XANES of intracellular local areas in a human HeLa cell at the phosphorus K absorption edge

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Introduction

XANES of biological specimens provides a unique method for molecular imaging using resonance absorption peaks assigned to a specific chemical bond with the aid of soft X-ray microscopy. DNA and its related compounds such as nucleotides have a sharp and intensive resonance peak at the phosphorus K absorption edge[1], suggesting the possibility of sensitive mapping of P-containing molecules. In the present study, we have obtained XANES of local regions with the area of 0.5 square microns in a human HeLa cell that were derived from a number of contact images around the P-K absorption edge. Furthermore, from these XANES spectra distribution of P-containing molecules was elucidated using a computer program that was developed for this purpose.

Materials and Methods

Contact X-ray microscopy using an electronic zooming tube was used to obtain X-ray images of human cancer HeLa cells that are subjected to critical point drying at the P-K absorption edge at the resolution about 0.5μm[2]. BL-11B beamline was used for this energy region. XANES of local areas in a HeLa cell were obtained from 13 X-ray images around the P-K edge including a XANES peak energy. From the ratio of absorption between at the peak energy and at the energy just below the peak for every pixel that constitute the images, distribution of P-containing molecules was obtained.

Results and Discussion

Figure 1 shows a group of HeLa cells taken at 2140eV. Small white areas of 0.5μm square were sites of XANES measurement. Absorption spectra for these areas were shown in Figure 2. XANES in the areas of 1, 2 and 3 exhibited the similar profile to that of DNA shown by the dotted line. In contrast spectra of area A and B, which locate outside the cells, exhibited rather broad structure. Figure 3 shows the result of DNA mapping of HeLa cells. This image indicates that DNA and P-containing molecules distribute throughout the cells with nearly uniform density. These cells appear to be densely packed, because optical microscopic observation showed dense and uniform morphology compared with other interphase cells. Further experiment is being planned in which spectra of nuclear and cytoplasmic region could be discriminated from the spectral profile.

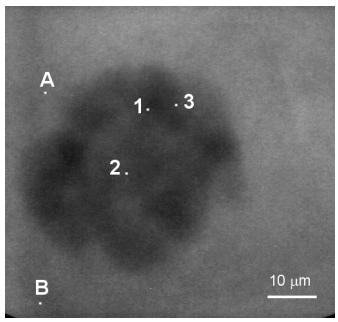

Fig. 1. Soft X-ray image of HeLa cells at 2140eV. Areas of 1, 2, 3, A and B with 0.5 square microns are the sites for XANES.

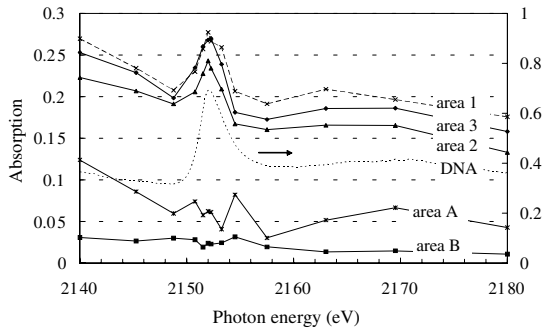

Fig. 2. XANES spectra of local areas in a HeLa cell at the P-K absorption edge. Dotted line shows a spectrum of calf thymus DNA.

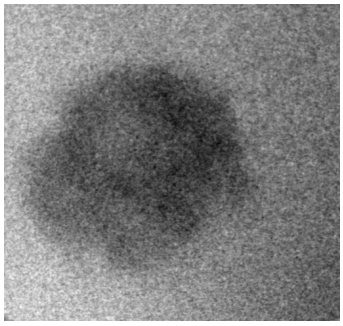

Fig. 3. Image of P-containing molecules in HeLa cells.

References

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