ESRF	**Subcellular distribution of iron in dopaminergic neurons following exposure to Parkinson's disease inducing neurotoxins, and iron chelators.**	**Experiment number**: MD282
Beamline: ID22	**Date of experiment**: from: 03/05/2007 to: 08/05/2007	**Date of report**: 04/09/2007
Shifts: 15	Local contact(s)**:** Sylvain BOHIC	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*Ortega Richard, CNAB, CNRS, Université de Bordeaux 1, Gradignan, France

*Carmona Asuncion, CNAB, Université de Bordeaux 1, Gradignan, France

*Devès Guillaume, CNAB, CNRS, Université de Bordeaux 1, Gradignan, France

*Bohic Sylvain, INSERM, ESRF, Grenoble, France

*Cloetens Peter, ESRF, Grenoble, France

Parkinson's disease (PD) is a neurodegenerative disorder that leads to the progressive loss of dopaminergic neurons in the substancia nigra pars compacta. This phenomenon is still unexplained, even though some metals, and especially redox metals such as Mn and Fe are suspected to play a major role in the etiology of PD. The aim of this experiment was to determine the chemical element mapping of dopaminergic neurons, PC12 cultured cells, at the sub-cellular level when neurons are treated with neurotoxins related to Parkinson's disease, such as manganese. We were especially interested in determining the cellular manganese distribution because at high concentrations manganese has been reported to cause Parkinsonian-like symptoms, known as '*manganism*', and this element is also considered like as potential environmental risk factor for PD and its related disorders (Martin, 2006). PC12 cells exposed to other neurotoxic compounds were also prepared for this experiment but the analyses focused mainly on manganese exposed cells as the results were very interesting and the analysis time limited.

In recent experiments at ESRF (experiment reports MD80 and MD178) we performed X-ray fluorescence imaging of chemical elements on PC12 cells with a 90 nm spatial resolution. Nano-chemical imaging indicates that iron accumulates into dopamine vesicles, within the cytosol, neurites, and distal ends of cultured dopaminergic cells (Ortega et al., 2007). Micro-XANES experiments revealed that iron was exclusively present as Fe(III) in cells (Bacquart et al., 2007). These results suggest that Fe is bound to dopamine in neurosecretory vesicles of normal dopaminergic neurons. Our hypothesis, as well as others (Lashuel et al., 2006), is that dopamine-iron complexes may wrongly relocate to the cytosol in PD dopaminergic neurons leading to cell death through redox cycling due to the oxidative stress induced by the highly reactive iron-catechol compounds.

In this experiment, a setup for high spatial resolution X-ray fluorescence microanalysis based on a Kirkpatrick-Baez lens and a piezo sample stage was used on ID22 beamline, thus leading to the obtention of a pink photon beam at the same time highly spatially resolved (220 nm x 80 nm v x h beam size) and with a high flux of photons (10^{11} ph/s). The characteristics of the beam fullfilled the requirements for mapping of biological trace elements (in the µg/g range) at a sub-micrometer scale (size of most intracellular organites). The energy of the incoming X-ray beam was set at 16.8 keV allowing to map all trace elements in cells.

X-ray fluorescence microanalysis of PC12 cells enabled to define the element distributions in the main cellular compartments : nucleus, cytosol, neurite outgrowths and distal ends. Figure 1 shows an example of analysis: K, Cl and Zn are found in all cellular compartments, with a higher Zn content in the nucleus, whereas Fe is found in small structures in the cytosol confirming the results obtained in previous

experiments (Ortega et al., 2007). Manganese accumulates in a specific region of cell cytosol, near the nucleus. In figure 2, and for about 10 examples more obtained during the experiment (not shown), a similar distribution was observed showing the same peri-nuclear accumulation of manganese.

The perinuclear localisation of manganese suggests that this element accumulates within the Golgi apparatus and/or the endoplasmic reticulum wich are usually located in one side of the cell, close to the nucleus. This result should now be confirmed by further experiments using PC12 cells labelled with fluorescent markers specific of Golgi apparatus and of the endoplasmic reticulum. If the accumulation of managanese in those compartments is proven it would be of paramount importance to understand the neurotoxicity of manganese. A similar interaction with Golgi apparatus and the endoplasmic reticulum has been evidenced for alpha synuclein mutant proteins and linked to the pathogenesis of PD (Cooper et al., 2006). The alteration of Golgi apparatus and endoplasmic reticulum functions would result in a deffective accumulation of dopamine in cells (outside neurovesicles) explaining the specificity of dopaminergic cells neurodegeneration in PD (Lashuel et al., 2006).

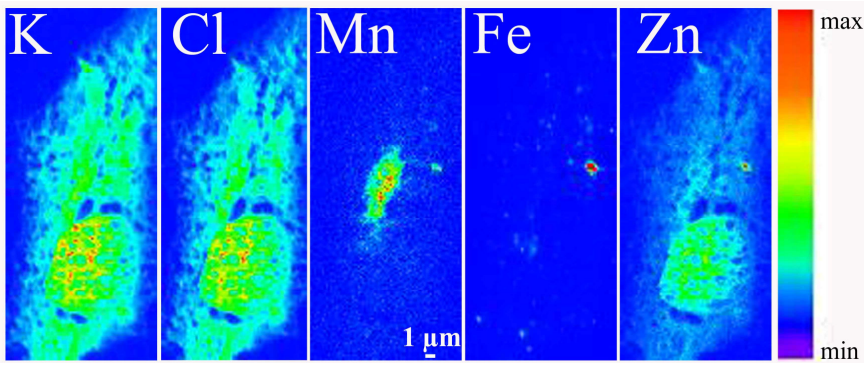

Figure 1: elemental distribution in a PC12 cell exposed to 100 µM of $MnCl_2$ during 24 hours. Scan size is 15 x 35 µm. Color bar ranges from blue to red (min to max) is proportional to the number of X-rays detected.

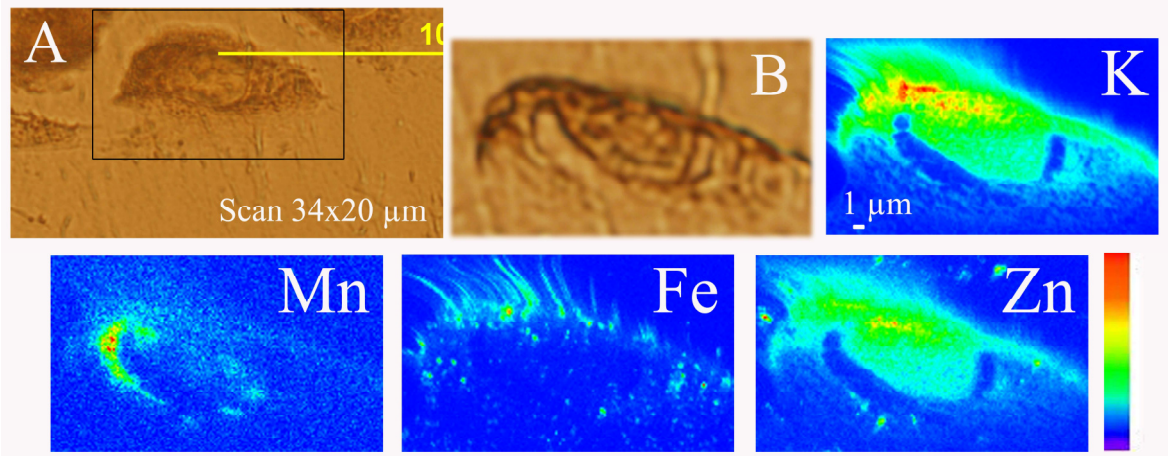

Figure 2: elemental distribution in a PC12 cell exposed to 100 µM of $MnCl_2$ during 24 hours. Scan size is 34 x 20 µm. **A:** is the image of cell before irradiation and **B:** after. Note that the intense X-ray beam leads to sample damage, the nucleus and cytosol are separated during irradiation. Color bar ranges from blue to red (min to max) is proportional to the number of X-rays detected.

Bacquart T., Devès G., Carmona A., Tucoulou R., Bohic S., Ortega R. (2007) Subcellular speciation analysis of trace element oxidation states using synchrotron radiation micro-X-ray absorption near edge structure. *Analytical Chemistry (in press, available on line).*

Cooper, A.A., et al. (2006) Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. *Science* 313, 324–328.

Lashuel H.A., Hirling H. (2006) Rescuing defective vesicular trafficking protects against alpha-synuclein toxicity in cellular and animal models of Parkinson's disease. *ACS Chemical Biology,* 1, 420-4.

Martin C.J. (2006) Manganese neurotoxicity: connecting the dots along the continuum of dysfunction. *Neurotoxicology*. 27347-9.

Ortega R., Cloetens P., Devès G., Carmona A., Bohic S. (2007) Iron storage in neurovesicles revealed by chemical nano-imaging. *PLoS One (http://www.plosone.org/).*