	Experiment title:	Experiment number:
	METAL RELEASING STUDIES OF Cd, Zn METALLOTHIONNEIN USING NITRIC OXIDE	SC 926
Beamline: ID26	**Date of experiment:** from: 21 November 2001 to:27 November 2001	**Date of report:**
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Names and affiliations of applicants (* indicates experimentalists):

Dr. Jose Angel MARTIN-GAGO*

ICMM-CSIC, cantoblanco-Madrid-Spain

Dr Elena CASERO-JUNQUERA*,Dr. Encarna LORENZO, Dr Felix PARIENTE*: Universidad autónoma de Madrid- Campus cantoblanco-SPain

Report:

Due to the important biological role of the nitric oxide (NO) in the human body our group has been working in the development of several electroanalytical strategies for its determination including amperometric sensors and biosensors. In order to continue with this research field, we wanted to employ metallothioneins based biosensors for the direct determination of nitric oxide.

Metallothioneins (MT) constitute a class of low-molecular-weight proteins that are characterize by a high content of cysteine and capacity to bind metal ions, such as Zn(II) and Cd(II). The protein is formed by two clusters, one with four bivalent ions and other with three ions, all of them bound by four thiolate ligands arranged in tetraedral symmetry. These proteins are able to react with NO through the sulfhydryl groups of the cysteines.

The main aim of the project was to perform a complete study by means X-Ray absorption (NEXAFS and EXAFS) of the competitive mechanism between the metals in the MT protein (Cd and Zn) and the NO molecule from a solution in order to know how the interaction of NO with MTs takes place.

Experimental procedure and results we have obtained are summarized as follows:

1. We have recorded the X-ray adsorption after Zn edge of the MT protein in a diluted solution (around 800 µM) (Figure 1).

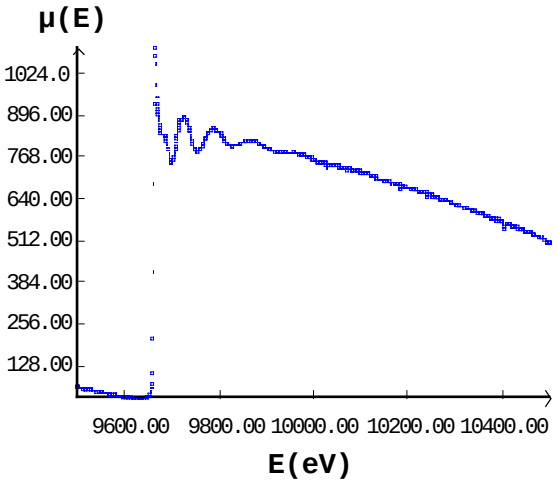

Figure 1

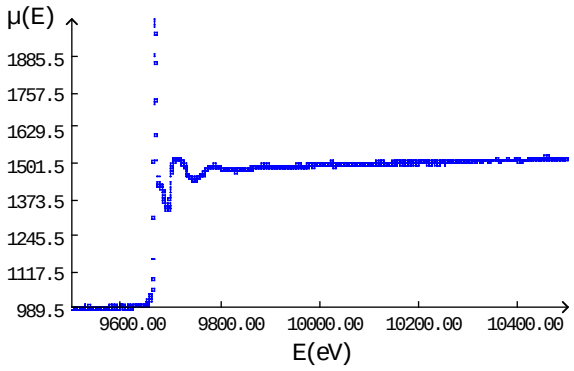

Figure 2

2. Upon addition of NO from a saturated solution (1.9 mM) the new absorption signal above Zn edge was recorded (Figure 2).

3. The experimental and simulated Fourier filtered EXAFS spectra of both samples are shown in Figures 3A and 3B. The structural parameters resulting of analysis are summarized in Table 1.

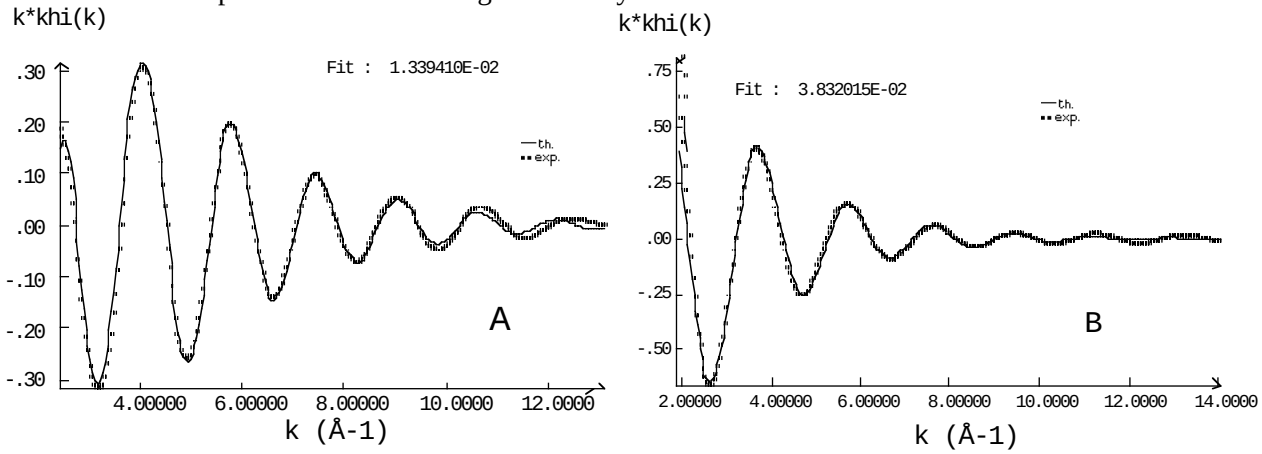

Figure 3

	R_O (Å)	N_O	s (Å)
MT	2.33	4.0	0.081
MT + NO	2.09	5.8	0.090

Table 1

As can be seen, as the nitric oxide is added to the MT solution, there is a local Zn environment change from a tetrahedral symmetry (data obtained from Figure 3) to an octahedral symmetry corresponding to an isolated Zn ion in the solution (data obtained from reference spectra, not shown). This quantitative result is directly verified by using the XANES part of the spectrum as a fingerprint. We have contacted to Maurizio Benfatto ´s group to reproduce by multiple scattering procedures the obtained spectra. A detailed Analysis in this direction is in progress.

4. In addition, the metal interchange between metal ions in solution (Fe^{3+}; Cu^{2+}) and the Zn^{2+} of the MT was studied. The analysis is in progress, but we expect to obtain a description of a competitive mechanism among the different metals.

Summarizing, The project was performed according the proposed objectives, the data analysis is in progress and we have got the first group of results that will be publish soon.