmium and mercury. Each of these proteins is characterized by its low molecular weight (~6,000), a high cysteinyl content, and the ability to bind substantial amounts of metal ions, such as Cd, Zn, Cu, Ag, Co and Hg. Results obtained from a variety of spectroscopic studies clearly indicate that the metal ions are bound to the protein *via* the sulphur atoms of the cysteinyl residues. However, a definitive structural characterization of a metallothionein has not yet been achieved. To provide further information concerning the details of metal attachment to metallothionein, we have recorded the copper and zinc K-edges for pig liver metallothionein containing copper and zinc, and the K-edge of zinc for sheep liver metallothionein 2 containing zinc, and rabbit liver metallothionein containing zinc or zinc and cadmium. Freeze-dried samples were employed and X-ray absorption spectra were recorded for these materials at room temperature and 77K.

For zinc, the EXAFS data are consistent with back-scattering from, essentially, a single shell of our sulphur atoms at *ca.* 2.28 Å. The chemical interpretation of this information is that zinc is bound by the sulphur atom of four cysteinyl groups, as $Zn(Scys)_4$. Similar conclusions have been reached for the immediate environment of the copper atoms in pig liver metallothionein containing *ca.* six copper and two zinc atoms per protein molecule. The Cu–S distance is *ca.* 2.22 Å and there appear to be three sulphur atoms at this distance.

An assessment of the quality of the interpretation of the EXAFS data obtained for zinc and copper in metallothionein has been obtained by graphically displaying the variation of the function

$$r_g = 1/\Sigma_k[k^2(\psi_T(k) - \psi_E(k))]^2$$

for different values of the parameters used to interpret the EXAFS data. The reciprocal of the simple least-squares function was chosen since it produces clearer visual displays than the function itself; thus, the optimum interpretation of the experimental data is when r_g is maximised. An iterative procedure was adopted, refining (R and E_0) and (N and α) sequentially, until reasonable self-consistency was obtained. The final value of R (Zn–S) was obtained by plotting a slice through the r_g *versus* (R, E_0) profile at the optimum value of E_0; the uncertainty in this value being taken as the width of this peak at 70% height.