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XANES of carboxy and cyanomet-myoglobin The role of the distal histidine in the bent Fe–C–O configuration

A. Bianconi[1], A. Congiu-Castellano[1], A. Giovannelli, M. Dell'Ariccia[1], E. Burattini[2], P. J. Durham[3], and G. M. Giacometti[4]

[1] Dipartimento di Fisica, Università "La Sapienza", I-00185 Roma, Italy
[2] CNR-INFN, Laboratori Nazionali di Frascati, I-00044 Frascati (Roma), Italy
[3] SERC, Daresbury Laboratory, Daresbury, Warrington WA4 4AD, UK
[4] Dipartimento di Biologia, Università di Padova, I-35131 Padova, Italy

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Abstract. The ligand bonding geometry of carboxy- and cyanomet-myoglobin (MbCO and MbCN) has been measured by the XANES method (X-ray Absorption Near Edge Structure). A comparison between the ligand bonding geometry of carboxy- and cyanomet-myoglobin and of chelated protoheme methyl ester shows that the bent Fe–C–O configuration is the same in both systems. Therefore, we suggest that this configuration is not associated with any steric constraint imposed by the side chains of the aminoacid residues at the distal side of the heme pocket.

Key words: Myoglobin, XANES, synchrotron radiation, protein structure

Introduction

Our knowledge of protein structure is largely based on X-ray diffraction methods applied to protein crystals. However, two problems are associated with protein crystallography: *i)* the necessity of growing suitable crystals and *ii)* the possibility that the crystal lattice interactions induce significant strain on the single macromolecule which may effect the active site geometry and thereby its functional properties. Several spectroscopic techniques, such as two-dimensional high resolution NMR and resonance Raman can provide information on the structural and dynamic properties of proteins in solution. Among such techniques, X-ray absorption spectroscopy using an intense synchrotron radiation source has grown in importance as it provides information on specific structural parameters barely accessible to other spectroscopic techniques. EXAFS (Extended X-ray Absorption Fine Structure) provide the pair distribution function around a selected metal atom (Eisenberger et al. 1978), XANES (X-ray Absorption Near Edge Structure) (Bianconi et al. 1982, 1983) provides information on the higher order pair correlation functions of atomic distribution near a selected atom.

Here we report the quantitative application of this method for determination of the differences of the ligand bond angle between myoglobin and chelated protoheme. It is well established that the Fe–C–O and Fe–C–N configurations are bent and/or tilted in carboxy-myoglobin (MbCO) (Hanson and Schoenborn 1981) and cyanomet-myoglobin (MbCN) (Bretscher 1968) as judged by X-ray crystallography (Heidner et al. 1976; Deatherage et al. 1976). In contrast, the Fe–C–O and Fe–C–N bonds are usually considered to be linear in simple chemical compounds (Hoard 1975; Peng and Ibers 1976; Traylor 1981).

We have recently shown (Bianconi et al. 1985) that the Fe–C–O bond angle can be measured in MbCO, both in crystalline and solution phases with an accuracy of $\Delta\theta = 10°$ and it has been found to be $\sim 150°$. We have shown that the Fe–C–O is also bent in a chelated protoheme (Bianconi et al. 1985). Here we present a careful comparison between the XANES spectra of myoglobin and of the chelated protoheme to determine small variations of the ligand bond angle in the two systems. The comparison between myoglobin and chelated protoheme, where the distal histidine is absent, allows us to determine the role of the steric effect of distal histidine on the bent ligand configuration.

Materials and methods

The XANES measurements were performed at the Frascati "wiggler" beam line using synchrotron radiation monochromatized with a Si(111) channel-cut crystal and 0.5–1 mm exit slits. The absorption spectra of the protein samples in solution were calculated from the measured energy transmission. The

zero of the energy scale was carefully fixed at the absorption threshold of the Fe-metal K-edge as the first maximum of its derivative spectrum. The pre-edge absorption background was subtracted in all spectra. The absorption coefficient was normalized, in all spectra, to the atomic absorption above the absorption jump. This was obtained by extrapolation toward lower energy of a linear fit of EXAFS oscillations in the range 50–150 eV, above the absorption threshold. This normalization procedure allows for quantitative comparison between different spectra. Accumulation of up to three scans and integration time of 10 s/point were used to collect the data. An energy resolution of about 1 eV was obtained and energy shifts larger than 0.2 eV could be measured.

MbCO and MbCN samples at ~ 10 mM concentration were prepared from sperm whale metmyoglobin, obtained from sigma, and used without further purification. Optical spectra were recorded on a Cary 219 spectrophotometer before and after the exposure to X-rays in order to show that the samples had suffered no damage during irradiation.

Results

In Fig. 1 we report the comparison between the myoglobin spectra and the corresponding experimental XANES spectra of the CO and CN derivatives of chelated protoheme methyl ester (CPME), as obtained by Bianconi et al. (1985). The identity of the XANES patterns for myoglobin and CPME indicates unequivocally that the bonding angle is the same in myoglobin and in the model compound.

The theoretical Fe K-XANES of the porphyrin and its ligand were obtained by the full multiple scattering approach already used for $Fe(CN)_6$ clusters (Bianconi et al. 1982) and deoxy-myoglobin (Bianconi et al. 1984). In this approach the absorption cross section for one electron transitions from a core level, $\Phi_c(r)$, at energy ε_c to a state in the continuum at energy $E = \varepsilon_c + \omega$ is calculated in the real space.

$$W_c(\omega) = -2 \sum_{LL'} m(E)\, \mathrm{Im}[\tau^{00}_{LL'}(E)]\, m^*(E)\, \theta(E-\mu)\,,$$

where μ is the Fermi level, L stands for the pair (l, m) of angular momentum quantum numbers, $m(E)$ is the dipole matrix element, which is an atomic quantity and $\tau^{00}_{LL'}$ is the multiple scattering matrix. The matrix contains all the effects of the surrounding atoms. In the Green's function formalism, τ^{00} gives the sum of all scattering paths for the photoelectron which begin and end on the photoabsorption site at the origin. This method uses a muffin-tin form for the potential of each atom (Durham et al. 1982).

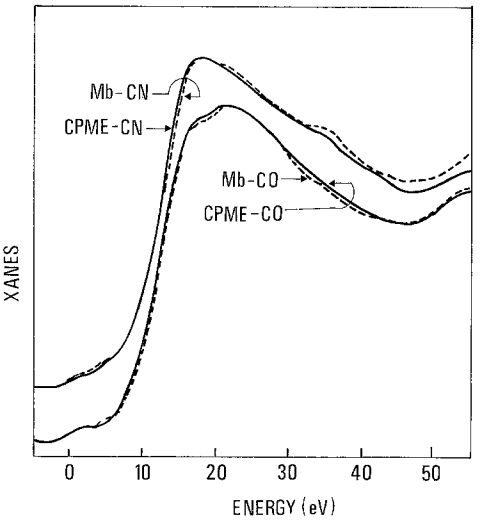

Fig. 1. Experimental XANES spectra of MbCO and MbCN are compared with experimental XANES spectra of chelated protoheme-CO (CPME-CO) and chelated protoheme-CN (CPME-CN)

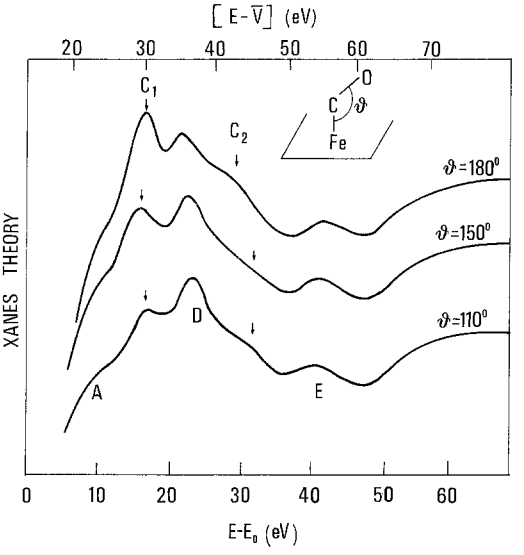

Fig. 2. Theoretical unpolarized spectra showing the effect of different bond angles $\theta = 180°$ (linear configuration), $\theta = 150°$ and $\theta = 110°$

The theoretically expected Fe–K XANES for the heme-group are reported in Fig. 2 for three different configurations of the Fe–C–O angle. It may be observed that the main effect in going from a linear ($\theta = 180°$) to a bent configuration consists in the relative intensity change of peak C_1, with respect to peak D. In fact, the peak C_1 is essentially due to reflections from the ligand molecule, whereas peak D is determined by multiple scattering in the heme plane (Bianconi et al. 1985).

By comparison with theoretical XANES, the ratio of peak C_1 to peak D in the experimental XANES of carboxy-myoglobin and cyanomet-myoglobin allows us to calculate a Fe−C−O bonding angle of $\theta = 150°$ in MbCO and a linear ($\theta = 180°$) configuration in MbCN. The latter finding is in accord with a previous XANES unpolarized calculation for a linear Fe−C−N configuration in cyanomet-hemoglobin (Durham et al. 1983) and with the crystallographic results of Deatherage et al. (1976).

Discussion

Although oxygen is the physiological ligand for myoglobin and hemoglobin, carbon monoxide has been extensively used to probe the structure-reactivity relationship in hemoproteins because it shows several advantages when compared to oxygen. Among them we may recall the increased stability towards oxidation (Antonini and Brunori 1971) and the high photosensitivity of the heme-CO complex which allows one to study the dynamics of the heme reactions using simple photolysing methods (Hoffmann et al. 1978; Brunori and Giacometti 1981). However carbon monoxide is considered a somewhat special case for it tends to bind with a "linear" geometry when compared to O_2 and NO, which are "bent" ligands. The commonly accepted assumption that the Fe−C−O bond is linear and parallel to the normal axis of the heme plane in unconstrained model compounds has raised a number of difficulties in explaining the origin of the bent configuration in myoglobin. On the basis of available structural information a mutual steric interaction between the metal bound CO and the residues on the distal side, such as His E7 and Val E11 has been claimed (Baldwin 1980) and this steric distortion from the "physiological" linear configuration has been proposed as the main reason for the slow association rate constant (Moffat et al. 1979). Although suggestive from a physiological point of view, the hypothesis of a distal steric discrimination between CO and O_2 has been criticized after the observations that increasing the distal steric hindrance in model compounds affects rate constants for both CO and O_2, decreasing them by the same factor (~ 400 fold) (Traylor et al. 1981). Moreover, the myoglobin from the mullusc Aplysia limacina, which is characterized by a more open heme cavity and by having a different distal residue (*Lys* instead of *His*) (Bolognesi et al. 1985) displays exactly the same rate constant for CO binding as sperm whale myoglobin (Wittenberg et al. 1965).

The relation between CO bonding geometry and affinity has also been the subject of several investigations. Romberg and Kassner (1979) studied the NO and CO affinities for horse myoglobin and *N*-methylimidazole protoheme (*N*MeIm−FePP) claiming evidence for steric interaction of the ligand with the distal residue in myoglobin. In contrast, Traylor and Berzinis (1980), using their synthetic heme models conclude that bending of the Fe−C−O bond does not correlate with affinity. However, it is worth observing that the relation between ligand binding geometry and functional parameters has mostly been discussed in terms of steric distortion from a 'putative' linear configuration of Fe−C−O in solution. Thus La Mar et al. (1978) have tried to use the $J(^{13}C-^{57}Fe)$ coupling constant from NMR experiments to characterize the CO bonding geometry, suggesting the same linear configuration in *N*MeIm−FePP and Mb. Collman et al. (1983) have found no simple correlation between the bond configuration and CO stretching frequency. Moreover they showed that the value of $J(^{13}C-^{57}Fe)$ cannot be used to infer bond angle variations but they agreed that a similar bond configuration can be deduced in MbCO and in the model compound.

The long standing controversy on the origin of the bent Fe−C−O configuration in myoglobin seems therefore to find a simple resolution in our results using the XANES technique. The results disprove the idea of a linear CO bond and give evidence for a similar bent configuration in myoglobin and unhindered heme models.

This finding is in accord with recent results which indicate that proximal effects are mainly responsible for the kinetic and thermodynamic changes in the reaction of hemoproteins with CO (Giacometti et al. 1977; Coletta et al. 1985).

Our results can also be reconciled with a theoretical analysis of the coordination modes of CO to Fe in porphyrin (Hoffmann et al. 1977). In this molecular orbital calculation it has been shown that the linear Fe−C−O geometry is stable for a $(Fe-C-O)_{n=6}$ electronic configuration where n is the number of d electrons in $Fe^{2+}(3d^5)$ together with those electrons in CO which occupy the σ^* or π^* levels. A bent Fe−C−O configuration can be determined by an increase of the occupation of the π^* orbital of CO. In fact, for $(Fe-C-O)_{n=7}$ the stable configuration is bent. Charge transfer to C−O can occur by a distortion of the heme changing the mixing of heme Fe−C−O orbitals i.e. delocalization and back donation from (heme π) and Fe(3d) to CO(π^*). The Fe−C−N configuration is found to be linear both in myoglobin and in the protoheme, in agreement with the stable $(Fe^{3+}CN^-)_{n=6}$ electronic configuration. However it is important to point out that XANES is very sensitive to the variation of the angle but is much less sensitive to the tilting off the axis normal to the heme.

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