ESRF	**Experiment title:** Imaging modern and antique microbe-mediated processes in mineralized environments (II)	**Experiment number**: *ME-824*
Beamline: ID22	**Date of experiment**: 3 to 13 November 2004	**Date of report**: December 2005
Shifts: 26	**Local contact(s)**: P. Bleuet, S. Bohic	*Received at ESRF:*

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The aim of this Long Term Project was to bring together a variety of specialists of different scientific horizons to image and quantify living and fossil biomaterial in mineralized environment and fluid inclusions using a variety of tracers such as sulfur, lipids, selenium, arsenic and iron. By exploring the full spectrum of microbial ecology in extreme environments, this has provided important new insights into life-sustaining processes in the rock record and contribute to the understanding of the environmental conditions and the origin of life on the Primitive Earth.

We have obtained a total of 44 shifts on ID21 and 82 shifts on ID 22 from April 2004 to December 2005.

On ID 22, the 82 shifts were dedicated to:

I) Fluid inclusion studies: Development and applications. This study is part of the PhD of Jean Cauzid who was supported by a CNRS-ESRF grant current 2002-2005. Jean Cauzid has been hired as a postdoctorate on ID22 this year for a tree year period starting January 2006 (see report 5-13 decembre 2005)

II) Intracellular XANES spectroscopy at iron K edge on frozen hydrated neuron cells (this report)

III) 3D imaging of microorganisms in mineralized samples (see report 5-13 decembre 2005)

II - Intracellular XANES spectroscopy at iron K edge on frozen hydrated neuron cells (15 shifts of 3-13 november 2004)

The redox cycling of Fe is suspected to play an important role in the etiology of neurodegenerative diseases such as in Parkinson's disease. In a previous experiment at ESRF (MD80) we revealed that iron distribution in cultured dopamine neurons was not homogeneous but exhibited a diffused distribution in the nucleus and a granular localisation within the cytosol. The aim of this experiment

was to determine iron oxidation state in these two intracellular compartments (nucleus and cytosol) of cultured dopamine neurons using XANES spectroscopy at Fe K edge on beamline ID22. In addition, in order to compare the effect of sample preparation and storage conditions on iron oxidation state the cells were either (1) maintained frozen hydrated in anoxic environment, or (2) freeze dried and stored in air, the first protocol being safer to preserve native oxidation state, the second one enabling an easier microscopic recognition of cellular compartments.

Cell cultures

PC12 dopamine neuron rat cells were exposed *in vitro* to 50 or 300 µM Fe during 24 h. Samples were processed following two distinct protocols (1) cryofixation into liquid nitrogen chilled isopentane and storage in liquid nitrogen until analysis; (2) cryofixation into liquid nitrogen chilled isopentane and freeze dried overnight at -35°C and storage in a dessicator.

XANES Spectroscopy and X-ray fluorescence imaging

ge ring mode	3 fill mode

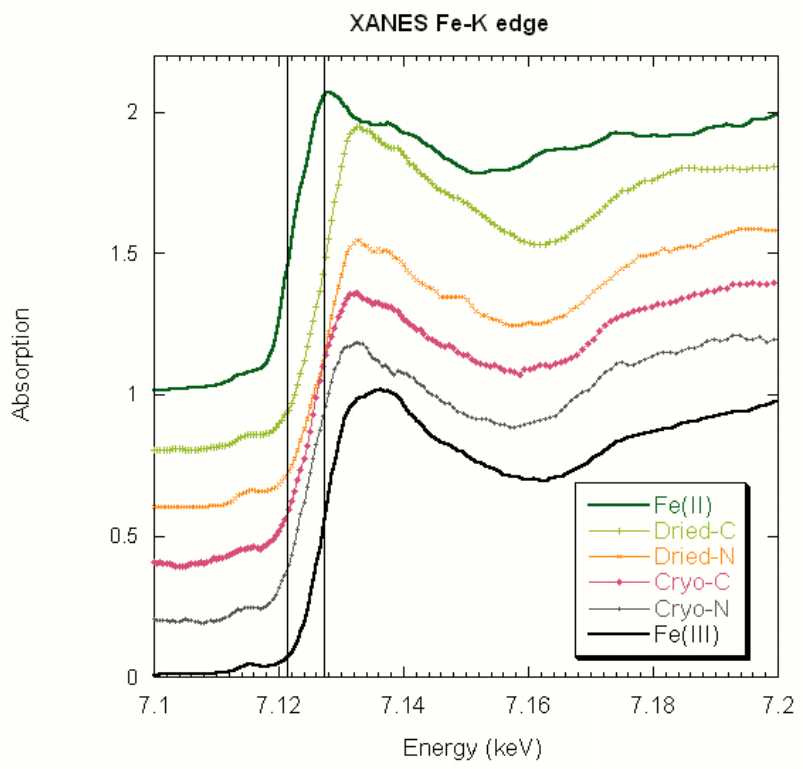
gy	ulator U23d	ulator U42u		3 (ph/s)
V (XRF)		1		0^9
keV (XANES)	2	6	1	0^8
n size	µm² @ 14keV using KB optics			

XANES parameters
pre-edge : -75 eV ; 1 eV / step; 5 s / step
edge : start -30 eV ; stop +30 eV ; 0.5 eV / step ; 5 s / step
postd edge: k = 2.806663 ; end k = 6 ; k step = 0.03 ; 5s / step

Analyses were performed at 100 K using a liquid nitrogen cryojet for sample preparation (1) or at room temperature for sample preparation (2).

Data treatment was performed using Axil software.

Results and discussion



XANES spectra at Fe K edge for the reference compounds (ferrous iron Fe(II); ferric iron Fe(III)) and cellular samples (freeze dried (Dried), and frozen hydrated (Cryo) cells, nuclear zone (N) and cytosol (C).

The 5eV energy shift found between Fe(II) and Fe(III) absorption edge of reference compounds is in good agreement with the results published in the literature. Our results demonstrate that iron is found in its trivalent state in both cellular compartments (cytosol and nucleus) independently of sample process, storage, and temperature analysis conditions.

Conclusion

This experiment allowed the characterization *in situ* of Fe oxidation state in cultured neuron intracellular compartments. Fe(III) was found in all cases, either in the cytosol or in the nucleus. The lack of toxicity observed during Fe exposure up to 300 µM during 24h confirms that when Fe is stored intracellularly in its Fe(III) form it is harmless for neurons. However redox processes involving Fe are suspected to play an important role in the etiology of neurodegenerative diseases such as Parkinson's disease. This original experimental model could now be used to check for the effect of neurotoxins on Fe reduction involved in neurodegenerative diseases.

Publications

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