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Resonant Scattering in Macromolecular Structure Research. Applications in Molecular Biology and Material Science

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This report concerns the X-ray resonance scattering work carried out using the instrument X15 which receives synchrotron radiation from the storage ring DORIS. X15 covers a wavelength range from 0.6 to 3.3 Å. It therefore accesses resonance scattering at the K-absorption edges of the elements with Z = 20 (Ca) to Z = 42 (Mo) and at the L_3-absorption edges from Z = 50 (Sn) to all heavier atoms. The instrument consists of a double monochromator with a vertical offset of the beam by 1.22 m and a camera of 10 m length. A multiwire proportional chamber (from A. Gabriel) at the end of the evacuated beam line detects the scattered X-ray photons on its area of 200 mm × 200 mm. The distances between sample and detector may vary in discrete steps from 0.37 m to 7.2 m. The extreme resolution in physical space from 3 Å to 3000 Å at the short and long wavelength side respectively makes X15 suitable for structure research of both low molecular weight organic compounds and macromolecular structures.

Resonance X-ray scattering starts there, where the structure-resolving power of the X-ray absorption fine structure ends, *i.e.* at about 3 to 4 Å*. The dispersion of the resonance scattering factors f′ and f″ is strongest in the near edge region. It is also there that the chemistry of the excited atom may modify significantly the dispersion. We define the Scheme (see below). Any question of this Scheme relies on the preceding one being answered, at least experimentally ('heard, but not fully understood'). Resonance X-ray scattering starts from the measurement of the absorption spectrum of the atom chosen as a label. The absorption spectrum does not need to be analyzed further except that it is the basis for the calculation of the resonant scattering factors f′ and f″. For semicrystalline and amorphous systems described by

$$\rho_\lambda(\vec{r}) = \sum_{n=1}^{N} f_{o,n}\delta(\vec{r} - \vec{r}_n) + \{f_o + f'(\lambda) + if''(\lambda)\} \times \times \sum_{m=1}^{M}\delta(\vec{r} - \vec{r}_m)$$

the dispersion of the scattering intensity will be

$$I_\lambda(h) = I_O(h) + f'(\lambda)I_{OR}(h) + \{f'^2(\lambda) + f''^2(\lambda)\}I_R(h)$$

Compare: 4b and 4a. The three basic scattering functions I_O, I_{OR} and I_R — differing in dimensions — can be determined by measurements of I(h) at three (in practice: 20) different wavelengths in the near edge region. The cross term $I_{OR}(h)$ is usually most easily measured, as its dispersion function $f'(\lambda)$ differs strongly from the always predominant dispersion of the absorption and, to a smaller extent, fluorescence. Very often it is the only resonant term which can be obtained in practice. Examples are:

– Iron in ferritin, dispersion of the radius of gyration of the ferritin molecule, (Stuhrmann, 1980).

– Iron in hemoglobin, distances between the iron atoms of the four subunits of oxyhemoglobin, (Stuhrmann and Notbohm, 1981).

– Zinc and mercury in the native and ligated Hg-derivative of ATCase (Moody).

– Terbium bound to tRNA (Rigler).

– Cobalt in copper (Gerstenberg).

– Zinc in aluminium (Kostorz).

– Distribution of ions around polyelectrolytes. Cs and I in DNA (Oberthür), Cs, Rb and Br in polyacrylic acid (Ragnetti), Cs, Ba and I in *E. coli* ribosomal 50S subunit (Nierhaus), binding of Ba ions to LDL and of Eu ions to DPPC vesicles (Laggner), Er in DPPC membranes (Büldt).

– Bromine as a probe for the distribution of epibromhydrin in a semicrystalline copolymer PEO-epibromhydrin (Strobl, Urban).

*Resolution in physical space.

Question:	Measurement of:	Information:
1. Who are you?	Ionization energy	analytical
2. How are you? (valency, covalency)	X-ray absorption near edge structure, XANES	chemical range: ~1 Å
3. What and where are your nearest neighbours?	extended X-ray absorption fine structure, EXAFS	chemical and structural range: ~4 Å
4a. From excited atom to excited atoms	X-ray resonance scattering in the near edge region	structural range: ~100 Å
4b. From excited atoms to silent atoms		
5. From excited atom to another excited atom feeling different (different XANES)	X-ray resonance scattering with more detailed dispersion analysis	structural and chemical range: ~100 Å

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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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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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*