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Determination of Phospholipid Regiochemistry by Ag(I) Adduction and Tandem Mass Spectrometry

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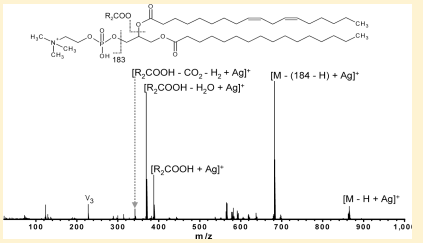
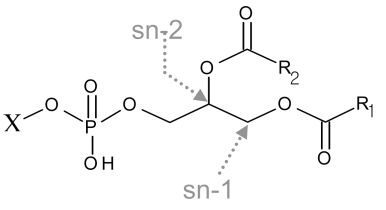
Supporting Information

ABSTRACT: Collision-activated dissociation (CAD) and infrared multiphoton dissociation (IRMPD) of Ag-adducted phospholipids were investigated as structural tools. Previously, determination of the acyl chains at the two phospholipid esterification sites has been performed based on the R_1COO^-/R_2COO^- ratio in negative ion mode CAD tandem mass spectrometry. However, the observed product ion ratio is dependent on the extent of unsaturation of the fatty acyl group at sn-2 as well as on the total chain length. Similarly, in positive ion mode CAD with/without alkaline or alkaline earth metal adduction, the ratio of product ions resulting from either R_1COOH or R_2COOH neutral losses is dependent on the nature of the phospholipid polar headgroup. Ag^+ ion chromatography, in which silver ions are part of the stationary phase, can provide information on double bond number/distribution as well as double bond configuration (cis/trans) because of interaction between Ag^+ ions and olefinic π electrons of fatty acids and lipids. We hypothesized that interactions between double bonds and Ag^+ may be utilized to also reveal phospholipid esterification site information in tandem mass spectrometry. CAD and IRMPD of Ag-adducted phospholipids with unsaturated fatty acids (R_xCOOH, $x = 1$ or 2) provided characteristic product ions, $[R_xCOOH + Ag]^+$, and their neutral losses. The characteristic product ions and their abundances do not depend on the type of polar headgroup or the number of double bonds of unsaturated acyl chains. Tandem mass spectrometry of Cu-adducted phospholipids was also performed for comparison based on the Lewis acid and base properties of Cu^+ and phospholipid double bonds, respectively.



Phospholipids, present in all organisms, are the building blocks for cellular membranes and are involved in a diverse number of other functions, from compartmentalization of cytoplasm to cell signaling.[1−7] Differentiation between the sn-1 and sn-2 positions of phospholipids (their structure is shown in Scheme 1) is important from both a biochemical and nutritional viewpoint because cell signaling through phospholipids and absorption of polyunsaturated fatty acids into the body are related to the position of the acyl chains on the glycerol backbone.[8−11] Classical methods for phospholipid analysis rely heavily on thin-layer chromatography (TLC) to separate and identify the various phospholipid classes with relatively nonspecific detection techniques, such as visualization of phosphorus by spray reagents (e.g., primuline, which fluoresces under a UV lamp).[12] Phospholipids extracted from TLC plates are amenable to gas chromatography (GC) or GC/mass spectrometry (MS) following hydrolysis of the fatty acyl group and chemical derivatization. Such analysis allows identification of the specific fatty acids in each phospholipid class.[13] Fast atom bombardment (FAB), matrix-assisted laser desorption/ionization (MALDI), electrospray ionization (ESI), and desorption electrospray ionization (DESI)-MS can directly analyze phospholipids as intact molecules and preserve the information inherent in their chemical structures.[3,14−21] It has been shown that determination of the acyl chains at sn-1 versus sn-2 can be made from the ratio of R_1COO/R_2COO in negative ion mode collision-activated dissociation (CAD) tandem mass spectrometry, but the observed ion ratio is dependent on the extent of saturation of the fatty acyl group at sn-2, the total chain length, the collision energy, and the nature of the polar headgroup.[13,15,17,22] ESI-MS is superior to FAB-MS because the former technique does not suffer from some of the problems associated with the latter, including complication of spectra due to matrix ions and molecular ion fragmentation

Scheme 1. Structure of Phospholipids



$X = C_2H_4NH_2$	Phosphatidylethanolamine (PE)
$C_2H_3NH_2COOH$	Phosphatidylserine (PS)
$C_2H_4N(CH_3)_3^+$	Phosphatidylcholine (PC)

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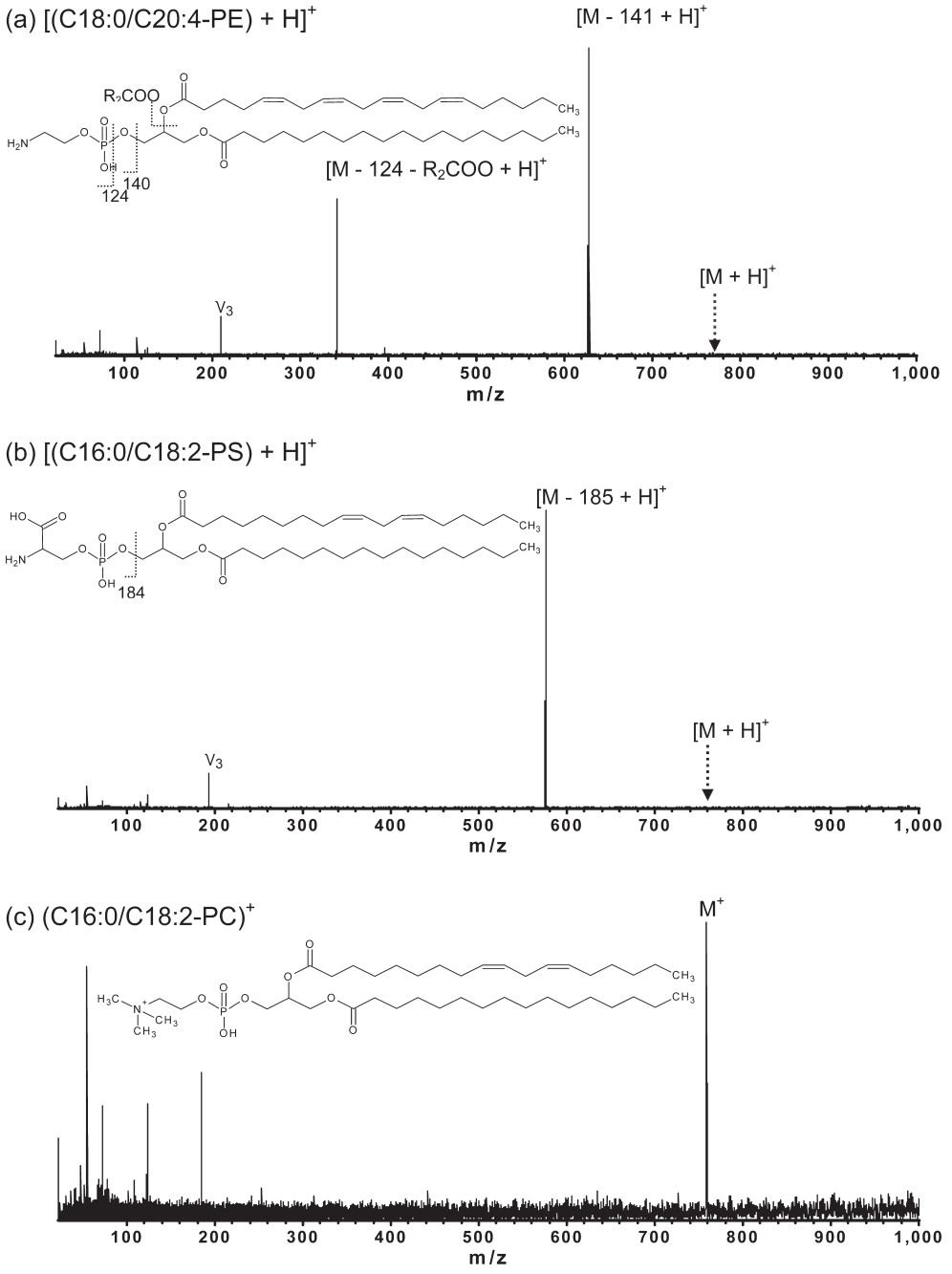

Figure 1. CAD of protonated/non-silver-adducted phospholipids (a) CAD of $[(C18:0/C20:4\text{-PE}) + H]^+$ (−20 V collision voltage), where the combined loss of 124 and R_2COO produces an even-electron product ion, (b) CAD of $[(C16:0/C18:2\text{-PS}) + H]^+$ (−25 V collision voltage), (c) CAD of $(C16:0/C18:2\text{-PC})^+$ (−20 V collision voltage). v_3 indicates the third harmonic of the most abundant ion in each spectrum.

during the ionization process.[3,13,23] Alkali metals, alkaline earth metals, and divalent metal ions have been used to form metal−phospholipid complexes for structural determination of phospholipids. [24,25] Positive ion mode CAD of alkali metal-adducted phosphatidylserine resulted in greater abundance of product ions from cleavage at the sn-2 position compared to the sn-1 position.[24] For alkaline earth metals and divalent metal ions, however, it was reported that CAD product ion abundances from cleavage at the sn-1 and sn-2 esterification sites may change depending on the types of metals and phospholipids.[25]

Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry[26] can provide improved molecular identification due to its accurate mass capabilities[27] and additional MS/MS fragmentation techniques, including infrared multiphoton dissociation (IRMPD),[28,29] electron-induced dissociation (EID),[30−33] and electron capture dissociation (ECD).[34−37] These techniques can often provide complementary structural information compared to CAD for the characterization of various kinds of biomolecules.[30,34−36,38−41] However, there has been very limited application of alternative MS/MS fragmentation techniques available in FT-ICR mass spectrometry to phospholipids.[42] James et al.

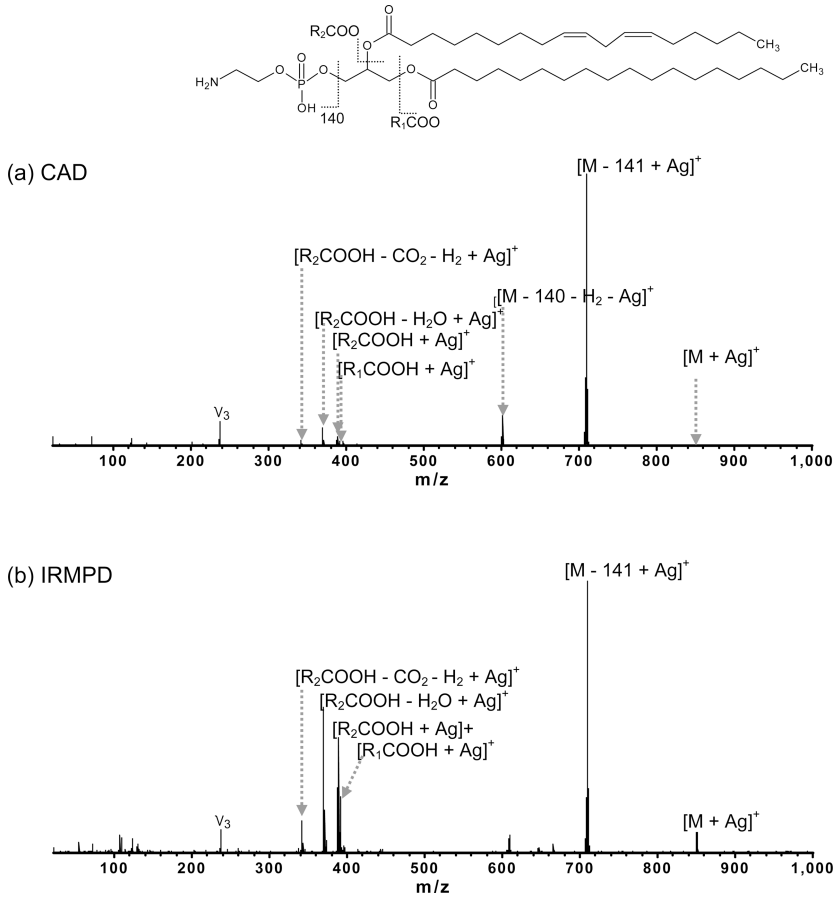

Figure 2. CAD (−35 V collision voltage) and IRMPD (7.5 W laser power, 750 ms irradiation) of Ag-adducted phosphatidylethanolamine, [(C18:0/C18:2-PE) + Ag]$^+$. ν_3 indicates the third harmonic of the most abundant ion in each spectrum.

applied ECD to complexes of the form $[\text{metal}^{II}(L)_n]^{2+}$ where L corresponds to diacylglycerophosphocholine. In these experiments, the ratio of sn-1/sn-2 acyl chain loss was dependent on the number (n) of lipids complexed with metal ions.[42] IRMPD has been shown to be an efficient fragmentation technique for nucleic acids, phosphopeptides, and phosphate-containing metabolites due to strong phosphate absorption at the 10.6 μm wavelength typically used.[28,30,43−46] Thus, IRMPD may also have advantages over CAD for structural characterization of phospholipids.

Silver high performance liquid chromatography (Ag-HPLC) has been utilized to separate various fatty acid methyl esters, triacylglycerols, and lipids based on number, geometry, and position of double bonds.[9,47−49] In addition to Ag-HPLC, various kinds of silver ion chromatography have been widely applied to all lipid classes. For many years, Ag-TLC has been one of the key separation techniques used in lipid analysis.[47,48] Furthermore, atmospheric pressure column chromatography, HPLC, and supercritical fluid chromatography (SFC) have also been used in silver ion mode for lipid analysis.[47,48] In most cases, silver ion chromatography involves column support materials to which silver ions can bind, typically silica gel.[47,48] The retention mechanism in silver ion chromatography is based on the interaction between Ag^+ ions and olefinic π electrons of unsaturated organic molecules.[47−49] We hypothesized that such interactions, which have previously been utilized to cationize organic molecules,[50,51] may reveal esterification site information in tandem mass spectrometry of Ag-adducted phospholipids.

The relative ion abundance changes with types of headgroup, the extent of unsaturation, and types of metals in tandem mass spectrometry of alkali, alkaline earth, or divalent metal-adducted phospholipids may cause confusion when characterizing the regiochemistry of phospholipids.[24,25] Thus, a more consistent method is desired for straightforward determination of phospholipid acyl chains. To our knowledge, the present work represents the first application of Ag-adduction to obtain esterification site information that differentiates the two acyl chains of phospholipids by tandem mass spectrometry. Both IRMPD and CAD were used for fragmentation of Ag-adducted phospholipids. Because Ag(I) and Cu(I) have similar Lewis acid properties, Cu(I)-adducted phospholipids were also analyzed for comparison.

EXPERIMENTAL SECTION

Sample Preparation. Phospholipids used in this work include phosphatidylethanolamine (C18:0/C18:2-PE and C18:0/C20:4-PE), phosphatidylserine (C16:0/C18:2-PS and C18:0/C18:1-PS), and phosphatidylcholine (C16:0/C18:1-PC, C18:1/C16:0-PC, and C16:0/C18:2-PC). Phospholipids are designated as follows: C_1:d_1/C_2:d_2-PL, where C_1 and C_2 are the number of carbon atoms in the fatty acyl chains of the sn-1 and sn-2 positions, respectively, d_1 and d_2 are the number of double bonds of the sn-1 and sn-2 fatty acyl chains, respectively, and PL is the abbreviation of each type of phospholipid.[52] Phospholipids were purchased from Avanti Polar Lipid (Alabaster, Al). Silver acetate and copper acetate used for formation of phospholipid adducts

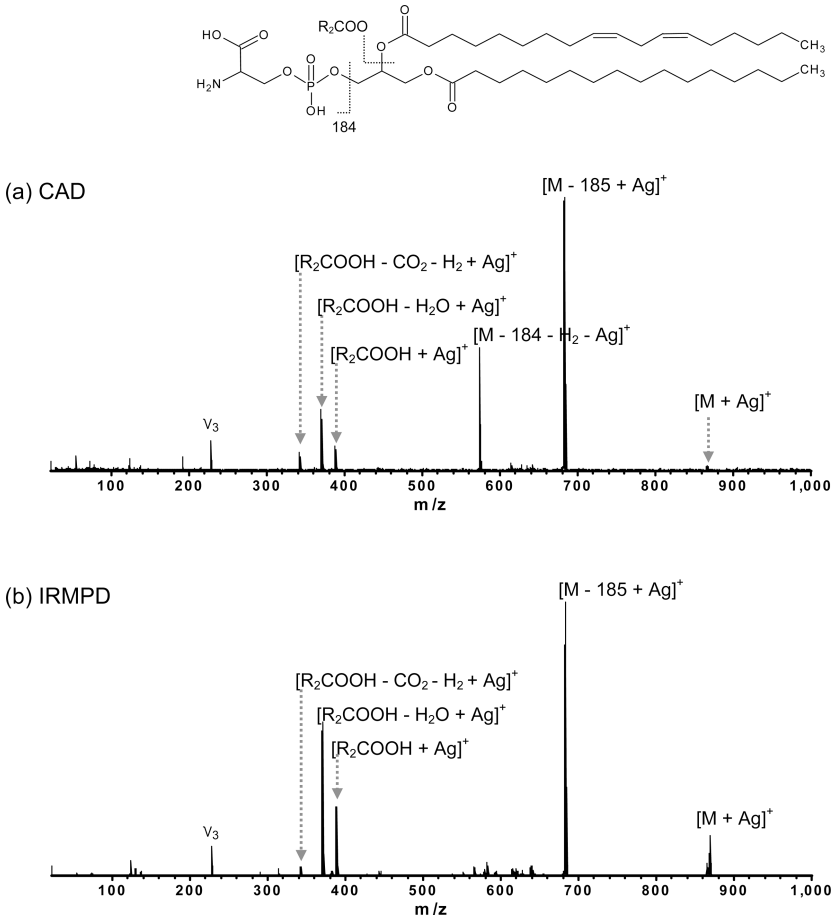

Figure 3. CAD (−35 V collision voltage) and IRMPD (7.5 W laser power, 300 ms irradiation) of Ag-adducted phosphatidylserine, $[(C16:0/C18:2\text{-PS}) + Ag]^+$. ν_3 indicates the third harmonic of the most abundant ion in each spectrum.

were purchased from Sigma-Aldrich (St. Louis, MO). Phospholipids (130−140 μM) were mixed with 2 mM silver acetate (or copper acetate) in water/methanol (20:80) with 1% chloroform. Ag-adducted phosphatidylserine solutions were made with methanol containing 0.1% formic acid because of the acidity of phosphatidylserines. All sample solutions were freshly made at least 30 min prior to MS analysis.

Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. Singly charged Ag-adducted phospholipids, $[M + Ag]^+$ (or $[M - H + Ag]^+$ for PC, which contains a fixed positive charge), were generated by external ESI at 70 μL/h (Apollo II ion source, Bruker Daltonics, Billerica, MA). All experiments were performed with a 7 T quadrupole (Q)-FT-ICR mass spectrometer (APEX-Q, Bruker Daltonics) as previously described.[53] All data were obtained in positive ion mode.

Briefly, ions produced by ESI were mass-selectively externally accumulated in a hexapole for 0.2−2 s,[54,55] transferred via high voltage ion optics, and captured in the ICR cell by dynamic trapping. This accumulation sequence was looped three times to improve precursor ion abundance. In MS/MS experiments, $[M + Ag]^+$ (or $[M - H + Ag]^+$ for PC) was mass selectively externally accumulated and CAD was performed at a collision cell DC offset of 20−35 V with Ar as collision gas. IRMPD was performed inside the ICR cell with a 25 W, 10.6 μm, CO_2 laser (Synrad, Mukilteo, WA). The laser beam was deflected by two mirrors for alignment through a hollow dispenser cathode to the center of the ICR cell. The beam entered the vacuum system through a BaF_2 window. Photon irradiation was performed for 300−750 ms at 5−7.5 W laser power. All mass spectra were acquired with XMASS software (version 6.1, Bruker Daltonics) in broadband mode from m/z 21 to 1000 with 256K data points and summed over 5−10 scans, except for Cu(I)-adducted phospholipids, where 20−30 scans were summed. Data processing was performed with MIDAS analysis software.[56] Internal calibration was performed with $[M - \text{headgroup} + Ag]^+$ and $[R_2COOH - H_2O + Ag]^+$ as calibrants. All product ions were assigned within 10 ppm mass accuracy. The numerical mass value (e.g., 141 and 185) used in product ion labels denotes the mass of a fragment resulting from heterolytic cleavage, unless otherwise stated.

RESULTS

CAD of Protonated/Non-Silver-Adducted Phospholipids. CAD of protonated/non silver-adducted phospholipids generally resulted in headgroup loss, as shown in Figure 1. Singly protonated phosphatidylethanolamine and phosphatidylserine provided headgroup losses with or without the combined loss of R_2COOH where R_2 is the sn-2 acyl chain. Similar results from CAD of protonated phosphatidylserine have been reported by others.[24] Product ions were rarely observed for phosphatidylcholine, even at higher collision energy where precursor ion abundance was depleted by more than 90%. However, the different behavior for PC may be explained by the absence of a mobile

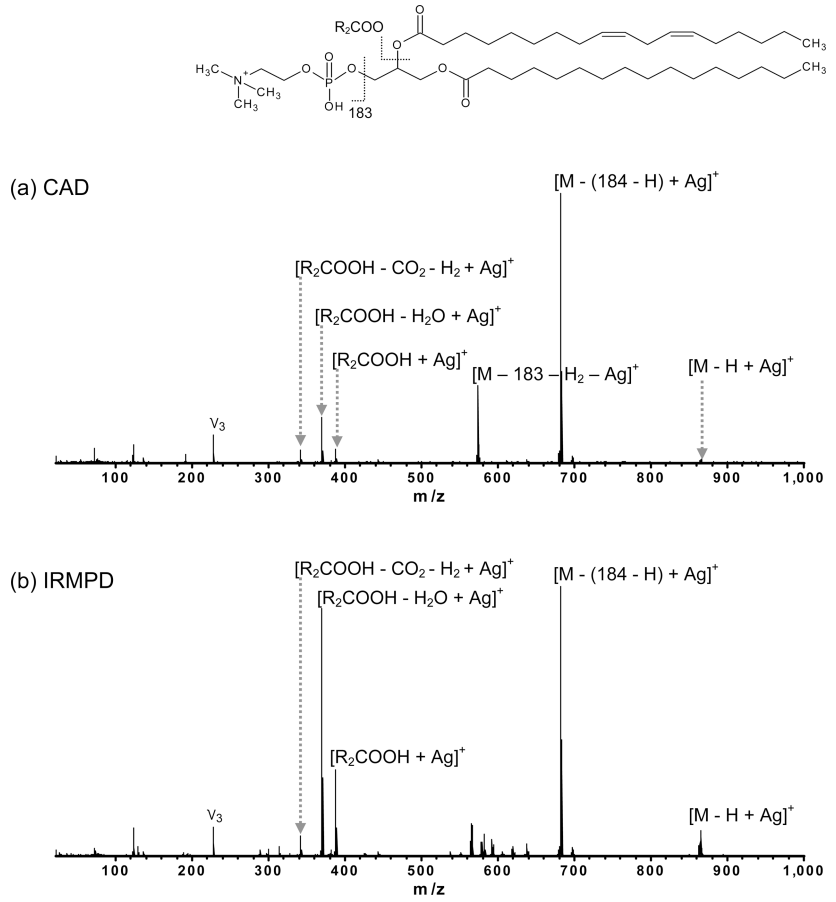

Figure 4. CAD (−35 V collision voltage) and IRMPD (7.5 W laser power, 450 ms irradiation) of Ag-adducted phosphatidylcholine, $[(C16:0/C18:2\text{-}PC) - H + Ag]^+$. ν_3 indicates the third harmonic of the most abundant ion in each spectrum.

proton due to the fixed charge on the PC headgroup. Thus, esterification site information could not be deduced from CAD of protonated phospholipids, although the phospholipid type could be deduced from the headgroup losses. IRMPD of protonated phospholipids resulted in very similar spectra as CAD of the same species; thus, IRMPD data are not shown.

CAD and IRMPD of Ag-Adducted Phospholipids. Figure 2 shows CAD and IRMPD of Ag-adducted phosphatidylethanolamine, $[(C18:0/C18:2\text{-}PE) + Ag]^+$. Headgroup loss, $[M - 141 + Ag]^+$, from precursor ions was the most dominant fragmentation pathway in both CAD and IRMPD. $[R_2COOH + Ag]^+$ was also observed along with accompanying water loss, $[R_2COOH - H_2O + Ag]^+$. In addition, $[R_2COOH - CO_2 - H_2 + Ag]^+$ was observed, even though this product ion was not as abundant as $[R_2COOH + Ag]^+$ and $[R_2COOH - H_2O + Ag]^+$. Neutral losses from $[R_2COOH + Ag]^+$ were only observed for the sn-2 acyl chain of Ag-adducted phosphatidylethanolamine, $[(C18:0/C18:2\text{-}PE) + Ag]^+$. $[R_1COOH + Ag]^+$ was also observed, both in IRMPD and CAD but with very low abundance. In addition, no neutral losses were observed from $[R_1COOH + Ag]^+$. Another phosphatidylethanolamine lipid, $[C18:0/C20:4\text{-}PE] + Ag]^+$, with a different fatty acyl group attached to the sn-2 position, was also investigated and showed behavior similar to that of $[(C18:0/C18:2\text{-}PE) + Ag]^+$ in both CAD and IRMPD (the IRMPD spectrum is shown in Figure 5a).

We believe that silver adducts form in ESI of Ag(I)-containing phospholipid solutions. Thus, Ag^+ should be interacting with the sn-2 acyl chain (which contains double bonds),[9,47−49] consistent with the observation of the $[R_2COOH + Ag]^+$ product ion. By contrast, the sn-1 acyl chain does not contain double bonds and $[R_1COOH + Ag]^+$ was not always observed. Even when $[R_1COOH + Ag]^+$ was observed, its abundance was much lower than other product ions. Generally, $[R_1COOH + Ag]^+$ was observed when a less unsaturated fatty acyl chain was present at the sn-2 esterification site as shown in Figures 2 and 5. For example, $[R_1COOH + Ag]^+$ was observed for C18:0/C18:2-PE (Figure 2) but not for C18:0/C20:4-PE. Stronger interactions between Ag^+ ions and organic molecules are expected with increasing numbers of double bonds.[48] Thus, observation of $[R_1COOH + Ag]^+$ could be explained by relatively weak interaction between Ag^+ ions and less unsaturated R_2COOH acyl chains, compared to stronger interaction between Ag^+ and more unsaturated sn-2 fatty acyl groups. In some cases, other minor neutral losses, such as $[R_2COOH - H_2 + Ag]^+$, were also observed with relatively low abundances. On the basis of the tandem mass spectra shown in Figure 2, $[R_2COOH + Ag]^+$ and related neutral losses could be used as characteristic product ions to characterize phospholipid structures. More phospholipids were examined to further verify this hypothesis.

CAD and IRMPD spectra of Ag-adducted phosphatidylserine, $[(C16:0/C18:2\text{-}PS) + Ag]^+$, are shown in Figure 3. Similar results were observed as in CAD and IRMPD of Ag-adducted phosphatidylethanolamine (Figure 2). Headgroup losses, with or without Ag adduction, from precursor ions were very abundant in both CAD and IRMPD of $[(C16:0/C18:2\text{-}PS) + Ag]^+$. Again, $[R_2COOH + Ag]^+$, $[R_2COOH - H_2O + Ag]^+$, and

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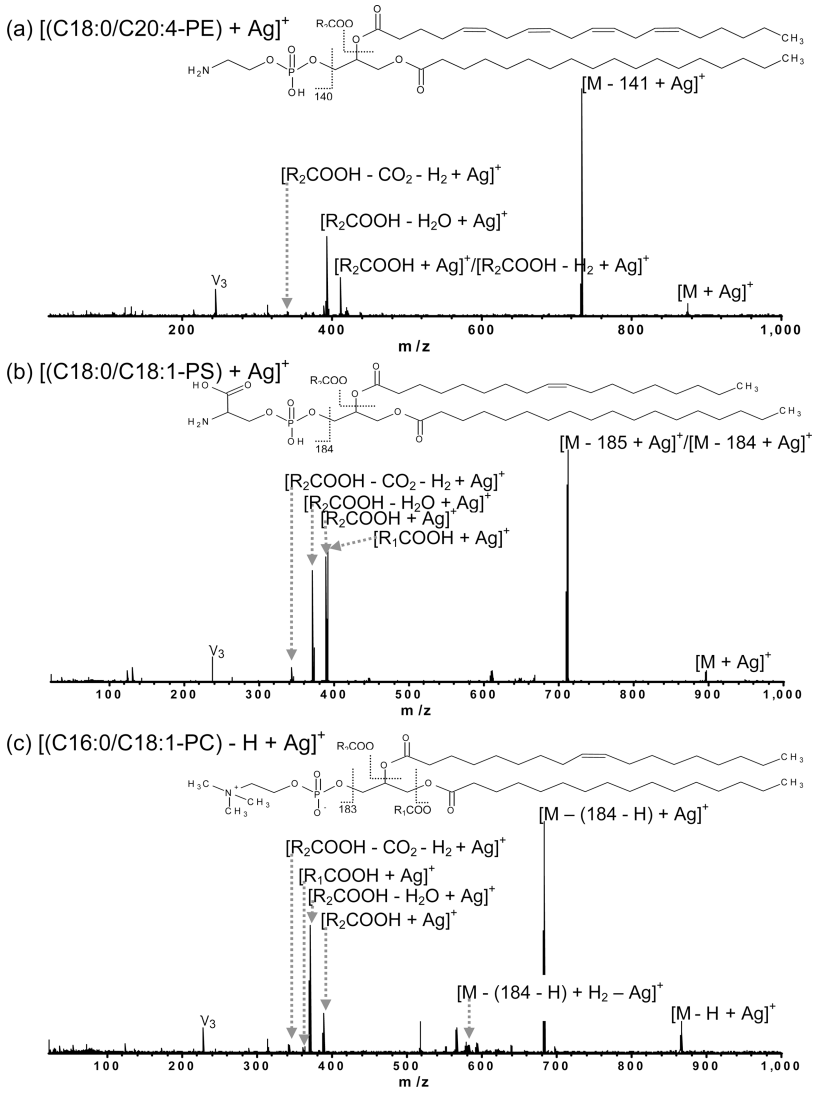

Figure 5. IRMPD of Ag-adducted phospholipids: (a) $[(18:0/C20:4\text{-PE}) + Ag]^+$ (5 W laser power, 300 ms irradiation), (b) $[(C16:0/C18:1\text{-PS}) + Ag]^+$ (7.5 W laser power, 600 ms irradiation), (c) $[(C16:0/C18:1\text{-PC}) - H + Ag]^+$ (7.5 W laser power, 450 ms irradiation). ν_3 indicates the third harmonic of the most abundant ion in each spectrum.

$[R_2COOH - CO_2 - H_2 + Ag]^+$ were observed with very similar relative abundances as observed in CAD and IRMPD of phosphatidylethanolamine. CAD and IRMPD of phosphatidylcholine, $[(C16:0/C18:2\text{-PC}) - H + Ag]^+$, followed the same trend as the other types of phospholipids investigated. As shown in Figure 4, characteristic product ions containing the sn-2 acyl chain ($[R_2COOH + Ag]^+$, $[R_2COOH - H_2O + Ag]^+$, and $[R_2COOH - CO_2 - H_2 + Ag]^+$) were observed along with abundant headgroup losses, with or without Ag adduction, from precursor ions.

Further examination of Figures 2 to 4 reveals that low m/z regions, where characteristic product ions are observed such as $[R_2COOH + Ag]^+$, are more abundant in IRMPD spectra compared to CAD. Precursor ion activation may be more efficient in IRMPD than CAD because of high IR absorption by phosphate groups and the longer interaction time between molecular ions and IR photons.[30,43,45] Thus, IRMPD may be preferred over CAD to ensure observation of all characteristic product ions, including $[R_2COOH + Ag]^+$ and related neutral losses, for characterization of phospholipid esterification sites. Additional IRMPD spectra of other Ag-adducted phospholipids are shown in Figure 5. Characteristic product ions observed in IRMPD can be used to identify the sn-1 and sn-2 acyl chains of each phospholipid. Other product ions such as $[R_1COOH + Ag]^+$ were also observed. However, these species were not dominant and not consistently observed for all phospholipids. Thus, such product ions may not be useful for phospholipid structural characterization. On the other hand, $[R_2COOH + Ag]^+$ and related neutral losses were observed for all investigated phospholipids with unsaturated acyl chains at the sn-2 esterification site. Potential low abundance $[R_1COOH + Ag]^+$ product ions should not cause confusion when analyzing unsaturated phospholipid sn-2 acyl chains because no neutral losses were observed from $[R_1COOH + Ag]^+$. Figure 6 shows IRMPD of Ag-adducted phosphatidylcholine (C18:1/C16:0-PC) which has an unsaturated acyl chain at the sn-1 position. As expected, $[R_1COOH + Ag]^+$, $[R_1COOH - H_2O + Ag]^+$, and $[R_1COOH - CO_2 - H_2 + Ag]^+$ were observed, presumably due to interaction between Ag^+ and the double bond in the sn-1 acyl chain. However, the water loss peak, $[R_1COOH - H_2O - H + Ag]^+$, is much less abundant than $[R_1COOH + Ag]^+$.

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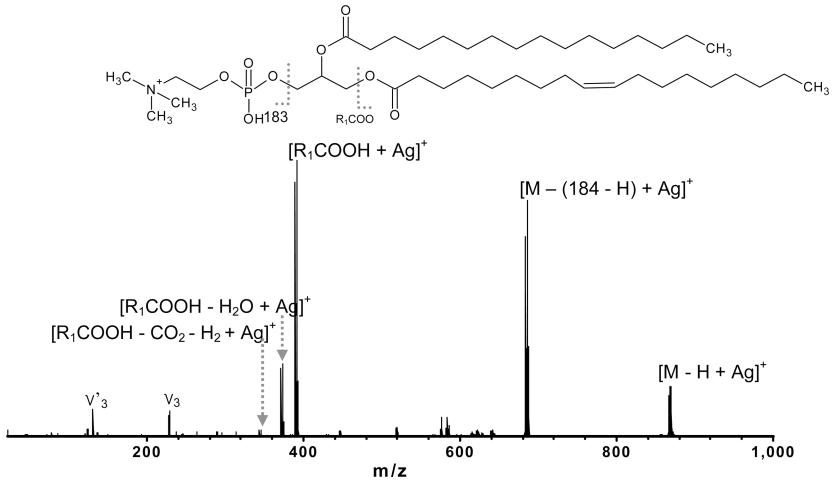

Figure 6. IRMPD of Ag-adducted $[(\text{C18:1/C16:0-PC}) - \text{H} + \text{Ag}]^+$ (7.5 W laser power, 300 ms irradiation). ν'_3 and ν_3 indicate the third harmonics of the two most abundant ions.

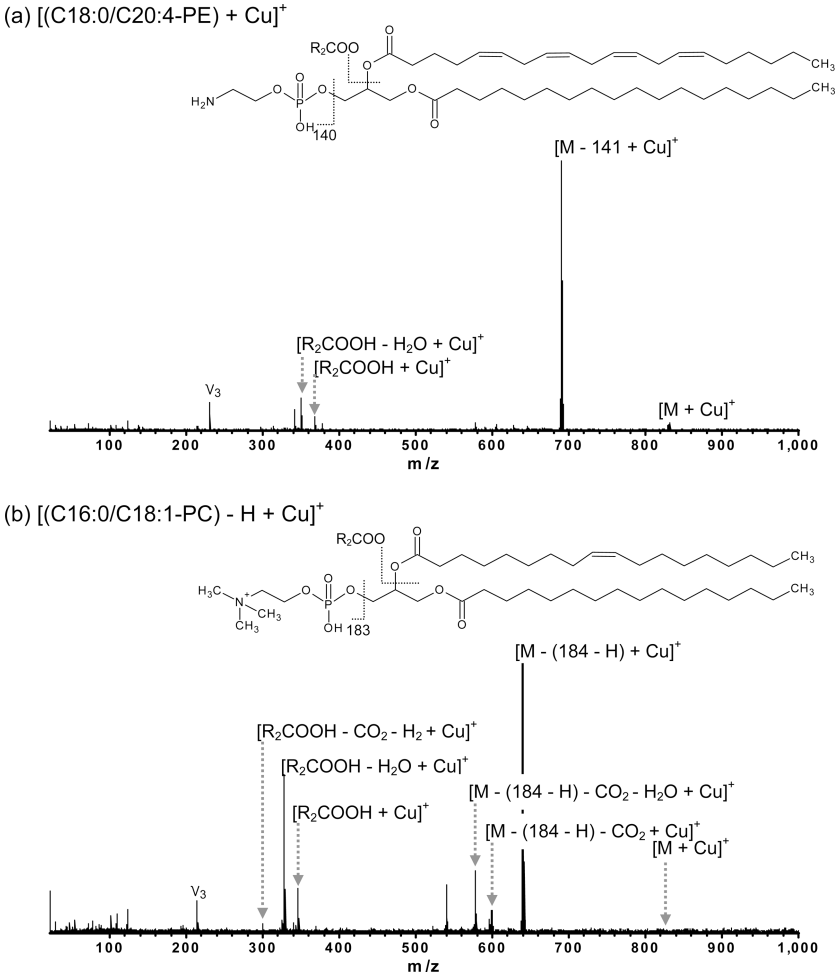

Figure 7. IRMPD of Cu-adducted phospholipids (a) $[(\text{C18:0/C20:4-PE}) + \text{Cu}]^+$ (7.5 W laser power, 300 ms irradiation), and (b) $[(\text{C16:0/C18:1-PC}) - \text{H} + \text{Cu}]^+$ (7.5W laser power, 300 ms irradiation time). ν_3 indicates the third harmonic of the most abundant ion in each spectrum.

Hsu et. al used Li^+ and Na^+ to form adducts with phosphatidylserine.[24] CAD of Li^+- or Na^+-adducted phosphatidylserine yielded mainly headgroup loss and R_xCOOH (x = 1, 2) losses, where greater relative ion abundances of R_2COOH loss over R_1COOH loss allowed identification of the fatty acyl group attached at the sn-2 site of phosphatidylserine.[24] However, the abundance ratios of product ions formed via loss of R_2COOH and R_1COOH, respectively, in CAD of alkali metal-adducted

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phospholipids change depending on types of phospholipid and types of alkali metal adduct for the same type of phospholipid..[25] In addition to alkali metals, alkaline earth and divalent metals (Met) such as Co^{2+} have been applied for phospholipid structural characterization via characteristic $[M-R_2COOH+Met]^+$ and $[M-R_1COOH+Met]^+$ product ion abundances in CAD.[25] However, the ratios of $[M - R_2COOH + Met]^+/[M - R_1COOH + Met]^+$ were still dependent on phospholipid and metal type. By contrast, fragmentation patterns reported here from tandem mass spectrometry of Ag-adducted phospholipids were not dependent on types of phospholipid. In CAD and IRMPD of Ag-adducted phospholipids, the activation energy did not appear to affect characteristic fragmentation patterns: lower activation energy only had an influence on fragmentation efficiency. The most abundant characteristic product ion ($[R_2COOH - H_2O + Ag]^+$), where R_2COOH is unsaturated, was observed at an activation energy lower than that of other characteristic fragments such as $[R_2COOH + Ag]^+$, which started to appear as the CAD and IRMPD activation energy increased (see Supporting Information Figures 1 and 2). The relative abundances of characteristic product ions did not change with activation energy. Thus, CAD and IRMPD of Ag-adducted phospholipids could be very useful for differentiating esterification sites. The characteristic product ions, $[R_xCOOH + Ag]^+$, $[R_xCOOH - H_2O + Ag]^+$, and $[R_xCOOH - CO_2 - H_2 + Ag]^+$ reveal R_xCOOH unsaturation. IRMPD appeared more beneficial for phospholipid structural characterization because of increased ion abundances at the lower m/z region where characteristic product ions are located. In addition to these characteristic product ions, abundant headgroup loss enabled us to identify the types of phospholipid, and other fatty acid fragments provided further confirmation about the fatty acyl groups attached at each esterification site. As a result, extensive structural characterization of phospholipids appears possible from CAD and IRMPD of Ag-adducted species. One exception may be phospholipids containing only saturated acyl chains because of the low abundance of saturated silver-adducted product ions. However, >80% of phospholipids include unsaturated acyl chains in biological systems.[57] In addition, unsaturated acyl chains have drawn much more attention than their saturated counterparts because of their biological effects.[58−60] Thus, CAD and IRMPD of Ag-adducted phospholipids should be a valuable approach in biomedical research related to phospholipids.

CAD and IRMPD of Cu-Adducted Phospholipids. Ag^+ ion has a soft acid property based on the hard and soft acid and base (HSAB) principle, and Ag^+ interacts well with olefinic π electrons of double bonds, which have a soft basic property.[9,47−49] Thus, metals such as Cu^+, which also has a soft acid property,[61] are expected to provide results similar to those of Ag-adducted phospholipids in MS/MS.

CAD and IRMPD of Cu-adducted phospholipids were examined. Overall, results were observed similar to those of Ag-adducted phospholipids, as anticipated. Figure 7 shows IRMPD of two phospholipids (phosphatidylethanolamine, $[(C18:0/20:4\text{-PE}) + Ag]^+$, and phosphatidylcholine, $[(C18:0/C18:1) - H + Ag]^+$). CAD yielded similar fragmentation patterns but with poorer fragmentation efficiencies compared to IRMPD, particularly in the lower m/z region (data not shown). No Cu^+ complex was observed for phosphatidylserine. Thus, Ag^+ appears more suitable for phospholipid characterization because of its higher ability to form complexes with phospholipids.

CONCLUSION

CAD and IRMPD of Ag-adducted phospholipids were used to differentiate fatty acyl groups attached to the sn-1 and sn-2 esterification sites. CAD and IRMPD of Ag-adducted phosphatidylethanolamine mostly provided abundant headgroup loss, $[M - 141 + Ag]^+$. In addition to headgroup loss, $[R_xCOOH + Ag]^+$ was observed along with accompanying neutral losses, $[R_xCOOH - H_2O + Ag]^+$ and $[R_xCOOH - CO_2 - H_2 + Ag]^+$, where R_xCOOH is unsaturated. Other types of phospholipids, including phosphatidylserine and phosphatidylcholine, were also examined, and their CAD and IRMPD spectra showed similar fragmentation patterns. Thus, $[R_xCOOH + Ag]^+$ and its related neutral loss peaks obtained from CAD or IRMPD of Ag-adducted phospholipids may be used as characteristic product ions to differentiate sn-1 and sn-2 esterification sites of phospholipids. Interaction between Ag^+ ion and olefinic π bonds of fatty acyl groups of phospholipids are believed to be crucial for revealing esterification sites of phospholipids. This explanation may be supported by IRMPD of Cu-adducted phospholipids, where similar fragmentation patterns were observed but with lower fragmentation efficiencies. Headgroup losses were consistently observed along with $[R_xCOOH + Ag]^+$ and its neutral losses. Thus, complete structural characterization of phospholipids is possible with CAD and/or IRMPD of Ag-adducted phospholipids.

ASSOCIATED CONTENT

Supporting Information. IRMPD of $[(C16:0/C18:1\text{-PC}) - H + Ag]^+$ (Figures S1 and S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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