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LIGAND BONDING ANGLE IN HEMOPROTEINS BY XANES SPECTROSCOPY

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Summary

The X-ray Absorption Near Edge Structure (XANES) spectra of hemoglobin, myoglobin and model compounds, have been measured at the wiggler beam line of the Frascati Synchrotron radiation facility. The XANES spectra give a direct structural probe for the spatial arrangement of two diatomic ligand molecules (O_2 , CO and CN) bonded to metal ion in the proteins in solution.

1. Introduction

The determination of the bonding geometry of diatomic molecules in the active site is fundamental to the understanding of the structure-activity relationships in natural hemoproteins. The Fe K-XANES extending over 50 eV can be interpreted by the full multiple scattering theory^(1,2). Two structures identified at ~ 17 eV and ~ 30 eV above the K threshold can be attributed to multiple scattering resonances determined by the diatomic molecules along the normal to the heme plane. Here we report two of the quantitative applications of this method: i) the determination of the ligand bond angle in MbCO in solution phase⁽³⁾; ii) the comparison between MbCO, MbCN and CO, CN-chelated protoheme where the distal histidine is absent in order to determine the role of the steric effect of distal histidine on the bent ligand configuration^(4,5).

2. Experimental

The Xanes measurements were performed at the facility of Frascati using the wiggler beam line monochromatized by a Si(111) channel cut single crystal. The absorption spectra were collected in transmission using

solutions samples of the proteins at concentrations ranging from 4 to 8 mM. Hemoproteins in the ligated forms have been prepared by standard method.

3. Result and discussion

XANES spectroscopy applied to MbCO in solution shows that the Fe-C-O configuration is bent at $\sim 150^\circ$ as occurs in the crystal. Fig. 1a shows several theoretical angular resolved spectra for two polarizations. The

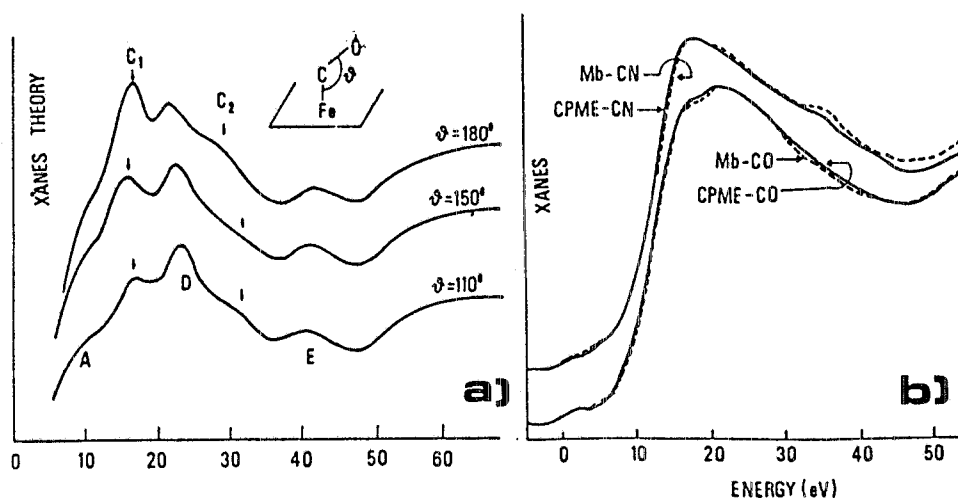


Fig. 1 - a) Theoretical XANES spectra for two polarization.
b) Experimental XANES of the myoglobin and chelated protoheme.

features BDE can be obtained if the multiple scattering pathways within a cluster of 30 atoms are considered. The features C_1 and C_2 show a large variation by changing the CO bonding geometry from linear configuration to a bent configuration ($\theta=180^\circ, 150^\circ, 110^\circ$). The best agreement between theoretical and experimental spectra was found for Fe-C-O configuration at $\theta=150^\circ$. The comparison between the XANES spectra of the myoglobin and the corresponding XANES spectra of chelated protoheme Methyl Ester show that the bonding angle is the same (Fig. 1b). This result contributes to solve the long standing controversy in explaining the origin of the bent configuration in myoglobin. In fact the XANES technique, disproving the commonly accepted assumption of a linear CO bond in unconstrained model, gives evidence that the bent CO configuration is not associated with any steric constraint.

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