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TDXAS study of the conformational landscape of MbCO

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We have developed a new experimental approach, Temperature Derivative X-ray Absorption Spectroscopy (TDXAS), to measure the conformational landscape of a metalloprotein and how it depends on the selected conformational coordinate of the metal active site structure. We have recorded the temperature variation of a selected feature of the X ray Absorption Near Edge Structure (XANES) at the iron K edge of carbonmonoxy-myoglobin (MbCO) of a photoproduct and we have extracted the distribution functions g(H) of activation enthalpy barriers H for CO recombination for different conformational coordinates.

Keywords: protein dynamics, temperature derivative spectroscopy, conformational landscapes.

1. Introduction

There is a growing evidence that the protein fluctuations control the protein function. The protein is described as a flexible molecule which fluctuates within conformational substates separated by energy barriers (Frauenfelder *et al.*, 1991). Therefore the protein is characterised by a landscape of potential barriers that controls the protein fluctuations. This landscape changes in different solvents, with interactions with small molecules, and with the folding states (Natali *et al.*, 1998). Myoglobin has been taken as the model system for this purpose, and the first experiments to investigate this key point have used infrared spectroscopy studying the time evolution of the infrared response at a fixed wavelength or the evolution with temperature of excited intermediate states (Berendzen & Brauenstein, 1990). Recently studies of structural fluctuations by time resolved x ray crystallography have been reported (Srajer *et al.*, 1996; Lim *et al.*, 1995). XANES probing the metal site structure (Pin *et al.*, 1994; Bianconi *et al.*, 1995) can be used to provide complementary and unique information on the conformations of the active site and its dynamics (Della Longa *et al.*, 1994).

Here we report the determination of the landscape of potential barriers for the CO recombination in myoglobin using a novel approach: temperature derivative x-ray absorption spectroscopy (TDXAS). Temperature derivative x-ray absorption spectroscopy (TDXAS) consists in measuring the derivative of a population of excited states with respect to the temperature following the evolution of a selected peak in XANES spectra. The temperature derivative of the observable is directly proportional to the temperature derivative of the population, dN/dT, of a set of protein substates characterised by a conformational parameter that controls the particular selected peak in the XANES spectrum. The characteristic of XANES peaks at the Fe K-edge of hemo proteins is that they are determined by features of the local structure in a range of 5 Å around the Fe site. In fact they arise from multiple scattering resonances (shape resonances) that are a direct probe of the different aspects of the heme structure (bond lengths and angles) and they have been well identified in systematic experimental and theoretical investigations (Pin *et al.*, 1994). Therefore we can select different conformational parameters of the local structure by following each of the XANES peaks. The results on CO recombination in myoglobin shows different landscapes of potential barriers selecting the Fe-Np distances and CO bonding angle as conformational coordinates.

2. Materials and Methods

The solution of horse-heart myoglobin was treated with sodium dithionite and saturated with carbon-monoxide (CO) to obtain the MbCO state.

The X-ray absorption spectra of myoglobin have been measured at the ESRF synchrotron radiation facility in Grenoble on the beam line BM29. The 6 GeV storage ring was operating in hybrid mode with a typical current of 160 mA. We have used a fixed-exit monochormator with a double Si(311) crystal. X-ray fluorescence was detected by a 13 elements Canberra ultra pure Ge array detector with energy resolution of 170 eV at the Fe K_α fluorescence peak. Photodissociation was performed at T_i=17K by continuous illumination. For dynamical measurements the single-energy X-ray absorption detection, performed while heating the sample in a wide T range using the method developed by Filipponi *et al.* (1996). Data have been recorded at fixed energy values (E_1=7.123 KeV and E_2= 7.147 KeV) in the temperature range 17-125K.

The sample temperature was ramped linearly with the heating rate β=1 K/min. Below 180 K the CO recombination process can be described as a first order transition: between the state Mb*, with the iron atom far from the porphyrin plane, and the MbCO state with planar configuration. The transition between these two states is observed following the variation of a spectroscopic observable: the absorption A. The time kinetics of the observable is correlated with temperature derivative The change in the observable with time is thus directly proportional to the temperature derivative of the population, dN/dT, which for a given model can be related to parameters such as activation enthalpy and entropy. Starting from the definition of a first order process we can write dN/dt = -KN(t) where K is the rate coefficient of the transition; by changing the variable from t to T, and integrating over the full range of temperature we obtain:

$$\frac{dN}{dT} = -\frac{N_i}{\beta} K \exp\left(-\int_{T_i}^{T} \frac{K}{\beta} dT'\right) \quad (1)$$

Using for K the Arrhenius expression, and solving numerically the Eq. 1, we obtain the following expression for a process governed by a single energy barrier:

$$-\frac{dN}{dT} = \frac{N_i}{\beta}\left[K \exp\left(\left(-\frac{A}{T_0}\right)\left(\left(T^2 E\left(\frac{H}{RT}\right) - T_i^2 E\left(\frac{H}{RT_i}\right)\right)\right)\right)\right] \quad (2)$$

with

$$E\left(\frac{H}{RT}\right) = a\frac{H}{RT}\exp\left(-b\frac{H}{RT}\right)$$

where a and b are fitting parameters.

The object of our study is an ensemble of proteins, which are in different conformational substates, with different enthalpy barriers. Therefore we fitted our experimental derivative spectra whit a linear combination of Eq. 2.

$$-\frac{dN}{dT}=\sum_{j=1}^{m} g(H_j)\left(-\frac{dN}{dT}\right)_j \qquad (3)$$

$g(H_j)$ is therefore the contribution of each enthalpy barrier to the whole process, and the enthalpy distribution function is obtained plotting the fitted values of $g(H_j)$. The evidence for the substates is then shown with a gaussian fit.

3. Results and Discussion

Fe K-edge XANES spectra of carbonmonoxymyoglobin as well as the photodissociated Mb* are plotted in (Fig. 1a). The Mb*-MbCO XANES difference spectra are also reported (Fig. 1b) . We have focused our study on the features A* and C_2 (E_{A*}=(11.6±0.5) eV and E_{C2}= (33.5±0.5) eV). The intensity of the A* peak in the XANES difference spectrum, provides information on the distance between the iron atom and the pyrrolic nitrogens of the porphyrin ring. The C_2 peak is due to the contribution of axial scattering of the photoelectron pathway, and provides information on the Fe-C-O bonding angle. Therefore the variation of the intensity of the A* and C_2 peaks are optimal probes to investigate the dynamics of the CO-recombination process.

Inside the cryostat some nitrogen is present: because its transition is around 77 K, the temperature dependence of the XANES peaks could be perturbed. To avoid this effect data are normalised by subtracting the F* pattern: in fact, in correspondence of (49.5±0.5) eV, the Mb* and MbCO XANES spectra show the same intensity.

We report the temperature derivative of N(T) for the A* and C_2 features (Fig. 2 a and 2b). The enthalpy distribution functions have been obtained as the distributions of the weight of the sum of the $g(H_J)$, theoretically calculated, used to fit the derivative pattern of N(T). Then we use a linear combination of gaussian functions to find out the principal contributions to the enthalpy distribution. In Fig. 3a the gaussian fits relative to the feature A* is shown, while Fig. 3b is referred to C_2 peak.

The distribution relative to the A* feature of the native protein has been fitted using a linear combination of three gaussian functions, but only a principal state, centred at 62K, which corresponds to (13±1) KJ/mol, is evident, while the gaussian fit the C_2 peak shows the presence of three states centred around (9±1)KJ /mol, (13±1) KJ /mol which seems to be the main peak, and a better resolved one around (19±1)KJ /mol.

By following the temperature dependence of a X-ray absorption peak intensity at a fixed energy value, is therefore possible to monitor processes as CO-recombination, providing information on the structural changes associated with the process under study.

In this particular case, the different shapes of enthalpies distribution, (related to the A* and the C_2 peaks) suggest to us that the protein reponse to the CO-photodissociation event, is not homogeneous. The distribution relative to the C_2 peak shows three states for the rebinding process at 9 KJ/mol, 13 KJ/mol and 19 KJ/mol.

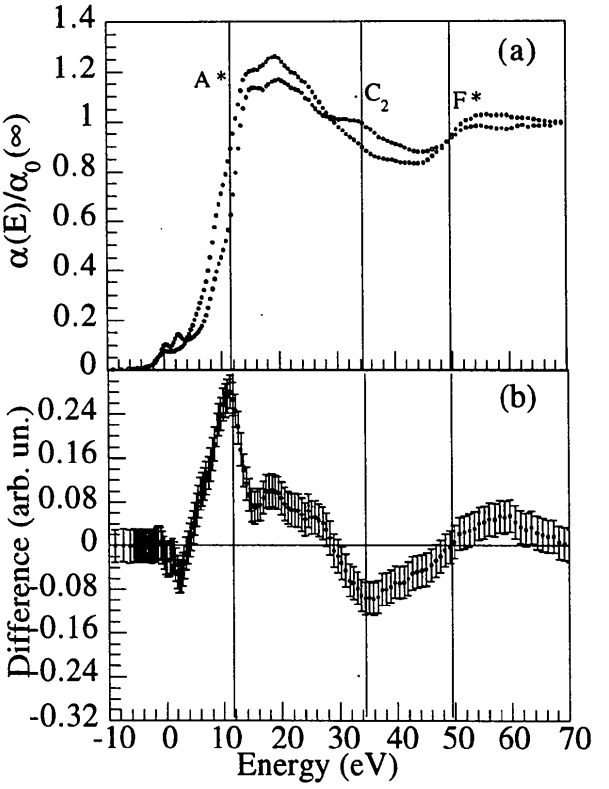

Figure 1
(a) Fe K-edge XANES spectra of native horse MbCO (open circles) and Mb* (filled circles) measured at 17 K. (b) XANES difference spectrum .

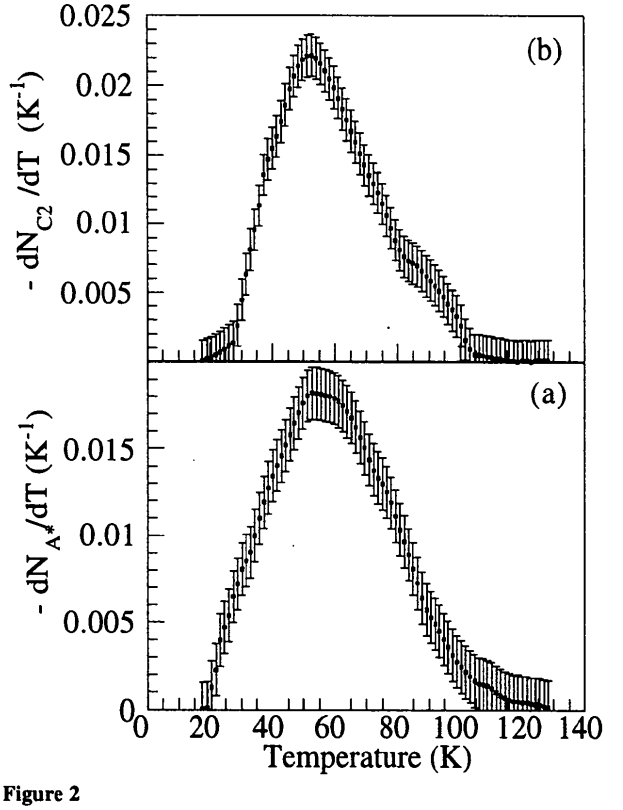

Figure 2
(a) Temperature derivative of N(T) relative to the A*. (b) Temperature derivative of N(T) relative to the C_2 feature.

A previous experiment of TDS performed with FTIR measurements showed three rebinding enthalpy barriers: 9.5 KJ/mol, 10.8 KJ/mol, 17.8 KJ/mol for A_0, A_1, A_3 states respectively (Frauenfelder *et al.*, 1991). Therefore following the XANES peak C_2 we obtain comparable results.

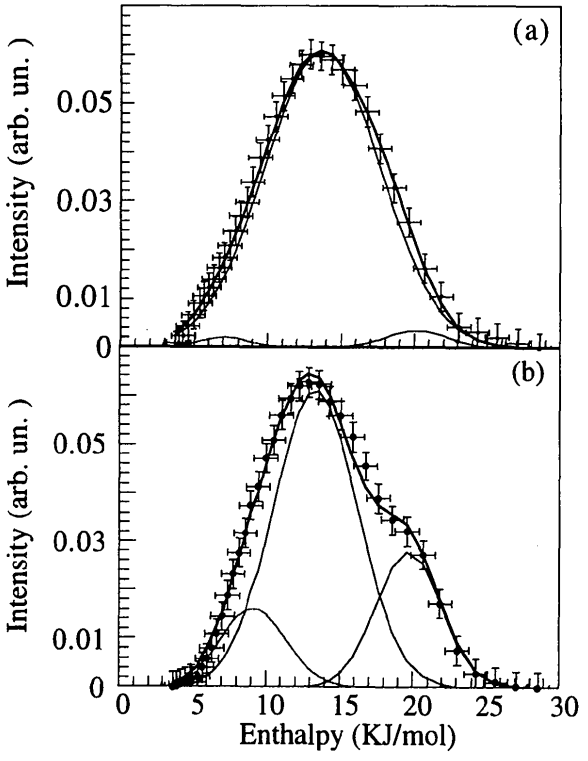

Figure 3
(a) The enthalpy distribution function relative to the A* feature of the myoglobin is shown (symbols). The distribution function is obtained using the weight of each $g(H_J)$: these theoretical functions are used to fit the derivative function of N(T). The solid line represents the fit of the distribution function, obtained as the sum of single gaussian curves (thin line). (b) The enthalpy distribution function (symbols) relative to the C_2 peak, as for the A*, of the myoglobin is shown. The single gaussian curves (thin lines) of the fit and their linear combination (solid line) are shown, too.

The only peak of the A* enthalpy distribution function is centred at an energy point which lies between the two main peaks of C_2 feature, allowing us to state that the Fe reaches the planar configuration independently from the CO recombination mode. Moreover the low energy state relative to C_2 feature, can be associated with a van der Waals interactions of the CO molecule with the heme plain, which take place before the chemical recombination.

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