X-Ray Micro-imaging of Vanadium Uptake in *Dictyostelium discoideum* at BL12

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Abstract

Micro-distribution of vanadium in *Dictyostelium discoideum* was investigated with the use of soft X-ray microscope at BL12. Amoebae of *Dictyostelium* were stained with $VOSO_4$ solution after fixation. Since vanadium has a strong absorption L-edge in water window region, cells imaged at different wavelengths, 2.3 nm (below the L-edge) and 2.5 nm (above the L-edge) show high contrast in vanadium distribution in the cell. Using 0.2M $VOSO_4$, a nucleus and cytoskeltons were clearly seen by vanadium-enhanced contrast.

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1. Introduction

Soft X-ray microscope is a powerful tool to investigate whole, hydrated cells up to 10 μm thickness with much higher resolution than optical microscope. Because of its short wavelength, sub-optical spatial resolution can be obtained. Since cells are imaged in the wavelength region of X-ray transmissible “water window”, where organic material absorbs approximately one order of magnitude more strongly than water, and then chemical contrast enhancement agents are not required to view the cellular structures. However, for the understanding of physiologically important processes in living cells, special labeling is also useful. To visualize the label in TEM, colloidal gold conjugated antibodies and silver enhanced gold labeling are often used. The labeling techniques have also been applied to X-ray microscopy [1-2]. Among them, to visualize intracellular structure by the soft X-ray microscope, vanadium is particularly attractive as a contrast enhancement reagent because it has a strong absorption L-edge (2.43 nm [3]) in water windows. In order to use vanadium as the labeling agent, it is necessary to perform specific labeling operation using vanadium to cells.

In this report, we show the first results of vanadium-enhanced cell images by a soft X-ray transmission microscope at BL12, and we examine the staining condition of vanadium to be used as a probe to cells. Multi-wavelengths observations at 2.3nm (below the vanadium L-edge) and 2.5nm (above the vanadium L-edge) were performed in order to examine the labeling power of vanadium.

In the past report, mouse fibroblast cells incubated with vanadyl sulfate were imaged, using soft X-ray scanning microscope. Vanadium staining was clearly seen around and in the cell [4]. The study indicates that vanadium is useful for labeling with the use of X-ray microscope. However as vanadium is highly toxic to biological specimen, it easily kills important cellular functions, such as (Na^+-K^+)-ATPase activity [5-7]. Genotoxicity of vanadium compounds and the influence in yeast were also reported [8]. On the other hand, it is reported that vanadium stimulated DNA synthesis in mouse, similar to the effect of colchicine [9]. Although we have to be careful in using vanadium, we are also confident the use of vanadium gives powerful tool in the study of subcellular structure and function of the cell. Based on these understanding, at first we started examining specific labeling power of vanadium on fixed cells. *Dictyostelium* amoeba proliferates rapidly by binding fission during growth phase. After growth period, they aggregate to form multicellular structures and develop into fruiting bodies. Because its culture is easy and the patterning and differentiation in the development are simple, it is commonly used in the fields of biogenetics and cell biology. *Dictyostelium* cell was used as a sample cell and vanadyl sulfate was used as a staining solution agent.

2. Materials and Methods

2.1 Sample preparation

Cell culture and fixation. *Dictyostelium discoideum* Ax-2 was used. Amoebae, cultured in axenic medium (HL5), were collected into centrifuge tubes and washed with 0.01M phosphate buffer (Sörensen phosphate buffer, pH 7.4). After removing phosphate buffer as much as possible, 1% glutaraldehyde dissolved in 0.1 M phosphate buffer pH7.4 was added in the tubes as a fixative. The cell was broken easily. Then the cell suspension in the tube was mixed gently. The suspension was incubated at room temperature for 1 hour. After fixation, the cells were washed with 0.1M phosphate buffer.

Staining. The buffer was removed as much as possible. The fixed cells were stained with 0.2M, 0.5M, and 1M $VOSO_4$ dissolved in distilled water for 16h at room temperature, and washed 3 times with distilled water. The suspension was mixed gently and incubated at room temperature for 16 hours. After staining, the cells were washed with draft water.

2.2 X-ray microscope

All cell images shown here were observed by using a soft X-ray transmission microscope (BL12) [10-11]. Using an automated CZP positioning system, multi-wavelength observation is possible [12]. The cells were observed with two wavelengths; 2.3 nm (below the vanadium L-edge absorption line) and 2.5 nm (above the vanadium L-edge absorption line).

One drop of the cell suspension was placed on a copper mesh with a supporting film (plastic substrate 200-A, Ohken Shoji, Japan) and air-dried. Prior to X-ray observation, the cells were pre-observed by a light microscope. The first observation of the target cell was done at 2.3 nm and then at 2.5 nm at room temperature.

3. Results and discussion

Fig.1 shows a micrograph of an unstained *Dictyostelium* cell. Fig. 1 (a) is a light microscopic image and (b) and (c) are X-ray microscopic images. As shown in Fig. 1 (a), the cell size is approximately 9 μm in diameter. Heterogeneous intracellular structures were seen in the cell. In addition, at the center of the cell, we can observe a core which is probably ascribed to the nucleus (black arrow).

In Figs. 1 (b) and (c) X-ray dense areas were seen in the cell (white arrows). The contrast at 2.3 nm is higher than that at 2.5 nm. This is mainly due to X-ray absorption edge of oxygen (2.33 nm [3]). An occupied domain of these X-ray dense areas corresponds to that of the core seen in Fig. 1 (a).

Fig. 2 shows an image of *Dictyostelium* cell stained with 1 M vanadium. By comparing with X-ray micrographs in Fig. 1, Fig. 2 shows a striking increase in contrast of the cell. Since the contrast difference corresponds to the distribution of stained vanadium, this result reveals that vanadium is suitable to be used as a labeling probe. However, X-ray contrast level is so high that we can’t observe its intracellular structure.

Figs. 3 and 4 are images of *Dictyostelium* cell stained with 0.2 M vanadium. As shown in Fig. 3 (a), the cell size is approximately 9 μm in diameter, and at its center we can see a core, of which size is approximately 4.5 μm. In contrast, the cell size in Fig. 4 (a) is approximately 16 μm in diameter.

Figs. 3 and 4 (b) show X-ray micrographs taken at 2.3 nm and Figs. 3 and 4 (c) show X-ray micrographs taken at 2.5 nm. As shown in Fig. 3 (b), (c) and Fig. 4 (b), (c), it is obvious that vanadium labeling is effective to enhance the image contrast. The concentration of stain solution is so appropriate for the cells that intracellular structures are clearly revealed.

As shown in Figs. 3 (b) and (c), there is a high X-ray dense area of which size is 7.5 μm in diameter. Surrounding the area, filamentous structures were seen in the cytoplasm, which may be assigned to cytoskeletons

As shown in Fig. 4 (b) and (c), there are several X-ray dense parts in the cell. It is probable that they are identified as nuclei of a multinuclear cell. Surrounding them, cytoskeletons are also clearly seen. By comparing with images at 2.5nm, contrast due to cytoskeletons at 2.3 nm is enhanced, and we can observe fine structures in it (Fig. 5, white arrow). The size is about 0.2 μm (Fig. 5).

These results indicate that a nucleus and a cytoskeleton are kinds of vanadyl binding sites. It is then very encouraging for vanadium to be used as a labeling agent. However, in order for vanadium to bind at the specific site, we have to improve staining solution.

As seen in Fig.4, the cell size in Fig. 4(c) is smaller than that in Fig. 4(a) and 4(b). To get full images, we have to irradiate the cell repeatedly, which induces remarkable radiation damage and then induces shrinking of the cell. To avoid such radiation damage, cryo-fixation should be used. At BL12, a new cryo-fixation apparatus and cryo-stage will be mounted at the next beam time. Using them, cells will hopefully be observed without such radiation damages.

4. Conclusion

Using the soft X-ray microscope at BL12, we have successfully observed the effect of the contrast enhancement by vanadium labeling on *Dictyostelium discoideum*. Using 0.2M $VOSO_4$ staining solution, a nucleus and cytoplasm of the cell were visible. Especially, details of cytoskeleton were clearly seen. Further experiments are planned to allow labeling of living cells with improved vanadium-based stain solution and observing with a new cryo-fixation apparatus and the cryo-stage.

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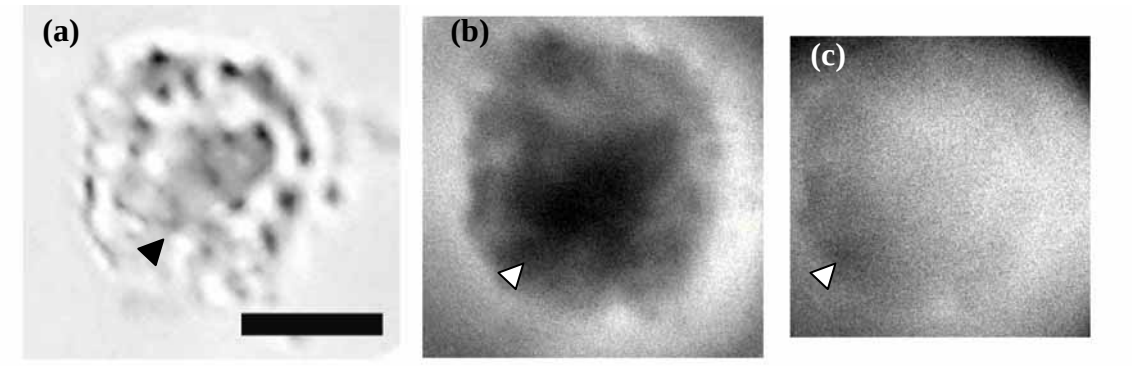

Fig. 1 Light microscopic and X-ray microscopic images of *Dictyostelium* cell (a) light microscopic image, (b) X-ray image at 2.3 nm and (c) at 2.5 nm. Exposure time was 60 s. Scale bar is 5 μm.

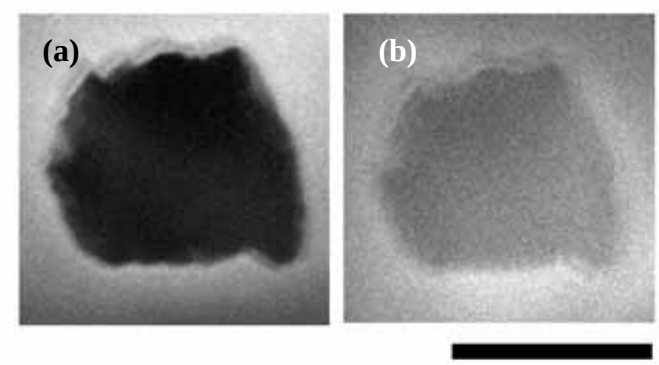

Fig. 2 X-ray microscopic images of *Dictyostelium* cell stained with 1M $VOSO_4$ solution at (a) 2.3 nm (a) and at (b) 2.5 nm. Exposure time was 60 s. Scale bar is 5 μm.

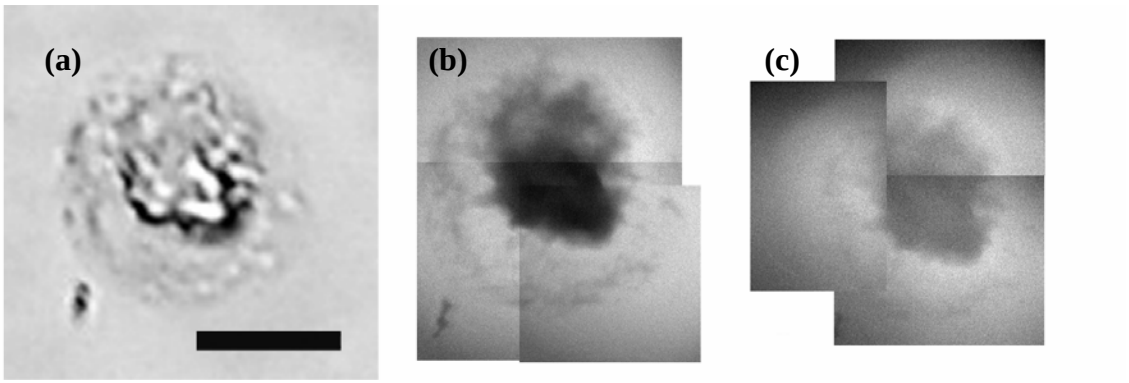

Fig. 3 Light and X-ray microscopic images of *Dictyostelium* cell stained with 0.2M $VOSO_4$ solution. (a) light microscopic image, (b) X-ray image at (b) 2.3 nm and (c) at 2.5 nm. Exposure time was 60 s. Scale bar is 5 μm.

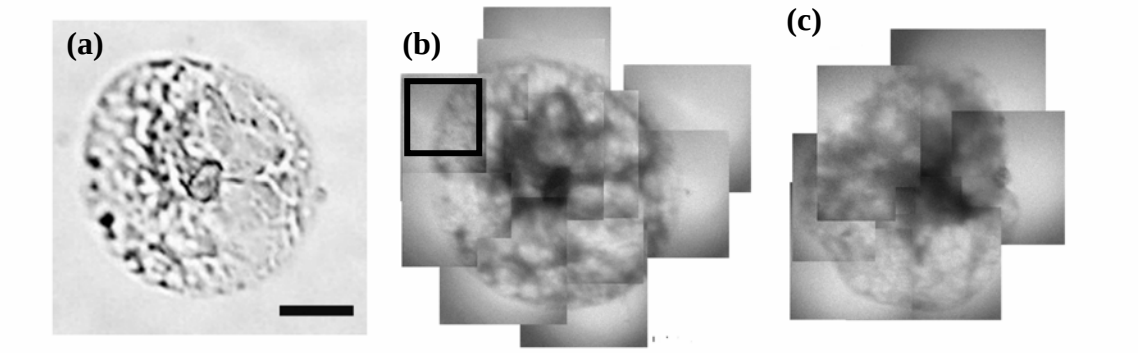

Fig. 4 Light and X-ray microscopic images of *Dictyostelium* cell stained with 0.2 M $VOSO_4$ solution. (a) light microscopic image, (b) X-ray image (b) at 2.3 nm and (c) at 2.5 nm. Exposure time was 60 s. Scale bar is 5 μm.

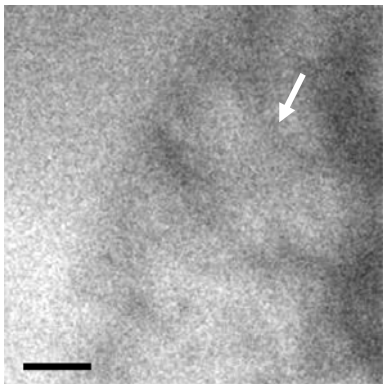

Fig. 5 A digitally magnified image of square in Fig. 4 (B). Exposure time was 60 s. Scale bar is 1 μm.

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