Imaging of Br-labeled fatty acids in mammalian cells by scanning X-ray fluorescence microscopy

Fatty acids are taken up by cells and incorporated into lipids such as neutral lipids and glycerophospholipids (GPLs). GPLs are major constituents of cellular membranes of the various organelles, e.g. Golgi, ER, nucleus and lipid droplets (Fig. 1). There are more than 1,000 molecular species of GPLs having different polar head groups and fatty acid compositions. Fatty acids are efficient energy sources by themselves or components of cellular membranes. Such versatile functions of fatty acids are essential for cell survival, growth, and movement, and are related to diseases. Fatty acids have been well analyzed by mass spectrometry. However, intracellular imaging of fatty acids and GPLs has not been successful using conventional methods. Labeling with large molecules as chromophore may interfere with metabolism as a result of steric hindrance. Isotope labeling of fatty acids in combination with positron emission tomography and matrix-assisted laser desorption ionization or desorption electrospray ionization imaging mass spectrometry are inadequate to determine the intracellular localization of fatty acids owing to their low resolution (>5 μm). To overcome this issue, we employed the single-element labeling of fatty acids combined with scanning X-ray fluorescence microscopy (SXFM, Fig. 2(b)) technology at SPring-8 **BL29XUL**, which enables the imaging of multiple intracellular elements at the sub-organelle level by a sub-100-nm X-ray beam focusing system [1-3].

We prepared Br-palmitic acid (PA) and Br-stearic acid (SA), which possess Br at position Δ12 (Fig. 2(a)). Cells were treated with Br-SA for 24 h and subjected to SXFM (Fig. 2(b)). The Br-SA SXFM spectrum of cells showed that the Br X-ray fluorescence lines with different electron shells (Br-Kα and Br-Kβ) increased in a manner dependent on the Br-SA concentration, whereas the X-ray fluorescence energies of the rest of the elements including zinc (Zn) were relatively stable [4]. While untreated control cells showed background Br signals owing to the use of a serum-containing culture medium, Br-SA-treated cells exhibited more than 10-fold higher Br contents, which enabled mapping images ready for SXFM. The concentration range of bromine according to the results of SXFM was 0.0013–10 fg/μm^2. Spectra of Br-Kα and Zn-Kα were mainly used for mapping images. Mapping data (500 nm/pixel) indicated that Br signals were mostly detected in the perinuclear region of the cytoplasm in both Br-fatty acid (FA)-treated cells (Fig. 2(c)). The Br signals tended to be clustered on one side of the nucleus (arrows). In contrast, Zn signals were observed in the entire cellular region but mainly were concentrated in the nucleus, whereas the signals did not exceed the background levels in ethanol (EtOH)-treated cells. We observed cells for 72 h after Br-FAs addition. The cell number increased logarithmically and Br signals remained in the perinuclear region *via* three passages. Analyses using inductively coupled plasma mass spectrometry combined with column separation suggested that Br-FAs were mainly metabolized into GPLs in cells. Further liquid chromatography mass spectrometry showed that Br-PA and Br-SA produced different species of Br-labeled phosphatidylcholines, one of the major components of GPLs [4]. These data suggested that Br-FAs were metabolized in

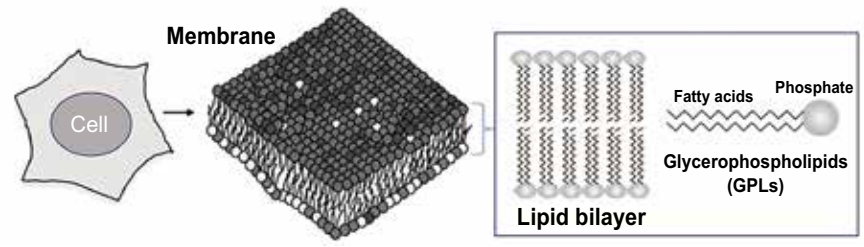

Fig. 1. Schematic structures of saturated glycerophospholipids (GPLs) in cells. GPLs are the most abundant lipid in the cellular membrane. Palmitic acid and stearic acid used in this study are the components of GPLs.

cells without alternation of cellular functions. Higher-resolution imaging (250 nm/pixel) revealed spotlike Br signals in the cytoplasm of cells, which were distinct from the Zn signals (arrows, Fig. 3). The size of the spotlike Br signals was <1 μm in diameter and the bromine signals were well co-localized with signals by staining of endoplasmic reticulum (ER) marker dye rather than mitochondrial one [4], which is consistent with previous findings that GPLs are produced by enzymes at ER.

In this study, we established a novel procedure for the imaging of intracellular FAs by single-element labeling combined with SXFM technology. The application of this new technology to unsaturated fatty acids will contribute to understanding of the dynamics of eicosanoids or other polyunsaturated fatty acid (PUFA)-derived lipid mediators in various fields of physiology and pathology, including inflammation, neurodegenerative diseases, and cardiovascular diseases.

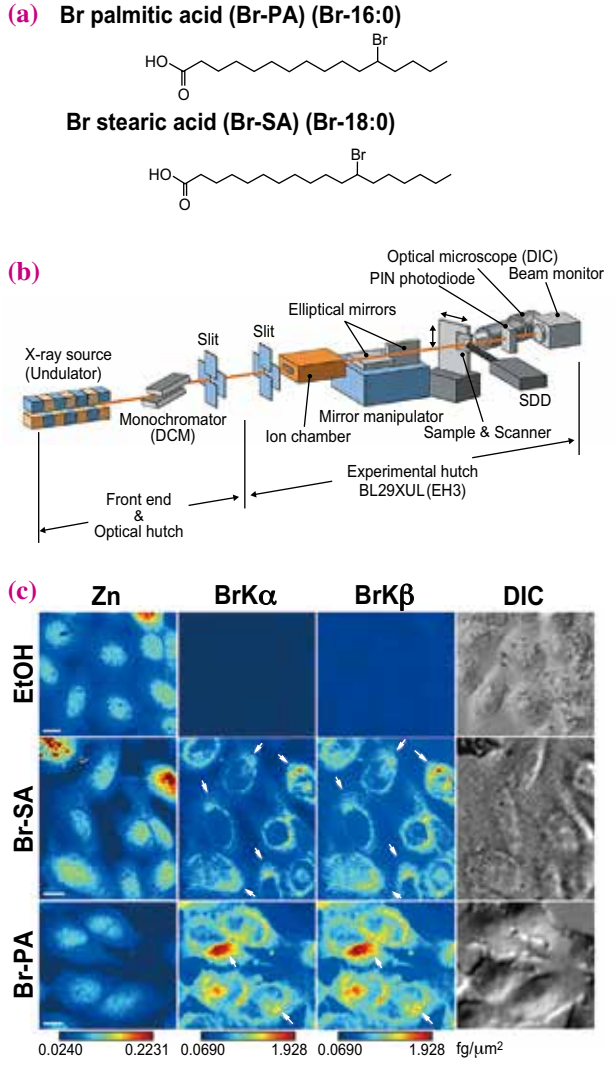

Fig. 2. X-ray fluorescence images of Br-labeled fatty acids. **(a, b)** Schematic views of the Br-labeled fatty acids and SXFM. **(c)** Zn- and Br-mapping images of Br-SA-, Br-PA-, and EtOH-treated CHO-K1 cells treated for 24 h. Arrows, clustered Br signals; BrKα and Brkβ, bromine X-ray emission lines; DIC, differential interference contrast image. A brighter color indicates higher signal intensity. Color bar, fg/μm^2; bar, 10 μm.

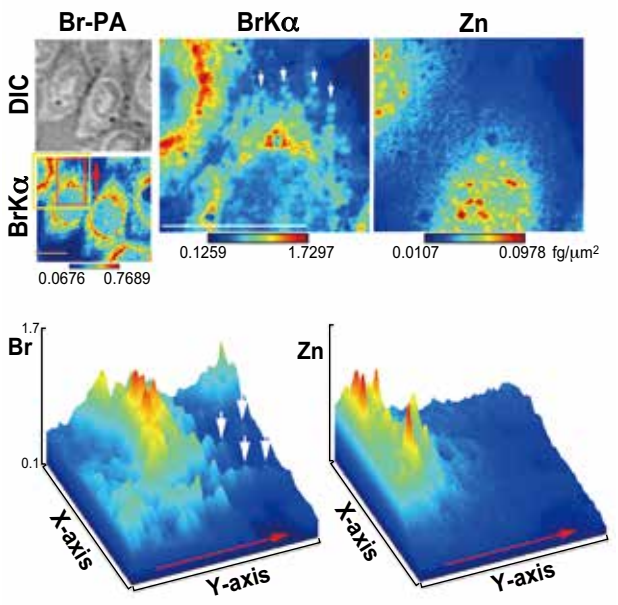

Fig. 3. Higher-resolution X-ray fluorescence images. Top, Br-PA. Bottom, surface plot generated on the basis of the red area in the top images. The yellow-framed area was measured at a higher resolution. Red arrows, direction presented in the surface plots; white arrows, spotlike Br distribution. A brighter color indicates higher signal intensity. Color bar, fg/μm^2; bar, 10 μm.

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