

X-Ray absorption near edge structure (XANES) for CO, CN and deoxyhaemoglobin: geometrical information

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We use the recently developed multiple scattering theory to give a quantitative analysis of the X-ray absorption near edge structure (XANES) of haemoglobin and some of its substituents. We demonstrate that the XANES may contain information not provided by the extended X-ray absorption fine structure (EXAFS) part of the spectrum about the coordination geometry around the Fe atom, and in particular discuss the sensitivity of the XANES to the orientation of the CN group in HbCN. The anisotropy of the system leads to a strong dependence of the calculated spectrum on the polarisation of the X-rays. We show how this effect can be exploited in further XANES structural studies.

Key words: XANES/haemoglobin/near edge spectra/synchrotron radiation

Introduction

During the past decade extended X-ray absorption fine structure (EXAFS) spectroscopy has been extensively used to probe the environment of metal atoms in a variety of biological systems. Even though accurate information has been obtained regarding the distance, nature and number of ligands surrounding the metal site (Teo and Joy, 1981; Garner and Hasnain, 1981), such studies have yielded little geometrical information on the ligands because the electron scattering in the EXAFS region is predominantly due to a single scattering process and is spherically averaged (Lee and Pendry, 1975). It has been realised for some time that detailed information about the coordination geometry may be available in the near edge absorption region (XANES) where multiple scattering of the excited electron confers sensitivity to the angular, as well as radial, distribution of the neighbouring atoms (Bianconi, 1981). However, to date, it has not been possible to analyse the XANES spectra in a complex biological system, mainly due to a lack of proper methods for reliable interpretation of the experimental results. We have recently developed a multiple scattering theory for the calculation of near edge spectra (Durham *et al.*, 1981) which is now applied to haemoglobin (Hb) and some of its substituents to provide the first quantitative discussion of the XANES of a biological molecule. Our aim is to investigate the sensitivity of the XANES to small changes in local structure around the metal atom. The initial calculations are encouraging, and we suggest that this method may give a new probe for haemoglobin and other metalloproteins with regard

to: (a) the effective charge on the iron; (b) the geometry of the ligands; and (c) the displacement of the Fe atom from the porphyrin plane. In particular, we predict that the spectra for crystalline samples will show a strong dependence on the polarisation of the X-rays, and indicate how this effect can be exploited to determine ligand bonding geometries.

Results and Discussion

XANES spectra associated with Fe K-edge were recorded both in the fluorescence and transmission modes at Stanford and Frascati Synchrotron Radiation Laboratories. Spectra for deoxy Hb were recorded at both Laboratories (Morante *et al.*, 1983; Perutz *et al.*, 1982), picket fence [Fe²⁺ (TPinPP) (2-Melm).EtOH] at Stanford (Perutz *et al.*, 1982), CN and CO-bound Hb at Frascati (Morante *et al.*, 1983). After the XANES experiments, the samples were checked spectrophotometrically to ensure that neither oxygenation nor oxidation had occurred (Morante *et al.*, 1983).

The experimental data for Hb, HbCO, HbCN and the picket fence (PF) are shown in Figure 1, together with the first derivatives of the Hb spectrum. In each case, five features (A–E) are observed, showing significant differences in relative intensities. In addition, a large edge shift (~4 eV) is observed in the case of deoxy Hb compared with HbCN, HbCO and other liganded forms (Morante *et al.*, 1983; Eisenberger *et al.*, 1976).

Our calculations use the multiple scattering cluster method of Durham *et al.* (1981), which has been successful in analysing the XANES of ferro- and ferricyanide complexes (Bianconi *et al.*, 1982). We used the same scattering amplitudes for Fe, C, N atoms here as in our earlier work (Bianconi *et al.*, 1982). In principle, these amplitudes should be calculated afresh for each new system, but past experience leads us to believe that this will give only minor changes in the XANES. Our cluster consisted of up to three atomic shells of the porphyrin group, together with the imidazole group and, in some calculations, a CN ligand coordinating the central Fe atom. The geometry of our cluster represents an average over the atomic positions determined for Hb by Fermi (1975), and is idealised in that the porphyrin group is taken to be planar and 4-fold symmetrical. We have tested the sensitivity of the calculated XANES to various values of the Fe–N_p distance and will report on this in another paper. Accurate atomic positions (if these are known) and scattering amplitudes should certainly be used in a definitive analysis of the XANES of Hb and related systems, but our aim in this first report is to see if it is feasible to analyse the XANES usefully within the current theoretical framework.

Figure 2 shows the theoretical spectra for one to three shells of a cluster modelling Hb and PF, for each case calculations are presented for $E \parallel n$ and $E \perp n$, where n is normal to the porphyrin plane. For three shells, a calculation averaged over the two polarisations is also given which can be compared with the experimental curves shown in Figure 1, all the spectral features (A–E) are clearly shown. The separation for these features are compared with those observed for the PF complex in Table I. We regard the agreement of theory with

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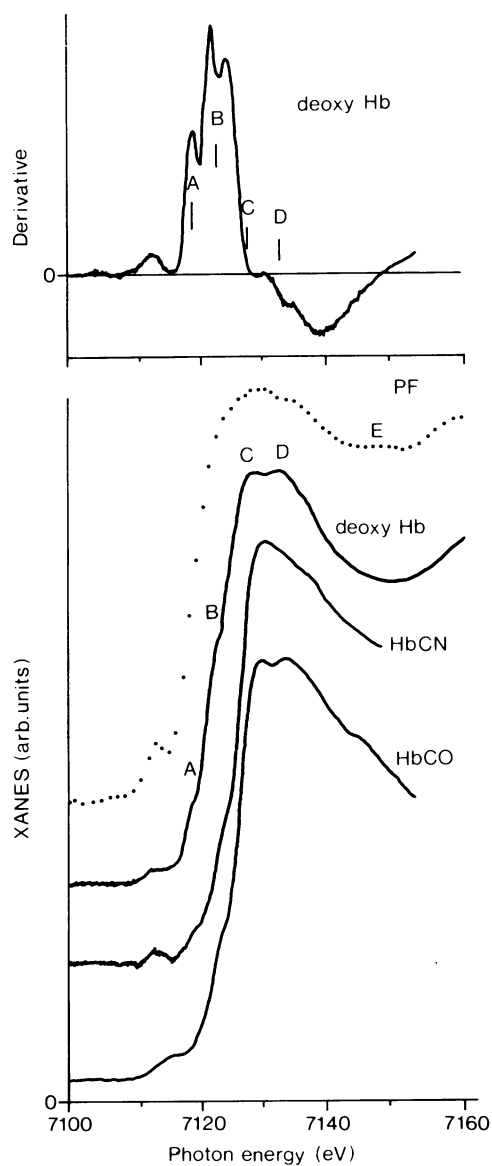


Fig. 1. Experimental K-edge XANES for PF, deoxy Hb, HbCN and HbCO. The derivative spectrum is also shown for deoxy Hb.

experiment, both for the general shape of the spectra and for the relative energies of the main features, as very encouraging. The fundamental theoretical model underlying our calculations rests on assumptions [in particular, the muffin-tin approximation (Durham *et al.*, 1981)] which are appropriate to close-packed crystals, but which might be expected to break down for the open covalent systems studied here. It seems that this is not the case for electron energies relevant to the XANES where the atomic geometry seems, therefore, to play the major role.

Figure 2 clearly shows the necessity of including scattering by third shell atoms; the 1-shell calculations are clearly inadequate. This was also found to be the case in EXAFS for $E < 200$ eV (Perutz *et al.*, 1982). This large anisotropy of the system leads to a very strong polarisation dependence in the calculated spectra, and it would be very interesting to try to observe this in single crystal experiments. [Such effects have been seen in the XANES of Cu-plastocyanins (Scott *et al.*, 1983)].

Figure 2 also shows calculations for a similar cluster, but

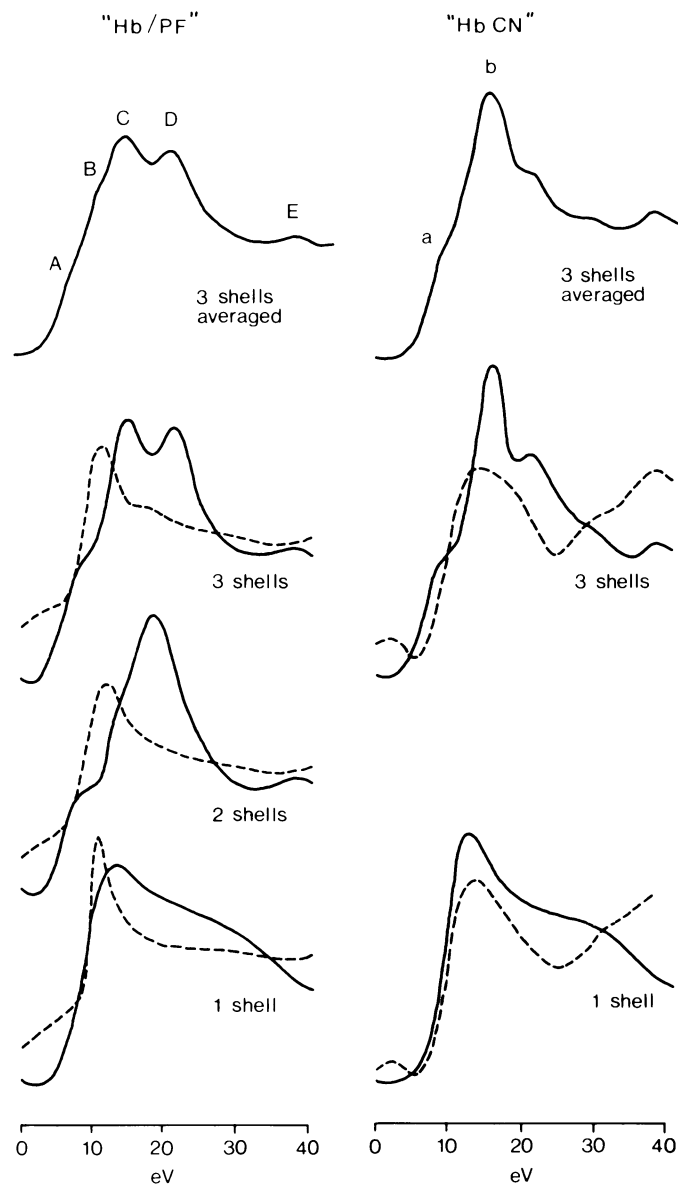


Fig. 2. Calculated XANES for atomic geometries close to deoxy Hb and PF ('Hb/PF') and HbCN ('HbCN'). Full curves: photon polarisation parallel to the porphyrin plane. Dashed curves: polarisation normal to the plane. In the upper curves the polarisation is averaged.

Table I. Energy positions (in eV) of spectral features (see Figures 1 and 2)

	A	B	C	D	E
Theory ('Hb/PF')	7.1	11.4	15.2	21.5	38.6
Experimental (deoxy Hb)	7.7	11.4	15.6	21.0	37.7 ^a

^aThis value is taken from the data on the PF compound.

The energy scale is that used in the calculations and the experimental and theoretical spectra have been aligned so that peak B occurs at the same energy in each.

with a vertical (i.e., normal to the porphyrin plane) CN ligand bonded to the Fe atom ($R_{\text{Fe}-\text{C}} = 1.8 \text{ \AA}$, $R_{\text{C}-\text{N}} = 1.134 \text{ \AA}$). The addition of the ligand causes quite large changes in the XANES, especially if the photons are polarised perpendicular to the plane.

Next, we studied the effect of ligand bonding angle on the

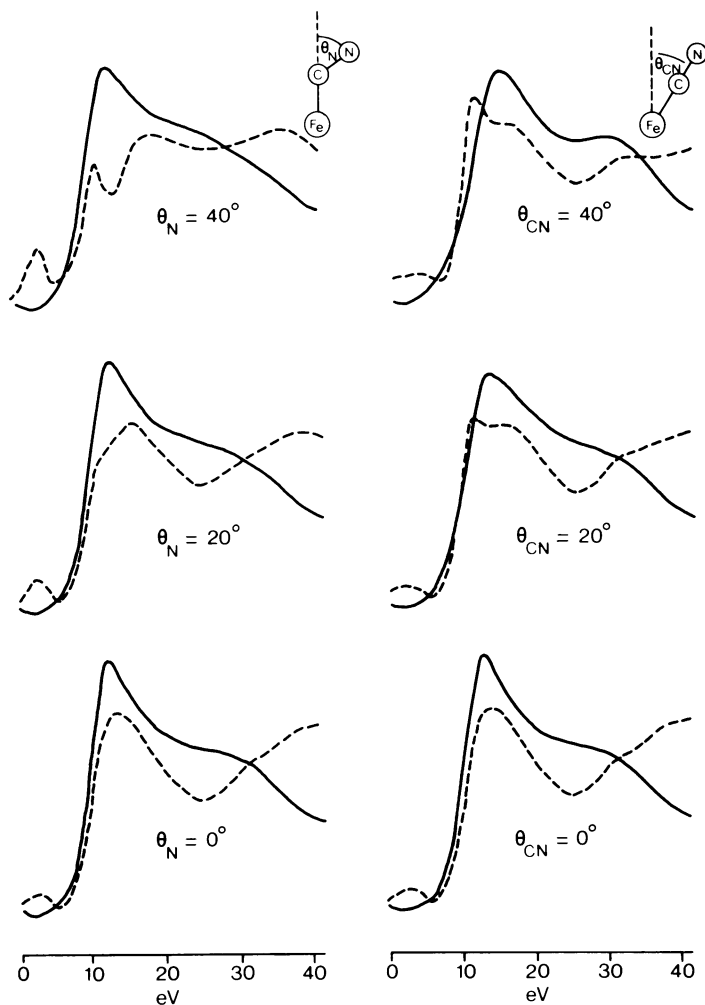


Fig. 3. Calculations for the 1-shell 'HbCN' cluster for different CN bonding geometries. Polarisation as in Figure 2.

near edge structure. CO and CN binding are expected to be similar; the Fe-C_t distance is ~0.10 and 0.2 Å for CO- and CN-bound Hb, respectively, and the Fe-N_p distance is expected to be the same (~1.98 Å) in the two cases. Detailed calculations were performed to understand the cause for differences in the experimental data (Figure 1b). The difference in the experimental data is large, and cannot be accounted for either by the difference in scattering (from the O and N atoms of the C=O and C≡N groups) nor by the small difference in Fe-C_t distance. Calculations with different bonding geometry show larger changes, similar to those observed in the experimental data.

Figure 3 shows a series of calculations on a 1-shell cluster (for computational economy) in which the CN group assumes different angles with respect to the porphyrin plane. For vertical CN coordination, these simplified calculations predict a single, rather sharp peak similar to the measured HbCN spectrum, which splits up as the CN group is tilted, rather like the HbCO XANES spectrum in Figure 1. This is consistent with recent neutron diffraction data on CO-myoglobin which has suggested an angle of 24° for the linear Fe-CO. We note that the calculations in Figure 3 do not show any significant difference between bent and co-linear Fe-C-N bonding, but this could show up in a full 3-shell calculation.

The calculations in Figure 3 show that the changes in the XANES as the CN group is tilted are strongly polarisation

dependent. An interesting way of determining the ligand angle would thus be to measure the XANES as a function of photon polarisation in oriented single crystals (Hanson and Schoenborn, 1981; Stöhr *et al.*, 1981), and, if successful, this would establish the technique quantitatively. However, this work shows that the real advantage of XANES over other experimental methods (e.p.r., optical spectroscopy, diffraction, etc.) is the possibility of finding information on ligand bonding geometries also in solutions close to the *in vivo* state.

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