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Mass spectrometry imaging of intact cholesterol in a mouse esophagus tissue section and mouse zygotes using VUV laser desorption/ionization method

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ABSTRACT

A new instrument based on VUV laser desorption/ionization (VUVDI) method was used to measure the mass spectrum of the cholesterol standard and the mass spectrometry images (MSI) of intact cholesterol in a mouse esophagus tissue section and mouse zygotes. The VUVDI mass spectrum of the cholesterol standard shows dominant peaks from the parent molecules. In the case of the mouse esophagus tissue section, the ion images with a lateral resolution of ~ 4 μm reveal that the cholesterol populates mainly in the mucosae. The experimental results indicate that the VUVDI method may provide a new MSI tool to measure the intact cholesterol in tissues and cells.

1. Introduction

Cholesterol plays an important role in cell membranes and tissues [1–3]. Its spatial distribution controls many functions of the cells, for example, endocytosis, cell migration and cell signaling. The abnormal cholesterol distribution in human brain relates to Alzheimer disease [2,3]. Therefore, a proper method for measuring the cholesterol distribution is essential for these studies.

Mass spectrometry imaging (MSI) techniques can be used to measure spatial distribution of cholesterol in tissues and cells [4]. Matrix-assisted laser desorption ionization (MALDI) is a powerful tool among various MSI methods [4–8]. In 2005, Jackson et al. had measured the MALDI mass spectra of a rat cerebellum, which showed a dominant peak at *m/z* 369.3 $[M{-}OH]^+$ [7]. In 2009, Murphy et al. measured the cholesterol distribution in a mouse kidney section using MALDI-MSI method, showing nearly a uniform distribution of cholesterol [8]. However, the lateral resolution of the MALDI imaging method is usually worse than 10 μm due to the size of laser spot and the size of the matrix crystal, which hinders a full understanding of the functions of cholesterol in tissues and cells. Recently, a spatial resolution of 1.4 μm was achieved, however, such method has not been applied to detect cholesterol [9].

Another commonly used method in MSI is secondary ion mass spectrometry (SIMS). SIMS can detect protonated ions, parent ions (cationized with Na^+ or K^+) and fragment ions of intact lipids with lateral resolution in the lower end of micrometer when using cluster ion beams [10–21].

In 2004, two groups measured the cholesterol ion images of mouse brain tissue sections by using Au_3^+ primary ion beams [11,12]. The possible extensions of this method to other tissues and even cells are interesting because the cholesterol concentration in brain is relatively high and thus easy to measure. In 2008, Ewing et al. had measured the cholesterol ion images of macrophage cells by using In^+ primary ion beam, and they found that the lateral distribution of cholesterol with reasonable signal to noise ratios could only be measured via the cholesterol fragments, *m/z* 147 [15]. Cell imprints were used to detect cholesterol ion images of cells, for example, by transferring cells to silver foils that form favourable adduct ions, the silver surface was then analyzed by SIMS [10,20].

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However, change of molecular distribution is unavoidable in the transferring process [10,20].

The SIMS method has been used to measure the ion images of dehydrated cholesterol $[M+H-H_2O]^+$, however, they may also arise from the fragments of cholesteryl esters [21,22]. Therefore, a method that can obtain the parent ions of intact cholesterol rather than its dehydrated fragments would be useful.

The laser desorption/ionization (LDI) method is another tool to measure the mass spectrometry (MS) images, however, it often produces high level of molecular fragmentation [23–26]. Nevertheless, LDI method has the advantage of requiring no matrix. Recently, an extreme ultraviolet (EUV) laser (46.9 nm) has been used as a desorption/ionization source (EUVDI) and was applied to MSI with a lateral resolution of 0.1 μm [26]. Because of the large photon energy of the EUV laser (26.4 eV/photon), the level of fragmentation of EUVDI for alanine sample is similar to that of SIMS using primary Bi_3^+ ion beam [26].

Instead of using EVU laser, we reported a new MSI instrument using a VUV laser (125.3 nm or 9.9 eV) as a desorption/ionization (VUVDI) source with an ablation spot of $2 \times 5\ \mu m^2$ or equivalent to an ablation circle of ϕ 4 μm [27]. The levels of molecular fragmentations for several standard molecules were found to be lower than those of SIMS method using primary Bi_3^+ beam [27]. In this work, the VUVDI instrument was used to measure the MS images of a mouse esophagus tissue section and mouse zygotes. The measured MS images illustrate that the VUVDI method is capable of detecting intact cholesterol molecules in these two samples.

2. Experimental

2.1. Instrumentation

The VUVDI-MSI instrument has recently been described in detail [27]. Therefore, only a short description is provided here. A VUV laser at 125.3 nm was generated by four-wave mixing method using three collimated laser beams and a heated Hg cell. The temperature of the Hg cell was kept at ~500 K. The three fundamental laser beams were fixed at the wavelengths of 631.2, 404.4, and 255.0 nm with pulse energies of 6, 8, and 3 mJ/pulse, respectively. The repetition rate of the VUV laser was 20 Hz. The VUV laser was separated from the fundamental laser beams using a coaxial lens configuration. The ablated spot size is ~ϕ 4 μm. The intensity of the VUV laser was ~ 500 mJ/cm^2 at the focus spot. The ions ejected from the sample surface were extracted and identified by a linear time-of-flight (TOF) mass spectrometer. The ions were extracted by delayed high voltage pulses. A Z-stack microchannel plates (MCP) was used as the ion detector. The TOF mass spectrum of each laser pulse was recorded by a digital oscilloscope and transferred to a PC. The spatial resolution was achieved by scanning the sample with a 3-D linear piezo stages. The fly-back mode, i.e., scanning in one direction, was used to scan the samples. The positive ion peaks at m/z: 275, 303, 319 and 445 from Nile red mass spectrum were used for mass calibration [26]. The resolution of the mass spectra was ~100 at m/z 386, and the mass accuracy was about ± 1 Da at m/z 386. A reflectron time-of-flight mass spectrometry will be installed in the near future and the mass resolution and mass accuracy of the instrument would be improved.

2.2. Cholesterol standard preparation

The cholesterol standard was purchased from Sigma Aldrich (St. Louis, MO, USA). Its aqueous solution was prepared using distilled water as solvent (Milli-Q water purification system, Millipore), ~1 mg/mL, and the solutions were then pipetted into a droplet (~0.5 μL) on a cleaned quartz plate ($2 \times 35 \times 0.5$ mm^3), allowing the solvent to evaporate. The samples were immediately introduced into the vacuum chamber for analysis.

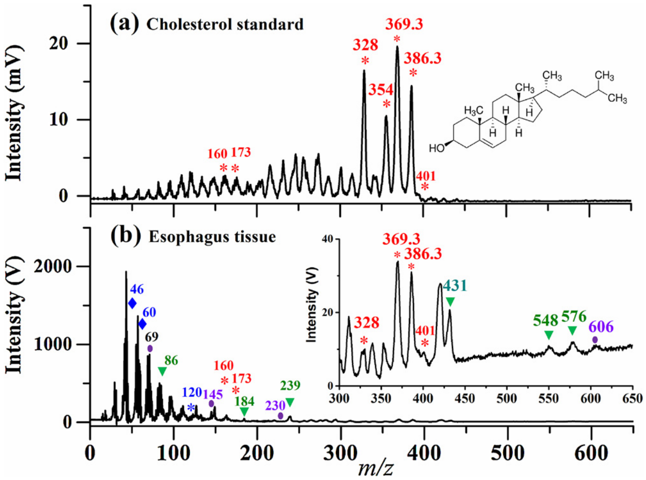

Fig. 1. (a) VUVDI mass spectrum of the cholesterol standard averaged by 16 laser pulses. (b) Total VUVDI mass spectrum of the mouse esophagus tissue section by summing all the measured mass spectra at each pixel of the mass spectrometry image. The peaks marked by *, ▼, ◆ and ● are tentatively assigned from fragments of cholesterol, lipids, amino acids and unassigned molecules, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

2.3. Mouse esophagus tissue section preparation

A fresh-frozen mouse esophagus tissue sample from adult male Wistar rats (Vital River Laboratory Animal Technology Co., Ltd, Beijing, China) was cut into sections of 12 μm thickness with a cryo-microtome (CM 1860 UV, Leica Microsystems, Wetzlar, Germany) at −24 °C and thaw-mounted onto a quartz plate. One adjacent tissue section was fixed in acetone and then were dyed with H&E for histological observation. The cryo-sections were stored at −80 °C until VUVDI-MSI analysis. Prior to VUVDI-MSI, tissue sections were dried in vacuum at −20 °C for 10 min and at room temperature for 30 min to avoid humidity condensation on the sample surface. Optical image of the tissue section was obtained before the VUVDI-MSI experiment with a custom-made microscope (Dayueweijia Technology Co., Ltd, Beijing, China).

2.4. Mouse zygotes preparation

The sample preparation method for the mouse zygotes was published elsewhere [28]. Briefly, the analyzed zygotes were collected from 3 to 4 weeks C57BL/J old females with 8 weeks C57BL/J old males (JAX, America) according to guidelines of Institutional Animal Care and Use Committee (IACUC) of Tsinghua University, Beijing, China. The harvested zygotes were first placed onto the surface of a cleaned quartz and then the distilled water was dropped on the zygotes to remove the unnecessary matrix. The water was removed immediately to avoid collapsing the cell membranes. The samples were introduced to the vacuum chamber for analysis after drying at room temperature.

3. Results and discussion

3.1. VUVDI mass spectra

Fig. 1a shows the measured VUVDI mass spectrum of the cholesterol standard. There are four dominant peaks at m/z 328, 354, 369 and 386 in the spectrum, in which m/z 386 was from the parent ion of cholesterol ($[M]^+$). The strong peak at m/z 386 is in contrast