Published in final edited form as:

Anal Chem. 2017 September 05; 89(17): 9452–9458. doi:10.1021/acs.analchem.7b02271.

Trimethylation Enhancement Using ^{13}C -Diazomethane (^{13}C -TrEnDi): Gas-Phase Charge Inversion of Modified Phospholipid Cations for Enhanced Structural Characterization

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Abstract

Methylation of phospholipids (PL) leads to increased uniformity in positive electrospray ionization (ESI) efficiencies across the various PL sub-classes. This effect is realized in the approach referred to as “trimethylation enhancement using ^{13}C -diazomethane” (^{13}C -TrEnDi), which results in the methyl esterification of all acidic sites and the conversion of amines to quaternary ammonium sites. Collision induced dissociation (CID) of these cationic, modified lipids enables class identification by forming distinctive head-group fragments based on the number of ^{13}C atoms incorporated during derivatization. However, there are no distinctive fragment ions in positive mode that provide fatty acyl information for any of the modified lipids. Gas-phase ion/ion reactions of ^{13}C -TrEnDi-modified PE, PS, PC, and SM cations with dicarboxylate anions are shown to charge-invert the positively charged phospholipids to the negative mode. An electrostatically-bound complex anion is shown to fragment predominantly via a novel head-group dication transfer to the reagent anion. Fragmentation of the resulting anionic product yields fatty acyl information, in the case of the glycerophospholipids (PE, PS, and PC), via ester bond cleavage. Analogous information is obtained from modified SM lipid anions via amide bond cleavage. Fragmentation of the anions generated from charge inversion of the ^{13}C -TrEnDi modified phospholipids was also found to yield lipid class information without having to perform CID in positive mode. The combination of ^{13}C -TrEnDi-modification of lipid mixtures with charge inversion to the negative ion mode retains the advantages of uniform ionization efficiency in the positive ion mode with the additional structural information available in the negative ion mode without requiring the lipids to be ionized directly in both ionization modes.

TOC image

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INTRODUCTION

Phospholipids (PL) are associated with eukaryotic cell structure, signaling, and energy storage^{1,2,3,4,5} and constitute a complex mixture of heterogeneous molecules that contain a polar phosphate head group.^{3,4,6,7,8,9} PL classes are determined based on the functionality attached to the phosphate head group. The identities and abundances of PLs are dynamic and reflect *inter alia* the state and progression of lipid-related disease. Mass spectrometry (MS), due to its high specificity, is often used to characterize cellular PL composition and electrospray ionization (ESI) is a popular approach for generating PL ions. Shotgun lipidomics, which involves the application of ESI-MS to PL mixtures without the use of chromatography, is a high-throughput technique for PL mixture analysis.^{5, 9,10,11,12} However, ionization efficiency is highly variable for the various PL classes. Sphingomyelin (SM) and phosphatidylcholine (PC) ionize particularly efficiently in positive mode due to the presence of a quaternary ammonium in their phosphocholine head groups. Phosphatidylethanolamine (PE) and phosphatidylserine (PS) ionize moderately well in positive mode due to the basicity of the primary amine but can also ionize via deprotonation in negative mode under basic solution conditions. Relative to PC and SM cations, however, PE and PS are more prone to suffer from ionization suppression in positive mode.

Ideally, a method would allow for the efficient and comparable detection of SM, PC, PE, and PS lipids in the same ionization polarity. Solution additives have been used in the negative ESI of these PL classes to provide a one-shot method as well as structurally informative fragmentation via tandem mass spectrometry. Acetate or formate salts, for example, have been used to enhance the ionization efficiencies of SM and PC lipids in the negative mode since collision induced dissociation (CID) of anionic PL species provides structurally informative fragments and simple tandem mass spectra.^{5,12,13,14} However, these gas-phase adducts often have low stability and can inadvertently form isobaric species when applied to complex mixtures.^{15,16,17,18} An alternative approach has been to derivatize PLs prior to ionization both to improve the ionization responses of PE and PS lipids in the positive mode^{2,19} and to facilitate the distinction of isobaric species^{10,12,16}. One such derivatization technique is trimethylation enhancement using ¹³C-diazomethane (¹³C-TrEnDi) modification. TrEnDi covalently modifies carboxylate, phosphate, and primary amine functional groups via ¹³C-methylation. Primary amines are therefore converted to quaternary amines, which allows for the formation of a fixed-charge functionality on the head group of PEs and PSs lipids.^{6,7} The fixed-charge allows for uniform ionization via ESI of SM, PC, PE, and PS in the positive mode. In addition, ¹³C-TrEnDi provides distinction between PE and PC/SM lipid head groups by adding four ¹³C-methyl groups to PE and one ¹³C-methyl group to PC/SM. Thus, the head group cation formed via CID of the ¹³C-TrEnDi-modified PE versus ¹³C-TrEnDi-modified PC/SM cations are 202 Da and 199 Da in mass, respectively.⁷ Furthermore, ¹³C-methylation of the phosphate group reduces metal ion

adduction, thereby minimizing the distribution of analyte signal among peaks with different counter-cations.^{8,11,20,21}

¹³C-TrEnDi has been shown to provide enhancement in the sensitive and selective identification of PL class, number of carbons, and degree of unsaturation in the positive ion mode. However, it does not readily allow for the identification of fatty acyl composition via CID. Furthermore, the methylation of the acidic sites on PLs inhibits negative ion formation via anion adduct attachment. Charge inversion through gas-phase ion/ion reactions has been shown to be efficient and useful in terms of probing PL structure. For example, PC and PE cations have been shown to react in the gas-phase with dicarboxylate anions to form a negatively-charged, electrostatically-bound complexes that can be further probed via CID to determine the identity and regiochemistry of fatty acyl groups.^{22, 23} Unmodified PC cations undergo charge inversion via combined methyl cation transfer and proton transfer whereas unmodified PE cations undergo charge inversion via double proton transfer. Hence, the charge inversion process was shown to separate isomeric PE and PC cations. This method has also been shown to increase the sensitivity of shotgun lipidomics analyses of PEs and PCs as charge inversion allows for a significant decrease in chemical noise found when analyzing crude mixtures.

This work was motivated by the possibility for combining the advantages of ¹³C-TrEnDi-modification of PLs for uniform cation generation with gas-phase charge inversion to enable enhanced structural characterization via anion CID. We show here that novel charge inversion chemistry occurs for each of the ¹³C-TrEnDi-modified PL classes using a dicarboxylate reagent anion and that the resulting anions yield the desired fatty acyl composition information upon CID. This charge inversion approach is characterized for ¹³C-TrEnDi-modified PL standards and for their unmodified counterparts. The method was applied to cations obtained from nano-ESI of ¹³C-TrEnDi-modified HeLa cell extracts in order to show the relatively uniform efficiency of gas-phase charge inversion of the methylated lipids and separation of isobaric species in the complex mixture.

EXPERIMENTAL SECTION

Materials

Methanol, ethanol, ammonium hydroxide, and acetonitrile were obtained from Fisher Scientific (Pittsburgh, PA). 1,4-phenylenedipropionic acid (PDPA) was purchased from Sigma-Aldrich (St. Louis, MO). A solution of ~220 μ M PDPA was made in 49.5/49.5/1 (v/v/v) acetonitrile/methanol/ammonium hydroxide. The phospholipid standards used were: 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (POPS), and N-palmitoyl-D-erythro-sphingosylphosphorylcholine. All standards were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). TrEnDi-modified PL running solutions were made in ethanol at 1–2.5 μ M. Unmodified PL running solutions were made in ethanol at 2–15 μ M. Shorthand phospholipid nomenclature was adopted from Liebisch *et al.*²⁴ and is described briefly in Supplemental Information.

¹³C-TrEnDi Modification

Standard samples were derivatized as previously published.^{6,7} Each ¹³C-TrEnDi-modified extract and unmodified counterpart was shipped under N₂ gas and on dry ice to West Lafayette, Indiana, USA. Dried samples were reconstituted in ethanol for analysis.

Mass Spectrometry

All experiments were performed on a TripleTOF 5600 triple quadrupole/time of flight mass spectrometer (SCIEX, Concord, ON, Canada) modified to perform ion/ion reactions similarly to modifications previously made on an AB SCIEX Q-STAR quadrupole/time of flight mass spectrometer.²⁵ tmPL cations and PDPA dianions were alternatively pulsed via nano-electrospray ionization (nESI) emitters. tmPL solutions were sprayed first for 100–500 ms. tmPL cations were then mass selected in transit in Q1, and stored in the q2 reaction cell. The PDPA solution was then sprayed for 50–100 ms via negative nESI. [PDPA-2H]^{2–} ions were isolated in transit in Q1, then transferred to q2. Ions were stored for 5–30 ms. Isolation of product ions was performed in q2 by application of a high amplitude excitation at the resonant frequency of the unwanted ions. Complex ions were fragmented via CID using a single frequency resonance excitation at a q-value of 0.2 to ensure a stable trajectory for fatty acyl fragment ions. Second generation fragment ions were fragmented using a second resonance excitation event, where the RF frequency was changed to ensure the ion of interest was placed at a q-value of 0.15.

RESULTS AND DISCUSSION

Charge Inversion of ¹³C-TrEnDi-modified Glycerophospholipids

Sensitivity and structural elucidation of glycerophospholipids via ESI-MS has been previously shown to be enhanced from in-solution modification.^{16,19} TrEnDi-modification, for instance, has been shown to increase sensitivity of PE and PS lipids by producing a fixed charge moiety and phosphate ester to consolidate the signal of any given glycerophospholipid.^{6,7} ¹³C-TrEnDi-modification of glycerophospholipids involves the ¹³C-methylation of primary amines and acidic moieties. Scheme S-1 shows unmodified, generic glycerophospholipids and sphingomyelin with their ¹³C-TrEnDi-modified counterparts. The number of ¹³C additions is determined by the addition of methyl groups. For instance, PCs only have one site of modification, the phosphate group, and thus contain only one ¹³C, while PEs and PSs undergo four and five ¹³C-methyl additions, respectively. Ion-trap CID of ¹³C-TrEnDi-modified phospholipids (tmPL) provides for selective identification of the modified lipid head group. However, there is little information relating to the fatty acyl chains attached to those head groups. Positive nESI of standard solutions of tmPC, tmPE, or tmPS resulted in a dominant cationic peak (Supplemental Figure S1(a–c)) and CID of the cations, [tmPC]⁺, [tmPE]⁺, and [tmPS]⁺, yields ¹³C-TrEnDi-modified head group cations at *m/z* 199, 202, and 260, respectively (Supplemental Figure S1d–f). Structurally informative peaks pertaining to the fatty acyl chains attached to the glycerol backbone are known to be obtained from CID of the negatively charged glycerophospholipids.^{13,14,15,19,20,22,23,24} Conventionally, solution additives, such as ammonium acetate can be used to generate adduct anions in the negative mode. A single acetate adduction at the quaternary ammonium site neutralizes the ¹³C-TrEnDi-modified species and there are no other sites for the

adduction of a second acetate. Negative nESI of a tmPL solution containing ammonium acetate, therefore, does not result in a $[\text{tmPL} + 2\text{CH}_3\text{COO}]^-$ ion.

Gas-phase ion/ion reactions can be used to charge invert the tmPC, tmPE, tmPS, and tmSM cations to the negative ion mode. Figure 1 shows the post-ion/ion reaction product ion spectrum for cations of each tmPL class with PDPA dianions. In each case, a fragile complex is generated that fragments into a major product. Scheme 1 summarizes the reaction of a generic ^{13}C -TrEnDi-modified glycerophospholipid cation with $[\text{PDPA} - 2\text{H}]^{2-}$ in the gas-phase resulting in an electrostatically bound complex of the form $[\text{tmPL} + \text{PDPA} - 2\text{H}]^-$. A double cation transfer process leads to charge inversion of the lipid. The dominant process involves the transfer of the head group as a dication to the PDPA dianion producing a negatively charged, ^{13}C -methylated phosphatidic acid (^{13}C -mePA). This process is consistent with previous work showing that gas-phase reactions of onium cations and carboxylates results in favorable transfer of large alkyl cations compared to methyl cation transfer.²⁶ In Scheme 1, it is indicated that a methyl cation from the quaternary ammonium group neutralizes one of the carboxylate groups of PDPA and an alkyl cation from the remainder of the head group neutralizes the other PDPA carboxylate group thereby generating a neutral diester. A minor product is also observed in Figures 1a and 1b from a dication transfer process involving two methyl cation transfers from the $[(\text{tmPC}/\text{tmPE} 16:0/18:1) + \text{PDPA} - 2\text{H}]^-$ complexes. However, this minor process is not observed in Figure 1c, which summarizes the reaction between $[(\text{tmPS} 16:0/18:1)]^+$ and $[\text{PDPA} - 2\text{H}]^{2-}$. In the case of the tmPE cation, two ^{13}C -methyl groups are transferred to generate a $[\text{tmPE} - 2(^{13}\text{CH}_3)]^-$ species whereas the tmPC cation transfers a ^{13}C -methyl group and a ^{12}C -methyl group to form the $[\text{tmPC} - ^{13}\text{CH}_3 - \text{CH}_3]^-$ product ion. The products were confirmed to originate from fragmentation of the complex for the $[(\text{tmPC}/\text{tmPE} 16:0/18:1) + \text{PDPA} - 2\text{H}]^-$ ions via isolation and subsequent CID of the complex found in each reaction spectrum (Supplemental Figure S-2). There was so little signal from the complex $[(\text{tmPS} 16:0/18:1) + \text{PDPA} - 2\text{H}]^-$ peak remaining in Figure 1c that an analogous experiment could not be performed. Signals due to $[\text{PDPA} - 2\text{H} + ^{13}\text{CH}_3]^-$ arising from single methyl cation transfer, which is the major competing neutralization pathway, are highlighted in Figure 1 primarily to indicate how little this process occurs for each of these tmPL cation classes. The peaks labelled with a circle (●) arise from ion/molecule reactions during the between the highly abundant PDPA reagent dianions and adventitious neutral species during the anion accumulation and mutual storage times.

Mass differences between the ^{13}C -mePA product anion and the complex can be used to identify ^{13}C -TrEnDi-modified glycerophospholipid classes in the gas-phase. Figure 1a shows a mass difference of 307 Da between the complex and the major product ion, which is indicative of a tmPC and, based on the m/z of the cation and/or complex peak, the standard can be assigned as tmPC 34:1. The class data can be obtained in similar fashion from the reactivity of tmPE in Figure 1b, with a mass shift of 310 Da from the complex. The mass measurement of the cation or complex anion can then be used to determine the ions to arise from tmPE 34:1. Figure 1c provides similar information for tmPS where the mass shift is 369 Da and the mass measurement determines the peak to be due to tmPS 34:1. This is information that could be deduced from fragmentation in the positive mode. However, the gas-phase charge inversion process allows for further structural information to be obtained.

CID of either dication transfer product originating from fragmentation of the complex results in fatty acyl information, (see Scheme 2). The CID product ion spectrum of $[(\text{tmPC } 16:0/18:1) - (\text{CH}_3)_3\text{NCH}_2\text{CH}_2]^-$, the major product formed from the gas-phase reaction of $[(\text{tmPC } 16:0/18:1)]^+$ and $[\text{PDPA} - 2\text{H}]^{2-}$, is shown in Figure 2a. The spectrum contains dominant fragments of the fatty acyl product anions. The product ion masses indicate that the fatty acyl chains on this tmPC standard are 16:0 and 18:1, thus allowing for the identification of the standard to be tmPC 16:0_18:1. Their abundance ratio confirms their glycerol chain position, C18:1, having the highest abundance, is on the sn2 position, while C16:0 is on the sn1 position, further characterizing the lipid as tmPC 16:0/18:1. Figures 2b and 2c provide similar information for tmPE and tmPS, respectively. CID of their major head group transfer products obtained post-ion/ion reaction provide fatty acyl fragment anions for C16:1 and C18:1. The ratio of the fatty acyl fragments confirm the identities of the standards as tmPE 16:0/18:1 and tmPS 16:0/18:1, respectively. This information can also be obtained for tmPC and tmPE via CID of the minor products formed post ion/ion reaction of $[\text{tmPC/tmPE}]^+$ with PDPA dianions (Supplemental Figure S-3).

Charge Inversion of ^{13}C -TrEnDi-modified Spingomyelin (tmSM)

^{13}C -TrEnDi modification of SM results in two derivatized products, the ^{13}C -methylation of the phosphate group (tmSM), and, to a lesser extent, the ^{13}C -methylation of both the hydroxyl group on the sphingoid base and the phosphate group (tm*SM). The positive nESI of the ^{13}C -TrEnDi modified SM d18:1/16:0 standard solution shows both modification products (Supplemental Figure S-4a). Fragmentation of the tmSM d18:1/16:0 and tm*SM d18:1/16:0 cations are shown in Supplemental Figures S-4b and c, respectively. In both cases, CID of the precursor cation predominantly forms the ^{13}C -methyl-esterified phosphate head group cation with little structural information. There is also no evidence of an unmodified SM head group, which suggests that tmSM d18:1/16:0 is dominated by ^{13}C -methylation of the phosphate group with very little or no sole methylation of the hydroxyl group of the sphingoid base. The post-ion/ion reaction product ion spectrum of the tmSM d18:1/16:0 cation with PDPA dianion is shown in Figure 3a. Similar to the glycerophospholipid reactivity noted above, a complex (viz., $[(\text{tmSM d18:1/16:0}) + \text{PDPA} - 2\text{H}]^-$) is observed along with evidence of fragmentation of the complex via head group transfer to give the product $[(\text{tmSM d18:1/16:0}) - (\text{CH}_3)_3\text{NCH}_2\text{CH}_2]^-$, which is a ^{13}C -methylated ceramide-1-phosphate (^{13}C -meC1P) anionic product. Evidence for double methyl cation transfer in the form of one ^{12}C -methyl transfer and one ^{13}C -methyl transfer to PDPA ($[(\text{tmSM d18:1/16:0}) - ^{13}\text{CH}_3 - \text{CH}_3]^-$) is also observed as a minor process. Proposed structures of these products can be found in Supplemental Scheme S-2. The neutralization pathways, the formation of $[\text{PDPA} - 2\text{H} + \text{CH}_3]^-$ and $[\text{PDPA} - 2\text{H} + ^{13}\text{CH}_3]^-$ were also observed to be negligible. $[(\text{tmSM d18:1/16:0}) - (\text{CH}_3)_3\text{NCH}_2\text{CH}_2]^-$ and $[(\text{tmSM d18:1/16:0}) - ^{13}\text{CH}_3 - \text{CH}_3]^-$ are products formed from fragmentation of the complex (Supplemental Figure S-5). The mass difference between the major product ion and the complex is 307 Da, the same as tmPC above. In order to distinguish between tmSM and tmPC, the nominal mass of the precursor cation or the complex must be determined to be even or odd, respectively. Note that this is the opposite for their unmodified counterparts. For instance, the even nominal mass of the complex in Figure 3a (i.e., 938 Da) confirms that the peak is tmSM 34:1. Figure 3b depicts the fragmentation of $[(\text{tmSM d18:1/16:0}) -$

$(\text{CH}_3)_3\text{NCH}_2\text{CH}_2]^-$, which results in amide cleavage confirming the standard to be tmSM d18:1/16:0. Isolation and CID of the minor product (see Supplemental Figure S-6b) results in a dominant peak for the demethylated phosphocholine head group anion and cleavage of the amide bond resulting in a demethylated sphingosine-1-phosphocholine anion, providing the same information. Proposed structures for these fragments are provided in Supplemental Scheme S-3. Comparing the CID of the products, $[(\text{tmSM d18:1/16:0}) - (\text{CH}_3)_3\text{NCH}_2\text{CH}_2]^-$ and $[(\text{tmSM d18:1/16:0}) - ^{13}\text{CH}_3 - \text{CH}_3]^-$, shows that CID of the major product lacks a head group fragment ion because the trimethylethanaminium functionality has transferred to the PDPA dianion and thus the head group is absent from the precursor ion (Supplemental Figure S-6). Analysis of the low-abundance derivatization product tm*SM d18:1/16:0 undergoes a similar reaction, providing the same information, see Supplemental Figure S-7 and S-8.

Analysis of ^{13}C -TrEnDi-modified HeLa Cell Extract Cations via Gas-Phase Charge Inversion

Both modified and unmodified PL (i.e., tmPL and PL) cations have been shown to undergo gas-phase charge inversion in reactions with doubly-deprotonated PDPA, although largely by different mechanisms. The anions generated from the PL and tmPL cations from the various standards were found to provide the same fatty acyl chain information (Supplemental Figures S-7 to S-10). The methylation process converts PC, PE, and SM lipids to a common head-group and PS lipids to a head-group with a carboxylate methyl ester. For all modified PLs, transfer of the head-group as a dication is the major charge inversion process. Minimal evidence (much less than 1% relative abundance) for single methyl cation transfer (as reflected by $[\text{PDPA-2H}+\text{CH}_3]^-$) was evident in the post-ion/ion reaction data (see Figure 1 and Figure 3a), which suggests that little neutralization of the tmPL cations takes place in ion/ion reactions with PDPA dianions. The ^{13}C -TrEnDi-modification strategy is intended to equalize ionization response for the various PL classes and, for similar reasons, likely tends to equalize charge inversion efficiency.

A HeLa cell extract was modified in solution via ^{13}C -TrEnDi, reconstituted in ethanol, and subjected to nESI in the positive mode resulting the spectrum, in blue, in Figure 4. The cationic distribution is that of the ^{13}C -TrEnDi-modified phospholipids (tmPLs), of the form $[\text{tmPL}]^+$, found in the extract. The entire population was charge inverted by reacting the cations with PDPA dianions. The ion/ion reaction product ion spectrum, insert on the bottom spectrum, shows two dominant distributions, viz., the complexes $[(\text{tmPL} + \text{PDPA} - 2\text{H})^-]$ and partial head group transfer products $([(^{13}\text{C-mePA} - \text{H})^-]/[^{13}\text{C-meC1P} - \text{H})^-])$. The butterfly plot comparing the distribution obtained via positive nESI (blue) versus the head group transfer distribution (red) shows that the relative abundances observed in the positive ion data are reproduced with good fidelity in the charge inversion data. The two spectra are aligned by shifting the anion spectrum up by 87 Da, which is the mass of the head-group loss associated with PCs, the most abundant PL class in the mixture. A similar plot was made comparing the $[\text{tmPL}]^+$ distribution and the $[(\text{tmPL} + \text{PDPA} - 2\text{H})^-]$ distribution in Supplemental Figure S-13 using the mass shift associated with the addition of the PDPA dianion, which also shows very similar relative abundances in the cation and charge inversion data. Signal-to-noise calculations further confirm that ion/ion reaction results in an increase in signal-to-noise ratio of roughly a factor of 3. For instance, the largest peak [tmPL

775]⁺ at m/z 775.671 in the positive nESI spectrum has a signal-to-noise of 5900. In the post ion/ion reaction spectrum the tmPL 775 ion predominantly results in [(tmPL 775) + PDPA – 2H][–] at m/z 995.719 and [¹³C-mePA 775 – H][–] at m/z 688.526, which have a combined signal-to-noise ratio of 16100.

The most effective way to deal with mixtures of isobaric and isomeric components from different PL classes is to perform an isolation step in the positive mode and to interrogate the charge inversion products via CID. An example of this process is provided here. In positive mode, two peaks (see Supplemental Figure S-14a) at m/z 828.744 and m/z 828.814 were observed within the m/z 828–829 window. Fragmentation of the cations in this window (Supplemental Figure S-14b) identified the tmPE 38:6 and tmSM 42:2 species as comprising these peaks, respectively. More information can be obtained using gas-phase charge inversion. Figure 5a shows the most abundant products formed via ion/ion reactions of the cations in the isolation window at m/z 828.7 ($\pm$ 0.2) reacted with [PDPA – 2H]^{2–}. A low abundance complex [(tmPL 828) + PDPA – 2H][–] can be seen at m/z 1048.7. Classes can be separated post-ion/ion reaction following partial head group loss. The class of any given peak can be deduced based on the mass difference between head group transfer signal and that of the electrostatically-bound complex. Mass differences from the complex of 369, 310, or 307 Da correlate with tmPS, tmPE, or tmSM/tmPC, respectively. For the tmPL cations at nominal m/z 828, the charge inversion results (Figure 5a) show that there are two dominant peaks that are 310 Da and 307 Da from the intact complex, which enables the identification of the species in the original cationic isolation window to be tmPE 38:6 and tmSM 42:2, respectively. The tmSM is confirmed by the fact that the cation and complex are of even m/z . The head group transfer anions can be further interrogated via CID. To increase the intensities of the head group transfer peaks, the complex ions were fragmented using a single frequency CID with a high enough amplitude to fragment both complex ions. Another waveform was then used to perform CID of the [tmPE 38:4 – (¹³CH₃)₃NCH₂CH₂][–] ion. The product ion spectrum in Figure 5b shows the product ions of fatty acyl anions C18:0 and C20:4, which identifies the lipid component as tmPE 18:0_20:4. The ratio of the fatty acyl peak intensities further identifies the peak as being predominantly composed of the tmPE 18:0/20:4 species. Alternatively, Figure 5c shows the CID spectrum of the tmSM 42:2 partial head group transfer anion, which results in a peak at m/z 393, which arises from the cleavage of the amide bond resulting in a ¹³C-methylated sphingosine-1-phosphate anion. This fragment is indicative of the sphingoid base on tmSM 42:2 which is d18:1. Therefore, the lipid can be identified as tmSM d18:1/24:1.

CONCLUSIONS

¹³C-TrEnDi modification has been already been shown to lead to uniform ionization efficiencies for various PL classes.^{6,7} Fragmentation of tmPLs in positive mode results in a dominant head group product that facilitates PL class identification. However, only limited information regarding fatty acyl chain composition can be obtained from the cations. tmPL cations undergo charge inversion to the negative mode via gas-phase ion/ion reactions with PDPA dianions. Upon CID, the anions that result from head-group dication transfer to the reagent dianion provide structural information that includes the fatty acyl chain lengths, degrees of unsaturation on each chain, and their locations along the glycerol backbone.

Charge inversion of unmodified PL cations takes place via either double proton transfer (e.g., PEs) or combined proton and methyl cation transfer (e.g., PCs) but, despite these different mechanisms, the resulting anions provide the same fatty acyl chain structural information. The charge inversion method was applied to cationic peaks from a ^{13}C -TrEnDi modified HeLa cell extract showing that isobars can be separated based on the mass difference of the partial head group transfer from the electrostatically bound complex and that detailed fatty acyl chain information could be obtained from the respective anions generated from the head group transfer. Overall, this work demonstrates that the advantages of ^{13}C -TrEnDi modification of PC, PS, PE, and SM lipids in positive ion mode can be combined with the desirable structural characterization capability afforded by anion CID via the use of gas-phase charge inversion chemistry.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by the National Institutes of Health under Grant GM R37-45372 and Sciex. JCS acknowledges support from the National Science and Engineering Council of Canada (NSERC).

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Figure 1.

Ion/ion reaction between the reagent dianion $[PDPA - 2H]^{2-}$ and a) ^{13}C -TrEnDi-modified PC 16:0/18:1 ions $[(tmPC\ 16:0/18:1)]^+$, b) ^{13}C -TrEnDi-modified PE 16:0/18:1 ions $[(tmPE\ 16:0/18:1)]^+$, and c) ^{13}C -TrEnDi-modified PS 16:0/18:1 ions $[(tmPS\ 16:0/18:1)]^+$. Circles (●) indicate ions that were present prior to cation injection.

Figure 2.

Ion trap CID of a) $[(\text{tmPC } 16:0/18:1) - (\text{CH}_3)_3\text{NCH}_2\text{CH}_2]^-$, b) $[(\text{tmPE } 16:0/18:1) - (^{13}\text{CH}_3)_3\text{NCH}_2\text{CH}_2]^-$, and c) $[(\text{tmPS } 16:0/18:1) - (^{13}\text{CH}_3)_3\text{NCH}(\text{COO}^{13}\text{CH}_3)\text{CH}_2]^-$. Lightning bolt (⚡) signifies activated ions.

Figure 3.

Ion/ion reaction between the ^{13}C -TrEnDi-modified SM d18:1/16:0 ions [(tmSM d18:1/16:0)]⁺ and the reagent dianion [PDPA – 2H]²⁻. b) Ion trap CID of isolated [(tmSM d18:1/16:0) – (CH₃)₃NCH₂CH₂]⁻ resulting from ion trap CID of isolated electrostatically-bound complex, [(tmSM d18:1/16:0) + PDPA – 2H]⁻. Lightning bolt (⚡) signifies activated ions. Circles (●) indicate ions that were present prior to cation injection.

Figure 4.

Butterfly plot containing the positive nESI spectrum (blue) and partial head group transfer product distribution (red) obtained from ion/ion reaction from a ^{13}C -TrEnDi-modified HeLa cell extract. The bottom mass scale is shifted up by 87 Da relative to the top mass scale to account for the loss of the head group of a tmPC cation, the most abundant class of tmPL cation in the HeLa sample.

Figure 5.

a) Mutual Storage of isolated m/z 828 ions $[tmPL\ 828]^+$ and $[PDPA - 2H]^{2-}$, b) CID of $[PE\ 38:4 - (^{13}CH_3)_3NCH_2CH_2]^-$, c) CID of $[SM\ 42:2 - (CH_3)_3NCH_2CH_2]^-$.

Scheme 1.

Ion/Ion reaction scheme for the gas-phase charge inversion of ¹³C-TrEnDi modified glycerophospholipids.

Scheme 2.

Proposed structures of dominant fragments formed via ion-trap CID of gas-phase charge inversion products resulting from the reaction between [PDPA – 2H]²⁻ and ¹³C-TrEnDi-modified glycerophospholipid cations.