

# Visualization of phosphatidylcholine, lysophosphatidylcholine and sphingomyelin in mouse tongue body by matrix-assisted laser desorption/ionization imaging mass spectrometry

Hirofumi Enomoto · Yuki Sugiura · Mitsutoshi Setou · Nobuhiro Zaima

Received: 28 November 2010 / Revised: 13 March 2011 / Accepted: 16 March 2011 / Published online: 7 April 2011  
© Springer-Verlag 2011

**Abstract** The mammalian tongue is one of the most important organs during food uptake because it is helpful for mastication and swallowing. In addition, taste receptors are present on the surface of the tongue. Lipids are the second most abundant biomolecules after water in the tongue. Lipids such as phosphatidylcholine (PC), lysophosphatidylcholine (LPC) and sphingomyelin (SM) are considered to play fundamental roles in the mediation of cell signaling. Imaging mass spectrometry (IMS) is powerful tool for determining and visualizing the distribution of lipids across sections of dissected tissue. In this study, we identified and visualized the PC, LPC, and SM species in a mouse tongue body section with matrix-assisted laser desorption/ionization (MALDI)-IMS. The ion image constructed from the peaks revealed that docosahexaenoic acid (DHA)-containing PC, LPC, linoleic acid-containing PC and SM (d18:1/16:0), and oleic acid-containing PC were mainly distributed in muscle, connective tissue, stratified epithelium, and the peripheral nerve, respectively. Furthermore, the distribution of SM (d18:1/16:0) corresponded to the distribution of nerve tissue relating to taste in the stratified epithelium. This study represents the first visualization of PC, LPC and SM localization in the mouse tongue body.

**Keywords** Imaging mass spectrometry · Mouse tongue body · Phosphatidylcholine · Lysophosphatidylcholine · Sphingomyelin · Matrix-assisted laser desorption/ionization

Published in the special issue *Biomedical Mass Spectrometry* with Guest Editors Hisao Oka and Mitsutoshi Setou.

H. Enomoto · Y. Sugiura · M. Setou · N. Zaima (✉)  
Department of Molecular Anatomy,  
Hamamatsu University School of Medicine,  
1-20-1 Handayama, Higashi-ku,  
Hamamatsu, Shizuoka 431–3192, Japan  
e-mail: zaimanobuhiro@gmail.com

## Introduction

The mammalian tongue is one of the most important organs during food uptake, because it is helpful for mastication and swallowing. In addition, there are various type of taste receptors on the taste bud of the tongue, and the receptors play an essential role in taste perception. A lot of taste buds are present in the papillae, which are localized on the mouse tongue body [1, 2]. Lipids play fundamental roles in the maintenance of cell structure and in the mediation of cell signaling. Polyunsaturated fatty acid (PUFA) in phospholipids (PLs) are released in hydrolysis by enzymes such as phospholipase A2 (PLA2) and then converted into bioactive lipids in response to extracellular stimuli [3]. Bioactive lipids mediate important biological processes [4]. In addition, lysophosphatidylcholine (LPC) produced from the hydrolysis of phosphatidylcholine (PC) by PLA may also act as a signal mediator. Lipids are the second most abundant species in the tongue, suggesting that they play fundamental roles in various tongue functions.

Mass spectrometry (MS) is regarded as one of the most powerful methods for lipid analysis due to its high sensitivity [5]. Using soft ionization techniques, nearly all lipid classes can be analyzed very sensitively without major fragmentation. Traditional methods of lipid analysis, such as liquid chromatography (LC)/MS, are very useful methods for the identification and quantification of lipids [6–8]. However, these traditional methods require extraction after homogenization, and thus spatial information on the lipid species in the tissue is lost. Direct molecular analysis of biological tissue using MS provides valuable information [9–12]. Imaging mass spectrometry (IMS) of biological tissues, using matrix-assisted laser desorption/ionization (MALDI), is a powerful tool for visualizing the distributions of various molecules without requiring anti-

bodies, staining or complicated pretreatment [9]. Thus, IMS is applicable to the imaging of biomolecules [13–18], biomarker discovery [19–21] and drug delivery [22, 23] in tissues. IMS can separate multiple lipids according their mass-to-charge ratios ( $m/z$ ), and visualize the tissue distribution of each molecule [10, 22]. Furthermore, the use of multistage MS ( $MS^n$ ) allows the structures of the visualized molecules to be identified [24, 25]. In particular, MALDI-IMS is the only analytical method that can visualize lipid molecular species, because other imaging techniques such as immunohistochemical approaches using antibodies are unable to distinguish between a range of lipid molecular species created by varying the structure of the fatty acid moiety.

In this study, we identified and visualized PC, LPC and sphingomyelin (SM) in mouse tongue body with MALDI-IMS. This work represents the first visualization of the distributions of specific lipids in mouse tongue body.

## Materials and methods

### Chemicals and reagents

Glass slides (Fisherbrand Superfrost Plus microscope slides) were purchased from Thermo Fisher (Waltham, MA, USA). Methanol was purchased from Kanto Chemical Company Inc. (Tokyo, Japan), and 2,5-dihydroxybenzoic acid (DHB) was purchased from Bruker Daltonics (Bremen, Germany). All chemicals used in this study were of the highest purity available.

### Sample preparation for LC/ESI (electrospray ionization)-MS/MS

Ten milligrams of homogenized tongue were collected in glass vials for lipid extraction, and total lipids were extracted by the Folch method [26]. ESI-MS/MS analysis was performed using a model 4000 Q-TRAP quadrupole linear ion trap hybrid mass spectrometer (Applied Biosystems/MDS Sciex, Concord, ON, Canada) with an Acquity ultra-performance liquid chromatograph (LC) (Waters, Milford, MA, USA). The samples were resolved on an Acquity UPLC BEH C18 column (1.0×150 mm i.d., 1.7  $\mu$ m particles) and then subjected directly to ESI-MS/MS analysis. The samples (10  $\mu$ L) were injected directly by the autosampler and separated using a step gradient with ratios of mobile phase A (acetonitrile:methanol:water = 19:19:2 v/v/v, containing 0.1% formic acid and 0.028% ammonium) to mobile phase B (isopropanol, 0.1% formic acid, and 0.028% ammonium) of 95:5 (0–5 min), 70:30 (5–40 min), 50:50 (40–90 min), and 95:5 (90–120 min), flowing at a rate of 70  $\mu$ L/min and with a column temperature of 30 °C.

### Precursor ion scanning for specific PL classes

To identify specific PL classes, precursor ion scanning of the polar head groups of the PCs, LPCs and SMs ( $m/z$  184) was performed using the 4000 Q-TRAP instrument; this method is effective at detecting these lipids because characteristic fragment ions are generated by collision-induced dissociation (CID) [27]. An appropriate collision energy is crucial to the sensitive identification of focused molecules, and therefore the optimal conditions for detecting the appropriate precursor ion were determined by analyzing standard lipids beforehand.

### Preparation of mouse tongue sections

The care and use of laboratory animals were performed in accordance with the Animal Experiment Regulations at the Hamamatsu University School of Medicine, which follow the *Guidelines for the Proper Conduct of Animal Experiments* from the Science Council of Japan. Female C57BL/6 (40 wk) mice were purchased from SLC (Shizuoka, Japan) and provided with a standard diet (Oriental Yeast, Tokyo, Japan) and tap water ad libitum. The mice were euthanized and the tongue bodies were isolated immediately. The tongue sample was frozen in liquid nitrogen and stored at –80 °C without any fixation. Tissue sections were prepared as previously described [24], with slight modifications. Briefly, 8- $\mu$ m sections were obtained using a cryostat (Cryocut CM 1850; Leica Microsystems, Wetzlar, Germany). The sections were attached to a glass slide and then was washed for 10 s with 10 mM ammonium formate solution to remove endogenous sodium and potassium.

### Imaging mass spectrometry

MALDI-IMS analyses were performed using a linear quadrupole ion-trap LTQ-XL mass spectrometer (ThermoFisher) equipped with a 337 nm  $N_2$  laser at a repetition rate of 60 Hz. Sample preparation was performed as described previously [24]. Briefly, 50 mg/ml DHB in methanol/water (7:3, v/v) was used as a matrix. The DHB matrix solution (500  $\mu$ L) was sprayed uniformly over the sections with a 0.2-mm nozzle caliber airbrush (Procon Boy FWA Platinum; Mr. Hobby, Tokyo, Japan). After the glass slide had dried, it was affixed to the mass spectrometer target plate. Data were acquired with a step size of 100  $\mu$ m in the positive ion mode. The laser energy was set to 20  $\mu$ J with the auto gain control (AGC) mode.  $m/z$  values in the range of 450–1000 were measured. ImageQuest software (ThermoFisher) was used to create two-dimensional ion-density maps. Normalization of the peak intensity and adjustment of the color scale were also performed using this software. After analysis by MALDI-IMS, the tissues on the glass

slide were immersed for 10 s in methanol to wash out the DHB matrix, and then the section was subjected to hematoxylin-eosin (HE) staining. The ion image of potassium-adducted SM in the stratified epithelium was clearer than that of the proton-adducted one, and the IMS analysis of SM was done without washing using 10 mM ammonium formate solution.

#### Tandem mass spectrometry (MS/MS)

MS/MS was performed on mouse tongue body sections using the LTQ-XL mass spectrometer. The selected precursor ions and the product ions obtained by collision-induced dissociation (CID) were ejected from the ion trap and analyzed. Collision energy was set to 30% of the maximum available energy for the LTQ-XL, where 100% represents the energy required to completely fragment the precursor ion formed from the peptide, M-R-F-A [28]. The laser energy was set to 20  $\mu$ J.

## Results and discussion

#### Identification of PL species by LC/ESI-MS/MS

Total lipids were extracted from the homogenized tongue in the vials, and the molecular species compositions of specific PL classes were selectively identified by MS/MS scans, as provided by the LC/ESI-MS/MS system. The top twenty-three lipid species in terms of intensity are shown in Table 1. Because we scanned for lipids containing a phosphocholine head group, the lipids detected in this experiment were PCs, LPCs and SMs, and the number of species of each type of lipid were sixteen, five and two, respectively. Interestingly, PCs that contain docosahexaenoic acid (DHA) and arachidonic acid (AA) were detected as the major lipid species. Futo et al. [29] reported that the PLs made up 71–73% of the total lipids in mouse tongue, and the most abundant PL-containing fatty acid was DHA. It is known that organs such as the brain, heart and retina are rich in PLs that contain DHA, and that DHA plays fundamental roles in the functions of these organs [24, 30]. PUFA may play important roles in the mouse tongue body. Next, we performed MALDI-IMS in order to investigate the distributions of the PC, LPC, and SM species in mouse tongue.

#### Mass spectra obtained from the section

Figure 1a shows an optical image of an HE-stained mouse tongue body section after raster laser scanning. Although the mouse tongue section was damaged by

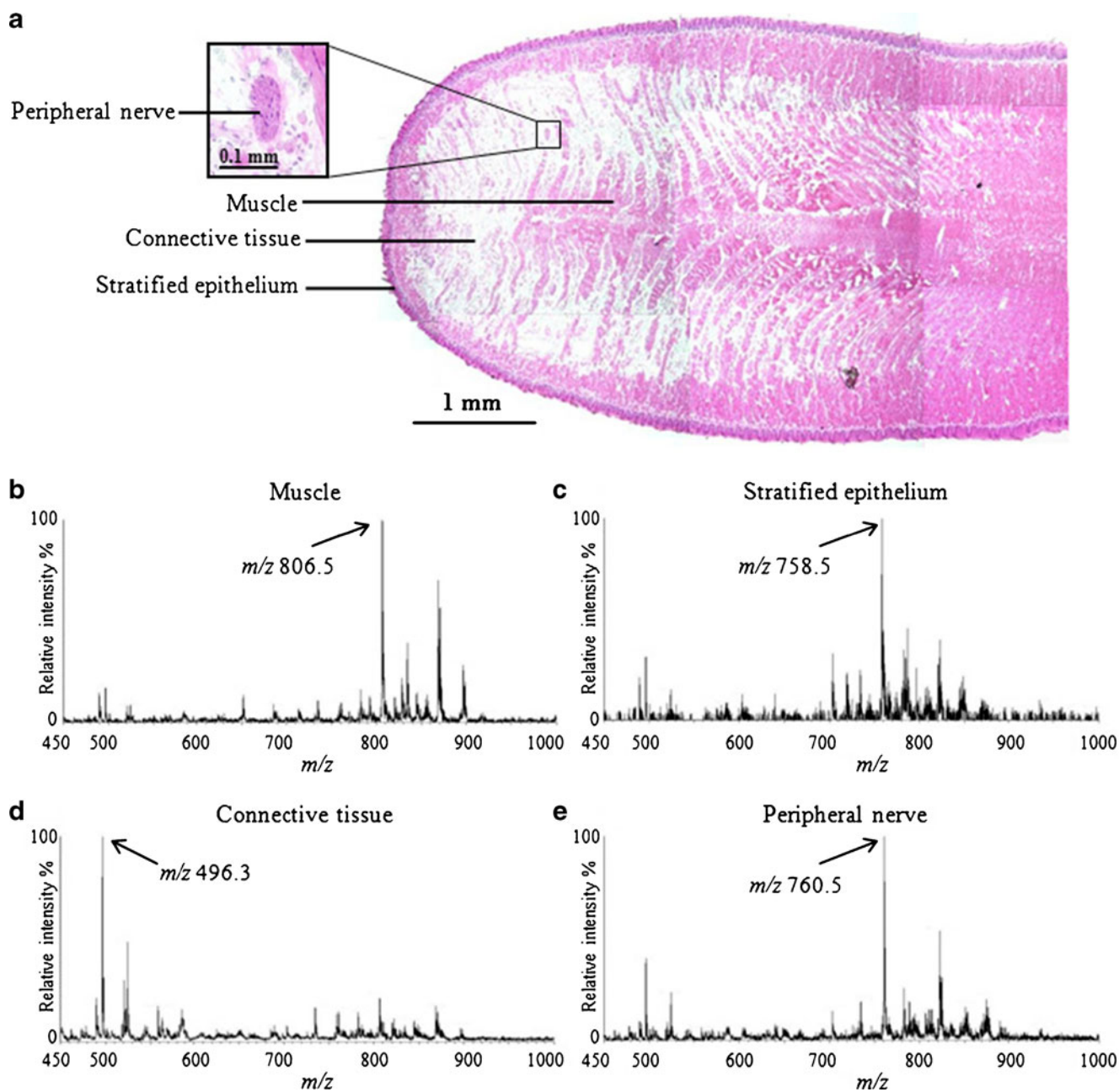
**Table 1** Molecular species of PCs, LPCs, and SMs in mouse tongue body, as identified by LC/ESI-MS/MS

Molecular species	Relative abundance	SD
PC (diacyl 16:0/22:6)	100.0	7.1
PC (diacyl 18:0/22:6)	88.9	5.2
PC (diacyl 16:0/18:2)	84.2	6.1
PC (diacyl 16:0/18:1)	79.5	3.6
PC (diacyl 18:0/20:4)	65.5	4.1
PC (diacyl 16:0/20:4)	63.2	5.5
PC (diacyl 16:0/16:0)	53.7	3.8
PC (diacyl 18:0/18:2)	52.8	2.9
PC (diacyl 18:1/18:2)	34.7	3.2
PC (diacyl 18:1/20:4)	27.3	1.7
PC (diacyl 18:2/22:6)	20.6	2.4
PC (diacyl 18:2/18:2)	15.7	1.3
SM (d18:1/16:0)	15.3	1.5
PC (diacyl 18:1/22:6)	13.7	0.9
SM (d18:1/18:0)	12.1	1.8
PC (diacyl 16:0/16:1)	8.7	0.8
PC (diacyl 18:0/18:1)	5.7	0.5
PC (diacyl 18:1/18:1)	2.9	0.2
LPC (1-acyl 18:0)	1.2	0.1
LPC (1-acyl 16:0)	0.9	0.1
LPC (1-acyl 18:2)	0.3	0.0
LPC (1-acyl 22:6)	0.2	0.0
LPC (1-acyl 18:1)	0.1	0.0

The extracted total lipid mixture from the mouse tongue body was subjected to precursor ion scanning of  $m/z$  184.1 to detect PCs, LPCs and SMs. The relative ion counts of each PC, LPC, and SM species are presented (the most intense species is considered to have an intensity of 100)

SD; standard deviation (n=3)

the laser scanning, the damage was not serious and thus allowed for the observation of the HE-stained tissue in a low-power field ( $\times 40$ ). Four major tissues were identified from observations of the HE-stained tongue section, and the name of each was indicated on the optical image (Fig. 1a). Mass spectra recorded for each tissue of the section are presented in the panels of Fig. 1 as follows: **b** muscle, **c** stratified epithelium, **d** connective tissue, **e** peripheral nerve. The mass spectra were obtained from each tissue using ImageQuest software, and the mass range  $m/z$  450–1000 is displayed. Mass spectral analysis of different tissues revealed that each tissue had a unique lipid MS profiles. The  $m/z$  value of the most intense peak detected was:  $m/z$  806.5 for muscle;  $m/z$  496.3 for connective tissue;  $m/z$  758.5 for stratified epithelium; and  $m/z$  760.5 for peripheral nerve, respectively. The major peaks of each tissue were identified by MS/MS analysis.



**Fig. 1** a–e Mass spectra obtained from the mouse tongue section. The analyzed section was stained with HE (a). The spectra recorded for each tissue are presented in the panels as follows: **b** muscle, **c** connective

tissue, **d** stratified epithelium, and **e** peripheral nerve. The mass spectra were obtained from representative points in each tissue using ImageQuest software, and show the mass range  $m/z$  450–1000

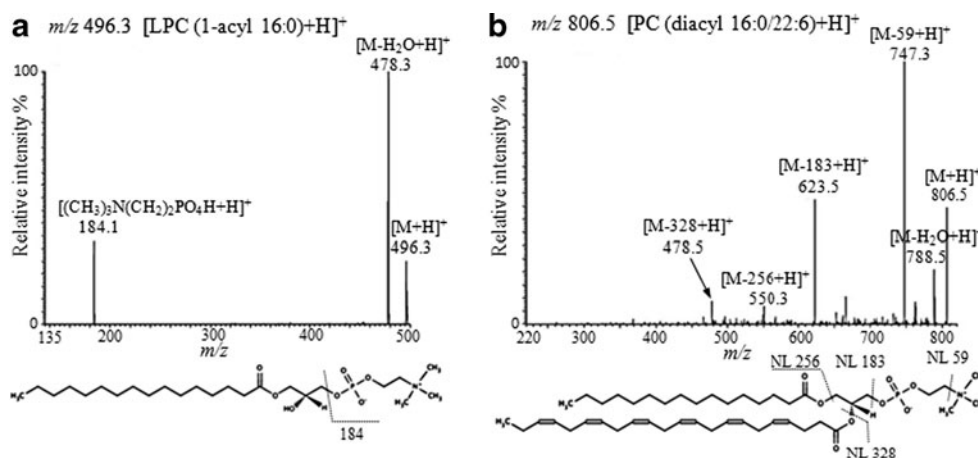
### Molecular identification

We performed MS/MS analysis on the precursor ion from each tissue to identify the biomolecules, and representative MS/MS spectra are shown in Fig. 2. The MS/MS spectrum of the  $m/z$  496.3 ion from each tissue shows an intense ion at  $m/z$  184.1 (Fig. 2a) which we assigned to the phosphocholine product ion generated from LPC. Thus, we can assign  $m/z$  496.3 to the  $[M+H]^+$  ion of LPC (1-acyl 16:0) [25, 31]. Similarly, as the ions in the section at  $m/z$  520.3,

524.3 and 558.3 also yielded the characteristic phosphocholine product ion at  $m/z$  184.1 (Table 2), we assigned these to the  $[M+H]^+$  ions of LPC (1-acyl 18:2), LPC (1-acyl 18:0), and the  $[M+K]^+$  ions of LPC (1-acyl 18:2).

The ion in the tissue with  $m/z$  806.5 was assigned to the  $[M+H]^+$  ion of PC (diacyl 16:0/22:6), because neutral losses of 59 and 183 Da in the MS/MS spectrum are indicative of the presence of a phosphocholine group, and because of the neutral losses of a 1-acyl fatty acid and a 2-acyl fatty acid, respectively (Fig. 2b) [25, 31]. In general,

**Fig. 2 a–b** Representative MS/MS spectra of  $[M+H]^+$  ions from the section. Precursor ions were: **a**  $m/z$  496.3 and **b**  $m/z$  806.5



saturated and unsaturated fatty acids were esterified at the *sn*-1 and *sn*-2 positions, respectively [32, 33]. Similarly, as the ions in each tissue with  $m/z$  734.5, 758.5, 760.5, 782.5, 784.5, 786.5, 788.5, 810.5, 828.5, 834.5, 844.5 and 856.5 showed neutral losses of 59 and 183 Da, and of a 1-acyl fatty acid and a 2-acyl fatty acid (Table 2), respectively, we

assigned these to  $[PC \text{ (diacyl } 16:0/16:0)+H]^+$ ,  $[PC \text{ (diacyl } 16:0/18:2)+H]^+$ ,  $[PC \text{ (diacyl } 16:0/18:1)+H]^+$ ,  $[PC \text{ (diacyl } 16:0/20:4)+H]^+$ ,  $[PC \text{ (diacyl } 16:0/20:4)+H]^+$ ,  $[PC \text{ (diacyl } 18:1/18:2)+H]^+$ ,  $[PC \text{ (diacyl } 18:0/18:2)+H]^+$ ,  $[PC \text{ (diacyl } 18:0/18:1)+H]^+$ ,  $[PC \text{ (diacyl } 18:0/20:4)+H]^+$ ,  $[PC \text{ (diacyl } 16:0/20:4)+K]^+$ ,  $[PC \text{ (diacyl } 16:0/22:6)+Na]^+$ ,  $[PC \text{ (diacyl$

**Table 2** The top seven molecular species in each tissue

Tissue	$m/z$	Molecular species	Fragment ion for assignment, $m/z$
Muscle	806.5	PC (diacyl 16:0/22:6)+H	478, 550, 623, 747
	834.5	PC (diacyl 18:0/22:6)+H	506, 550, 651, 775
	828.5	PC (diacyl 16:0/22:6)+Na	500, 572, 645, 769
	496.3	LPC (1-acyl 16:0)+H	184
	782.5	PC (diacyl 16:0/20:4)+H	478, 526, 599, 723
	844.5	PC (diacyl 16:0/22:6)+K	516, 588, 661, 785
	856.5	PC (diacyl 18:0/22:6)+Na	528, 572, 673, 797
Connective tissue	496.3	LPC (1-acyl 16:0)+H	184
	524.3	LPC (1-acyl 18:0)+H	184
	520.3	LPC (1-acyl 18:2)+H	184
	806.5	PC (diacyl 16:0/22:6)+H	478, 550, 623, 747
	558.3	LPC (1-acyl 18:2)+K	184
	734.5	PC (diacyl 16:0/16:0)+H	478, 551, 675
	760.5	PC (diacyl 16:0/18:1)+H	478, 504, 577, 701
Stratified epithelium	758.5	PC (diacyl 16:0/18:2)+H	478, 502, 575, 699
	786.5	PC (diacyl 18:0/18:2)+H	502, 506, 603, 727
	782.5	PC (diacyl 16:0/20:4)+H	478, 526, 599, 723
	703.5	SM (d18:1/16:0)+H	520, 644
	496.3	LPC (1-acyl 16:0)+H	184
	784.5	PC (diacyl 18:1/18:2)+H	500, 502, 601, 725
	820.5	PC (diacyl 16:0/20:4)+K	516, 564, 637, 761
Peripheral nerve	760.5	PC (diacyl 16:0/18:1)+H	478, 504, 577, 701
	496.3	LPC (1-acyl 16:0)+H	184
	782.5	PC (diacyl 16:0/20:4)+H	478, 526, 599, 723
	524.3	LPC (1-acyl 18:0)+H	184
	734.5	PC (diacyl 16:0/16:0)+H	478, 551, 675
	788.5	PC (diacyl 18:0/18:1)+H	504, 506, 605, 729
	810.5	PC (diacyl 18:0/20:4)+H	506, 526, 627, 751

The MS/MS analysis was performed directly on each tissue of mouse tongue body

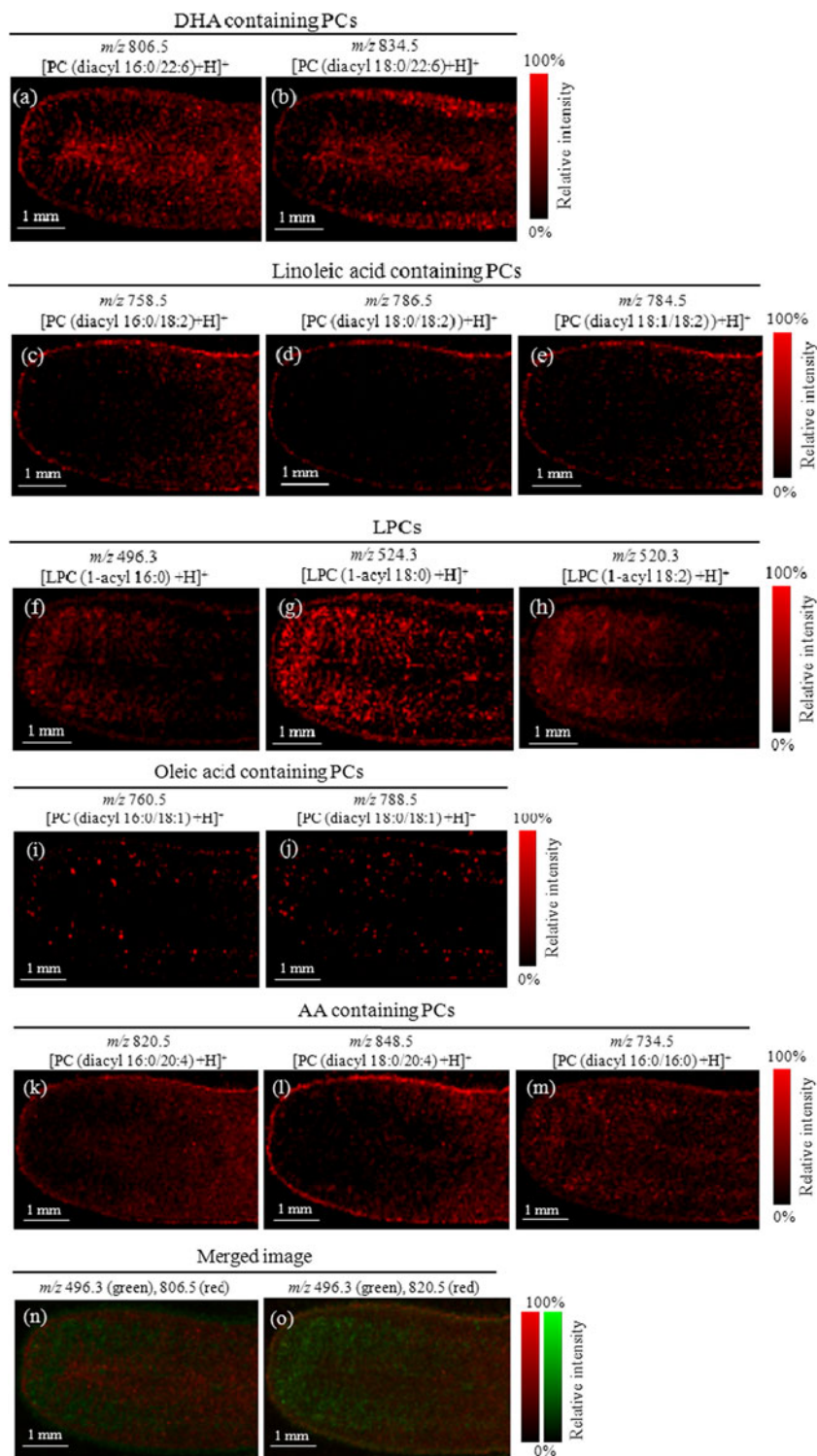
18:0/22:6)+H]<sup>+</sup>, [PC (diacyl 16:0/22:6)+K]<sup>+</sup> and [PC (diacyl 18:0/22:6)+Na]<sup>+</sup>.

The ion in the tissue at *m/z* 703.5 was assigned to the [M+H]<sup>+</sup> ion of SM (d 18:1/16:0) because neutral losses of 59 and 183 Da in the MS/MS spectrum are indicative of the presence of a phosphocholine group [25]. In addition,

SM was detected as an odd number by the nitrogen rule. These lipid molecular species identified by MS/MS analysis were reconfirmed by the *m/z* values obtained from the results of LC-ESI-MS/MS (Table 1).

The top seven representative molecular species identified in each tissue are summarized in Table 2. Lipids were

**Fig. 3 a–o** IMS analysis of LPC and PC. **a** [PC (diacyl 16:0/22:6)+H]<sup>+</sup>, **b** [PC (diacyl 18:0/22:6)+H]<sup>+</sup>, **c** [PC (diacyl 16:0/18:2)+H]<sup>+</sup>, **d** [PC (diacyl 18:0/18:2)+H]<sup>+</sup>, **e** [PC (diacyl 18:1/18:2)+H]<sup>+</sup>, **f** [LPC (1-acyl 16:0)+H]<sup>+</sup>, **g** [LPC (1-acyl 18:0)+H]<sup>+</sup>, **h** [LPC (1-acyl 18:2)+H]<sup>+</sup>, **i** [PC (diacyl 16:0/18:1)+H]<sup>+</sup>, **j** [PC (diacyl 18:0/18:1)+H]<sup>+</sup>, **k** [PC (diacyl 16:0/20:4)+H]<sup>+</sup>, **l** [PC (diacyl 18:0/20:4)+H]<sup>+</sup>, **m** [PC (diacyl 16:0/16:0)+H]<sup>+</sup>, **n, o** merged images



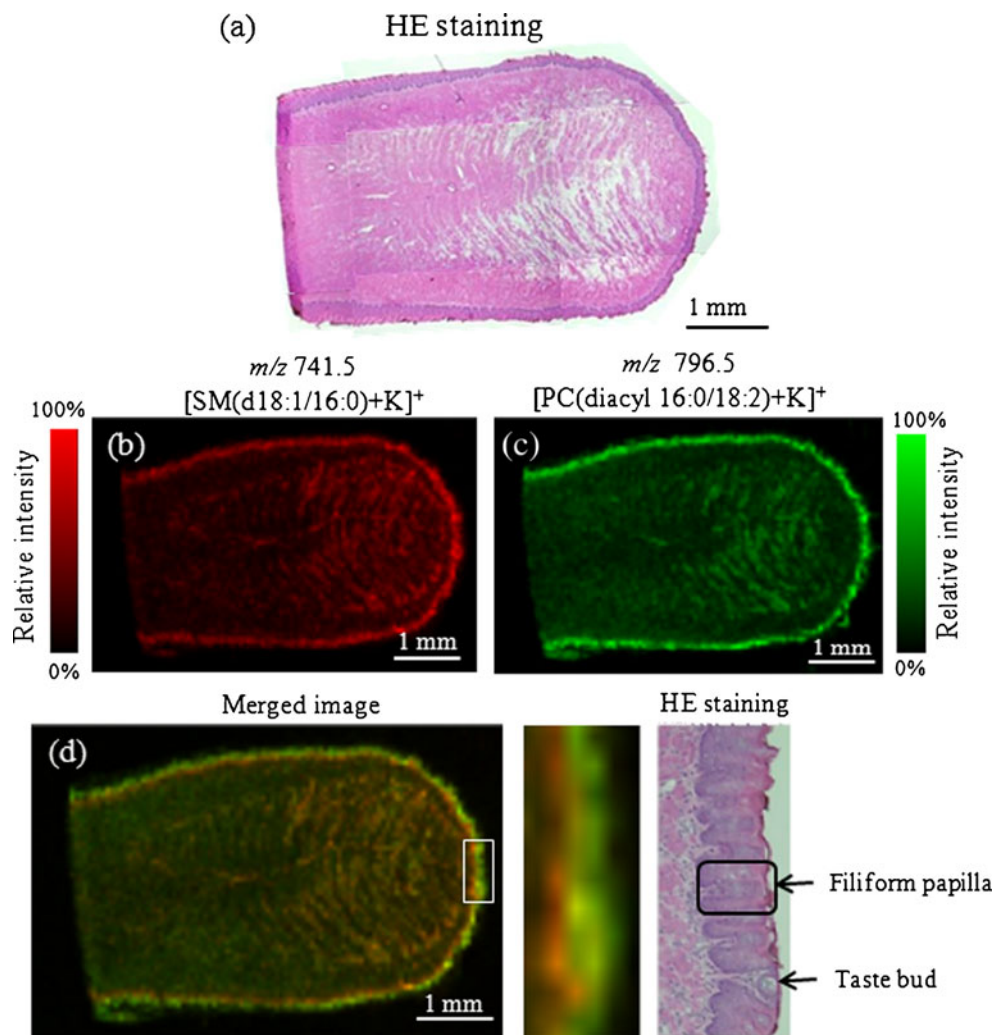
predominantly detected as protonated ions in each tissue by washing with ammonium formate. This result indicates that washing with ammonium formate is helpful for identifying the lipid species. Some lipids were characteristically detected in each tissue; the characteristic lipids in muscle were PCs that contain 22:6 (DHA); the characteristic lipids in connective tissue were LPCs; the characteristic lipids in stratified epithelium were PCs that contain 18:2 (linoleic acid) as well as SM (d18:1/16:0); while the characteristic lipids in peripheral nerve were PCs that contain 18:1 (oleic acid).

#### IMS analysis of PC and LPC

To investigate the distributions of PC and LPC in the tongue body section, we constructed ion images from the  $m/z$  value detected in each tissue using the ImageQuest software (Fig. 3). The ion images show the relative intensities of signals detected on the mouse tongue section. The ion images of PC (diacyl 16:0/22:6) and PC (diacyl 18:0/22:6) showed specific distributions in the muscle

(Fig. 3a–b). LPC (1-acyl 16:0), LPC (1-acyl 18:0) and LPC (1-acyl 18:2) were distributed in the connective tissue and stratified epithelium (Fig. 3c–e). PC (diacyl 16:0/18:2), PC (diacyl 18:0/18:2) and PC (diacyl 18:1/18:2) were mainly distributed in stratified epithelium (Fig. 3f–h). PC (diacyl 16:0/18:1) and PC (diacyl 18:0/18:1) were mainly distributed in peripheral nerve (Fig. 3i–j). Although LPCs comprised a minor proportion of the total lipids (Table 1), it was found that LPCs were most abundant in the connective tissue. PCs that contain AA, such as PC (diacyl 16:0/20:4) and PC (diacyl 18:0/20:4), were mainly distributed in muscle and stratified epithelium (Fig. 3k–l). On the other hand, PC (diacyl 16:0/16:0) that contains two saturated fatty acids (16:0, palmitic acid) was ubiquitously distributed in mouse tongue body (Fig. 3m). The unsaturated fatty acid-containing PC showed a specific distribution in each tissue, suggesting that the unsaturated fatty acids, especially PUFA, at *sn*-2 play specific roles in each tissue [3]. It was reported that the oleic acid-containing PC was predominantly detected in the PCs of mouse brain which mainly consisted of neurons [30]. In this study, the oleic acid-

**Fig. 4** a–d IMS analysis of SM (d18:1/16:0). The analyzed section was stained with HE (a). **b**  $[\text{SM}(\text{d}18:1/16:0)+\text{K}]^+$ , **c**  $[\text{PC}(\text{diacyl } 16:0/18:2)+\text{K}]^+$ . **d** The merged ion images of **b** and **c**



containing PCs were specifically distributed in peripheral nerve, suggesting that oleic acid is involved in the regulation of the neuronal functions of mouse tongue. Interestingly, DHA-containing PCs were strongly detected in the muscle tissue. We previously reported that retinal tissue, which undergoes strong oxidative stress, contains a large amount of DHA-containing PC [24]. The DHA in PCs may act as a protectant against external oxidative or chemical stress.

It is known that PLs are hydrolyzed to lyso-PLs with phospholipase A. The detected peaks from LPCs in the connective tissue suggest that this enzyme exists and acts in the tissue. LPC is produced by the hydrolysis of PC with an enzyme such as PLA2, and is changed to lysophosphatidic acid (LPA) by an enzyme such as lysophospholipase D (LPLD) [34]. It was suggested that the activity of PLA2 in the connective tissue was higher than that seen in any other tissue in the tongue body. Ion images of PC containing AA and DHA show their specific disappearance in connective tissue (Fig. 3n, o). Therefore, we can speculate that the increase of LPC in the connective tissue is due to the action of PLA2, which specifically hydrolyzes PC with AA and DHA at *sn*-2. The physiological significance of why LPCs were specifically distributed in the connective tissue is still not known.

#### IMS analysis of SM (d18:1/16:0)

To investigate the detailed distribution of SM (d18:1/16:0), which was detected in the stratified epithelium alone, we compared the ion image of the potassium-adducted SM with the same adducted PC (diacyl 16:0/18:2) that was a major PC in the tissue (Fig. 4). As shown in Fig. 4b-c, the SM was distributed in the stratified epithelium as well as the PC. However, from the merged image of the SM and the PC (Fig. 4d), a difference between these distributions in the stratified epithelium was clearly observable. The SM was distributed inside the PC in the stratified epithelium. When compared with the HE-stained section, it was noted that the distribution of PC (diacyl 16:0/18:2) corresponded to the region of filiform papillae. Neurons that sense the taste stimuli at the taste bud on filiform papilla were distributed in the epithelium region, and this distribution is likely to correspond to the distribution of SM in stratified epithelium. SM in the plasma membrane can be cleaved by neutral sphingomyelinases to generate ceramides and sphingosine-1-phosphate, which have been shown to play a variety of roles in cellular signaling processes [35]. SM (d18:1/16:0) may play an essential role as a source of signaling molecules involved in reception, such as taste, by the tongue body.

## Conclusion

We identified and visualized the lipid species in a mouse tongue body section with MALDI-IMS. Each tissue contained its own characteristic lipids: DHA-containing PC in muscle, LPCs in connective tissue, linolenic acid-containing PC and SM (d18:1/16:0) in stratified epithelium, and oleic acid-containing PC in the peripheral nerve. In addition, SM (d18:1/16:0) was found to be concentrated in the nerve tissue in the epithelium region. This work represents the first visualization of the localization of PC, LPC and SM in the mouse tongue body. In the future, this method will help us to combine the study of tongue functions such as taste and lipid research.

**Acknowledgements** This work was supported by the Program for Promotion of Basic and Applied Researchers for Innovations in Bio-oriented Industry (BRAINI) to N. Z.

## References

- Mistretta CM (1991) In: Getchell T, Doty R, Bartoshuk L, Snow J (eds) *Developmental neurobiology of taste: smell and taste in health and disease*. Raven, New York, pp 653–668
- Iwasaki S, Yoshizawa H, Kawahara I (1999) *J Anat* 195:27–38
- Yamashita A, Sugiura T, Waku K (1997) *J Biochem* 122:1–16
- Murakami M, Nakatani Y, Atsumi G, Inoue K, Kudo I (1997) *Crit Rev Immunol* 17:225–283
- Watson A (2006) *J Lipid Res* 10:2101–2111
- Costello CE (1997) *Biophys Chem* 68:173–188
- Han X, Gross RW (2005) *Mass Spectrom Rev* 24:367–412
- Zink KG, Mangelsdorf K (2004) *Anal Bioanal Chem* 380:798–812
- Frank J, Arafah K, Elayed M, Bonnel D, Vergara D, Jacquet A, Vinatier D, Wisztorski M, Day R, Fournier I, Salzet M (2009) *Mol Cell Proteomics* 8:2023–2033
- Sugiura Y, Setou M (2010) *J Neuroimmune Pharmacol* 5:31–43
- Svatos A (2010) *Trends Biotechnol* 28:425–434
- Zaima N, Hayasaka T, Goto-Inoue N, Setou M (2010) *Int J Mol Sci* 11:5040–5055
- Chen RB, Li LJ (2010) *Anal Bioanal Chem* 397:3185–3193
- Fuchs B, Süß R, Schiller J (2010) *Prog Lipid Res* 49:450–47
- Hardesty WM, Caprioli RM (2008) *Anal Bioanal Chem* 391:899–903
- Murphy RC, Hankin JA, Barkly RM (2009) *J Lipid Res* 50: S317–S322
- Stoeckli M, Chaurand P, Hallahan DE, Caprioli RM (2001) *Nat Med* 7:493–496
- Zaima N, Goto-Inoue N, Adachi K, Setou M (2011) *J Oleo Sci* 60:93–98
- Morita Y, Ikegami K, Goto-Inoue N, Hayasaka T, Zaima N, Tanaka H, Uehara T, Setoguchi T, Sakaguchi T, Igarashi H, Sugimura H, Setou M, Konno H (2010) *Cancer Sci* 101:267–273
- Shimma S, Sugiura Y, Hayasaka T, Hoshikawa Y, Noda T, Setou M (2007) *J Chromatogr B* 885:98–103
- Zaima N, Matsuyama Y, Setou M (2009) *J Oleo Sci* 58:267–273
- Reyzer ML, Hsieh Y, Ng K, Korfmacher WA, Caprioli RM (2003) *J Mass Spectrom* 38:1081–1092



23. Wang HYJ, Jackson SN, McEuen J, Wood AS (2005) *Anal Chem* 77:6682–6686
24. Hayasaka T, Goto-Inoue N, Sugiura Y, Zaima N, Nakanishi H, Ohishi K, Nakanishi S, Naito T, Taguchi T, Setou M (2008) *Rapid Commun Mass Spectrom* 22:3415–3426
25. Shimma S, Sugiura Y, Hayasaka T, Zaima N, Matsumoto M, Setou M (2008) *Anal Chem* 80:878–885
26. Folch J, Lees M, Sloane Stanley GH (1957) *J Biol Chem* 226:497–509
27. Taguchi R, Houjou T, Nakanishi H, Yamazaki T, Ishida M, Imagawa M, Shimizu T (2005) *J Chromatogr B* 823:26–36
28. Lopez LL, Tiller PR, Senko MW, Schwartz JC (1999) *Rapid Commun Mass Spectrom* 13:663–668
29. Futo T, Hitaka T, Mizutani T, Okuyama H, Watanabe K, Totsuka T (1989) *J Neurol Sci* 129:97–105
30. Sugiura Y, Konishi Y, Zaima N, Kajihara S, Nakanishi H, Taguchi R, Setou M (2009) *J Lipids Res* 50:1776–1788
31. Hayasaka T, Goto-Inoue N, Zaima N, Kimura Y, Setou M (2009) *Lipids* 44:837–848
32. Amate L, Ramirez M, Gil A (1999) *Lipids* 34:865–871
33. Yang LY, Kuksis A, Myher JJ, Steiner G (1995) *J Lipid Res* 36:125–136
34. Allenmark S, Sjodahl E, Sjodahl R, Tagesson C (1980) *Prep Biochem* 10:463–471
35. Hannun YA, Obeid LM (2008) *Nat Rev Mol Cell Biol* 9:139–150