Experiment Report MX 918

“XAS study of the active site of a bacterial NO sensor protein”

1. Methods

The Fe K-edge XAS spectra of ferric, ferrous, NO-bound and CO bound holo-DNR have been collected in fluorescence mode at ESRF-BM30B, Grenoble, by using a 30-elements ultra-pure Ge detector. The spectra were calibrated by assigning the first inflection point of the Fe foil spectrum to 7112. The energy stability of each spectrum was carefully assessed by checking the position of a glitch in the I_0 at 7220 eV. Data analysis with MXAN and GNXAS software packages are in progress, so that in this Experiment report we will show only a first qualitative analysis of the XANES data.

2. Results

In Fig. 1, the Fe K-edge XANES spectrum of the formally “ferric” holo-DNR is shown. This species evolves under X-ray irradiation toward a photoreduced one. Its derivative spectrum is shown in the inset. The spectrum displays some rather broad features (1-7 in the absolute spectrum and α-δ in the derivative spectrum), all typical of hexa-coordinated hemes and is reminiscent of the spectra reported for Fe(III)TPP(Imid)$_2$ and neuroglobin, both having a bis-imidazole Fe-heme [1, 2].

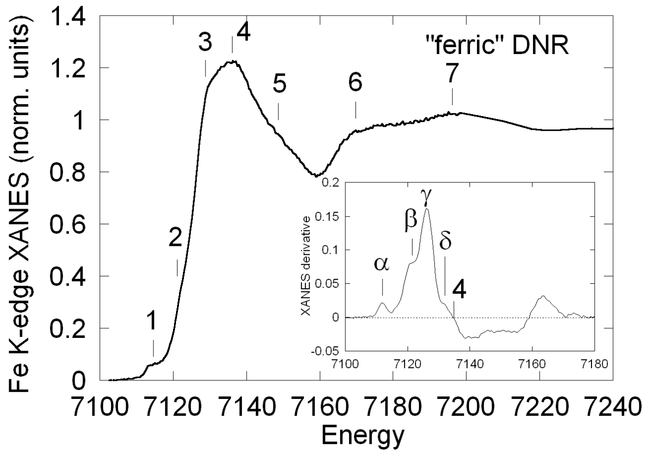

Figure 1. Fe K-edge XANES spectrum of ferric DNR. In the inset, the derivative spectrum at the edge is displayed. DNR was 1.6 mM, in 32 mM phosphate buffer, pH 7.4, 240 mM NaCl, gly 20%

The XANES spectrum of the chemically reduced (by sodium dithionite excess) ferrous DNR is shown in Figure 2 (lower curve, solid line) and compared with the formally ferric, photoreduced species (lower curve, dotted line).

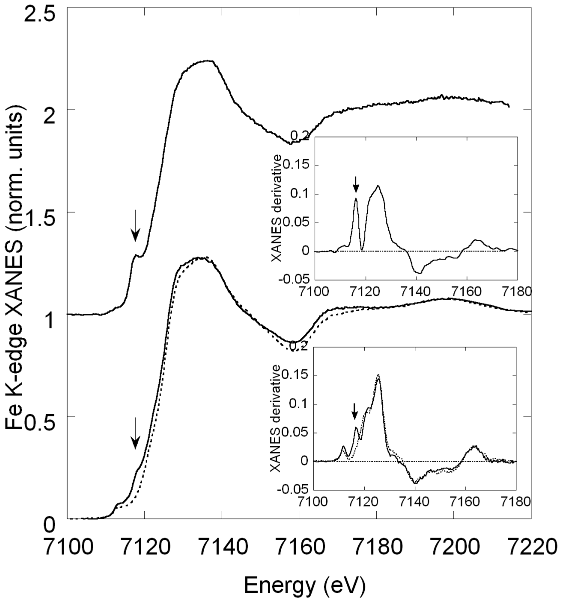

Figure 2. Fe K-edge XANES spectrum of ferrous DNR (lower curve, solid line), formally ferric, photoreduced DNR (dotted line). and Fe(II)-porphyrin in SDS micelle, a model for tetra-coordinate hemes (upper curve). In the insets, their respective derivative spectra are depicted. The feature at 7117 eV is put in evidence by an arrow.
DNR was 1.6 mM, in 32 mM phosphate buffer, pH 7.4, 240 mM NaCl, slight excess of sodium dithionite, gly 20%

The most important change is observed at 7117 eV (pointed out by an arrow), where a shoulder emerges in the low rising edge of ferrous NDR. The shoulder appears as a further peak in the derivative spectrum. This feature is typical (and very pronounced) in tetra-coordinated square-planar metallo-porphyrins (Zn, Cu, Fe, Ni) being assigned to a dipole allowed $1s \rightarrow 4p_z$ transition [3, 4]. Hemin dissolved in 10% sodium dodecyl sulphate (SDS) and reduced by sodium dithionite was used to represent the XANES spectrum of tetra-coordinate Fe(II)-protoheme in Figure 2, upper curve. The appearance of the shoulder at 7117 eV gives a clue for a partial destabilization of the axial ligation in ferrous DNR, resulting in an equilibrium between two species, an hexa- and a tetra-coordinate one.

Finally we also investigated the CO (data not shown) and NO bound form of this protein. As shown in figure 3, the NO bound form has been assigned to a five-coordinated species in agreement with previous investigation [5] and by comparison with model system.

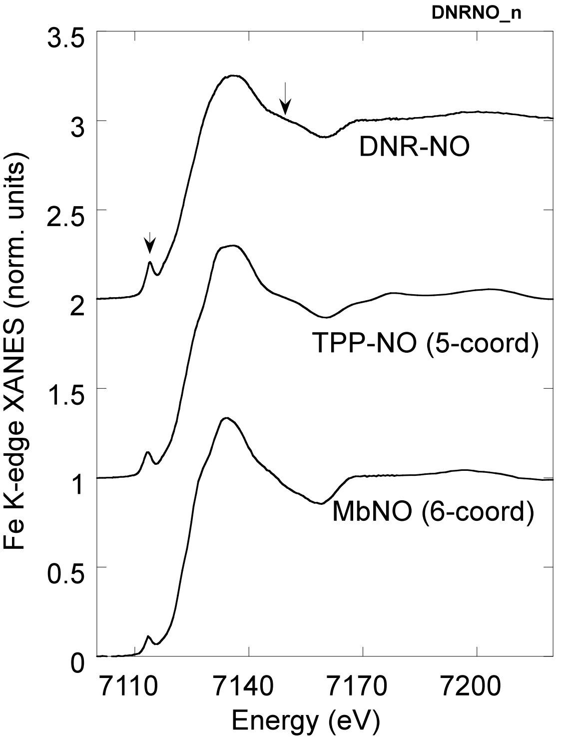

Figure 3. The Fe K-edge XANES spectrum of ferrous DNR-NO (upper curve) is compared with the five-coordinate TPP-NO derivative (intermediate curve) and the hexa-coordinate Mb-NO derivative (lower curve). The pre-edge peak intensity at 7114 eV and the feature emerging at 7150 eV (shown by the arrows) suggest that the heme iron of the DNR form is five-coordinate

It is important to note that the appearance of a percentage of four-coordinated species may have a fundamental biological role and it is a novel property for this class of protein sensors. Of course, a complete analysis with MXAN and GNXAS onto the XANES and EXAFS regime respectively is demanded. Moreover, further studies by means of XAS investigation onto single Histidine mutants are necessary to shed light onto the structural details of the heme site governing the biological function of this protein.

References

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