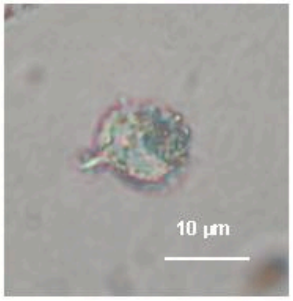


Figure 6.9a_1

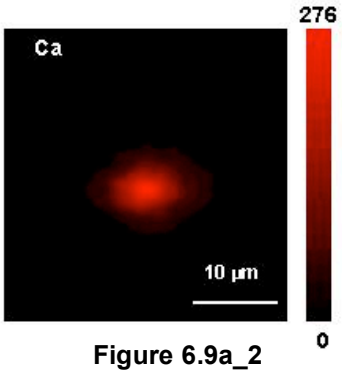


Figure 6.9a_2

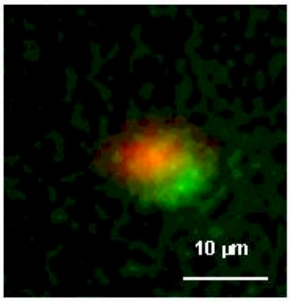


Figure 6.9a_5

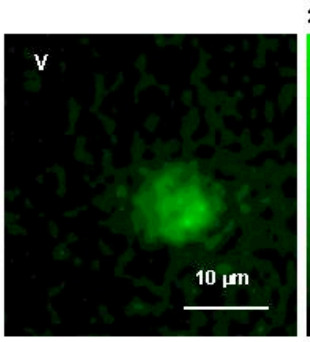


Figure 6.9a_3

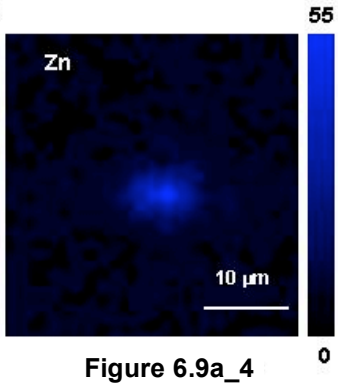


Figure 6.9a_4

Figure 6.9a_1-a_5. A microscopic photograph of a dried macrophage dried on PET film is shown in figure 6.9a_1. The elemental distribution of Ca, V and Zn within a single treated macrophage cultured in 0.04 g/L V solution for 0.5 h. are shown in figure 6.9a_2, a_3 and a_4, respectively. These images are matrices of 42 × 42 pixels of 1 µm resoltuion. In figure 6.9a_5, the images of Ca and V are overlapped.

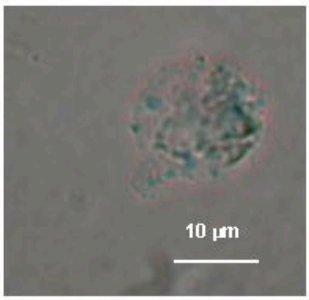


Figure 6.9b_1

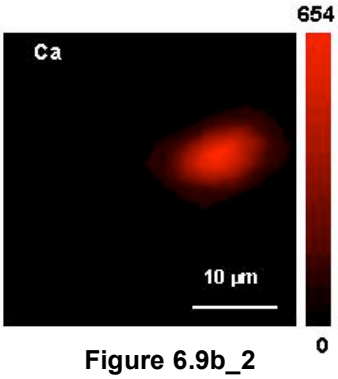


Figure 6.9b_2

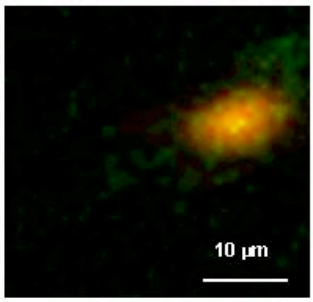
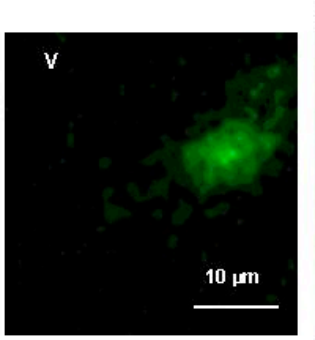


Figure 6.9b_5



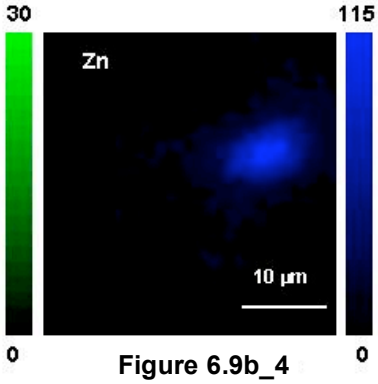


Figure 6.9b_3 Figure 6.9b_4

Figure 6.9b_1-b_5. A microscopic photograph of a dried macrophage dried on PET film is shown in figure 6.9b_1. The elemental distribution of Ca, V and Zn within a single treated macrophage cultured in 0.04 g/L V solution for 12 h. are shown in figure 6.9b_2, b_3 and b_4, respectively. These images are matrices of 42 × 42 pixels of 1 µm resoltuion. In figure 6.9b_5, the images of Ca and V are overlapped.

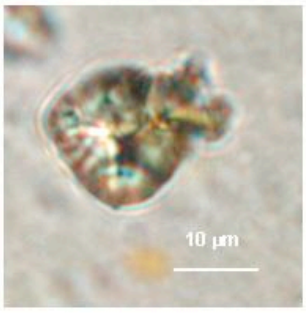


Figure 6.9c_1

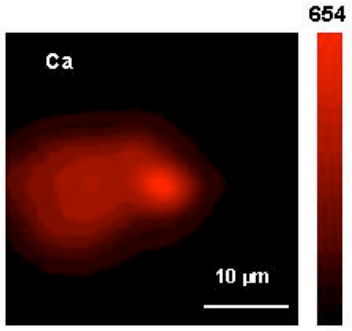


Figure 6.9c_2

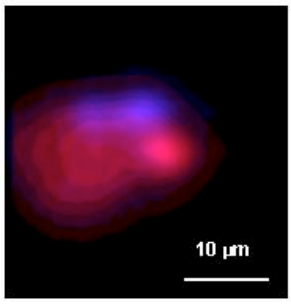


Figure 6.9c_5

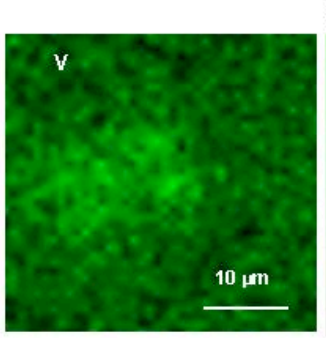


Figure 6.9c_3

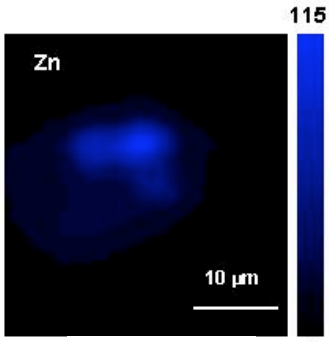


Figure 6.9c_4

Figure 6.9c_1-c_5. A microscopic photograph of a dried macrophage dried on PET film is shown in figure 6.9c_1. The elemental distribution of Ca, V and Zn within a single treated macrophage cultured in 0.04 g/L V solution for 24 h. are shown in figure 6.9c_2, c_3 and c_4, respectively. These images are matrices of 41 × 41 pixels of 1 µm resoltuion. In figure 6.9c_5, the images of Ca and Zn are overlapped.

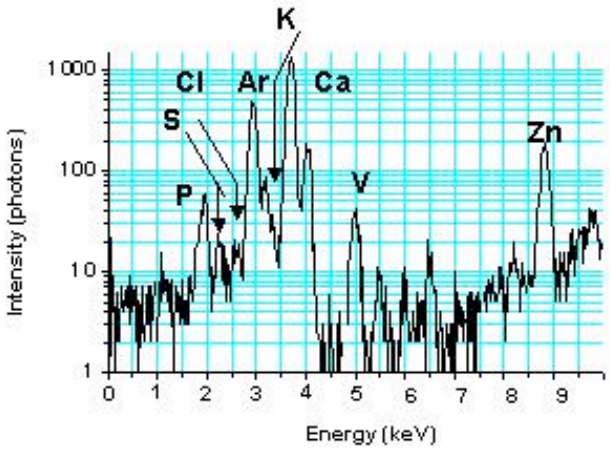


Figure 6.10. The spectrum was obtained from the cell shown in figure 6.9a with the highest density of Ca. The measurement time 200 sec and the incident x-ray energy was 14.3 keV.

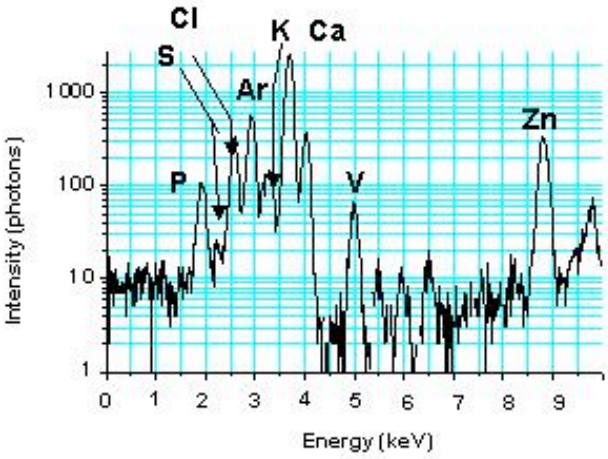


Figure 6.11. The spectrum was obtained from the cell shown in figure 6.9b with the highest density of Ca. The measurement time 200 sec and the incident x-ray energy was 14.9 keV.

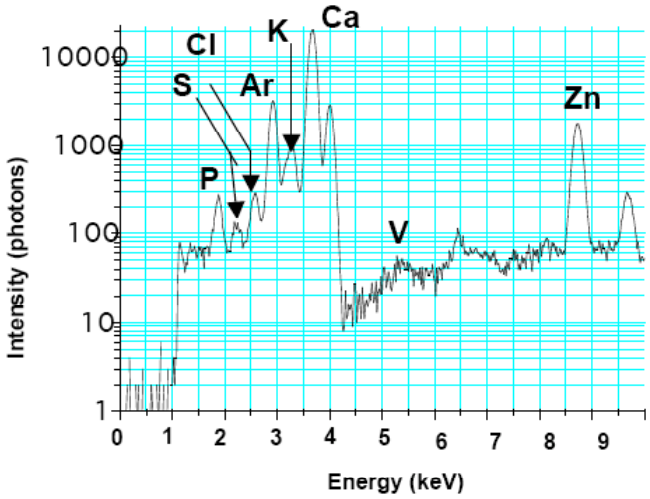


Figure 6.12. The spectrum was obtained from the cell shown in figure 6.9c with the highest density of Ca. The measurement time 200 sec and the incident x-ray energy was 14.3 keV.

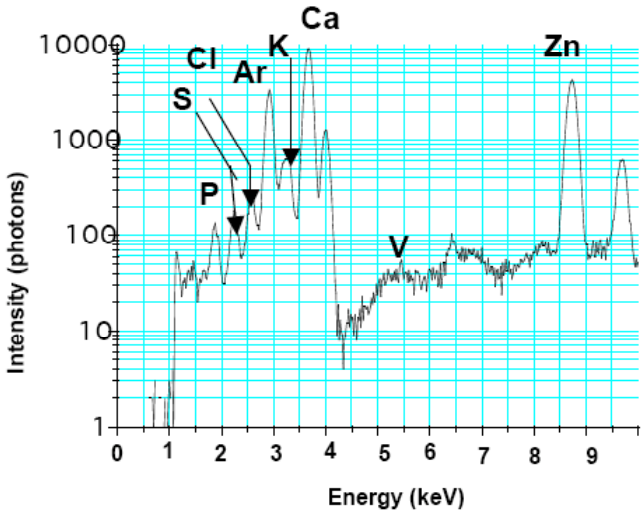


Figure 6.13. The spectrum was obtained from the cell shown in figure 6.9c with the highest density of Zn. The measurement time 200 sec and the incident x-ray energy was 14.3 keV.

6.3.2. Result of X-Ray Absorption Fine Structure Analysis

6.3.2.1. Culture in Cr Chloride Solution Environment

In order to compare the chemical state of Cr before and after internalization, x-ray absorption fine structure (XANES) spectrometry was employed. The spectra obtained in the fluorescence mode were obtained from the different cells shown in figure 6.14 and 6.15. The corresponding cells were cultured in Cr chloride solution environments (0.04 and 0.004 g/L) for 24 h. The K-edge XANES spectra of Cr within the cell under different conditions are shown in figure 6.16 together with the spectrum of Cr before internalization, as well as the reference spectrum, which is from Cr oxide (Cr_2O_3). The chemical structure around the element changes should be reflected in the x-ray absorption spectrum. It appears that the chemical states of Cr within the cells shown in figure 6.14 and 6.15 did not change because the three XANES spectra are virtually identical. While the distribution patterns and the densities of many intracellular elements changed significantly, it appears that the chemical structure around Cr atom within the cell does not change from the state before internalization.

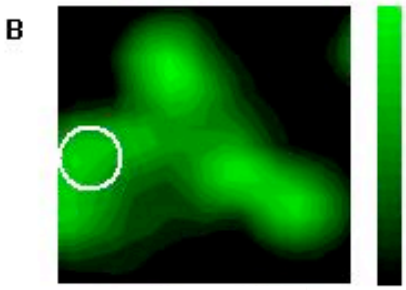


Figure 6-14

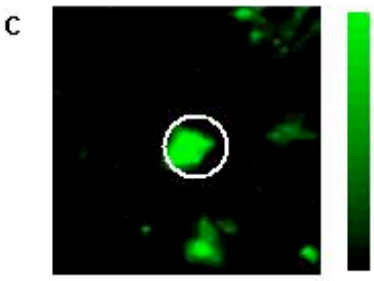
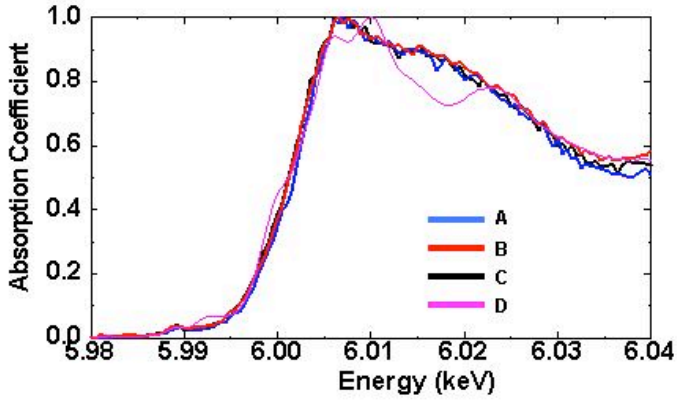


Figure 6-15

Figure 6.14 and 6.15. Cells cultured for 24 h. in $CrCl_3$ solution environment, 0.04 g/L for figure 6.14 and 0.004 g/L for figure 6.15.



A: Cr before uptake (0.04g/l)
B: Cr after uptake (0.04g/l) for 24 hours.
C: Cr after uptake (0.004g/l) for 24 hours.
D: Cr_2O_3

Figure 6.16. The XANES was obtained from the different cells shown in figure 6.14 and 6.15. These spectra were obatined in fluorescence mode. The K-edge XANES spectra of Cr before the uptake and the reference spectrum of Cr_2O_3 are also shown.

6.3.2.2. Culture in Fe Chloride Solution Environment

The XANES spectrum obtained from the cell cultured in an Fe solution environment (0.04 g/L) for 24 h. is shown in figure 6.17, together with the reference spectra from FeO and Fe_2O_3, and the spectrum of Fe before the internalization. Here, the absorption edge energy is defined at the point with half height to the maximum of the fluorescence yield. The change of the valence state induces shift of the absorption edge position. If the valence state changes from low to high, the absorption edge shifts to higher energy. Therefore, it is probable that the valence state of a portion of Fe internalized by the cell changes due to the chemical reaction within the cell. The pre-edge region of the spectrum shown in figure 4.33 is shown in figure 6.18. The

pre-edge structure of x-ray absorption spectrum reflects the arrangement of the atoms surrounding the Fe. The data suggest that the coordination geometry of the site occupied by the atom surrounding the Fe may have changed within 24 h. after the uptake of Fe into the cell leading to the transition of the valence state.

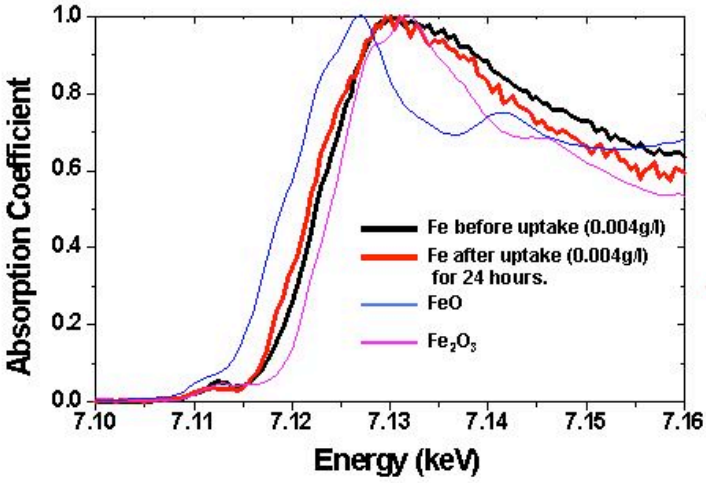


Figure 6-17

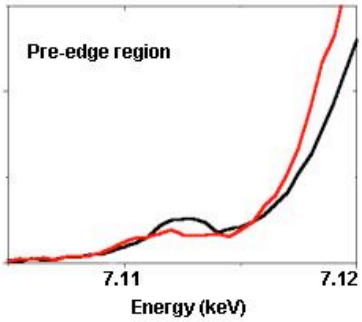


Figure 6-18

Figure 6.17 and 6.18. XANES spectrum obtained from the cell cultured in 0.04 g/L Fe solution environment for 24 h. is shown in figure 6.17. This spectrum was obatined in fluorescence mode. The reference spectra, which are FeO and Fe2O3, and the spectrum of Fe before the internalization are also shown. The pre-edge structure of the spectrum is shown in figure 6.18. It can be observed that there is a great difference between the two spectra in the pre-edge region.

6.3.2.3. Summary

The change of the chemical state of the metal up-taken by the cell is possibly induced by the defensive reactions of macrophages against foreign metal elements or by the offensive reaction of the up-taken metal elements against macrophages. Confirmation of this possibility requires further investigations into the information of the chemical state of foreign metal elements internalized within the cell. For now, one can only conclude that the chemical structure of the metal elements taken up by the cell is closely related to the cytoxicity and the cell's defense mechanisms.

6.3.3. Consideration About The Interactions Between Macrophages And Foreign Metal Elements

There are differences among the metal elements in the quantity internalized into macrophages. There are likely many factors in the interaction mechanisms between

macrophages and foreign metal elements. For example, a mutual simple collision, the charged surface of macrophages and the mediation of the chemical substances affect the contact between macrophages and metals. However, the reason why macrophages show different take-up activities for different metal elements may be due to the interaction between the cells and metal elements after contact.

Macrophages have a built-in strategy against foreign metal elements in order to defend themselves, the other cells and tissues. The uptake of foreign metal elements is induced after contact with them. Macrophages may have quantitative restrictions of the level of internalized foreign metal elements within their bodies. Therefore, the quantities of metals within the cells are different. Yet, the interference of the up-taking activity by foreign metals may be closely related to their mutual interaction. For some metallic elements, the take up is prevented in the early stage, and its level within the cell becomes a little due to the interference of the internalization. Why do the metal elements prevent macrophages from further activity after the internalization into the cells? Before considering this problem, it is necessary to consider the result of the excessive accumulation of Zn and Ca.

The density of Zn within the cell increased drastically when the rapid inflow of the foreign metal element was induced. The rapid inflow of Zn was observed in case of a V solution in spite of a little internalization. Why do macrophages need a lot of Zn within the cell when they are activated by foreign metal elements? It is probable that Zn plays an important role to promote the decomposition reaction of ATP (adenosine triphosphate). Macrophages need a lot of energy to realize the internalization and degradation of foreign particles and ions. ATP is produced in mitochondria, and a large amount of the energy used for various activities are generated by decomposing ATP. The generation of phosphoric acid is accompanied by decomposition process. By using EPMA imaging technique, it can be observed that the internalized Cr composed macromolecules including P. It is considered that the decomposition of ATP is required to generate phosphoric acid for composing macromolecules with metal elements. Therefore, a large amount of Zn may be required in order to support the decomposition of ATP in the process of the internalization and digestion of metal elements.

Normally, macrophages perform the activity of internalization, digestion and excretion of foreign bodies. In this process, the homeostasis of the elemental densities in the cells is kept within a certain range. However, in case of the rapid inflow of the specific metal element into the cell, a large amount of Zn may be induced to enter into the cell accompanied by the destruction of homeostasis of elemental densities. The excessive accumulation of Zn may induce the subsequent production of reactive oxygen

species (ROS). Free radicals are defined as atoms or molecules that contain one or more orbitals with a single unpaired electron. It is suggested that they tend to be highly reactive and capable of reacting with, and damaging, a variety of critical biologic molecules, including DNA, cytoskeletal proteins, and membrane lipids [1]. The mechanism of the increased free radical generation by Zn and the transport system for Zn is currently unknown, but it is suggested that Zn-induced production of ROS may be caused by the rapid entry of Zn into the cells [2-4].

It appears that mitochondrial dysfunction may be induced by the free radicals produced by the rapid entry and excessive accumulation of Zn within the cell. The subsequent reduction in ATP synthesis and rise in intracellular Ca concentration are accompanied by the impairment of mitochondrial functions. It is suggested that such a rise of the intracellular Ca concentration can result in activation of Ca^{2+}-dependent proteases, lipases, and endonucleases, with resultant damage to the cytoskeleton, cell membrane, and DNA, respectively [5]. Here, when macrophages were cultured in a V solution environment, the densities of Ca and Zn displayed the rapid increase. Macrophages need Ca within them in order to internalize and digest foreign bodies. However, mitochondrial dysfunction can not dispose with a rise of Ca concentration induced by the uptake of foreign metal element. Therefore, when the cell internalizes V, rapid entry of Zn into the cell and the subsequent mitochondrial dysfunction may be induced within the cell. Macrophages with mitochondrial dysfunctions can not avoid a rise of Ca density within the cell. If a rise of Ca density within the cell can not be relieved immediately, the further production of free radicals is induced and the subsequent mitochondrial dysfunction may be accelerated.

What is cytotoxicity? One can conclude that the strength of toxicity to macrophages equals the quantity of Zn entry per that of the internalized metal. If this value is very high, the subsequent process of the cell death may be induced rapidly in the early stage. However, the reason why each metal element has its own demand of Zn internalization for macrophages is still unknown. The chemical state of the metal element within the cell may be one of the important factors related to Zn internalization. It is necessary to do further investigations into this problem. The mechanism of the cell death after interactions with up-taken metal elements are considered here and shown in figure 6.19.

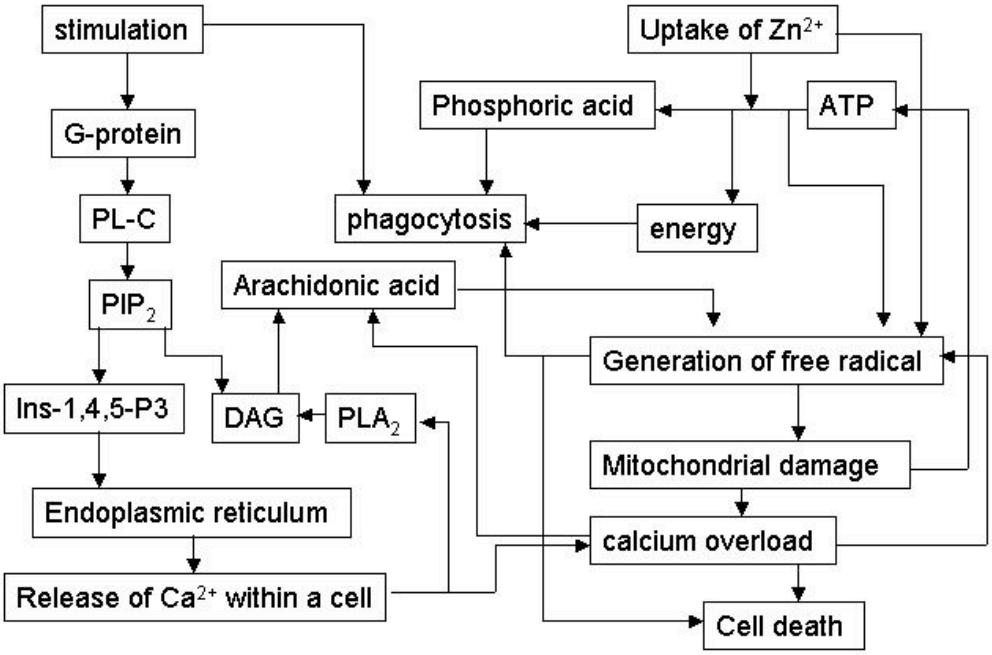


Figure 6.19. The schematic drawing of the defensive mechanism and process of cell death.

In this study, the elemental distribution patterns and densities were mainly obtained by SR-XRF. By using SR source, the trace elements, such as Zn and other metals, can be detected and visualized as the elemental images of the cell. The detailed elemental images visualizing the sub-cell structure can be obtained by employing EPMA imaging technique. However, not all of the trace elements included within the cell can be detected by employing this technique because of the relatively poor detection limit (> 0.1%). In order to obtain the trace and ultra-trace elemental distribution patterns of the sub-cell structure, a higher resolution for the SR microbeam is required. A resolution comparable to that obtaining in EPMA would enable better understanding of the roles of the ultra trace elements in single cells.

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