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Imaging MALDI Mass Spectrometry of Sphingolipids Using an Oscillating Capillary Nebulizer Matrix Application System

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Abstract

Matrix deposition is a critical step in tissue imaging by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). It greatly affects the quality of MALDI imaging, especially for the analytes (such as lipids) that may easily dissolve in the solvent used for the matrix application. This chapter describes the use of an oscillating capillary nebulizer (OCN) to spray small droplets of matrix aerosol onto the sample surface for improved matrix homogeneity, reduced crystal size, and controlled solvent effects. This protocol allows visualization of many different lipid species and, of particular interest, sphingolipids in tissue slices of Tay-Sachs/Sandhoff disease by imaging MALDI-MS. The structures of these lipids were identified by analysis of tissue extracts using electrospray ionization in conjunction with tandem mass spectrometry (MS/MS and MS³). These results illustrate the usefulness of tissue imaging MALDI-MS with matrix deposition by OCN for the molecular analysis in normal physiology and pathology. In addition, the observation of numerous lipid subclasses with distinct localizations in the brain slices demonstrates that imaging MALDI-MS could be effectively used for “lipidomic” studies.

Keywords

Imaging MALDI mass spectrometry; sphingolipids; oscillating capillary nebulizer; matrix application

1. Introduction

Imaging matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is a powerful tool that may be used to determine the spatial distribution and relative abundance of specific molecules in biological samples such as histological slices of tissues (1-4). Matrix deposition is a critical step in tissue imaging by MALDI-MS. It greatly affects the quality of MALDI imaging in terms of mass resolution, detection sensitivity, spatial resolution, and reproducibility. The effectiveness of the matrix is determined by the size, density, and homogeneity of the clusters/crystals that form on the surface. The extent to which deposition of the matrix perturbs the localization of molecules in the sample, such as the lateral diffusion of the analytes (especially lipids that can dissolve in the solvent used for matrix deposition), is also a critical factor for the success of imaging MALDI-MS experiments.

The oscillating capillary nebulizer (OCN) (5-7) is a low-cost device and is capable of providing a uniform matrix coating for accurate mass analysis, good sensitivity, and high reproducibility (8-10). It can generate small droplets/aerosols with a narrow size distribution (6) by nebulizing the matrix solution at the capillary tip. It can also effectively handle liquid

compositions from 100% aqueous to 100% organic (9). By controlling several parameters of OCN operation, the solvent content of the droplet approaching the sample surface can be manipulated (9) to reduce the analyte migration and enhance the matrix–analyte interaction. This feature demonstrates the strong potential of the OCN to improve the quality of data for imaging MALDI-MS. Furthermore, the OCN works well for both micro-flows ($\mu\text{l}/\text{min}$) and macro-flows (ml/min) with high transport efficiencies (5, 11), which can greatly minimize the time for matrix coating. This makes the OCN matrix application system very suitable for automated, high-throughput, and high-quality matrix deposition in imaging mass spectrometry of biological molecules.

This chapter describes the use of an oscillating capillary nebulizer (OCN) to spray small droplets of matrix aerosol onto the sample surface for improved matrix homogeneity, reduced crystal size, and controlled solvent effects. This protocol allows the visualization of many different lipid species and, of particular interest, sphingolipids in tissue slices of Tay-Sachs/Sandhoff disease by imaging MALDI-MS (12). The structures of these lipids were identified by analysis of tissue extracts using electrospray ionization in conjunction with tandem mass spectrometry (MS/MS and MS^3). These results illustrate the usefulness of tissue imaging MALDI-MS with matrix deposition by OCN for the molecular analysis in normal physiology and pathology.

2. Materials

2.1. Chemicals

1. 2,5-Dihydroxybenzoic acid (DHB).
2. Trifluoroacetic acid (TFA).
3. Sulfatides (Porcine Brain) (Avanti Polar Lipids, Inc., Alabaster, AL, USA).
4. Total ganglioside mixtures (Porcine Brain) (Avanti Polar Lipids, Inc., Alabaster, AL, USA).
5. Monosialogangliosides GM1, GM2, and GM3 (as NH_4^+ salts) (Matreya LLC, Pleasant Gap, PA, USA).
6. *Angiotensin* III. (Ang III) (Sigma Chemicals, St. Louis, MO, USA).
7. Fibrinopeptide B (*GluFib*) (Sigma Chemicals, St. Louis, MO, USA).
8. Adrenocorticotrophic hormone (ACTH) 1–24 (Sigma Chemicals, St. Louis, MO, USA).
9. Hematoxylin–Eosin (H&E) Staining Solution (VWR, West Chester, PA, USA).
10. HPLC grade acetonitrile (ACN).
11. HPLC grade methanol (MeOH).
12. Diethylaminoethyl cellulose (DEAE)-Sephadex A-25 (Pharmacia LKB Biotechnology, Uppsala, Sweden).
13. Nanopure water (18 M Ω).

2.2. Experimental Animals

The $\text{hexb}^{+/-}$ and $\text{hexb}^{-/-}$ mice (strain: B6; 129S-Hexbtm1Rlp, Jackson Laboratory, Bar Harbor, ME, USA) were obtained by crossing homozygous males with heterozygous females or by heterozygous mating (13). The hexb genotype was determined by PCR on mouse-tail DNA with primers B3 (5'-ATGTGGATGCAACTAACC-3') and B4 (5'-

AGGTTGTGCAGCTATTCC-3') that flank the disrupting MC1NeopolyA cassette in exon 13. The $hexb^{-/-}$ and $hexb^{+/-}$ male mice were sacrificed and dissected at 18.5 and 23 weeks, respectively. The whole procedure was conducted using protocols approved under license by the UK Home Office (Animals Scientific Procedures Act, 1986).

2.3. Experimental Instruments

1. Cryomicrotome (Cryo-Star HM560, MICROM, Walldorf, Germany).
2. Leica autostainer XL (Leica Microsystems, Bannockburn, IL, USA).
3. Nikon Eclipse E600 microscope (Nikon, Melville, NY, USA).
4. Stainless steel-polished blank MALDI sample plate (Applied Biosystems, Foster City, CA, USA).
5. Syringe pump (KD Scientific, Holliston, MA, USA).
6. 25 ml TLC reagent sprayer with standard ground glass joint (Kimble/Kontes, Vineland, NJ, USA).
7. Scanning electron microscope (SEM) (Nova Nanolab 200 system, FEI company, Hillsboro, OR, USA).
8. Voyager DE-STR MALDI-TOF mass spectrometer with a 337 nm N₂ laser (3 Hz) (Applied Biosystems, Foster City, CA, USA).
9. API 4000 QTrap tandem mass spectrometer (Applied Biosystems, Foster City, CA, USA).

2.4. Oscillating Capillary Nebulizer Setup

A diagram of the design and operation of the oscillating capillary nebulizer (OCN) matrix application system is shown in Fig. 7.1. The OCN sprayer (5, 7) consists of two coaxial fused silica capillary tubes (Polymicro Technologies, LLC, Phoenix, AZ, USA) that are friction-fit mounted with PEEK Sleeves (Upchurch Scientific, Oak Harbor, WA, USA) housed in a 1/16" stainless steel union tee (Swagelok, Solon, OH, USA). The inner capillary (i.d. 50 μ m, o.d. 150 μ m, length 80 mm) was used to transfer the matrix solution (signified by blue in Fig. 7.1) and the outer capillary (i.d. 250 μ m, o.d. 350 μ m, length 30 mm) allowed gas (signified by pink in Fig. 7.1) to pass through the annular space between the outer wall of inner capillary and the inner wall of the outer capillary to generate the oscillation of the inner capillary tip, which extends about 1 mm (R) from the outer capillary tip. The high-frequency oscillation induces the nebulization of the matrix solution and generates a fine and uniformly dispersed spray of matrix droplets/particles onto sample plates fixed on a xyz translation stage (Newport, Irvine, CA, USA) (see Note 1).

3. Methods

3.1. Preparation of Tissue Samples

1. The tissue samples were snap-frozen after dissection in liquid nitrogen and stored at -80°C .
2. Before tissue section, the tissues were first put into a sealed box with dry ice to equilibrate at respective temperature for 60 min.
3. The tissues were then transferred into cryomicrotome at -20°C for another 60 min.

¹An important feature of the OCN system is the ability to minimize the amount of solvent that comes into contact with the tissue. This serves to reduce analyte migration and matrix crystal size to minimize the loss of molecular spatial information.

4. Attach the tissues to the sample holder with small amount of optimal cutting temperature (OCT) compounds (Cryo-OCT Compound, Pittsburgh, PA, USA) at the back of tissue. No OCT compounds were used on the tissue area to be cut (*see* Note 2).
5. The tissue samples were sectioned as 10 μm slices at -18°C and thaw-mounted onto chilled MALDI plates ($\sim 5^{\circ}\text{C}$). The sample plates were sealed in Petri dishes and stored at -80°C .
6. Neighboring sections were also cut into 10 μm thickness under the same conditions and attached onto glass slides for histological staining.

3.2. Matrix Deposition

1. The tissue slices on the MALDI plates were taken out from the -80°C and brought into a desiccator at room temperature for 60 min before matrix coating.
2. Standard solutions were pipetted on different spots around the tissue slices for mass spectrometer calibration (*see* Note 3). For positive mode mass spectrometry operation, 1 μl of standard solution containing 5.0 pmol/ μl Leu-enkephalin, 1.0 pmol/ μl angiotensin II, 1.0 pmol/ μl angiotensin I, 1.0 pmol/ μl Glu-fibrinopeptide B, and 2.0 pmol/ μl ACTH (18–39 clip) in acetonitrile:water (50:50, v:v) was deposited on each spot. For negative mode mass spectrometry operation, 1 μl of standard solution containing 1.0 pmol/ μl sulfatides and 1.0 $\mu\text{g}/\mu\text{l}$ total gangliosides in methanol solution was deposited on each spot. The mass spectrometer was calibrated using the m/z values of the standard molecules.
3. The sample plate was then fixed on a xyz stage under OCN for matrix application.
4. The concentration of matrix solution, flow rate of matrix solution, nebulizing gas pressure, the length of inner capillary tip extending from the outer capillary tip, OCN–sample distance, and the moving speeds of the xy stages can be adjusted to control the size of matrix droplet and the surface wetting on the sample plate for optimized matrix coating (*see* Note 4).
5. The characteristics of the matrix crystals were observed using a scanning electron microscope (SEM) (*see* Note 5). The example results of matrix crystal formation using different matrix application techniques were shown in Fig. 7.2 (*see* Note 6).

²OCT compounds are not compatible with imaging MALDI-MS. Even small amounts of these compounds residing on a cutting blade may cause poor results. Precautions should be made to avoid the contamination of OCT compounds on studied specimens.

³The diffusion of the standard solution on sample plate should be tested first to determine the positions of standard spots to avoid the spreading of standard solution onto the tissue slices.

⁴It was determined that 30 mg/ml of DHB in acetonitrile:water (50:50, v:v, with 0.1% TFA) can form evenly distributed small (0.5–20 μm mean diameter) matrix crystals on the sample surface. Several different combinations of matrix solution flow rate, nebulizing gas pressure, and OCN–sample distance were evaluated for matrix deposition and have been reported (12). Briefly, the matrix molecules formed irregular clusters without obvious crystal boundaries when using a high flow rate with a low gas pressure and short sprayer–sample distance. This was anticipated because these conditions would likely produce big droplets with a high solvent content (9). A reduction of the matrix solution flow rate and an increase of gas pressure and sprayer–sample distance resulted in the matrix crystals becoming flat and rectangular in shape and having a size of several micrometers. This presumably occurs because the droplets are smaller and the solvent has more time to evaporate and begin to form DHB crystals in flight. At an even slower flow rate and higher gas pressure and sprayer–sample distance, the smallest needle-like matrix crystals were observed. A longer sprayer–sample distance ($> 8\text{ cm}$) was recommended to produce dryer DHB particles, which can minimize the analyte migration induced by the solvent and yield much greater signal response (15).

⁵The accelerator voltage of the SEM system was typically 5 kV. No carbon or gold was further coated to the matrix surface prior to the SEM analysis.

⁶The formation of matrix crystals on the sample surface can greatly affect the results of imaging MALDI-MS. In Fig. 7.2, it is clearly shown that the size and surface distribution of DHB crystals are dramatically different when using various matrix deposition protocols. SEM characterization of matrix crystals can be used to assist the optimization of matrix coating and improve the quality of the MALDI images.

6. The typical distance (L) between the OCN and the sample on the xyz translation stage is 8–20 cm depending on the flow rate of the matrix solution and gas pressure.
7. The matrix solution (30 mg/ml DHB in acetonitrile:water 50:50, v:v, with 0.1% TFA) was injected into the inner capillary of OCN through a 1 ml gastight syringe using a syringe pump with a flow rate $\sim 60 \mu\text{l}/\text{min}$.
8. The pressure of nebulizing gas (N_2) was adjusted to ~ 50 psi to generate a fine matrix aerosol which covers the selected sample area.
9. The sample plate was continually moved across the aerosol deposition area in the x direction (5 mm/s) and y direction (5 mm/s) to obtain an even matrix distribution throughout the sample surface.
10. The typical time of optimized OCN matrix coating for a 4 cm^2 sample is about 5 min with an estimated thickness of 10–20 μm (see Note 7).
11. After the matrix deposition is finished, the sample plate was further dried in a desiccator at room temperature for 10 min before being transferred into the imaging mass spectrometer.
12. The OCN system was flushed with nanopure water at a flow rate of $100 \mu\text{l}/\text{min}$ for 10–20 min to wash off any remaining residue (see Note 8).

3.3. MALDI Imaging Mass Spectrometry

1. The matrix-coated sample plate was loaded into the Voyager DE-STR MALDI-TOF mass spectrometer. The real-time video image of the tissue slice can be viewed via the video monitor. The tissue section boundaries were determined by their logical x and y coordinates on the MALDI plate.
2. Standard spots were used to optimize the instrument parameters for maximum sensitivity, resolution, and mass accuracy. The TOF mass spectrometer was operated in reflector mode with delayed extraction. The accelerating voltage, grid voltage, and delay time are typically 22 kV, 70%, and 400 ns, respectively. The laser intensity was checked daily to obtain the best signal-to-noise ratio. In negative mode, the laser intensity is usually a little bit higher (5–8%) than the positive mode. The acquisition mode was “manual” and the number of laser shots at each position was 10 for all acquisition methods (see Note 9).
3. Obtain an MALDI mass spectrum of the standard spot using the optimized acquisition method.
4. Load the *MALDI-MS Imaging Tool* software (free version at <http://www.maildi-msi.org>). Choose a calibrated mass spectrum of standard compounds as the “Data File” and select the optimized acquisition method as “Control File”.

⁷It was observed that a matrix thickness between 5 and 50 μm was sufficient for reasonable signal-to-noise ratio (s/n) of lipid ions in mouse brain tissue and other samples.

⁸To avoid a clogging problem with the OCN, it is suggested to pump the matrix solution for 2–3 min before turning on the nebulizing gas. If the flow of matrix solution needs to be stopped because of changing the syringe, adding more matrix solution, or switching to nanopure water when the matrix application is finished, turn off the nebulizing gas first and keep pumping the matrix solution for another 2–3 min after there is no gas flow through the OCN.

⁹The mass spectra and resulting ion images can be obtained with only 8–10 laser shots per spot in either the positive or the negative ionization mode. This suggests that the OCN matrix deposition system is able to generate a good matrix–analyte interaction, which promotes efficient laser desorption and subsequent ionization of lipids. Since the OCN system can yield mass spectra with high s/n ions via fewer laser shots, the data acquisition time was also greatly reduced, which is a considerable benefit for time-consuming imaging mass spectrometry experiments.

5. Define imaging area by setting the x and y coordinates of the tissue boundaries.
6. Set the step size of the laser rastering, which determines the pixel number of the MALDI image. Typically, a step size of 60 μm is used for brain tissue within the size range of 15–30 mm^2 (see Note 10).
7. Start the data acquisition and the *MALDI-MS Imaging Tool* creates an .img file.

3.4. Imaging Data Analysis

1. MALDI mass spectra of every individual pixel can be acquired by the *MALDI-MS Imaging Tool*. Using the “calculate image” function, an image of a selected m/z value can be visualized. The mass spectrum of a pixel can be displayed by *Data Explorer* (factory-equipped software on Voyager DE-STR MALDI-TOF mass spectrometer) when the point of interest is selected. The example results were shown in Fig. 7.3 (see Note 11).
2. MALDI images can be visualized using *Biomap* software package (free 3.7.4 version at <http://www.maldi-msi.org>) by loading the .img file (imaging MALDI-MS data).
3. MALDI images of all the m/z values can be viewed by the movie function (File/Export/Movie/). The MALDI images of selected m/z values were obtained by choosing the corresponding values of the data points (the N number) on the left panel.
4. The contrast of an ion image can be adjusted by changing the values of the slide bars on the left panel and the colors can be defined by clicking the “Set color table” button.
5. The display mode of the ion images was set to the default “interpolated” mode.
6. The ion images were saved using the export function of *Biomap* (File/Export/Image). The example ion images of sphingolipids in mouse brain of Tay-Sachs and Sandhoff disease model are shown in Fig. 7.4 (see Note 12).
7. The summed MALDI mass spectrum of the whole sample can be obtained by the plot function (Analysis/Plot/Global/Scan).

¹⁰The step size should be carefully selected. Although decreasing the step size may provide more details about the sample, it also causes longer data acquisition time, larger data files, and the possibility of matrix subliming off the sample in ultra-high vacuum (UHV) chamber, especially for samples prepared using dryer matrix coating conditions.

¹¹MALDI-MS spectra acquired from the $\text{hexb}^{-/-}$ mouse brain slices prepared by OCN matrix coating system showed several prominent ions of m/z 888.6, 1,132 and 1,383 (Fig. 7.3) localized in different regions of the brain.

¹²Sphingolipids (sulfatide, ganglioside GM2, and asialo-GM2 (GA2)) were distinctly visible in $\text{hexb}^{-/-}$ mouse brain samples by using OCN for matrix application. These ion images clearly demonstrate that the OCN system is useful for sample preparation for imaging MALDI-MS of lipids. The spatial distribution of sulfide subspecies ST d18:1/C22:0 (m/z 862.6), ST(OH) d18:1/h22:0 (m/z 878.6), ST d18:1/C24:1 (m/z 888.6), ST d18:1/C24:0 (m/z 890.6), ST(OH) d18:1/h24:0 (m/z 906.6), ST(OH) d18:0/h24:0 (m/z 908.6), and an unknown ion (m/z 868.6) displayed a remarkably similar pattern to the myelinated fiber (white matter) region of the cerebellum and a relatively even distribution in brain stem (c.f., H&E staining) (Fig. 7.4a–g). The localizations of potassiated ganglioside GA2 (d18:1/C18:0) (m/z 1,132), potassiated ganglioside GA2 (d20:1/C18:0) (m/z 1,160), ganglioside GM2 (d18:1/C18:0) (m/z 1,383), and ganglioside GM2 (d20:1/C18:0) (m/z 1,411), which are also known to accumulate in mice with this genetic defect (16), closely matched the granular cell region in cerebellum and produced no detectable ions in the brain stem region (Fig. 7.4 h–k). The imaging MALDI-MS results in Fig. 7.4 illustrate that various subcategories of sphingolipids are localized to specific regions of the brain. Therefore, this technology is a valuable complement to other types of “lipidomic” analysis, which uses homogenized extracts of the entire tissue which may miss potentially important regional changes in both the types and the amounts of the lipids present.

3.5. ESI Mass Spectrometry

1. Brain tissues were homogenized (10 mg/ml) in water on ice. The lipids were extracted and the acidic glycolipids recovered by batch elution from a DEAE column (14).
2. The extracts were dissolved in 1.0 ml of MeOH and introduced via syringe infusion (0.6 ml/h) into an API 4000 QTrap tandem mass spectrometer.
3. Precursor ion scans for m/z 96.9 in negative ion mode were used to determine the potential *N*-acyl chain length of sulfatide subspecies in each sample. The declustering potential (DP) was set to -220 eV and the collision energies were ranged from -100 to 120 eV. Precursor ion scans for m/z 290.1 in negative mode were used to identify the potential *N*-acyl chain length subspecies within each family of acidic gangliosides (i.e., GM1, GD1, GT1). These scans were performed with declustering potential of -70 to 100 eV (lower DP was required to reduce in-source fragmentation for species having multiple sialic acid residues). Collision energies ranged from -55 to 75 eV with lower collision energies used for species having increasing numbers of sialic acid residues because of the lability of these molecules toward fragmentation.
4. Ionization conditions were optimized for individual sulfatide or ganglioside subspecies and enhanced product ion (EPI) scans were selected to provide a greater diversity of product ions. EPI scans were performed with Q0 trapping set to "on," a linear ion trap fill time of 100 ms, and a scan rate of 1,000 amu/s. Example of ESI-MS/MS analysis of GM2 subspecies is shown in Fig. 7.5a (see Note 13).
5. The MS³ analysis is performed with the first mass analyzer (Q1) setting to "open" to pass a wide m/z window around the precursor ion of interest. This is transmitted to Q2 where it collides with a neutral gas and dissociates to various fragment ions. The linear ion trap (LIT) is then set to trap and hold a 2 m/z unit window centered on the product ion of interest. The selected m/z ions were fragmented further to secondary product ions, which are then scanned out of the LIT. The sphingoid base and fatty acid composition of each ganglioside can be successfully identified by MS³ analysis. An example of ESI-MS³ analysis of GM2 subspecies (1,410.9/592.60 transition) is shown in Fig. 7.5b (see Note 14).
6. Neutral glycosphingolipids were analyzed in positive ion mode as both (M+H)⁺ and (M+Na)⁺ species. Neutral glycosphingolipids fragment primarily via cleavage of carbohydrate groups. Potential subspecies were identified via neutral loss scans for hexose and *N*-acetylhexosamine (162 and 203 units, respectively). The parameters of EPI and MS³ scans for neutral glycosphingolipids were kept the same as those for acidic gangliosides.

¹³ESI-MS/MS analysis of the lipid extracts from the mouse brains was performed to confirm the structure of sulfatide, GM2, and GA2. For example, in negative ion mode, MS/MS of m/z 1,410.9 generates five major fragment ions corresponding to losses of different sugar moieties in the head group (Fig. 7.5a). The product ions of m/z 1,119.8, 916.8, 754.8, and 592.6 correspond to the Y-type glycosidic bond cleavage involving loss of NeuAc, NeuAc/GalNac, NeuAc/GalNac/Gal, and NeuAc/GalNac/Gal/Glc, respectively. The m/z 290.1 ions were produced by C-type cleavage and charge retention on the sialic acid with subsequent dehydration, which confirms the existence of a sialic acid moiety.

¹⁴An MS³ experiment was performed on the Y₀ fragment ion of m/z 592.6 to determine the ceramide backbone of the m/z 1,410.9 ion. The resulting MS³ spectra (Fig. 7.5b) showed secondary fragment ions of m/z 324, 308, 282, and 283, corresponding to S, T, U, and V + 16 fragments, respectively (17), revealing that the amide-linked fatty acid is stearate (C18:0). The ions of m/z 265 and 291 correspond to complementary P and Q fragments, respectively (17), showing that the sphingoid base backbone is d20:1. Thus, this major species in hexb^{-/-} mouse brain is ganglioside GM2 (d20:1/C18:0).

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Fig. 7.1. Schematic of the oscillating capillary nebulizer (OCN) matrix application system with matrix solution and nebulizing gas.

Fig. 7.2.

SEM images of DHB crystals formed from matrix solution (30 mg/ml DHB in acetonitrile:water 50:50, v:v, with 0.1% TFA) using different matrix coating methods. **(a)** Direct drying of 1 μ l matrix solution. **(b)** Matrix deposited using TLC sprayer (nitrogen pressure: 5 psi; sprayer-sample distance: 12 cm; spraying time: 10 s/cycle; drying time: 30 s/cycle; coating cycle: 30 times). **(c)** Matrix deposited using OCN sprayer (matrix solution flow rate: 60 μ l/min; nitrogen pressure: 50 psi; sprayer-sample distance: 10 cm; coating time: 5 min).

Fig. 7.3.

Imaging MALDI-MS data from *hexb*^{-/-} mouse brain (cerebellum and brain stem, 4.954 mm × 5.358 mm). The fine structures of cerebellum in the H&E-stained images are labeled as (1) molecular layer, (2) myelinated fiber (white matter), and (3) granular layer. The MALDI spectra present the ion yield from specific spots in (a) myelinated fiber (white matter) in negative ion mode, (b) granular layer region in negative ion mode, and (c) granular layer region in positive ion mode, respectively. The molecular distributions of m/z 888.6 ions, m/z 1,383 ions, and m/z 1,160 ions are shown in (a–c), respectively.

Fig. 7.4.

Selected ion images of various sphingolipid species from *hexb*^{-/-} mouse brain, which illustrate different histological localizations. (a) *m/z* 862.6 [ST d18:1/C22:0]; (b) *m/z* 878.6 [ST(OH) d18:1/h22:0]; (c) *m/z* 888.6 [ST d18:1/C24:1]; (d) *m/z* 890.6 [ST d18:1/C24:0]; (e) *m/z* 906.6 [ST(OH) d18:1/h24:0]; (f) *m/z* 908.6 [ST(OH) d18:0/h24:0]; (g) *m/z* 868.6 [unknown]; (h) *m/z* 1,383 [GM2 d18:1/C18:0]; (i) *m/z* 1,411 [GM2 d20:1/C18:0]; (j) *m/z* 1,132 [GA2 d18:1/C18:0+K]; (k) *m/z* 1,160 [GA2 d20:1/C18:0+K].

Fig. 7.5.
 (a) ESI-MS/MS spectrum of m/z 1,411 and (b) ESI-MS³ spectrum of m/z 1,410.9/592.6 transition.