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O₂ and CO Bonding Geometry in Heme-Proteins in Solution Investigated by XANES

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1 Introduction

The bonding angle of oxygen and carbonmonoxy molecules in oxygen carrier hemoproteins is a key parameter to describe the bond strength of the diatomic molecules to iron atom and therefore to understand the mechanism of reversible bonding and releasing of oxygen in hemoglobin. In spite of the large number of studies of haemoglobin the determination of oxygen bonding angle in the proteins in solution escapes the available experimental methods. A different bonding angle of oxygen in oxy-hemoglobin (HbO₂) and in oxy-myoglobin (MbO₂) single crystals has been recently reported [1]. The different bonding angle in the two hemoproteins which have different biological roles, transport and storage of oxygen molecules respectively, but similar local structure at the iron site has renewed the interest in this problem. We report the first application of the XANES (X-ray Absorption Near Edge Structure) spectroscopy to obtain information on the oxygen bonding geometry in the protein in solution (close to the "in vivo" situation) which cannot be studied by diffraction methods. The appealing aspect of XANES to study this problem is that the multiple scattering resonances of the photoelectron emitted at the iron site in the 10-50 eV energy range depend on the relative atomic positions of neighbour atoms and not only on the first order radial distribution function like EXAFS (Extended X-ray Absorption Fine Structure), therefore it is a direct structural probe of bonding angles [2-4].

2 Experimental

The XANES measurements were performed at the Frascati "wiggler" beam line using synchrotron radiation monochromatized by a Si(111) channel-cut single crystal; the protein concentration ranged from 4 to 10 mM in heme. Optical spectra of unirradiated and irradiated samples were used to control the possible radiation effects. The sample prepared at pH=7.2 was kept at constant temperature +10 C during the experiment. Hemoglobin sample was obtained from human adult blood and myoglobin was obtained from Sigma sperm whale myoglobin.

3 Results and discussion

Figure 1 shows the XANES of oxy-myoglobin and oxy-hemoglobins of adult human and carp fish. The zero of the energy scale is fixed at the Fe metal K-edge. There are no differences between the XANES spectra over a range of 50 eV within the experimental noise. The comparison between the XANES spectra of HbCO and MbCO, shown in Fig. 2 shows clearly a difference in the intensity of peak C between the two spectra.

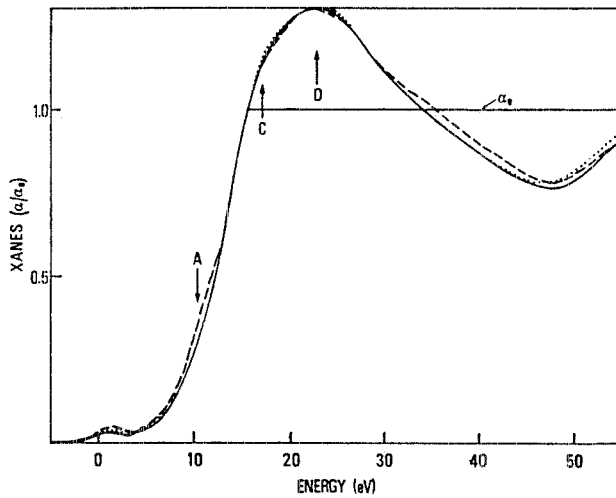


Fig.1 Experimental XANES spectra of oxy-myoglobin (solid line), human oxy-hemoglobin (dashed line) and carp fish oxy-hemoglobin (dotted line).

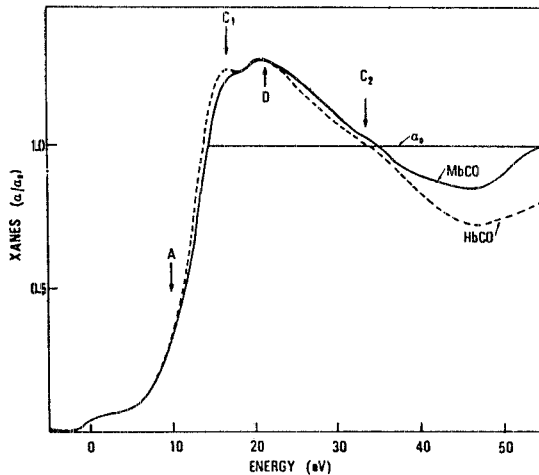


Fig.2 Experimental XANES spectra of HbCO (dashed line) and MbCO (solid line).

The similarity between the XANES of oxy-hemoproteins indicates similar local atomic arrangement near the Fe site in these proteins and therefore similar oxygen orientation in the protein in solution. This is in opposition with the situation in crystals where the oxygen bonding angle is different [1].

The XANES multiple scattering calculations have been performed starting from the coordinates of the atoms of MbO_2 and HbO_2 crystals. The multiple scattering resonances have been calculated for a photoelectron emitted by the Fe atom and reflected and transmitted by three shells of neighbour atoms forming a cluster of 29 atoms including the 4 nitrogens and 20 carbon atoms of the heme 3 atoms of the proximal histidine and the two oxygen atoms. The calculations are in good agreement with the energy position of the experimental features and only in qualitative agreement concerning the lineshape of the spectra. A large change of the XANES spectra for the variation of the oxygen bonding angle Fe-O-O between 115° and 156° , as it has been found in MbO_2 and HbO_2 crystals, is predicted by the calculations, mainly at the peak C in Fig. 3. Because we do not observe in the XANES spec-

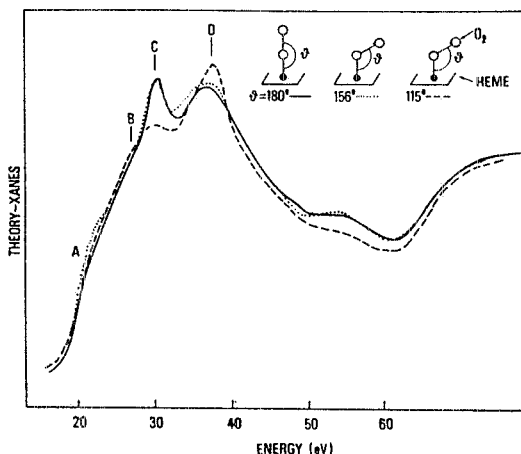


Fig.3 Theoretical XANES spectra calculated for different oxygen bonding angles.

tra a variation in this energy range we find no evidence of such a large variation of the oxygen bonding angle between HbO_2 and MbO_2 in solution. Evidence for different C-O bonding in MbCO in crystal and solution has been found by infrared vibrational spectroscopy [5].

Finally the structures C_1 and C_2 in MbCO and HbCO-XANES spectra in Fig. 2 have been assigned [3,4] to the strong π and σ multiple scattering resonances of the CO molecule. The photoelectron is trapped within the CO molecule like in electron scattering by CO in gas phase electron spectroscopy experiments at positive kinetic energies. The intensity of the resonances is strongly dependent on the Fe-C-O bonding angle because the photoelectron is strongly reflected toward Fe in the Fe-C-O colinear configuration. The weaker intensity of the C_1 peak in MbCO indicate a more tilted CO orientation (a smaller Fe-C-O angle) than in HbCO.

In conclusion we have shown that we can identify the resonances due to CO and O_2 in the XANES of heme-proteins which because of the short interatomic distance give strong multiple scattering resonances at ~ 20 eV and ~ 35 eV, above Fe-metal K-edge.

References

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