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Synchrotron Spectromicroscopy in Biophysics: Specificity of Metal Uptake by Neurons.

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Abstract. – Recent instrumentation advances have made it possible to apply experimental synchrotron radiation techniques of materials science to biophysics problems. We present the first results of a systematic photoelectron spectromicroscopy study of the interaction between metals and neurons *in vitro*. The main result is that aluminum is not uptaken by granule cells—the most common type of neurons—but it is selectively uptaken by cells such as Purkinje neurons and glial cells. On the contrary, granule cells are capable to uptake other metals like Ni and Co.

The needs of materials physics research have stimulated the rapid development of new and powerful microscopy techniques based on synchrotron radiation [1]. The performance levels are so good that these new tools can be exported to other research areas. Successful feasibility tests of photoelectron spectromicroscopy in biophysics have indeed been presented in the past three years [2].

We now discuss the first systematic results of a neuron biophysics study based on synchrotron photoelectron spectromicroscopy. The main objectives of the study were: 1) to detect the microscopic localization of different metals in neuron culture sample; 2) to test in this way the specific metal uptake mechanisms of different types of cells.

The most exciting results of this approach concern a basic difference between the biophysical mechanism of aluminum uptake and that of other metals. We believe that the importance of this result is enhanced by the suspected role of aluminum in neuropathologies

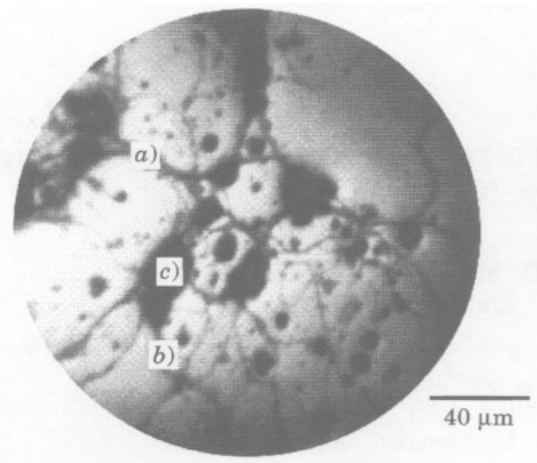

Fig. 1. – XSEM microimage of a portion of a granule cell culture, exposed to Ni ions. The labels *a*), *b*) and *c*) refer to the microscopic areas where the corresponding spectra of fig. 4 were acquired.

like the Alzheimer disease and the Guam syndrome [3]. In short, aluminum was never found in granule neurons that are the most common type of neurons in the brain. Aluminum was found instead in other cell populations like glial cells and GABAergic (Purkinje) neurons [4].

On the other hand, experiments on cobalt, nickel and other metals did reveal such elements in granule cells, so that we can conclude that the cell specificity is peculiar to aluminum and not a general mechanism for all metals. It should be noted that this difference would have been impossible to discover without the capability of synchrotron spectro-microscopy to perform microchemical analysis on a large number of cells in a relatively short time.

The specific experimental technique [5] was X-ray Secondary-Electron emission Microscopy (XSEM); in short, this consists of measuring X-ray absorption spectra by detecting the yield of secondary photoelectrons as a function of the photon energy [6]. The microscopy performance is obtained by processing the secondary photoelectrons with a magnifying electron lens system. This makes it possible to perform physico-chemical analysis on small areas, based on the absorption edges of specific elements. The same experimental system can also be used to obtain photoelectron micrographs and, therefore, to reveal the sample's morphology.

Figure 1 shows an example of XSEM micrograph of a portion of one of our cultures. Hundreds of images of these kinds have been taken on different kinds of cultures; digital subtraction processing of images taken at different photon energies has been used to quickly identify the elemental distribution [4]. Specific spectra have been taken from thousands of microscopic areas. The very small set of data presented in this letter is, therefore, representative of a much larger body of data.

Figure 2 summarizes the afore-mentioned properties of aluminum. We see here three spectra in the photon energy range of the Al $2p$ edge. No aluminum is detected in curves *a*) and *b*), that were selected among hundreds of spectra taken on granule cells. Curve *c*), instead, clearly shows the presence of aluminum in a Purkinje neuron.

These cultures had indeed been exposed to aluminum ions in solution at the end of their preparation procedure, which is described in detail in ref. [4]. In short, rat cerebellar cells were allowed to grow for several days on a gold-coated stainless-steel substrate, forming a neuron network. Then, each culture was exposed to an AlF_3 or Al citrate solution for a few

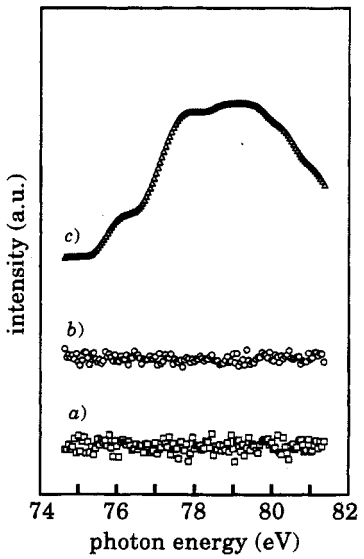

Fig. 2. – XSEM (X-ray absorption) spectra taken in three different $(3 \times 3)\ \mu m^2$ areas of cultures exposed to Al ions. Curves *a)* and *b)* are from granule cell cultures. Curve *c)* is from a Purkinje neuron culture, and clearly reveals the presence of Al from the Al $2p$ edge.

minutes. The aluminum not uptaken by the living cells with a biological active process was carefully washed out; the samples were then fixed and dehydrated. Similar processes were used for cultures exposed to other elements, using $NiCl_2$, $CoCl_2$ and other metal ion solutions. No traces of metal contamination were ever found outside the biological structures.

From a statistical point of view, we analyzed approximately 10^5 granule cells in samples exposed to aluminum, never finding evidence for this metal. These experiments were performed on cultures primarily containing granule cells. Such cultures typically include a small fraction of other types of cells. A systematic search detected aluminum in three of these minority cells, morphologically identifiable as Purkinje neurons or glial cells.

We then performed experiments on special cultures, grown to enhance the population of either Purkinje neurons or glial cells. In both cases, we found aluminum in nearly all of the cells; curve *c)* in fig. 2 is representative of these results, and was taken in a culture almost entirely formed by Purkinje neurons.

The first part of the synchrotron spectromicroscopy study, therefore, indicates that granule cells do not play a role in the Al uptake. The issue addressed by the second part was the possible generalization of this result to other metals.

Representative results are shown in fig. 3 and 4, and the results are clear: granule cells do uptake both cobalt and nickel (as well as zinc, iron and chromium). In fig. 3, spectra taken on portions of a granule-cell culture exposed to cobalt are compared to the top reference spectrum from $CoCl_2$. Figure 4 shows similar results for a granule cell culture exposed to $NiCl_2$.

In this last case, the microscopic areas correspond to the identically labeled spots in the micrograph of fig. 1, which refers indeed to the same sample. We may note from fig. 1 and 4 that Ni is only found in the fine structure of the granule neuron network and not on the cell bodies; a similar localization was found for all other metals (except of course Al).

These results show that synchrotron spectromicroscopy is well beyond feasibility tests in

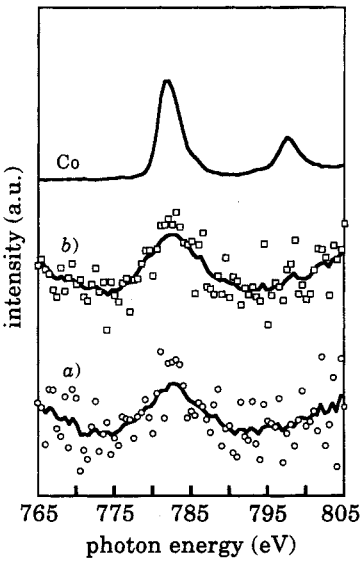

Fig. 3.

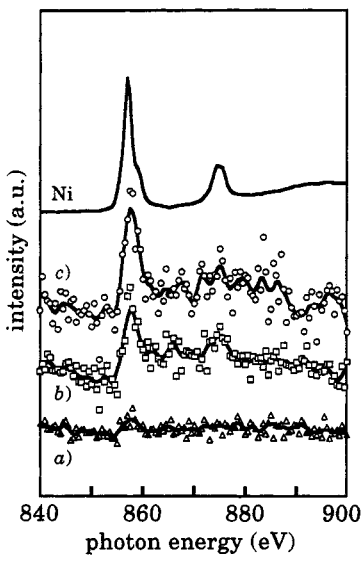

Fig. 4.

Fig. 3. – XSEM reference spectrum of Co from $CoCl_2$ (top) and (curves *a*) and *b*)) spectra from a granule cell culture exposed to Co ions. The Co $2p$ edge is clearly visible in all cases.

Fig. 4. – XSEM reference spectrum of Ni from $NiCl_2$ (top). Curves *a*), *b*) and *c*) are spectra from the granule cell culture exposed to Ni ions, taken in the similarly labeled spots of the micrograph of fig. 1. The Ni $2p$ edge is clearly visible in curves *a*) and *b*).

its applications to biophysics, and that it is already capable to detect peculiar mechanisms like the cell specificity of Al uptake thanks to rapid and systematic analysis of large numbers of cells.

The readers familiar with the materials science applications of techniques of this kind may wonder about the issue of surface sensitivity: is our experiment confined to the analysis of the cell membrane? The answer is negative: since we mostly detect low-energy secondary electrons [5], the electrons' escape depth is not extremely short. Being of the order of 50 Å, it exceeds the typical thickness of the cell's membrane (≈ 30 Å).

On the other hand, the fact that the probed depth does not greatly exceed the membrane thickness might lead to selective studies of the element distribution as a function of depth. We performed a first test in this direction, by artificially decapping cells [7] in a glial cell culture exposed to cobalt. Evidence of Co was not found in the portions of the cells with an undamaged membrane, whereas Co in the cytoplasm inside the cell was visible through the membrane's holes produced by decapping.

Finally, we evaluated the XSEM quantitative sensitivity by measuring the minimum detectable amount of phosphorus in granule cells from the P $2p$ spectral edge, and then the phosphorus concentration with ICP-AES (Inductively Coupled Plasma Atomic Emission Spectroscopy). The result was a phosphorus detection limit of 8 p.p.m.

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