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Tandem MS and C_{60} SIMS for the identification and characterization of lipids

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The tandem MS capability of a C_{60}-Qstar hybrid instrument was utilized for the characterization of glycerophosphocholine (GPC) lipids in positive ion mode SIMS. In general, the tandem MS of lipids is facilitated by cationization or reaction with alkali metal salts. Thus, the lipids were treated with LiTFA salt (lithiation) prior to mass spectrometric analysis. As a result of the sample treatment, cationized lipid molecular ions with lithium adducts were formed and were revealed by mass shifts of 6 Da relative to unreacted species in the SIMS spectra. The lithiated molecular ions of GPCs were then subjected to collision-induced dissociation (CID) in tandem MS mode. Upon CID, fragments specifying the fatty acid and head group composition of the GPC lipid were generated and could be used for its structural characterization and identification. In addition, a more significant observation was revealed in the time-of-flight-SIMS spectral data of the lithiated GPC. Here, we found that fragmentation was enhanced, and fragment ions similar to those produced by CID were present in the SIMS spectra. The implications of these results are discussed.

Keywords: tandem MS; C_{60} SIMS; lipids; glycerophosphocholine; CID; LiTFA; lithiation; alkali metal salts; cationization

Introduction

SIMS is playing an increasingly important role in the direct profiling of lipids in cells and tissues. With its capacity for high spatial resolution chemical imaging and molecular depth profiling, SIMS is rendered to be a distinctive and powerful tool for lipid analysis. However, as it is primarily employed as an *in situ* mass spectrometry technique, there are some challenges to be noted. For instance, the direct identification and characterization of individual lipid species in biological samples without prior separation or purification can be problematic because of the sheer number and structural complexity of lipids. Coupling SIMS with tandem mass spectrometry (MS) is a potential avenue for overcoming such challenges.

Tandem MS by collision-induced dissociation enables the identification and structural determination of lipids.[1,2] In positive ion mode mass spectrometry, the tandem MS of lipids is facilitated by the addition of alkali metal salts to the sample.[3,4] Salts of sodium, potassium, or lithium have been used for the structural characterization and identification of specific classes of lipids including glycerophospholipids, sphingolipids, and fatty acyls.[3–8]

In this study, we utilize lithium salts for the C_{60}-SIMS and tandem MS characterization of glycerophosphocholine (GPC) lipids. The specific instrument configuration of the C_{60}-Qstar that combines the benefits of ionization from a C_{60}^+ cluster ion source and the tandem MS capabilities of a commercial triple quadrupole orthogonal time-of-flight (TOF) mass spectrometer was employed for this application.[9] The work described here expounds upon information presented in both the TOF-SIMS and tandem MS spectrum of the synthetic GPC lipid standard, [PC 16:0/16:0] or 1, 2-dipalmitoyl-glycero-3-phosphatidylcholine (DPPC).

Experimental

The lipid standard, 1, 2-dipalmitoyl-glycero-3-phosphatidylcholine (DPPC) (catalog number 770355), was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). Lithium trifluoroacetate (LiTFA), chloroform and acetonitrile with 0.1% formic acid, were purchased from Sigma-Aldrich (St. Louis, MO).

For the control experiment, the lipid standard was prepared at a concentration of 5–10 mg ml^{-1} in chloroform solvent. For the lithiation experiments, lipid standards were reacted in lithium trifluoroacetate (LiTFA) solution. The LiTFA solution was prepared at a concentration of 1–2 mg ml^{-1} and dissolved in acetonitrile/water/formic acid (70/30/0.1, v/v/v). Standards were then prepared at a concentration of 5–10 mg ml^{-1} in the LiTFA solution. For SIMS analysis, both control and lithiated standards were placed on silicon substrates by the dried droplet method, and a drop of glycerol matrix was pipetted on the surface of the sample to enhance signal ionization and improve secondary ion yields.

C_{60} SIMS and tandem MS spectra were obtained on a hybrid SIMS C_{60}-Qstar instrument in the positive ion mode. The 20 keV C_{60}^+ direct current ion beam was focused through a 300-μm size aperture to a beam size of approximately 20 μm and a current of 100 pA. For SIMS tandem MS mode,

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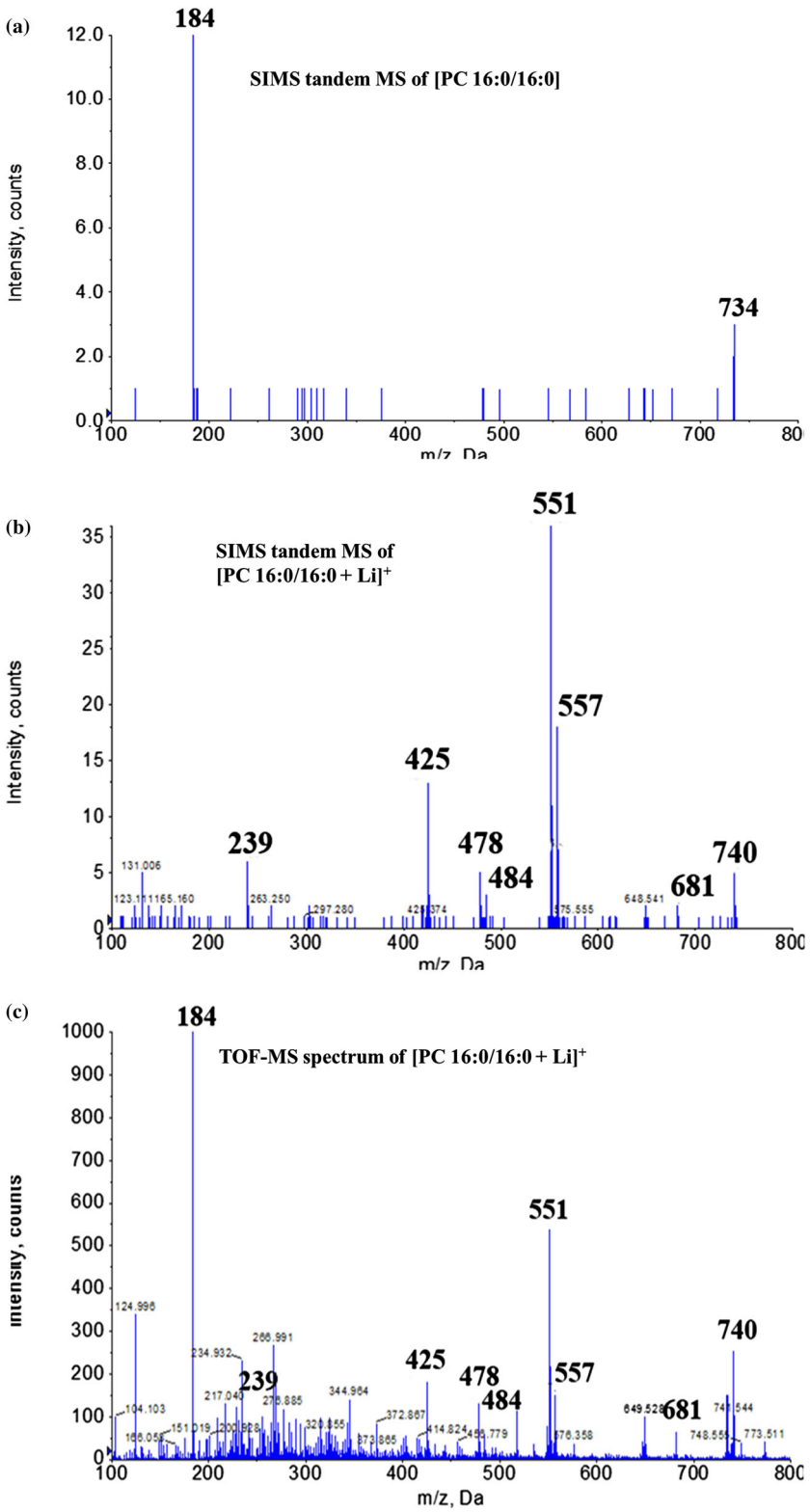

Figure 1. (a) Positive ion SIMS tandem mass spectrum of [PC 16 : 0/16 : 0], *m/z* 734, and (b) [PC 16 : 0/16 : 0 + Li]$^+$, *m/z* 740. (c) Positive ion TOF-MS spectrum of [PC 16 : 0/16 : 0 + Li]$^+$, *m/z* 740.

the precursor ion was selected with unit resolution in the Q1 mass filter. The collision energy used was 40 eV. Lipid fragmentation patterns observed in the product ion spectra were manually interpreted using reference tandem MS spectra found in the literature.

Results and discussion

The tandem MS of GPC lipids in positive ion mode mass spectrometry has predominantly been shown to generate headgroup fragments resulting in insufficient structural information. Treatment

with lithium salts enables the detection of both headgroup and fatty acid components of a GPC lipid, resulting in a more comprehensive structural characterization.[3,4] This detailed structural information for GPCs has primarily been obtained via collision-induced dissociation in the tandem MS of both electrospray ionization (ESI)[3,4,8] and matrix-assisted laser desorption ionization (MALDI).[10–13] In the present study, it was necessary to corroborate that the lithiation would be compatible with SIMS ionization and generate structurally relevant ions in the tandem mass spectra. As a starting point, the protocol was first developed with a synthetic GPC standard, [PC 16:0/16:0] or DPPC. For the lithiation experiments, LiTFA salt was used as it was found to produce the best results in terms of the number of relevant fragment ions generated in the tandem MS.[12]

The experimental protocol begins with a control set of experiments involving unreacted DPPC. Figure 1a illustrates the collision-induced dissociation (CID) of the $[M+H]^+$ ion of DPPC at *m/z* 734. The tandem mass spectrum of the protonated GPC reveals a prominent ion at *m/z* 184 indicative of the phosphocholine headgroup. However, identification of the fatty acid substituents is not possible as there are no other relevant ions in the tandem mass spectrum. The sample is then treated with LiTFA to reveal the lithiated adduct that exhibits a mass increase of 6 Da from its protonated counterpart resulting in *m/z* 740. Figure 1b illustrates the CID of *m/z* 740 that reveals additional ions that are representative of both the fatty acid and headgroup constituents of DPPC. Here, a more complex fragmentation pattern is observed. Table 1 lists all the fragments observed in the tandem MS spectrum in Fig. 1b. The fragment ions shown can be grouped to include those arising from loss of the headgroup and those reflective of fatty acid losses.

Headgroup moiety losses include the ion at *m/z* 681 that is consistent with loss of a trimethylamine to produce a fragment that is *m/z* 59 lower than the lithiated molecular ion. Another ion at *m/z* 425 reflects a combined loss of a trimethylamine plus one of the fatty acid constituents, palmitic acid. Other ions reflective of the headgroup include *m/z* 557 and 551, which are observed as a prominent pair of peaks separated by 6 Da in the tandem mass spectrum. This ion pair at *m/z* 557/551 is specific for DPPC and arises from the loss of phosphocholine (−183) and loss of the lithium salt of phosphocholine (−189), respectively. Such characteristic headgroup losses are indicative of a diagnostic fragmentation pattern that is unique to lipids containing a choline headgroup.

Table 1. Summary of fragments observed in the tandem mass spectrometry spectrum in Fig. 1b

Species	*m/z*
$([C_{15}H_{31}CO]^+)$	239
$([PC\ 16:0/16:0+Li]^+) - 59 - C_{15}H_{31}CO_2H$	425
$([PC\ 16:0/16:0+Li]^+) - C_{15}H_{31}CO_2Li$	478
$([PC\ 16:0/16:0+Li]^+) - C_{15}H_{31}CO_2H$	484
$([PC\ 16:0/16:0+Li]^+) - 189$	551
$([PC\ 16:0/16:0+Li]^+) - 183$	557
$([PC\ 16:0/16:0+Li]^+) - 59$	681
$[PC\ 16:0/16:0+Li]^+$	740

Apart from headgroup-related fragment ions, fatty acid information is obtained from ions at *m/z* 425, 478, and 484. The ion signals at *m/z* 484 and 478 correspond to the loss of palmitic acid and the lithium salt of palmitic acid, respectively. These fragments also appear as a pair of peaks separated by exactly 6 Da in the tandem mass spectrum. However, their signal intensities are much lower than those ions reflecting headgroup losses. Finally, the peak at *m/z* 239 represents the acyl ion from palmitic acid.

All fragment ions and peak assignments described and listed in Table 1 are in agreement with previous studies of lithiated DPPC obtained with tandem MS from both ESI[3,4] and MALDI.[12] The congruence of the tandem MS data between the different ionization modes is noteworthy as it implies that the available databases and spectral libraries of reference tandem MS data are equally accessible to lipid investigations involving SIMS as it is for other mass spectrometry methods.

While the SIMS tandem MS data is pertinent, a more significant observation is revealed in a comparison of the tandem MS spectrum to the C_{60} SIMS spectrum of lithiated DPPC shown in Figs 1b and 1c, respectively. Here, it is noted that all the structurally relevant and diagnostic ions identified in the tandem MS data are likewise present in the C_{60} SIMS spectrum. Prominent peaks in the TOF-MS spectrum include headgroup-associated fragment ions at *m/z* 184, 551, 557, and the lithiated molecular ion present at *m/z* 740. Less intense signals are observed for the fatty acid-related fragments at *m/z* 239, 425, 478, and 484. It is important to note that the ions at *m/z* 484, 557, and 740 resulted entirely from the lithium cationization and are not present in the C_{60} SIMS spectrum of the unreacted DPPC as illustrated in Fig. 2.

The observations described so far suggest that the nature of SIMS ionization is unique. The harder and more energetic SIMS-based ionization combined with the lithium cationization seems to generate a wider range of fragment ions similar to those found in tandem MS of lithiated lipids. This indicates that fragmentation processes occurring in SIMS sputtering and CID may be similar producing much the same ions in both the tandem MS and C_{60} SIMS spectra. Additional fundamental studies may provide insight into this aspect of SIMS. However, a more interesting aspect is the implication for lipid analysis and the opportunity to use these findings for the development of SIMS-based protocols targeted towards lipid characterizations and specific applications such as tissue imaging. In the case of lithiation, the resulting C_{60} SIMS spectrum is potentially information rich and could be used in GPC characterization studies. Because the ions that are generated resemble fragments produced by CID, the information obtained can be interpreted in a similar manner to tandem mass spectral data. For instance, it might be possible to identify a particular GPC solely based on prior knowledge of its characteristic and diagnostic fragmentation. In this regard, the identification of the specific GPC is not exclusively reliant on tandem MS, and the SIMS spectrum may by itself provide valuable information. Subsequently, the data obtained by C_{60} SIMS could be viewed as complementary to tandem MS. Thus, this approach may be particularly applicable to the initial examination of GPCs in the majority of TOF-SIMS instrumentation regardless of tandem MS capability.

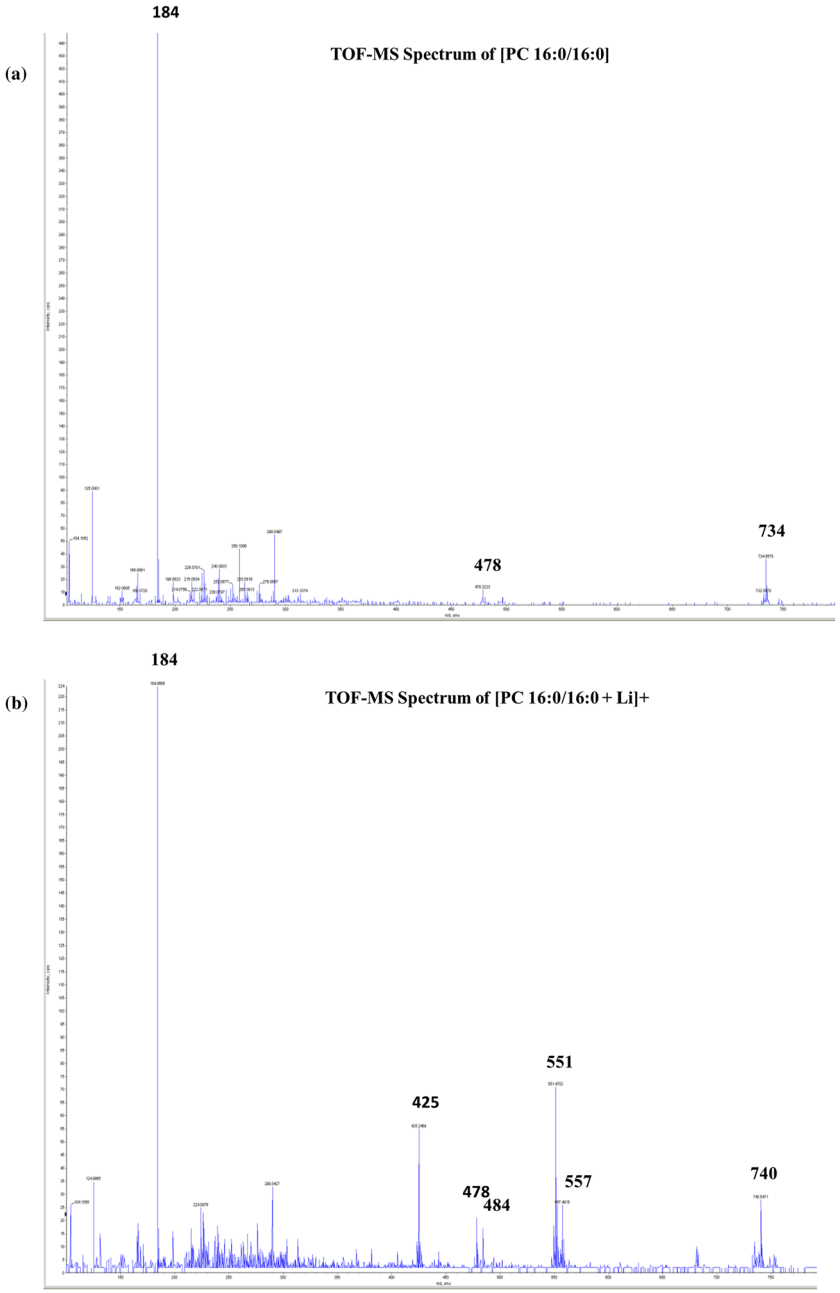

Figure 2. (a) Positive ion SIMS mass spectrum of [PC 16:0/16:0], *m/z* 734, and (b) [PC 16:0/16:0 + Li]$^+$, *m/z* 740.

Conclusion

An SIMS-based protocol has been successfully implemented for the characterization of lithiated GPC species. In this work, it was shown that the tandem MS of SIMS generates headgroup and fatty acid-related fragments that are useful in the structural characterization and identification of lithiated GPCs. We also discovered that similar ions were generated in the C_{60} SIMS spectrum of lithiated GPCs. Based on these findings, we propose specific applications for the SIMS analysis of lithiated lipids.

Acknowledgements

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