Lecture 6

Synchrotron Radiation Measurement

6.1. Sample Preparation

For treated macrophage samples, the cells were exposed to solutions containing different concentrations of Cr Chloride, Cr Oxide, V Chloride and Fe Chloride during culture. For untreated macrophage samples, the cells were cultured under normal conditions without metal uptake. The J774.1 mouse macrophages were provided by the RIKEN CELL BANK. The culture was carried out on 550 mm dishes in RPMI-1640 medium supplemented with 10 % Fetal Bovine Serum.

After exposure to metallic solutions, the cells were washed with the culture medium and separated by centrifuge for 5 min. After centrifugation, the cells were immersed in 70 % ethanol for 4 h. These procedures were repeated three times and then the cells were immersed in 100 % ethanol for 1 h. The ethanol solution containing the cells was pipetted on PET films and dried for XRF measurement.

6.2. Experimental Set-Up

The x-ray fluorescence spectra and elemental images were mainly obtained at beam line 4A in the High Energy Accelerator Institute in Tsukuba, using the x-ray beam emitted by the 2.5 GeV storage ring "Photon Factory". This beam line is a hard x-ray beam line with bending magnet and is dedicated to x-ray analysis in material science and biology, and ultra trace elemental analysis. A multilayer monochromator provides x-rays with energy that can be varied from 5.0 to 30 keV. The x-ray beam obtained in the experimental hutch has energy resolution $\Delta E/E$ of less than 1×10^{-4}, photon flux of $\sim 10^{11}$ photons/sec, and beam size of 1(V) × 3(H) mm^2. A platinum-coated plane mirror is used to reduce higher harmonics to less than 10^{-4}. A slits system is used to limit the vertical and horizontal beam width incident beam to improve resolution, and a KB (Kirkpartrick-Baez Optics) focusing mirror is located in front of the sample. The final beam size was 6 μm in diameter in this study. Fluorescent x-ray was detected by SSD (Si(Li)) in air.

Some of the x-ray fluorescence and most of the absorption spectra were measured at the Japan Synchrotron Radiation Research Institute using the x-ray beam emitted by an 8 GeV storage ring "SPring-8". Beam Line-39XU in SPring-8 was used to do XRF and XANES analysis. This beam line is a hard X-ray undulator beam line and is mainly used for studying x-ray absorption, x-ray microanalysis in material and biology, and

ultra trace elemental analysis. It consists of in-vacuum undulator with 32 mm period length and 140 period numbers and it generates a horizontally polarized x-rays beam. Combination of the undulator harmonics and a Si (111) double crystal monochromator provides x-rays with an energy range from 5.7 to 37 keV. The x-ray beam obtained in the experimental hutch has the energy resolution $\Delta E/E$ of less than 2×10^{-4}, photon flux of $\sim 10^{12}$ photons/sec, and beam size of 0.5(V) × 1.3(H) mm^2. A platinum-coated plane mirror is used to reduce higher harmonics to less than 10^{-4}. An incident beam slit is used to limit vertical and horizontal beam width to improve resolution and a pinhole 10 μm in diameter is located between the incident beam slit and the sample. Fluorescent x-ray was detected by SSD (Si(Li)) in vacuum.

6.3. Experimental Results

6.3.1. Elemental Images of Macrophages

Elemental distribution in untreated macrophages

A microscopic photograph of a macrophage dried on PET film is shown in figure 6.1a. An XRF spectrum measured at a point in the cell with a high density of Ca, Zn, K and P is shown in figure 6.2. The main intracellular elements detected were P, S, Cl, K, Ca and Zn. Ca has the highest intensity in the x-ray energy range of 1 keV to 10 keV.

The elemental distributions of Ca and Zn within a single untreated macrophage are shown in figures 6.1b and 6.1c, respectively. While the distribution of Ca and Zn in the macrophage cell are almost identical, the densities are completely different.

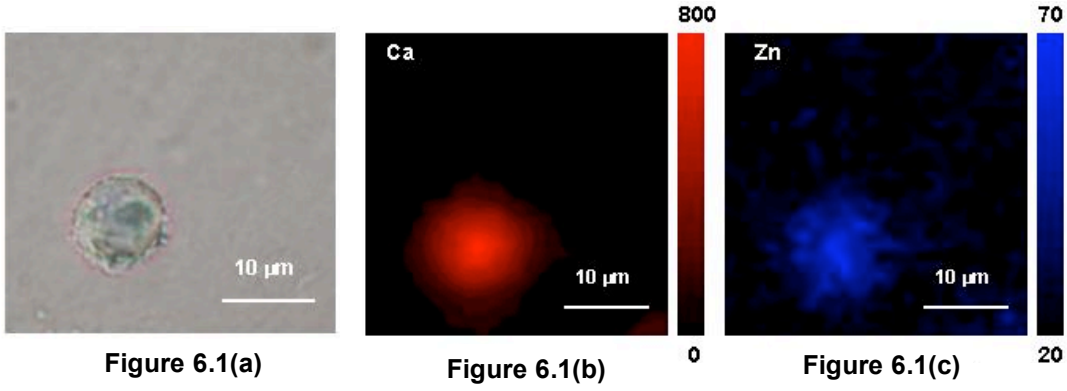

Figure 6.1(a) Figure 6.1(b) Figure 6.1(c)

Figure 6.1. A microscopic photograph of a macrophage dried on PET film is shown in figure 6.1a. The elemental distribution of Ca and Zn within a single untreated macrophage are shown in figure 6.1b and 6.1c, respectively. These images are matrices of 30 × 30 pixels of 1 μm resolution. The measurement time was 5 sec.

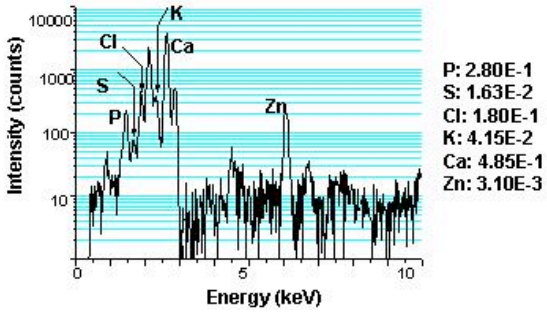

Figure 6.2. An XRF spectrum of a single untreated macrophage measured at the point with the highest intensity of Ca, Zn, K and P.

Exposure to Cr chloride solution

Elemental distribution in treated macrophages

Treated macrophages were cultured in a Cr chloride solution environment with different exposure times to investigate the differences in uptake of Cr and also the variations in the distribution of the internalized elements and intracellular elements. The exposure times were 0.17, 0.5, 4, 12, 24 and 48 h. Seven images for each sample of cells cultured in the Cr chloride solution environment showing the distributions of P, S, Cl, K, Ca, Cr and Zn were obtained with the exposure time as the variable. The images (elemental distributions) of Ca, Zn and Cr for four typical cases are shown in figures 6.3 a, b, c and d. The distributions of Ca, Zn and Cr that are shown in figures 6.3 a, b, c and d were measured in cells that were cultured in a Cr solution of 0.04 g/L medium for 0.17, 0.5, 4 and 24 h., respectively. These images are 1 μm resolution. The density of Ca, Cr and Zn are displayed as shades of green, red and blue respectively, as shown in figures 6.3 a, b, c and d. In figure 6.3 a_5 and b_5, the images of Ca and Cr are overlapped. The overlapping areas are expressed by their superimposed colors.

In the image shown in figure 6.3a, the ranges of the fluorescent x-ray intensities of Cr, Ca, and Zn are from 0 to 45, 0 to 107 and 0 to 26 counts, respectively. The measurement time was 5 sec. The distribution patterns shown in figure 6.3a are typical images that can be seen in the initial stage of the uptake of the foreign metal element. The experimental results shown in figures 6.3b and 6.3c reveal that Ca and Zn have almost identical distribution pattern to that of untreated cells, as can be seen by comparing with figure 6.3a.

In the image shown in figure 6.3b, the ranges of the fluorescent x-ray intensities of Cr, Ca and Zn are from 0 to 126, 0 to 966 and 1 to 155 counts, respectively. The measurement time was 5 sec. The distribution patterns shown in figure 6.3b are typical images observed when the uptake of Cr into the cell is increasing and the distributed Cr

populations within the cell are concentrated. It is assumed that this distribution pattern observed is independent of the exposure time. Ca and Zn are not always correlated. As shown in figure 6.4, Zn and Ca are completely separated from each other and Zn is localized near Cr in the cell.

In the image shown in figure 6.3c, the ranges of the fluorescent x-ray intensities of Cr, Ca and Zn are from 0 to 11, 0 to 21 and 0 to 18 counts respectively, obtained in 5 sec. When the uptake of Cr is at the low level, Cr, Ca and Zn have almost identical distribution patterns. However, the densities of Ca and Zn within the cell are much lower than those within the control (untreated) cells. The distributions of Ca and Zn become almost identical patterns independent of the exposure time for low level Cr uptake.

In the image shown in figure 6.3d, the ranges of the fluorescent x-ray intensities of Cr, Ca and Zn are from 0 to 7000, 0 to 280 and 0 to 180 counts respectively, obtained in 5 sec. The distribution patterns of Cr, Ca and Zn become identical with the high densities of these elements, when the uptake and accumulation of Cr are high. It is likely that the uptake of Cr was saturated and the cell division and other activities ceased.

When Cr chloride solution was exposed to macrophages, four typical distribution patterns of Cr, Ca and Zn were observed. In figure 6.3b, the densities of the intracellular Ca and Zn increased and their distribution patterns were localized within the cell. This pattern was unusual. A more typical pattern is shown in figure 6.3c, where Cr, Ca and Zn were not localized but distributed and their densities were low within the cell. This pattern was also observed for samples exposed to a Cr chloride solution (0.04 g/L) from 0.17 to 24 h. The meaning of this state on the process of phagocytosing foreign metal elements is considered at the end of this section.

Quantification of density of intracellular elements as a function of time and dose

For the quantification of the densities of the intracellular elements, XRF spectra were obtained at a point in each cell with the highest densities of the elements. The measurement time was 200 sec. for each point. In order to explain the increase or decrease in the density of the elements in relation to the uptake of Cr, it is useful to define a parameter, namely “relative density” for each element expressing the ratio of the intensities of that element in the treated macrophage divided by that in the untreated macrophage. Therefore, a relative density of less than 1 in a cell means that the density of that element in the treated cell decreases due to the uptake of Cr, and conversely a value greater than 1 corresponds to an increase.

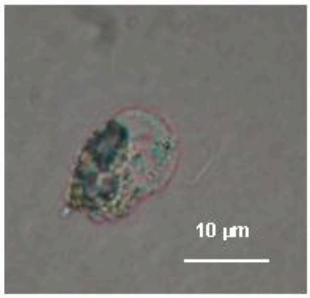

Figure 6.3a_1

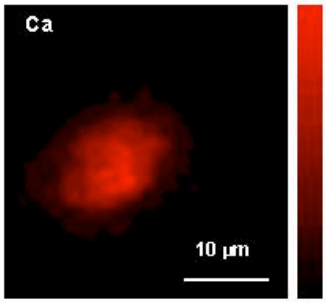

Figure 6.3a_2

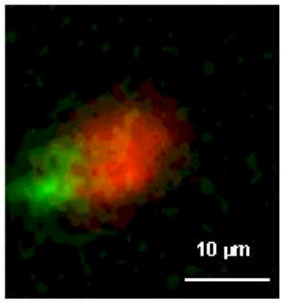

Figure 6.3a_5

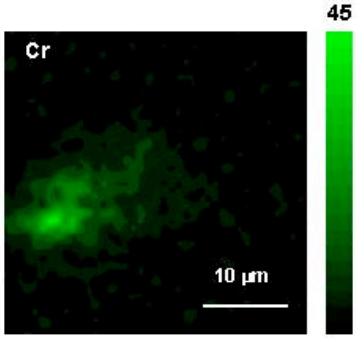

Figure 6.3a_3

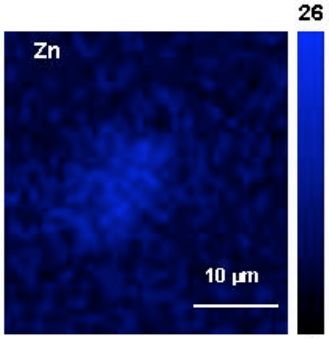

Figure 6.3a_4

Figure 6.3a_1-a_5. A microscopic photograph of a dried macrophage dried on PET film is shown in figure 6.3a_1. The elemental distribution of Ca, Cr and Zn within a single treated macrophage cultured in 0.04 g/L Cr solution for 0.17 h. are shown in figure 6.3a_2, a_3 and a_4, respectively. These images are matrices of 42 × 42 pixels of 1 µm resolution. In figure 6.3a_5, the images of Ca and Cr are overlapped.

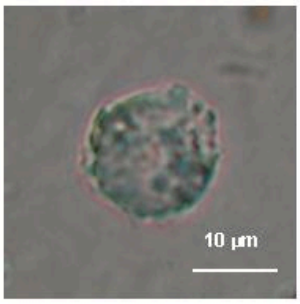

Figure 6.3b_1

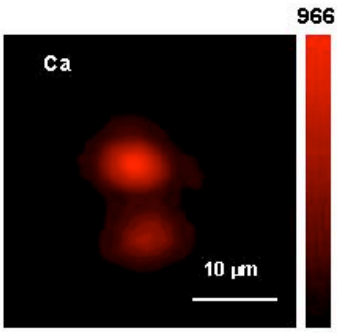

Figure 6.3b_2

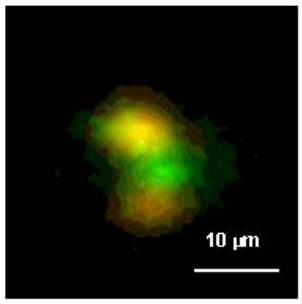

Figure 6.3b_5

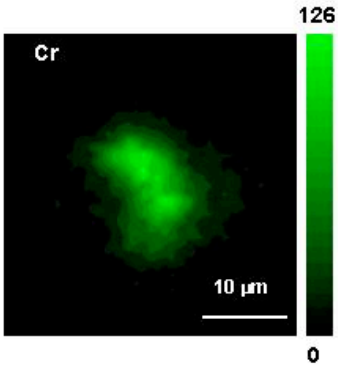

Figure 6.3b_3

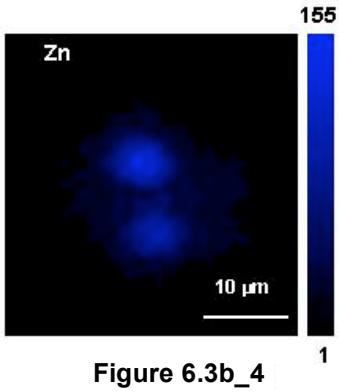

Figure 6.3b_4

Figure 6.3b_1-b_5. A microscopic photograph of a dried macrophage dried on PET film is shown in figure 6.3b_1. The elemental distribution of Ca, Cr and Zn within a single treated macrophage cultured in 0.04 g/L Cr solution for 0.5 h. are shown in figure 6.3b_2, b_3 and b_4, respectively. These images are matrices of 42 × 42 pixels of 1 µm resolution. In figure 6.3b_5, the images of Ca and Cr are overlapped.

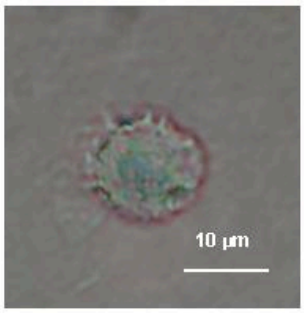

Figure 6.3c_1

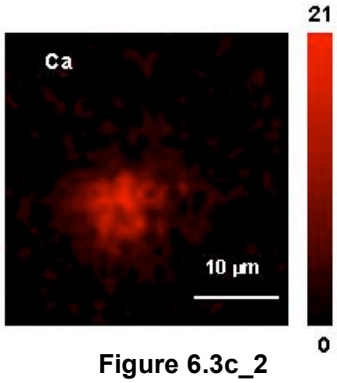

Figure 6.3c_2

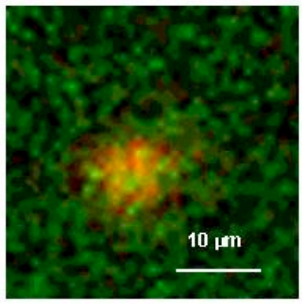

Figure 6.3c_5

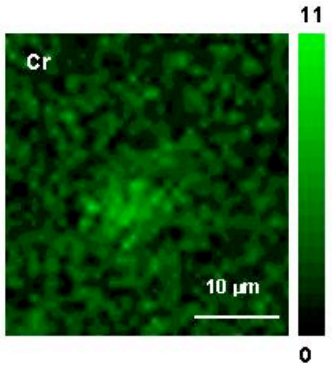

Figure 6.3c_3

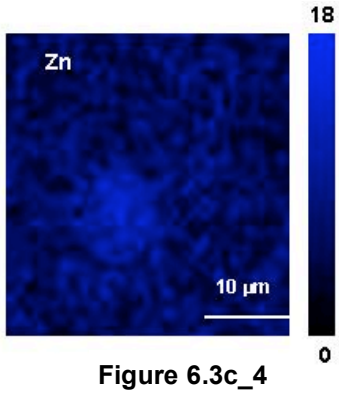

Figure 6.3c_4

Figure 6.3c_1-c_5. A microscopic photograph of a dried macrophage dried on PET film is shown in figure 6.3c_1. The elemental distribution of Ca, Cr and Zn within a single treated macrophage cultured in 0.04 g/L Cr solution for 4 h. are shown in figure 6.3c_2, c_3 and c_4, respectively. These images are matrices of 42 × 42 pixels of 1 µm resoltuion. In figure 6.3c_5, the images of Ca and Cr are overlapped.

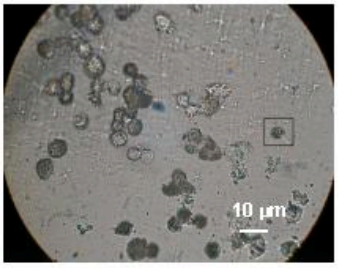

Figure 6.3d_1

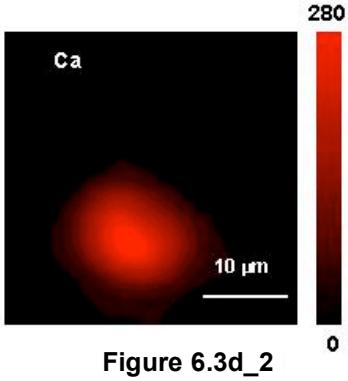

Figure 6.3d_2

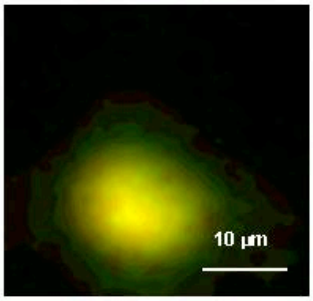

Figure 6.3d_5

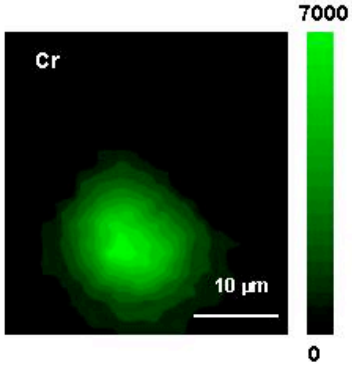

Figure 6.3d_3

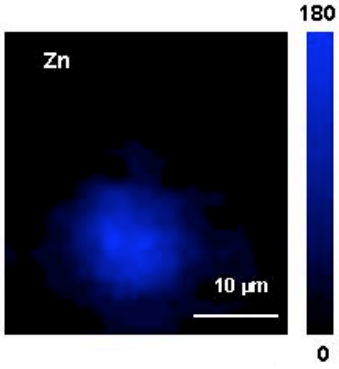

Figure 6.3d_4

Figure 6.3d_1-d_5. A microscopic photograph of a dried macrophage dried on PET film is shown in figure 6.3d_1. The elemental distribution of Ca, Cr and Zn within a single treated macrophage cultured in 0.04 g/L Cr solution for 24 h. are shown in figure 6.3d_2, d_3 and d_4, respectively. These images are matrices of 31 × 31 pixels of 1 µm resoltuion. In figure 6.3d_5, the images of Ca and Cr are overlapped.

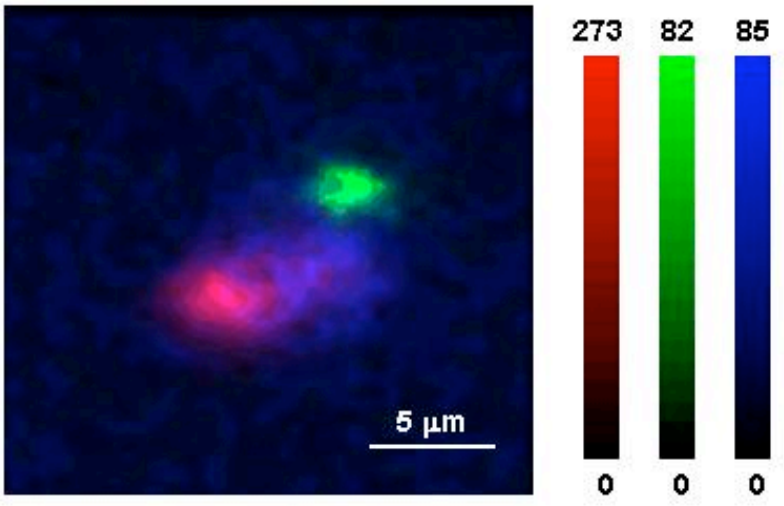

Figure 6.4. This image was obtained from a macrophage cell cultured in a Cr solution environment (2 mg/L medium) for 30 min. The ranges of measured fluorescent intensities are from 0 to 273 photons/sec. for Ca, from 0 to 85 photons/sec. for Zn and from 0 to 82 photons/sec. for Cr. Each range is divided into 20 levels. Each level has assigned a shade of red, blue and green respectively.

Effect of Cr uptake on P density

The spectrum shown in figure 6.5 was obtained from the cell shown in figure 6.3a with the highest density of Ca, cultured in a Cr chloride solution (0.04 g/L) for 0.17 h. The value of the relative density for P was 0.636. Values for other cells exposed to the same solution for the same time of 0.17 h. are almost identical. Although the cell was exposed to Cr chloride solution for only 0.17 h., the P may be emitted from inside the cell and bonding to some other molecules.

The spectra in figure 6.6 were obtained from the cell shown in figure 6.3b which has the highest densities of Cr and Ca and was cultured in a Cr chloride solution (0.04 g/L) for 0.5 h. The resulting relative density of P was 2.64. The amount of the internalized Cr was large in spite of the short exposure time. It appears that the rapid inflow of Cr into the cell induced subsequent inflow of P.

The spectra in figure 6.7 were obtained from the cell shown in figure 6.3c with the highest densities of Cr and Ca, cultured in a Cr chloride solution (0.04 g/L) for 4 h. The value of the relative density of P was 0.134. It appears that when the amount of the internalized Cr into the cell was small, the density of P was also small, and unrelated to the exposure time.

The spectra in figure 6.8 were obtained from the cell shown in figure 6.3d with the highest densities of Cr and Ca, cultured in a Cr chloride solution (0.04 g/L) for 24 h.

The value of the relative density of P was 1.74.

The following mechanism appears plausible. First, the density of P within the cell decreased after exposing the cell to a Cr chloride solution. If the up-taken Cr into the cell is low, the density of P within the cell may be maintained at low level independent of the exposure time. However, the intracellular density of P increases when the density of Cr within the cell is large regardless of the rate of inflow of Cr into the cell. It appears that the density fluctuation of P is correlated with the uptake of Cr into the cell.

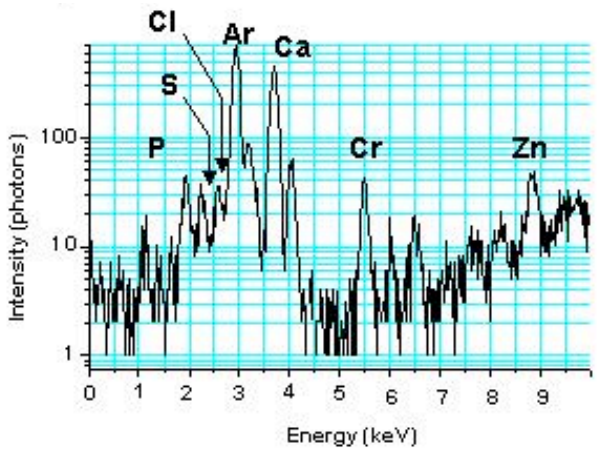

Figure 6.5. The spectrum was obtained from the cell shown in figure 6.3a with the highest density of Ca. The measurement time 200 sec and the incident x-ray energy was 14.3 keV.

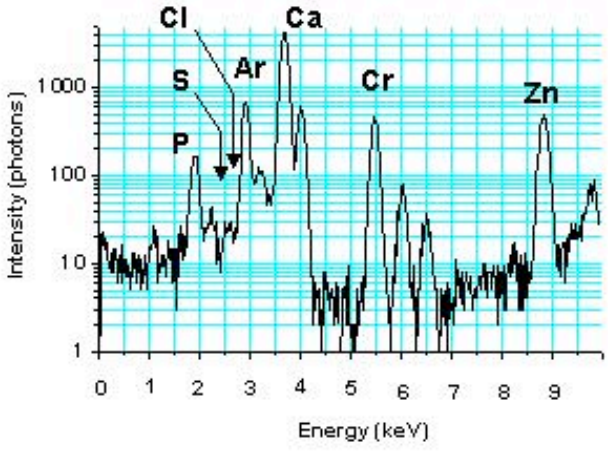

Figure 6.6. The spectrum was obtained from the cell shown in figure 6.3b with the highest density of Ca and Cr. The measurement time 200 sec and the incident x-ray energy was 14.3 keV.

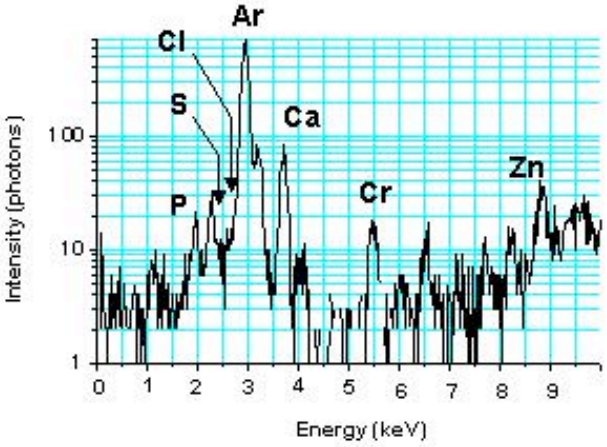

Figure 6.7. The spectrum was obtained from the cell shown in figure 6.3c with the highest density of Ca and Cr. The measurement time 200 sec and the incident x-ray energy was 14.3 keV.

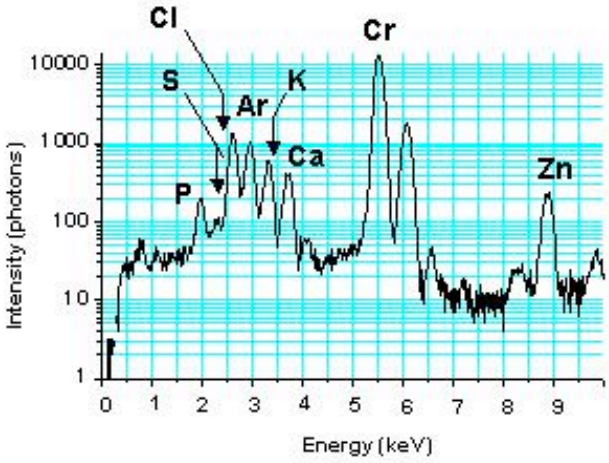

Figure 6.8. The spectrum was obtained from the cell shown in figure 6.3d with the highest density of Ca. The measurement time 200 sec and the incident x-ray energy was 14.3 keV

Effect of Cr uptake on K density

The value of the relative density of K within the cell shown in figure 6.3a was 0.690, suggesting that a part of the intracellular K was emitted out of the cell after exposing the cell to a Cr solution. The value for the cell shown in figure 6.3b was 1.29, indicating that the density of K within the cell increased because of the rapid inflow of Cr into the cell.

For the cell shown in figure 6.3c, the value was almost 0. It appears that almost all of the intracellular K was emitted from the cell when the density of Cr remained low level after exposing the cell to a Cr solution.

For the cell shown in figure 6.3d, the value was 5.28, suggesting that when the density of Cr within the cell steadily increased, the density of the intracellular K increased also. Here also, the density of K within the cell may be correlated with the value of the uptake of Cr into the cell. Furthermore, it is probable that the value of the re-uptake of K is related to the condition of the cell membrane affected by the injurious effect of Cr solution environment.

Effect of Cr uptake on Zn density

The value of the relative density of Zn within the cell shown in figure 6.3a was 0.837, indicating very little outflow of Zn from the cell. For the cell shown in figure 6.3b, the value was 7.78, suggesting that after the rapid inflow of Cr into the cell, the subsequent inflow of Zn into the cell occurred rapidly. Furthermore, the distribution of Zn was localized adjacent to the population of Cr. The value for the cell shown in figure 6.3c was 0.445, indicating that the rate of the reduction of Zn from inside the cell is smaller than that of P or K. For the cell shown in figure 6.3d the value was 2.56, suggesting that the subsequent inflow of Zn was induced by the slow uptake of Cr into the cell over a long time.

The outflow of Zn may be restricted to a certain range. It is possible that Zn is indispensable for the cell in order to dispose foreign bodies. Furthermore, it is probable that Zn performs central role in defense against the toxic effect of the foreign metal element because of the fact that the subsequent rapid inflow of Zn is induced by the rapid inflow of Cr into the cell.

Effect of Cr uptake on Ca density

The value of the relative density of Ca within the cell shown in figure 6.3a was 0.385. Although the exposure time was very short, a large amount of Ca was emitted out from the cell. The value for the cell shown in figure 6.3b was 3.60, indicating that the inflow of Ca into the cell was induced by the rapid uptake of Cr. When the uptake of Cr was very fast in a short time, the relative density of Ca exceeded 1, which is the value for the control cell.

For the cell shown in figure 6.3c the value was 0.0648, indicating a rapid reduction of Ca within the cell. This outflow of Ca is the largest compared to those of other elements that can be detected by SR-XRF. The value for the cell shown in figure 6.3d was 0.232. In this case, the uptake of Cr increased slowly over a long time. It appears that the re-uptake of Ca may have occurred after the outflow in the initial stage.

Summary

In this study, Kitamura and Ektessabi found that the uptake of Cr ions or complexes causes characteristic changes in the distributions and densities of the intracellular elements in macrophages according to the speed and quantity of the uptake of Cr into the cell. It is clear that P, K, Zn and Ca are closely related to the uptake of foreign Cr into the cell. They found a rapid Cr uptake in the case of the cell shown in figure 6.3b which was interpreted as an evidence that a part of the Cr added in the culture medium may be gathered temporally in a localized area around the cell in spite of the fact that the Cr solution was stirred. Therefore, macrophage cells in a highly condensed environment of Cr solution may be unable to avoid internalizing the high density of Cr. The fluctuations in the densities of the elements in the cells may be permitted within a certain range to sustain normal conditions after the uptake of foreign bodies. Yet, if the rapid inflow of Cr occurs, the rapid and excessive inflow of some elements may be induced in order to counteract the toxicity of Cr. From the results of SR-XRF analysis, it can be concluded that Ca, P and Zn play important roles in defense against the toxicity of Cr.

When the macrophage cells are cultured in a Cr chloride solution environment, the inflow of Cr occurs slowly. In the process of the uptake of Cr, the depletion of some elements such as Ca, K, P, S and Zn are observed within the cells. When the uptake and accumulation of Cr increase within the cells, the intracellular densities of P and Zn increase more than those in the control cells. However, the subsequent inflows of Ca, P and Zn were rapidly caused by the uptake of a large quantity of Cr in a very short time. These elements may be immediately required to defend against the foreign metal element.

Exposure to V Chloride Solution

Elemental Distribution in Treated Macrophages

Treated macrophages were cultured in a V chloride solution environment with different exposure times to investigate the differences in uptake of V and also the variations in the distribution of the internalized elements and intracellular elements. The exposure times were 0.5, 12 and 24 h. For each cell, seven images showing the distributions of P, S, Cl, K, Ca, Cr and Zn were obtained with the exposure time as a variable. The images showing the elemental distributions of Ca, Zn and V for three typical cases are shown in figure 6.9 a, b and c. The images for Ca, Zn and V shown in figure 6.9 a, b and c were from cells cultured in a V chloride solutions of 0.04 g/L medium for 0.5, 12 and 24 h, respectively. These images are 1 μm resolution, and the

ranges of densities of Ca, V and Zn are each divided into twenty levels assigned to shades of green, red and blue respectively, as shown in figures 6.9 a, b and c. In figure 6.9a_5 and b_5, the images of Ca and Cr overlap, and in figure 6.9c_4, the images of Ca and Zn overlap as well. The overlapping areas are expressed by their superimposed colors.

In the image shown in figure 6.9a, measured in 5 sec., the maximum counts of the fluorescent x-ray of V, Ca, and Zn are 27, 276 and 55 counts respectively. The distribution patterns shown in figure 6.9a are typical images observed for the early stage of the uptake of V. The distribution patterns of Ca and Zn are almost identical with those in the control cell, but with higher densities. When the cells are cultured in a V solution environment, the uptake of V may be induced in a very short time.

In the image shown in figure 6.9b, the maximum x-ray intensities of V, Ca, and Zn are 30, 654 and 115 counts respectively, measured in 5 sec. These are typical images seen at the state when the densities of Ca and Zn within the cell are increasing. The distribution patterns of Ca and Zn are almost identical with those in the cell shown in figure 6.9a. The quantity of V within the cell decreased compared to the early stage of the uptake of V.

In the image shown in figure 6.9c, the intensities for V, Ca, and Zn varied from 40 to 115, 11 to 4216 and 28 to 1164 counts respectively, also measured for 5 sec. These were cultured in V solution environment for a long time. The distribution patterns of Ca and Zn are separated and adjacent each other. However, the quantity of V within the cell is almost zero.

Effect of V Uptake on P Density

The spectrum in figure 6.10 was obtained from the cell shown in figure 6.9a with the highest density of Ca. The cell was cultured in V chloride solution (0.04 g/L) for 0.5 h. The value of the relative density (defined as previously as the ratio of the particular element in the treated to that in the untreated cells) of P was 1.227. For other similarly cultured cells, the value of the relative density of P varied widely. It was anticipated that the density of P and V within the cell may be closely related, however such was not the case. The rapid inflow of V may not induce similarly rapid increase of P within the cell.

The spectrum in figure 6.11 was obtained from the cell shown in figure 6.9b with the highest densities of Ca. This cell was cultured in the same V chloride solution (0.04 g/L) for 12 h. The value of the relative density of P was 2.1287.

The spectra shown in figure 6.12 and 6.13 were obtained from the cell shown in figure 6.9c with the highest densities of Zn and Ca. The cell was cultured in a V

chloride solution (0.04 g/L) for 24 h, and the value of the relative density of P was 0.5709. Almost nothing of the initially internalized V remained within the cell. It appears that the depletion of V may induce the outflow of P from inside the cell.

Effect of V uptake on K density

The value of the relative density of K within the cell shown in figure 6.9a was 0.7605, implying that part of the intracellular K may be emitted outside the cell after exposing the cell to a V solution. For the cell shown in figure 6.9b the value was 2.034. The density of K within the cell increased because of the rapid inflow of V into the cell.

For the cell shown in figure 6.9c the value was 1.898. It is probable that the value of the re-uptake of K is related to the condition of the cell membrane affected by the injurious effect of V solution environment.

Effect of V Uptake on Zn Density

The value of the relative density of Zn within the cell shown in figure 6.9a was 4.587. Apparently the rapid inflow of V into the cell was accompanied by rapid inflow of Zn. Furthermore, the distribution of Zn was localized adjacent to that of Ca. The intracellular V induced much larger uptake of Zn into the cell compared to the case of Cr chloride solution case.

The value of the relative density of Zn within the cells shown in figure 6.9b and 6.9c were 7.996 and 16.44, respectively. The depletion of P was observed when the outflow of the internalized V occurred from inside the cell. However, for Zn the density within the cell remained at a high level in spite of the depletion of V.

It is probable that Zn performs a central role to defense the toxic effect of the foreign metal element because of the fact that subsequent rapid inflow of Zn is induced in both cases where rapid inflows of Cr and V occur into the cell. After the outflow of V from inside the cell, the population of Zn remained within the cell. The inflow of Zn into the cell may be required as a defense against the toxic effect of V, but the accumulation of the high density of Zn after the depletion of V may be toxic to the cell. It appears that macrophages with the excessive accumulation of Zn within their bodies can not go back to the normal states. The excessive inflow of Zn beyond the permitted range to sustain normal conditions may be injurious to the cells.

Effect of V uptake on Ca density

The value of the relative density of Ca within the cell shown in figure 6.9a was 2.017. The rapid inflow of Ca may be induced by the rapid inflow of V into the cell. In

case of other cells exposed to the same solution for 0.5 h., the value of the relative density of Ca varied widely. It appears that a remarkable depletion of Ca within the cell was induced at the early stage of the uptake in both cases of Cr and V exposure. However, the inflow of Ca into the cell is induced by a small quantity of V internalized into the cell.

The values of the relative density of Ca within the cells shown in figures 6.9b and 6.9c were 2.946 and 4.289 respectively. The distribution pattern of Ca was localized and adjacent to the population of Zn. In case of other cells exposed to the same solution for 24 h., the value of the relative density of Ca varied widely. Depending on circumstances, although the relative density of Zn is much higher than 1 (within the control cell), the density of Ca within the cell is much less than in the control cell.

Summary

When the macrophage cells were cultured in Cr chloride and V chloride solution environments, there were some points in common concerning the changes of the elemental distribution patterns and the elemental density fluctuations within the cell. However, there are many different points that are very important in considering the differences of the toxic effect between Cr and V.

The outflow of V after initial uptake by the cell was observed in these experiments, posing a question. It is possible that the up-taking activity may be prevented by the unusual changes of the elemental densities within the cell. The first element that displays the rapid entry into the cell is Zn. Therefore, it can be concluded that the excessive uptake and accumulation of Zn within the cell prevent the cell from accumulating other elements. The maximum value of the relative density of Zn became 25.81 when the cells were cultured in V chloride solution environment for 24 h. It was observed that the inflow of a large amount of Zn was caused by a small amount of V within the cell. The excessive accumulation of Zn within the cell is considered to induce not only the blocking of the uptake but also an injurious effect to the cell. The localization and excessive accumulation of Ca within the cell was also observed in the area adjacent to the population of Zn. It is probable that the preservation of the high density of Ca within the cells may also be toxic.