methods

A simple desalting method for direct MALDI mass spectrometry profiling of tissue lipids

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Abstract Direct MALDI-mass spectrometry (MALDI-MS) profiling of tissue lipids often observes isobaric phosphatidylcholine (PC) species caused by the endogenous alkali metal ions that bias the relative abundance of tissue lipids. Fresh rat brain cryosections were washed with 70% ethanol (EtOH), water (H_2O), or 150 mM ammonium acetate (NH_4Ac), and the desalting effectiveness of each fluid was evaluated by MALDI-MS profiling of PC and sphingomyelin (SM) species in tissue and in the washing runoff. The results indicated that EtOH and H_2O only partially desalted the tissue lipids, yet both substantially displaced the tissue lipids to the washing runoffs. On the other hand, NH_4Ac effectively desalted the tissue lipids and produced a runoff containing no detectable PCs or SMs. NH_4Ac wash also unveiled the underlying changes of PCs and SMs in the infarcted rat cortex previously masked by edema-caused increase of tissue sodium. The MS/MS of an isobaric PC in the infarcted cortex revealed the precursor change as the result of NH_4Ac wash and confirmed the desalting effectiveness of such wash. Other than desalting, NH_4Ac wash also removes contaminants in tissue, enhances the overall spectral quality, and benefits additionally in profiling of biological molecules in tissue.—Wang, H-Y. J., C. B. Liu, and H-W. Wu. **A simple desalting method for direct MALDI mass spectrometry profiling of tissue lipids.** ***J. Lipid Res.*** **2011.** 52: **840–849.**

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Alkali metal ions such as potassium and sodium are essential constituents in all biological organisms. A slight change in the concentration of these ions will significantly impact the organisms in osmotic pressure and fluid volume regulation, threshold maintenance of the excitable membranes, and in other aspects of homeostasis. However, these alkali metal ions usually become the inevitable constituent of, and frequently the interference to, the mass spectrometry analysis of biological samples. Previous MS studies of biological lipids reported that phosphatidylcholines (PCs) were preferentially ionized and detected in their alkalinated forms (1–4). Therefore, the highly abundant endogenous potassium and sodium ions might have conceivably biased the mass spectrometry presentations of tissue PCs (3). Changes in tissue cations due to the pathological effects such as edema (5, 6) further enhanced the ionization of alkalinated PCs and SMs in the direct MALDI profiling (7). Adding lithium (1), potassium (3), or cesium (7, 8) in the MALDI matrix solution was attempted to circumvent this obstacle. However, protonated PCs and SMs were still easily detected with their alkalinated counterparts in most of the MALDI spectra acquired by this approach. Therefore, the profile of tissue lipids so revealed was still a biased pattern.

Desalting samples prior to subjecting them for instrument analysis has long been a standard procedure in sample processing for biological MS studies. Direct MALDI-MS profiling and imaging of tissue proteins and peptides generally adopt an ethanol wash step to remove the endogenous salts and other interference in tissue before spectrometrically analyzing the tissue sections (9, 10). However, it remains to be determined if this ethanol wash is suitable for tissue lipid studies. In this study, we evaluated the desalting effectiveness and the overall suitability of ethanol wash for tissue lipids. We also explored the possibility of desalting tissue lipids with water based on the general immiscibility between water and lipids. Meanwhile, the desalting effectiveness of ammonium acetate (NH_4Ac) solution isotonic to the mammalian physiological milieu was investigated. The profiles of PCs and SMs in tissue section and in the washing runoff were used together in the evaluation of each desalting fluid. It was rea-

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Abbreviations: DHA, dihydroxyacetophenone; EtOH, ethanol; H_2O, water; ITO, indium-tin oxide; NH_4Ac, ammonium acetate; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; S/N, signal-to-noise ratio.

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soned that a suitable desalting fluid should effectively remove the alkali metal ions bound to tissue lipids while minimally disturbing or displacing the tissue lipids to the washing runoff. The most suitable desalting fluid was further tested on infarcted rat brain sections where the edema-induced increase of tissue sodium masked the revelation of underlying changes of PCs and SMs by MALDI-MS. Comparison of the MALDI-MS/MS result of an isobaric PC in the infarcted cortex revealed the change of precursor by NH_4Ac wash and confirmed the desalting effectiveness of the chosen desalting fluid.

MATERIAL AND METHODS

Chemicals

MALDI matrix 2,6-dihydroxyacetophenone (DHA) was purchased from Fluka (Bauch, Switzerland). Ammonium acetate (ACS grade) was purchased from Sigma-Aldrich (St. Louis, MO). All the organic solvents were acquired from J. T. Baker or Mallinckrodt (Mallinckrodt Baker Inc., Phillipsburg, NJ). All the solvents were of HPLC grade except ethanol, which was 99.98%.

Animal handling and tissue collection

Animal care and use were in accordance with the US Public Health Service Policy on Humane Care and Use of Laboratory Animals. The experimental procedures were approved by the Institutional Animal Care and Use Committee of National Sun Yat-Sen University before the study began. Male Sprague-Dawley rats (280–350g) were euthanized with isoflurane and decapitated immediately after the spontaneous breathing had ceased. The brains were rapidly dissected from the cranium and snap-frozen in isopentane prechilled on dry ice. The frozen brains were quickly wrapped in aluminum foil and stored at −80°C until needed (11).

Surgical induction of ischemic stroke in rats, the neurological evaluation of the animals, and histological verification have been described previously (7). Rat brains were collected 24 h after stroke induction.

The frozen rat brains were cut into 14 μm coronal sections with a cryostat (Leica CM 3050, Nossloch, Germany) and collected on the glass slides coated with indium-tin oxide (ITO slides; 7-10 Ω, 2.5 cm × 7.5 cm) for mass spectrometry profiling. All the coronal sections were cut and collected serially from the region approximately 1.2 ± 0.2 mm rostral to Bregma (12) along the anterior-posterior axis. After a very brief thaw-mounting, the collected sections were immediately placed in a desiccator evacuated by a vacuum pump (RV5, Edwards, West Sussex, UK) for 15 min.

Tissue section desalting wash

Seventy percent (v/v) ethanol (EtOH), water (H_2O; 18.2 MΩ/cm, purified by Synergy Ultrapure Water System, Millipore Co.), and 150 mM NH_4Ac solution (pH = 6.9) (13) were selected as the desalting fluids for this study. Each desalting fluid was tested on three separate brain sections. Each brain section was drip-washed with 1 ml of cold (4°C) desalting fluid. During section washing, the ITO slide carrying the tissue section was slightly elevated at one end to create a downward slope and the desalting fluid was dripped slightly upstream to the tissue section. Dripping of the washing fluid was carefully oriented to ensure a complete and even coverage of tissue section by the desalting fluid. The washing runoff was collected for the subsequent lipid extraction and analysis. Usually 970–985 μL of runoff could be collected from the washing of one tissue section. Care was taken to avoid sloughing of tissue section and the debris by the dripping of washing fluid. The entire washing process was usually accomplished in 3 min. After washing, the tissue section was immediately dried in the vacuum dessicator for another 20 min to evaporate the remaining washing fluid and its constituents. Thereafter, the primary somatosensory cortex region was spotted with 0.1 μL of DHA solution (30 mg/ml in 70% acetonitrile) for MALDI-MS and MALDI-MS/MS analyses.

Analysis of lipids in the washing runoff

Lipids in the collected washing runoff were extracted with the method of Folch and colleagues (14). At the end of lipid extraction, the lower organic phase was transferred to a clean glass sample vial and the solvent was dried under a gentle stream of nitrogen. The dried content was reconstituted in 10 μL of mixture of chloroform and methanol (chloroform: methanol = 2:1; v/v), then mixed with equal volume of DHA solution. One microliter of this mixture was spotted on the stainless steel target plate for MALDI-MS analysis of lipids in the extracts of the washing runoffs.

Mass spectrometer and data processing

The analyses of PC and SM in tissue and in the desalting runoffs were carried out on a Bruker Autoflex III MALDI time-of flight (TOF)-TOF mass spectrometer equipped with a 355 nm Nd:YAG Smartbeam laser (Bruker Daltonics, Breman, Germany). The instrument was operated under positive ion mode for both MALDI-MS profiling and MALDI-MS/MS analyses. For MALDI-MS profiling, ions from 200 consecutive laser shots were collected under reflectron mode and summed into one spectrum. For MALDI-MS/MS analyses, ions from 2,000 consecutive laser shots for precursor and fragments each were collected and summed into one spectrum. The collected spectra were processed with FlexAnalysis 3.0 (Bruker Daltonics). The ion signal was deemed a true ion peak when its signal-to-noise (S/N) ratio was ⩾3. Designation of lipids follows that of the updated LIPID MAPS classification system (15).

RESULTS

In situ PC and SM profiles after the section were washed with EtOH, H_2O, and NH_4Ac

To evaluate the desalting effectiveness of these three fluids, we compare the profiles of PC and SM in the normal rat cortex with those from sections washed with EtOH, H_2O, and NH_4Ac. **Figure 1** shows a typical MALDI-MS profile of PCs and SMs in rat cortex that is dominated by the potassiated PC and SM species. However, the protonated and sodiated species are easily identified as well. **Table 1** lists the commonly encountered rat brain PC and SM species by MALDI-MS (7). We have noticed a few isobaric PC pairs among all the identified cortical PCs. **Figure 2A** shows the PC and SM profile in rat cortex after EtOH wash. It appears that the relative abundances of the cortical PC and SM species became aberrant after EtOH wash. For example, the tissue PC (32:0) (*m/z* 734.58) was almost as abundant as PC (34:1) (*m/z* 760.59) after EtOH wash. Their potassiated (*m/z* 772.54 and *m/z* 798.55, respectively) and sodiated counterparts (*m/z* 756.55 and *m/z* 782.62, respectively) were reduced by EtOH wash as well. Such a pattern could be the result of uneven removal of tissue sodium and potassium, the uneven removal of tissue PC and SM, or the combination

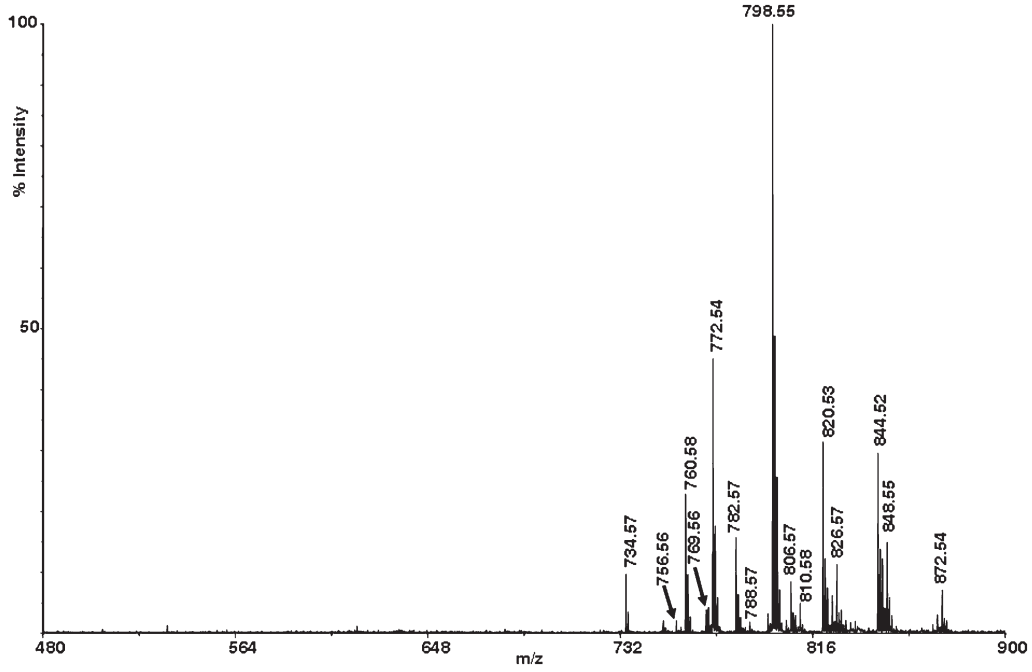

Fig. 1. MALDI-MS profile of PC and SM species in a normal rat cortex.

thereof, by EtOH wash. Although EtOH wash reduced the sodiated and potassiated PCs in tissue, this approach did not completely remove the sodium and potassium bound to tissue lipids.

We next tested whether H_2O is an effective desalting fluid for PC and SM in tissue. Figure 2B shows the cortical profile of PC and SM after the brain section was washed with H_2O where a significant reduction of sodiated and potassiated PCs was clearly noted. However, similar to the result of EtOH wash, H_2O wash could not completely remove the alkali metal ions in tissue. In addition, after H_2O wash, the protonated and potassiated SM (18:0) (*m/z* 731.60 and 769.56, respectively) were no longer detectable in tissue.

Evaluation of NH_4Ac as the desalting fluid for tissue lipids was carried out following the previous two tests. Figure 2C shows a typical MS profile of PC and SM in rat cortex after NH_4Ac wash. The spectrum was dominated by the protonated PC (32:0), PC (34:1), and PC (38:6) at *m/z* 734.57, 760.59, and 806.59, respectively, whereas their corresponding potassiated species were no longer detectable. Most of the sodiated PCs also disappeared after NH_4Ac wash. Nevertheless, the molecular ions at *m/z* 782.58 and *m/z* 810.63 that represent two isobaric PC pairs remained clearly visible. Because NH_4Ac wash essentially eliminated other highly abundant potassiated and sodiated PC species, these two potentially isobaric molecular species were likely the protonated PC species (see below). In addition to PC and SM, we also detected the low abundant lysophosphatidylcholine (LPC) 16:0 in tissue after NH_4Ac wash.

PC and SM in the washing runoff

Other than profiling the lipids in tissue, we also analyzed the PC and SM displaced to the runoff by the desalting washes. **Figure 3A** shows the PC and SM in the EtOH washing runoff of the section for Fig. 2A. Highly abundant PC and SM in their protonated, sodiated, and potassiated forms in Fig. 3A signified the undesirable yet significant displacement of tissue lipids to the runoff by EtOH wash. This result and the tissue lipid profile in Fig. 2A indicate that EtOH is ineffective as the desalting fluid for the direct profiling studies on tissue lipids.

TABLE 1. The theoretical *m/z* values of the commonly encountered lysophosphatidylcholine (LPC), phosphatidylcholine (PC), sphingomyelin (SM), and other small molecular species in rat brain section by MALDI-MS (7)

Species Assignment	Theoretical *m/z*
LPC (O-16:0)+H	482.36
LPC (16:0)+H	496.34
LPC (O-16:0)+Na	504.34
LPC (16:0)+Na	518.32
LPC (18:1)+H	522.36
LPC (18:0)+H	524.37
LPC (16:0)+K	534.30
LPC (20:4)+H or LPC (18:1)+Na	544.34
LPC (18:0)+Na	546.35
Heme $^+$	616.18
SM (18:0)+H	731.61
PC (32:0)+H	734.59
PC (32:0)+Na	756.55
PC (34:1)+H	760.59
SM (18:0)+K	769.56
PC (32:0)+K	772.53
PC (34:1)+Na or PC (36:4)+H	782.57
PC (36:1)+H	788.62
PC (34:1)+K	798.54
PC (38:6)+H	806.57
PC (38:4)+H or PC (36:1)+Na	810.60
PC (36:4)+K	820.53
PC (36:1)+K	826.57
PC (38:6)+K	844.53
PC (38:4)+K	848.56

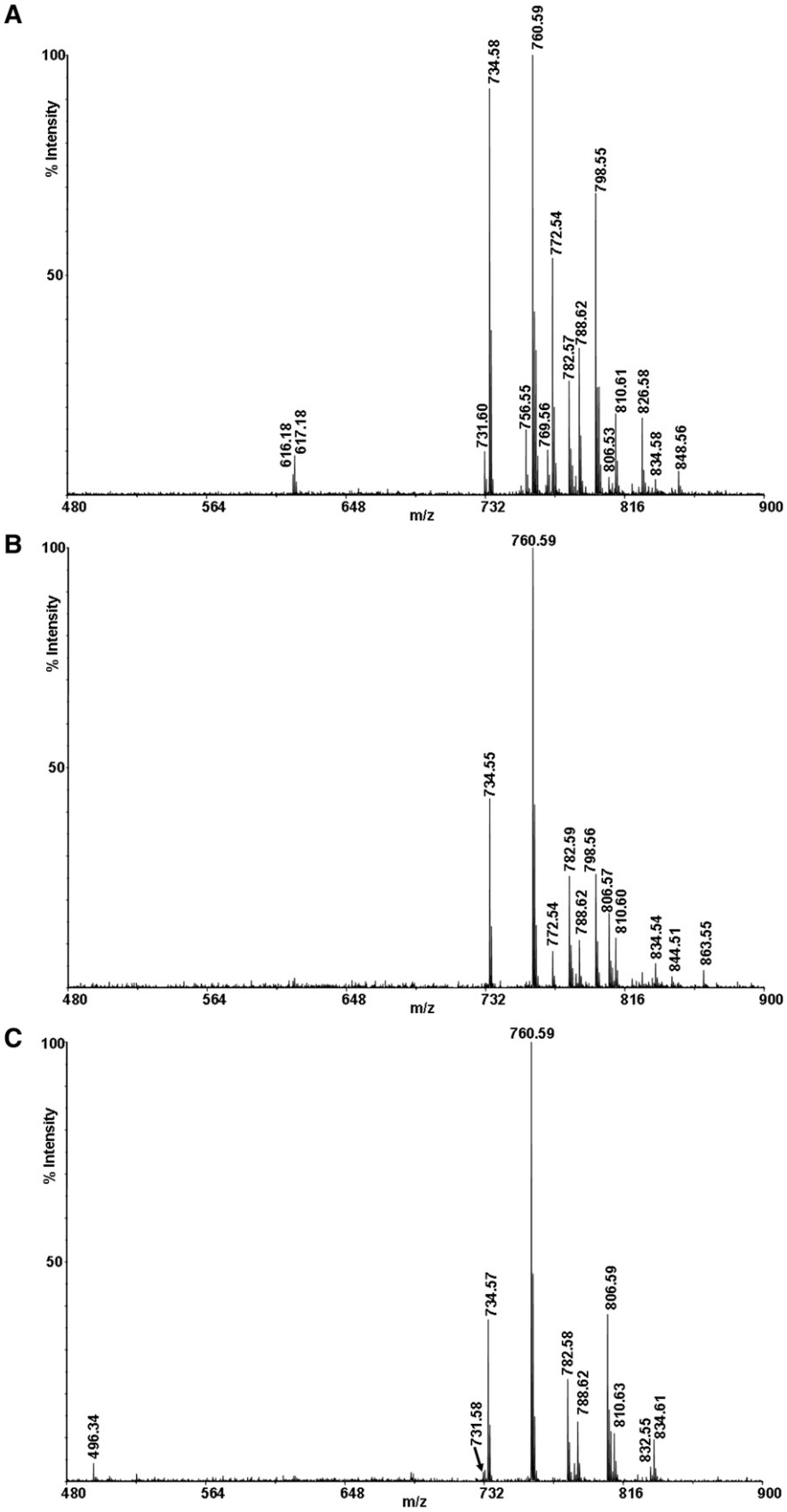

Fig. 2. MALDI-MS profile of PC and SM species in rat cortex after desalting wash. A: The PC and SM species in rat cortex after the brain section was washed with 70% ethanol (EtOH). B: The PC and SM species in rat cortex after the brain section was washed with water (H_2O; 18.2 MΩ/cm). C: The PC and SM species in rat cortex after the brain section was washed with 150 mM ammonium acetate (NH_4Ac; pH = 6.9).

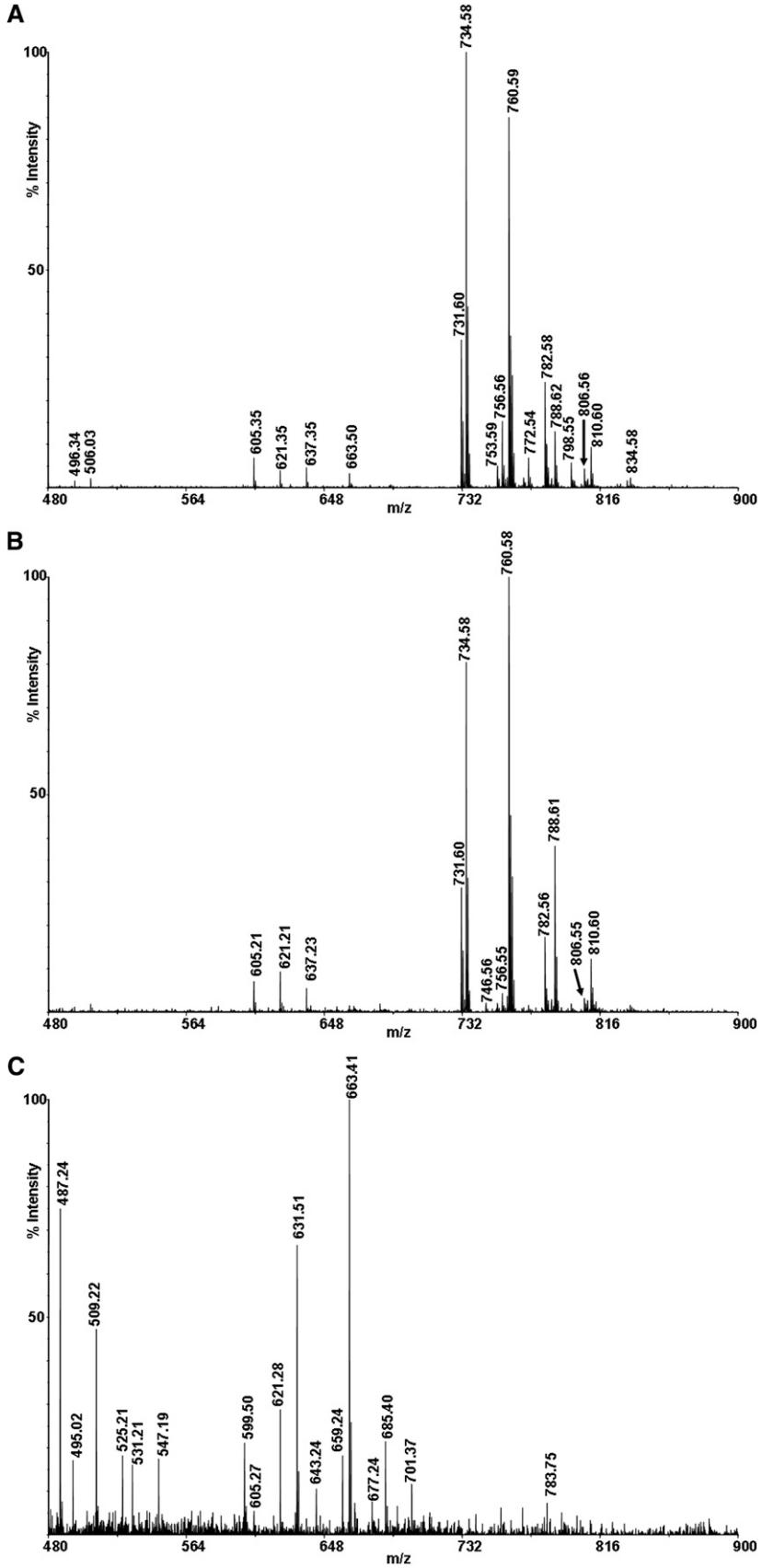

Fig. 3. MALDI-MS analysis of PC and SM species in the washing runoff of brain sections. A: The PC and SM species in the runoff of EtOH wash (from the section for Fig. 2A). B: The PC and SM species in the runoff of H_2O wash (from the section for Fig. 2B). C: The lack of PC and SM species in the runoff of NH_4Ac wash (from the section for Fig. 2C).

Figure 3B shows the PC and SM in the H_2O washing runoff of the section for Fig. 2B. It appears that several protonated PC species dominated the spectral profile. However, the collected runoff appeared slightly turbid. We were unable to prevent the emergence of such turbidity despite the most careful and gentle wash with H_2O. A brief centrifugation of the collected runoff produced a clear supernatant and a small amount of whitish sediment at the bottom of the container. It appeared that tiny fragments were sloughed off from the tissue section and contributed to the turbidity observed in the runoff even though the tissue section appeared intact after washing. Although the in situ lipid profile suggests that H_2O is a more effective tissue desalting fluid, high abundant PC and SM in the runoff and the formation of tissue debris by H_2O wash together have excluded H_2O as a suitable desalting fluid for the in situ lipid profiling studies.

Figure 3C shows the lipids in the NH_4Ac washing runoff of the section for Fig. 2C. It was noted that the overall spectral quality and the S/N ratio of the major peaks in Fig. 3C were far less desirable than those in Fig. 3A and B. It was likely due to the low ion abundance across the profiled mass range. However, unlike the previous results, no known PC or SM species were identified in Fig. 3C. This result and the tissue lipid profile in Fig. 2C strongly favor NH_4Ac as the suitable desalting fluid that effectively removes the sodium and potassium bound to the tissue lipids yet causes minimal or no displacement of the in situ PC or SM.

NH_4Ac desalting of infarcted rat brain section

To substantiate the desalting advantage of NH_4Ac, we profiled the cortical lipids in the infarcted rat brain section after it was washed with 1 ml of NH_4Ac. **Figure 4A** shows a typical profile of lipids in the infarcted rat cortex before NH_4Ac wash that is strongly biased toward the sodiated PCs and LPCs (7). It was difficult to discern the underlying changes of PCs by this profile due to the fact that the heightening of the signals for the sodiated PCs and the diminishing of the signals for the corresponding potassiated species were simultaneously shown in the profile. Figure 4B shows the typical lipid profile in the infarcted rat cortex from the NH_4Ac-washed section that was immediately adjacent to that of Fig. 4A. This profile clearly shows the increase in cortical LPC (O-16:0) (*m/z* 482.31), LPC (16:0) (*m/z* 496.34), LPC (18:1) (*m/z* 522.37), LPC (18:0) (*m/z* 524.38), LPC (20:4) (*m/z* 544.35), and LPC (22:6) (*m/z* 568.35) that is free from the interference of their alkalinated species. Figure 4B also reveals the stroke-mediated changes in the relative abundances of PC (34:1) (*m/z* 760.58), PC (36:4) (*m/z* 782.56), PC (38:6) (*m/z* 806.57), and PC (40:6) (*m/z* 834.59) in rat cortex. Upregulation of brain phospholipase A2 by ischemic stroke was previously associated with the reduction of PCs in the brain parenchyma (16, 17). Therefore, the profile of PCs in Fig. 4B nevertheless provides a snapshot of PCs remaining in the cortex after infarction. The runoff analysis of the section for Fig. 4B did not result in any identification of PCs or LPCs.

MS/MS of an isobaric PC in the infarcted cortex before and after NH_4Ac wash

To confirm the desalting effectiveness of NH_4Ac wash, we performed the MALDI-MS/MS analyses of an isobaric PC at *m/z* 810.60 from the infarcted cortex before and after the brain sections were washed with NH_4Ac. **Figure 5A** shows a typical MALDI-MS/MS result of the *m/z* 810.60 species before the section was washed with NH_4Ac. Theoretically, both the protonated PC (38:4) and the sodiated PC (36:1) could be isolated together as the precursor for the MS/MS analysis. Therefore, the MS/MS result is expected to show, among others, the diagnostic fragments that characterize the precursor as the sodiated PC. The moderately abundant *m/z* 751.50 fragment, an $[M-59]^+$ signature fragment for the sodiated PC precursor, and the two fragments at *m/z* 627.50 ($[M-183]^+$) and *m/z* 605.52 ($[M-205]^+$), plus the sodiated O,O'-dimethylenephosphoric acid ($[C_2H_5O_4PNa]^+$) at *m/z* 146.99 in Fig. 5A all supported the notion that the precursor contains a sodiated PC (18–21). The phosphocholine fragment (*m/z* 184.10) was generally regarded as the signature fragment of the protonated PC, although it could also be generated from the sodiated PC in MALDI-MS/MS. Nevertheless, we were surprised to see, even in low abundance, the potassiated O,O'-dimethylenephosphoric acid at *m/z* 162.98, from, presumably, a mixture of protonated and sodiated PC precursors. Other than the above-listed diagnostic fragments, several moderately abundant artifacts between *m/z* 630 and *m/z* 800 were also noted. This MS/MS result nevertheless shows the heterogeneity of the precursor in tissue before NH_4Ac wash. Figure 5B, on the other hand, illustrates a drastically different MS/MS result of the *m/z* 810.60 precursor from the infarcted cortex after the section was washed with NH_4Ac. The entire spectrum was dominated by the phosphocholine fragment (*m/z* 184.09). Although the $[M-183]^+$ fragment (*m/z* 627.53) was detected, it was relatively low in abundance. The $[M-59]^+$ ion, the $[M-205]^+$ ion, and the sodiated O,O'-dimethylenephosphoric acid ion mentioned above were no longer observed. Most of the artifacts between *m/z* 630 and *m/z* 800 in Fig. 5B were low abundant ions that together formed a different pattern from that in Fig. 5A. Together, the MS/MS profile in Fig. 5B clearly characterizes the precursor as a protonated PC and supports the notion of successful tissue desalting by NH_4Ac wash.

DISCUSSION

A desalting method suitable for direct profiling of tissue lipids not only effectively removes the alkali metal ions in tissue, it should also resolve the isobaric species caused by these endogenous ions. The use of such desalting method should also minimally disturb the tissue lipids and maintains the integrity of the tissue sections. In this study, we have demonstrated that NH_4Ac solution isotonic to the general mammalian physiological environment is best suited for the tissue desalting purpose among the three desalting fluids evaluated. The MS profile of the brain sections sufficiently washed with this solution shows a profile

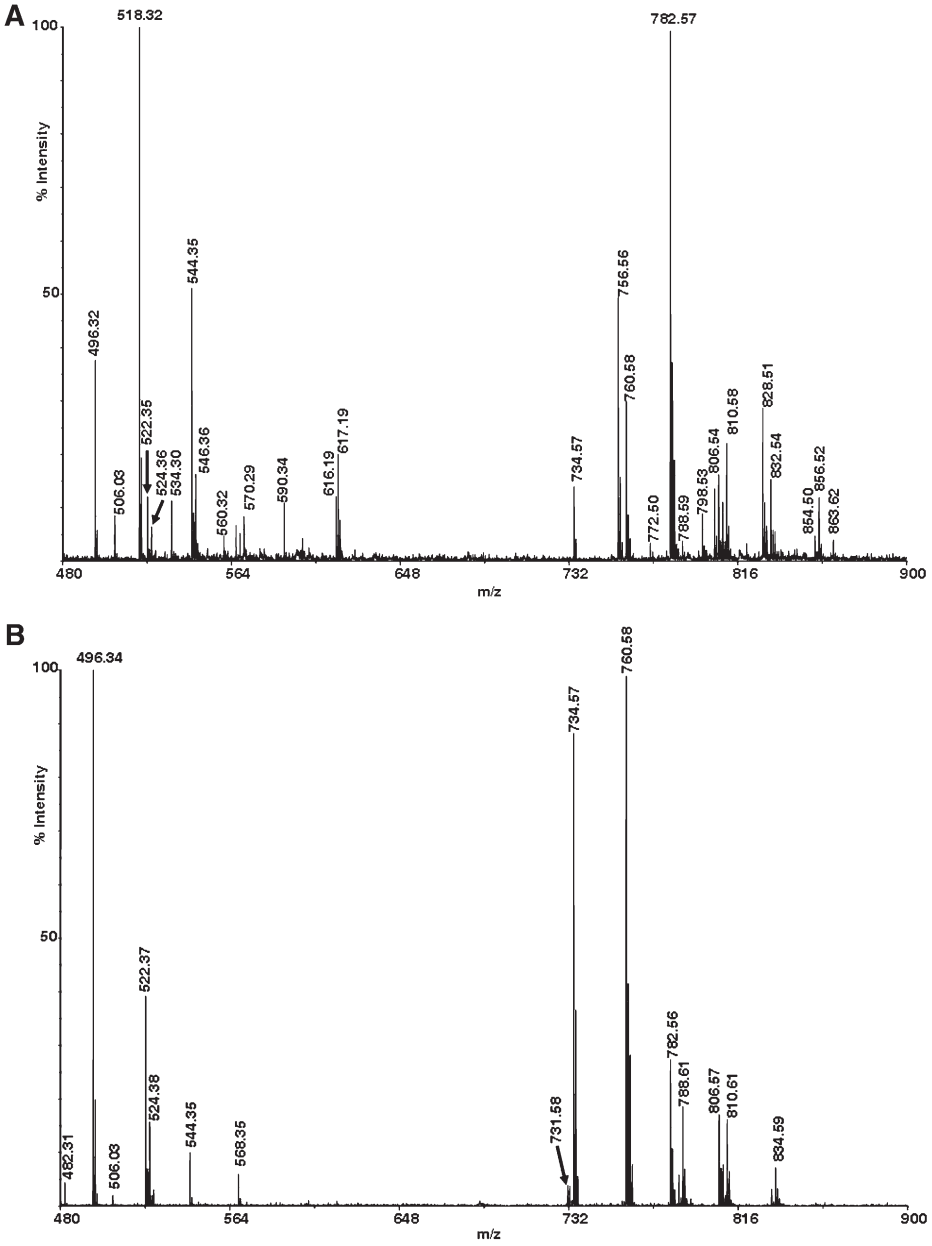

Fig. 4. MALDI-MS profiles of LPC, PC and SM species in the infarcted rat cortex. A: Profile of LPC, PC, and SM species in the infarcted rat brain section before it was washed with NH_4Ac. B: Profile of LPC, PC, and SM species in the infarcted rat brain section after it was washed with NH_4Ac.

of PCs and SMs devoid of potassiated and sodiated species. Analysis of its washing runoff reflects no detectable displacement of tissue PCs or SMs. This desalting wash not only simplifies the spectral profile of tissue PCs and SMs, it also improves the overall spectral quality and enhances the detection of low abundant LPC in the normal brain sections. With this desalting method, we are able to unveil the changes of brain PCs and SMs by ischemic stroke that would have been otherwise overwhelmed by the concurrent changes of tissue potassium and sodium due to edema in the infarcted area.

In mass spectrometry applications, solutions of volatile salts like NH_4Ac carry some advantages not found in other buffer salts commonly used in the biological laboratories. At a concentration isotonic to the physiological environment of the sample like that shown in this study, the ions in the solution maintain a proper osmolarity against the tissue section and conceivably compete with the endogenous ions in general for their effects on the biological molecules. This nature and the bulk removal of the displaced endogenous ions by wash will practically move the tissue alkali metal ions to the runoff. The residual volatile ions could then be easily eliminated from tissue under vacuum. In all our direct profiling of tissue lipids from sections washed with NH_4Ac, we have yet to detect any PC or SM species in their ammoniated forms, suggesting that the overall handling and processing of tissue section is adequate for the intended desalting purpose. Once the underlying lipid profile is revealed, the constituents of each PC species can be further revealed by MALDI-MS/MS with

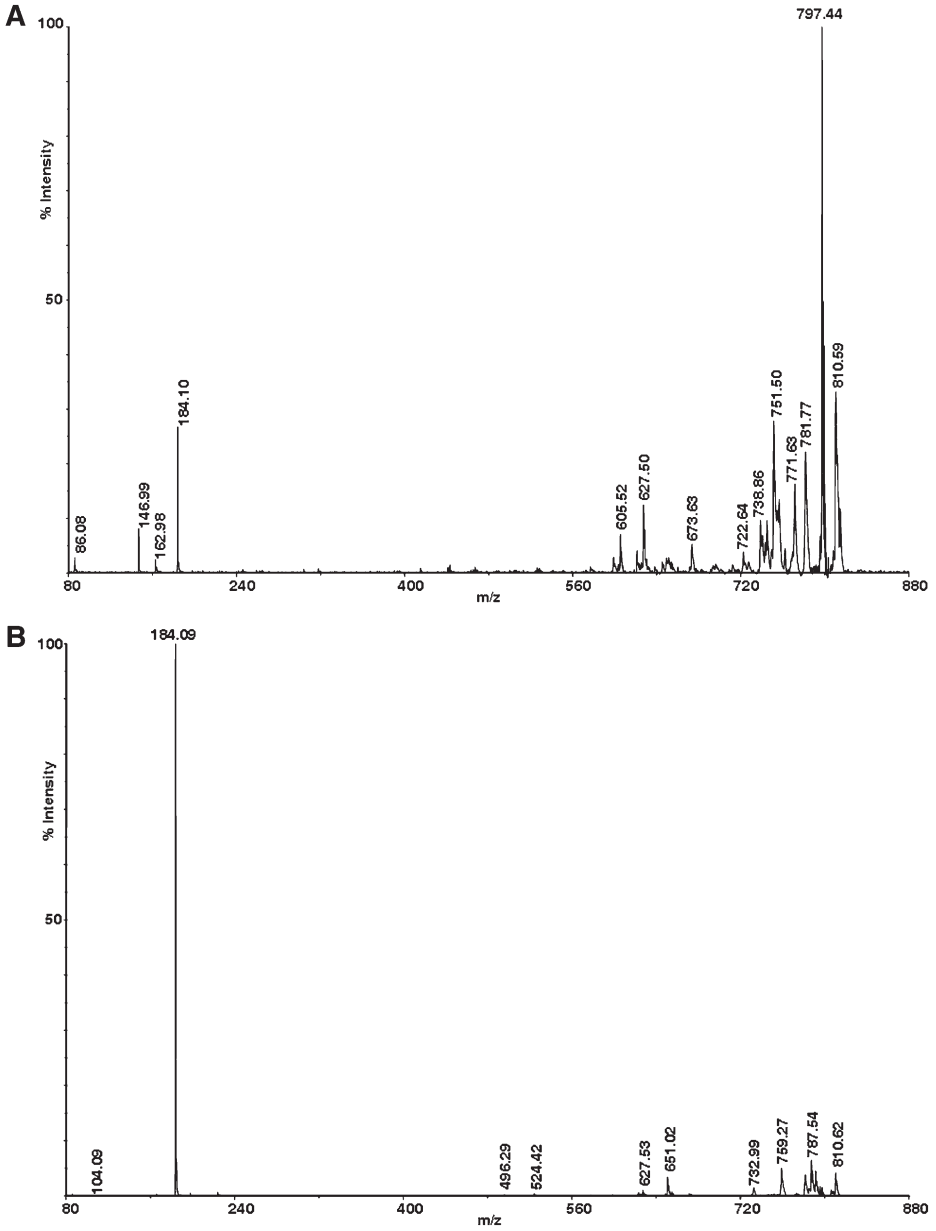

Fig. 5. MALDI-MS/MS of *m/z* 810.60 ion species from the infarcted rat cortex. A: MS/MS of *m/z* 810.60 species from the brain section before it was washed with NH_4Ac. B: MS/MS of *m/z* 810.60 species from the brain section after it was washed with NH_4Ac.

matrices containing exogenous alkali metal ions such as lithium or potassium.

In addition to NH_4Ac, we have also evaluated the desalting effectiveness of ammonium formate, another volatile salt regularly used in the mass spectrometry application. For brain sections washed with 1 ml of 150 mM ammonium formate (pH = 7.0), the lipid profiles in tissue section and in the runoff were essentially identical to those from sections desalted with NH_4Ac. Therefore, for the purpose of tissue desalting, these two ammonium salts appear practically interchangeable.

Other than the drip washing method used in this study, we have also tested two dip washing methods to desalt lipids in the normal rat brain sections. The sections were either completely immersed in a Coplin jar containing cold NH_4Ac for 30 s, or they were gently dipped three times of 10 s each in the cold NH_4Ac. Both dipping methods extensively desalted the tissue lipids. However, the potassiated PC 34:1 remained clearly visible after either dip washing (S/N ratio >7). When the dipping duration was extended, or the number of dippings was increased, the integrity of tissue section was always compromised. In lieu of such result, we did not pursue the desalting of the infarcted, spongiosis-prone rat brain sections with either dip washing method in fear of tissue loss during sample processing.

Among the sample processing methods used for the direct MS profiling or imaging of proteins and peptides in tissue, EtOH wash is generally considered a means of desalting and a way of fixation for sections cut from the

freshly frozen tissue blocks. In order to remove the lipid interference in tissue, sections are usually washed with more lipophilic solvents like hexane, chloroform, dichloromethane, acetonitrile, acetone, xylene, or the combination thereof, after the initial EtOH wash (9, 10, 22, 23). Due to its lack of efficiency, EtOH is seldom considered as a means of lipid removal in most of the proteomic studies. Although it is not our main purpose, the EtOH desalting results demonstrated in this study nevertheless agree with this notion. However, based on this conclusion, some trivial yet pertinent concerns were raised. First, in lieu of its desalting effectiveness, whether EtOH wash alone is an adequate desalting method for tissue proteomic studies may require further evaluation. Second, previous direct profiling studies of tissue PCs and SMs using MALDI matrices prepared in EtOH might have demonstrated the lipid profiles of inadvertently adulterated patterns of alkalination (1, 24, 25). Perhaps the more authoritative profiles of in situ PCs and SMs with their alkalination pattern should be obtained with sample processing methods that avoid the inadvertent interferences of any solvent (26).

Although H_2O appears as a more effective desalting fluid for tissue lipids than EtOH, its low osmolarity inadvertently ruptures cells (13) and disrupts the tissue integrity and subsequently results in sloughing of tissue debris. The disappearance of tissue SMs after H_2O wash is likely due to its slightly hydrophilic nature, which is evidenced by its slightly longer retention than PCs in the normal phase LC-MS/MS studies (27, 28).

Other than desalting tissue sections, we have also tested the same NH_4Ac solution for the removal of contaminants such as optimal cutting temperature (OCT) compound introduced during cryosections, or used it as a general means to enhance the ion signal for the in situ MS profiling. In both tests, the NH_4Ac solution delivered satisfactory results when the sections were carefully and sufficiently washed. We accidently discovered that incorporating this NH_4Ac wash into regular sample processing for other types of tissue profiling studies would often improve the S/N ratio in the subsequent mass spectrometry analysis. Therefore, this washing method may conceivably see further application in tissue-based studies and benefits in lipidomic, proteomic, and other studies of similar nature.

In conclusion, a simple, yet effective method to desalt the tissue sections with NH_4Ac solution that is suitable for direct mass spectrometry profiling of lipids in tissue has been demonstrated in this study. Tests of this desalting method on tissue subjected to pathological changes show the effectiveness and ruggedness of this method. The additional benefits in contaminant removal and signal enhancement by this method will conceivably benefit other tissue-based mass spectrometry studies in lipidomics and other "omics" type of research.

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