XAFS in a single macrophage cell

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In this paper, a novel approach using XAFS will be introduced for the study of the problem of cytotoxicity of metal ions. A micro beam from a SR source was used to measure the time-dependent distributions and chemical states of elements in mouse macrophages that were cultured in metallic solutions of different concentration. It is revealed that the concentrations and distributions of calcium, zinc and potassium in a single cell are closely related to the uptake of the metal. Using XAFS analysis, it is shown that metal elements absorbed by the cell interact with the cell and that the chemical state of the metal is changed.

Key words: chemical state; cytotoxicity; single cell

1. Introduction

The interactions and responses of cells to foreign metal elements have been widely investigated in the past decades. The interactions between cells and metal ions or particles, has been investigated (in-vivo and in-vitro), in the case of wear debris and corrosion in metal implants (Wang, J. C. et al. (1999); Ektessabi, A. et al. (1998); Ektessabi, A. et al. (1997); Wang, J. Y. et al. (1996); Ektessabi, A. and Wennerberg, A. (1995); Shanbhag, A. S. et al. (1994)). Phagocytic activity and the inflammatory reaction of macrophages are often discussed from a variety of points of view because of the fact that the macrophage is central to the direction of host inflammatory, immune and phagocytic processes (Kao, W. J. et al. (1999)). The problem of cytotoxicity has also been investigated in the interactions between alveolar macrophages and airborne dusts or fly ashes (Pinheiro, T. et al. (1999); Goegan, P. et al. (1998); Radloff, M. et al. (1998); Gercken, G. et al. (1996); Geertz, R. et al. (1994)).

In several previous studies, Ektessabi et al. (Ektessabi, A. et al. (1998); Ektessabi, A. et al. (1997); Ektessabi, A. and Wennerberg, A. (1995)) applied PIXE, micro-beam PIXE, and SR-XRF to the investigation of human tissues around total hip replacements where SUS316L and Ti-6Al-4V had been inserted into the human body for long periods of time. They showed that Fe, Cr, Ni, and Ti were released and distributed into the tissues around the total hip replacement in the forms of mechanical frictions and corrosion products from the implant. The characteristics of metal particles or ions, such as surface chemistry, surface morphology, net charge, porosity and degradation rate, are critical factors in the interaction of these particles with cells. These features can result in the initiation of various responses, such as preferential protein adsorption, complement activation, and cell recruitment (Meresse, S. et al. (1999); Granchi, D. et al. (1999) ; Wataha, J. C. et al. (1995)). However, the mechanisms of phagocytosis of macrophages and cytotoxicity to metal ions or particles are extremely complex, and no single model can fully account for the diverse structures and outcomes associated with particle internalization (Aderem, A. and Underhill, D. M. (1999); Underhill, D. M. et al. (1999)). While it is possible for each individual response to be identified and analyzed separately, this approach gives an analysis of only a relatively small component of the many factors controlling the overall host responses (Hunt, J. A. et al. (1997)). The aim of this study is to investigate the chemical state of phagocytosed metal elements and fluctuations in the densities and distributions of intracellular elements simultaneously by using SR-XRF imaging technique when macrophages are cultured in a metallic solution environment under differing conditions.

2. Method

The cells (treated macrophages) were cultured while being exposed to solutions containing different concentrations of chromium, iron and vanadium, and also cells without metal uptake (untreated macrophage) were cultured under normal conditions. The dosing solutions were prepared by dissolving $CrCl_3$, $FeCl_3$ and VCl_3 in culture medium (40, 2 and 0.1 mg/l). J774.1 mouse macrophages were provided by the RIKEN GENE BANK. These macrophages were cultured on 5 cm dishes in RPMI-1640 medium supplemented with 10% Fetal Bovine Serum. After exposure times of 10, 30 minutes, 4, 12, 24 and 48 hours, the cells were washed with culture medium and separated by centrifugation for 5 minutes. After centrifugation, the cells were immersed in 70% ethanol for 4 hours. These procedures were repeated three times and then the cells were immersed in 100% ethanol for 1 hour. The ethanol solution containing the cells were pipetted on PET films and dried for XRF and XAFS measurement.

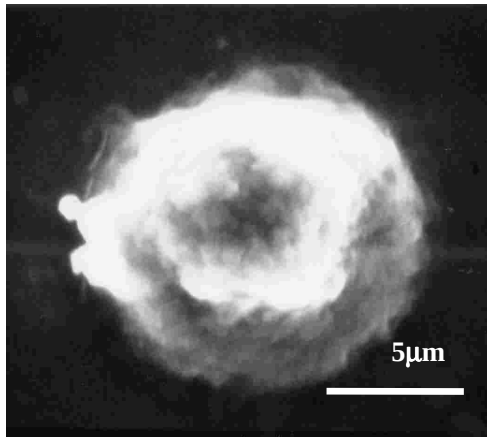

Figure 1
This image was obtained with a scanning electron microscope after measuring the elemental distribution by using SR-XRF.

The X-ray fluorescence and absorption spectra were measured at the Japan Synchrotron Radiation Research Institute using the X-ray beam emitted by 8 GeV storage ring “SPring-8”. The elemental distribution within a single cell was obtained in Photon Factory, High Energy Accelerator Research Organization, Tsukuba Japan.

3. Results

A SEM photograph of the single macrophage cell dried on the PET film is shown in figure 1. The intracellular elements in the untreated cell detected by XRF measurement were mainly, phosphorus, sulfur, chlorine, potassium, calcium and zinc. The distribution patterns of calcium and zinc in the macrophage cell are almost identical.

As for the cells cultured in the chromium solution environment, seven images for each cell - showing the distributions of phosphorus, sulfur, chlorine, potassium, calcium, chromium and zinc - were obtained with the exposure time and chromium concentration of the culture medium as variables. The image (elemental distributions) of calcium, zinc and chromium for the typical case are shown in figure 2. The distributions of calcium, zinc and chromium that are shown in figure 2 were obtained from cells that were cultured in a

chromium solution of 2 mg/l medium for 30 minutes. The ranges of density of calcium, chromium and zinc were each divided into ten levels. The levels of the measured and interpolated points were assigned to a corresponding shade of black, and are plotted as shown in figure 2.

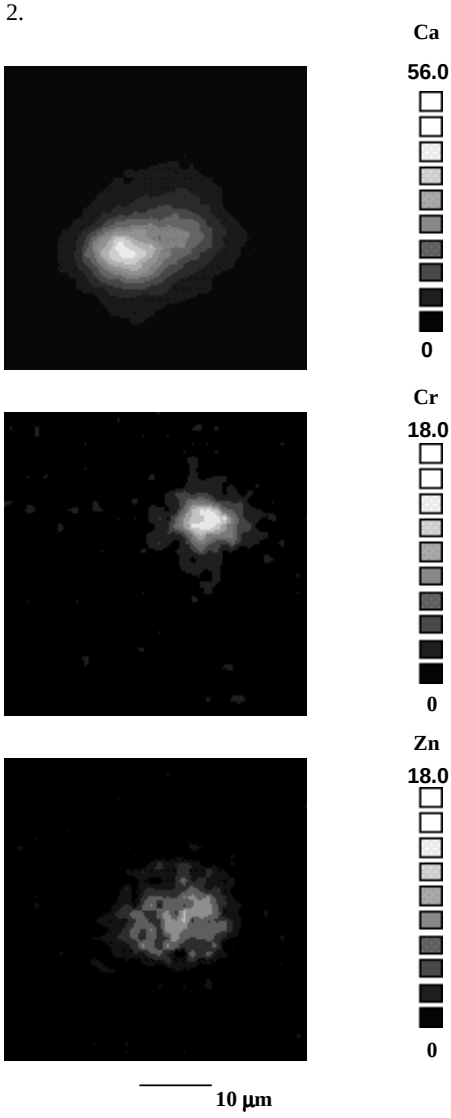

Figure 2
This image was obtained from a macrophage cell cultured in a chromium solution environment (2 mg/l medium) for 30 minutes. The ranges of measured fluorescent intensities are from 0 to 56 photons per second for calcium, from 0 to 18 photons per second for zinc and from 0 to 18 photons per second for chromium. Each range is divided into ten levels. Each level has been assigned a shade of red, blue and green respectively.

In the case of treated macrophages cultured in chromium solution environment (2 mg/l for 30 minutes), zinc, calcium and chromium are localized in different parts of the cell, showing a significant difference from that of the untreated cell.

The distribution of iron in macrophage cells cultured in iron solution environment (2 mg/l for 24 hours) is shown in figure 3-III. The K-edge XANES spectrum of iron phagocytosed in macrophage cells was collected in fluorescence mode and is shown in figure 3-I (spectrum B). The spectrum B in figure 3-I was obtained from the cell that is exhibited by the arrow in the image of figure 3-III. The spectrum A in figure 3-I was collected from $FeCl_3$ dissolved in culture medium (2 mg/l). The pre-edge peaks of these two spectra are shown in figure 3-II. The spectra C and D were collected from FeO and Fe_2O_3 respectively as reference samples. It can be considered that a part of the intracellular iron phagocytosed by the macrophage cell exhibits a different oxidation state. Furthermore, it is likely that the coordination geometry of the site occupied by the atom surrounding the iron may change within 24 hours after uptake of iron into the cell. The K-edge XANES spectra of chromium phagocytosed in macrophage cells were collected in fluorescence mode and are shown in figure 4 (spectra B and C). The spectrum A in figure 4 was collected from $CrCl_3$ dissolved in culture medium (40 mg/l). The spectrum D was collected from Cr_2O_3 as a reference sample. The spectra A, B and C shown in figure 4 are almost identical. It can be concluded that the changeability of the chemical state of the metal elements phagocytosed by the cell dependents on the type of metal element in the culture solution.

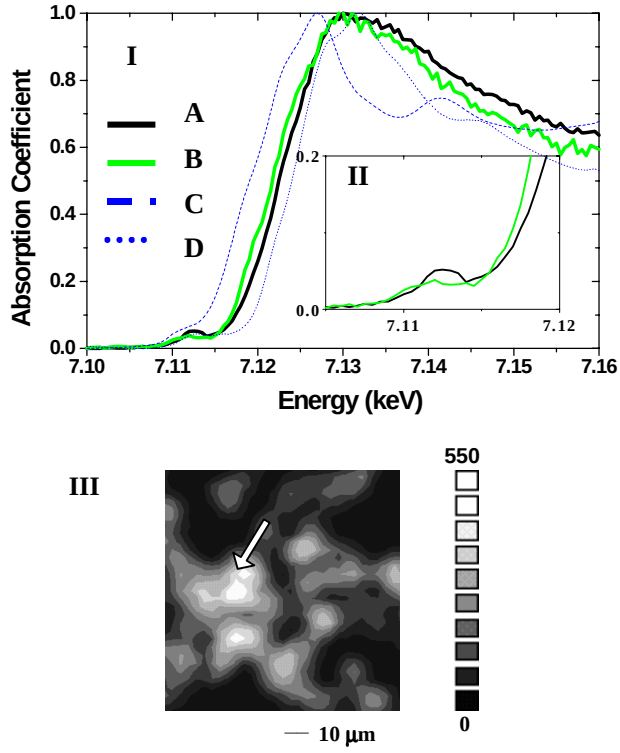

Figure 3
The distribution of iron in macrophage cells cultured in an iron solution environment (2 mg/l medium) is shown in figure III. The K-edge spectrum of iron in macrophage cells cultured for 24 hours in an iron solution environment was collected in fluorescence mode. The spectra A and D shown in figure I were obtained from FeO and Fe_2O_3 respectively. The spectrum B was obtained from $FeCl_3$ dissolved in a culture medium (2 mg/l medium). The spectrum C was obtained from the iron phagocytosed by the cell (arrow shown in figure III). The pre-edge region was shown in figure II.

4. Discussion

In this paper we employed a new approach to the investigation of the uptake of metal elements by cells using an XRF imaging technique and XANES spectrometry. Via this approach, we found that the uptake of metal ions or complexes caused characteristic changes in the distribution patterns of the intracellular elements and in the chemical states of the phagocytosed metal elements.

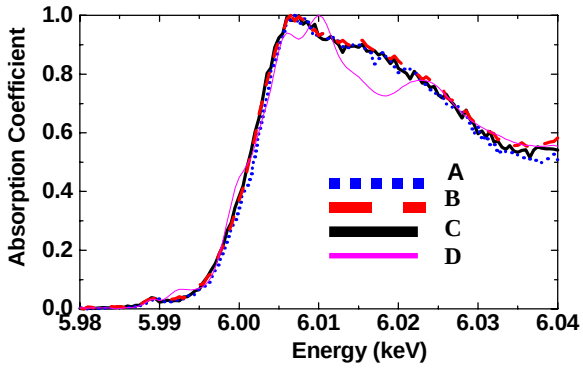

Figure 4
The K-edge spectra of macrophage cells cultured for 24 hours with two different concentrations of chromium solution were collected in fluorescence mode and shown in this figure. The concentrations of chromium in the solutions were 2 mg/l and 40 mg/l.

It is likely that the chemical states of the phagocytosed metal elements and the change in their chemical states are closely and complicatedly related to the cytotoxicity and the defensive mechanisms against the foreign metal elements. Furthermore, the density of the intracellular elements exhibited a characteristic change according to the concentration and the exposure time to the metallic solution. For example, the uptake of chromium at high doses causes an increase in the density of phosphorus and potassium by a factor of 2 to 5, and a decrease in the density of calcium by a factor of 2. The balance of the matrix elements in the cell is disturbed by exposing cells to the metallic solution. By using the focused micro beam from a synchrotron radiation source, new insights into the elemental balances in single cells and the density fluctuations of the matrix elements, which may be related to cell function or the process of cell death, can be obtained.

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