

Ca distribution in a human HeLa cell examined using XANES peak at the Ca-L absorption edge

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Introduction

XANES of biological specimens provides a unique and sensitive method for elemental imaging using resonance absorption peaks with the aid of soft X-ray microscopy. Ca has been one of major target elements because of its sharp and prominent XANES peaks at the L absorption edge[1] and biomedical significance of its distribution in tissues and cells. Several studies on the Ca distributions in calcified tissues have been conducted with scanning X-ray microscope at NSLS[1-4], but Ca mapping in a single mammalian cell has been scarcely reported.

In the present study, we applied contact soft X-ray microscopy to obtain Ca distribution in a human HeLa cell by calculating a ratio image between images at the peak and the bottom energy in XANES at the Ca-L absorption edge. The results suggested that Ca distributes predominantly in the nuclear region.

Materials and Methods

Contact X-ray microscopy using an electronic zooming tube was used to obtain X-ray images of human cancer HeLa cells treated with critical point drying at the Ca-L absorption edge at the resolution about 0.5 μm [5]. BL-11A beamline was used for this energy region.

XANES of CaCl_2 was measured to determine peak and bottom energies for taking X-ray images from 345 eV to 365 eV. CaCl_2 solution was placed on collodion thin film, which was followed by air-drying.

Results and Discussion

XANES of CaCl_2 at the Ca-L absorption edge was shown by dotted line in Fig. 1. Characteristic two peaks at 351.5 eV and 358.5 eV were observed (arrows "b", "d").

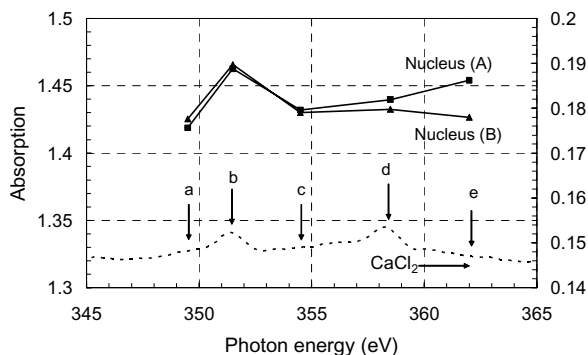


Fig. 1. XANES of nuclear micro-areas in a HeLa cell at the Ca-L edge. XANES of CaCl_2 is shown with dotted line.

To obtain Ca image, the ratio between an image at 351.5 eV denoted by arrow "b" and that at 349.5 eV denoted by arrow "a" was calculated as shown in the panel (b) of Fig. 2. The denser area has more Ca content. For identification of Ca distributed area soft X-ray image of cells at 349.5 eV was shown in Fig. 2 (a). Comparison of both images indicates that Ca seems to distribute around the nuclear region preferentially compared with cytoplasm.

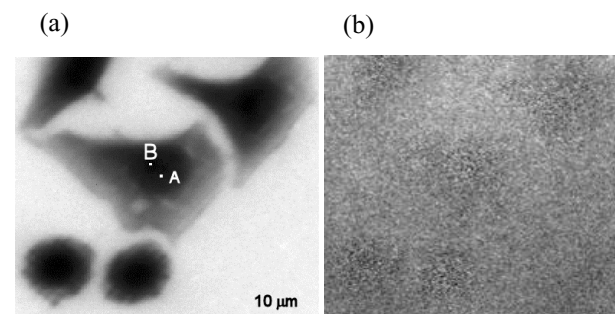


Fig. 2. Soft X-ray image of HeLa cells and Ca distribution. (a) X-ray image at 349.5 eV; (b) Ca image obtained by taking the ratio between images at 351.5 eV and 349.5 eV.

To confirm that the dense part is Ca rich region, absorption spectra of micro-areas with 0.645 μm square were measured, which were shown by solid lines in Fig. 1. The locations of two areas, "A" and "B" are from Fig. 2 (a). The spectra exhibited a peak at 351.5 eV, but showed slight (position "B") or no peak (position "A") at the energy of 358.5 eV indicated by arrow "d". Since Ca content in a cell is expected to be much smaller than that in calcified tissues, further repeated measurement is required to improve the accuracy of the intracellular localization of Ca.

References

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