Identification of Molecular Species of Phospholipids by combination of Neutral Loss Survey and MS^3

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Overview

Introduction:
We established the system for analyses of molecular species of phospholipids with neutral loss survey of the head group-relating mass values and succeeding MS^3 analyses by selecting the resulting product ions as precursor ions for MS^{3}analyses.

Methods:
The ESI-MS analyses were performed using a 4000QTRAP, quadrupole-linear ion trap hybrid mass spectrometer (Applied Biosystems/MDS Sciex, Concord, ON, Canada) or a LCMS-IT-TOF, time-of-flight-ion trap mass spectrometer (Shimadzu, Kyoto, Japan). The extracted phospholipids were subjected directly to ESIMS/MS or MS^3 analysis.

Results:
By the MS^{3}analyses of [M–CH$_3$]$^-$ (product ion of MS^2 step) obtained from PC or SM molecules, identification of the fatty acyl chains of PC, or sphingosine derivatives and their N-acyl species of SM can also be effectively obtained.
By using this combination of methods, most of the molecular species of the phospholipids could be identified separately even without pre-separation by LC.

Introduction

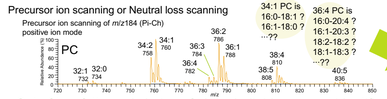
To elucidate the function of phospholipids, it is necessary to analyze not only their classes and subclasses but also molecular species. Recently, in the analysis of phospholipids, the application of mass spectrometry (MS) has become increasingly popular.

We found that electrospray ionization (ESI) MS^{3}analysis is effective for more detailed and accurate annotation of each molecular species. We established the system for analyses of molecular species of phospholipids with neutral loss survey of the head group-relating mass values and succeeding MS^{3}analyses by selecting the resulting product ions as precursor ions for MS^{3}analyses (Figure 3). This method can be effectively applicable without preliminary LC separation of phospholipid mixture.

Methods

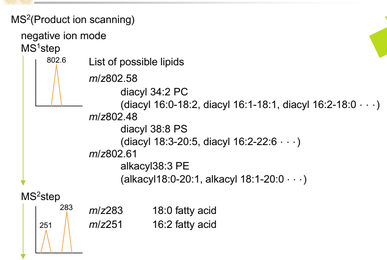
The total phospholipids were extracted from MDCK cells, rat liver (about 5g), pig liver, and calf serum (100μL) by Bligh and Dyer's method. The ESI-MS analyses were performed using a 4000QTRAP, quadrupole-linear ion trap hybrid mass spectrometer (Applied Biosystems/MDS Sciex, Concord, ON, Canada) or a LCMS-IT-TOF, time-of-flight-ion trap mass spectrometer (Shimadzu, Kyoto, Japan) with a LC-20AD VPμ; HPLC system combined with an SIL-20AC VP autosampler (Shimadzu, Kyoto, Japan). The extracted phospholipids were subjected directly to ESIMS/MS or MS^{3}analysis without LC separation by flow injection. The mobile phase composition was acetonitrile/methanol/water 6:7:2 (plus 0.1% ammonium formate) at a flow rate of 4μL min-1or methanol (plus 0.1% ammonium acetate) at a flow rate of 80μL min-1.

Figure 1. Difficult identification using class-specific detection



Set of two fatty acyl chains of phospholipids was not identified.

Figure 2. Difficult identification using MS^2



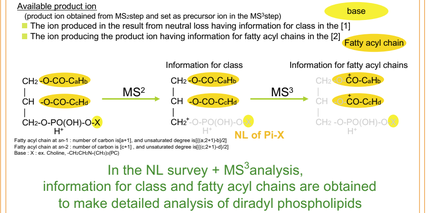
Diacyl 16:2-18:0 PC, diacyl 16:2-22:6 PS, and/or alkacyl18:0-20:1 ??

It was not easy to identify exact molecular species of lipids.

Figure 3. New method ---NL survey + MS^3---

[1] NL survey for specific detection of lipids by class(MS^{1}and MS^{2}step) → Information for class

[2] Second MS^{2}analysis of mass value corresponding to product ion obtained in the first MS^{2}analysis (MS^{2}step) → Information for fatty acyl chains



In the NL survey + MS^{3}analysis,
information for class and fatty acyl chains are obtained
to make detailed analysis of diradyl phospholipids

Results

Neutral loss scanning was applied for the selective detection of individual classes of phospholipids using a quadrupole-linear ion trap mass spectrometer (4000QTRAP). By using ammonium formate as an elution buffer, both phosphatidylcholine(PC) and sphingomyelin(SM) were detected as [M+HCOO]$^-$ions in the negative ion mode. Upon collisional activation, the [M+HCOO]$^-$ adduct ions underwent facile elimination of HCOO and CH$_3$ to yield an [M–CH$_3$]$^-$ion. By selecting the proper conditions for scanning for neutral loss of 60u (HCOO+CH$_3$), SM species were identified separately from PCs (Figure 4). Further, by selection of this [M–CH$_3$]$^-$ion as the precursor ion, the identities of the fatty acyl chains of PC species can be effectively obtained by MS^3 experiments (Figure 5). Furthermore, by the MS^3 analyses of [M–CH$_3$]-specifically obtained from SM molecules, identification of sphingosineor sphinganine derivatives and their N-acyl species can also be effectively obtained (Figure 5).

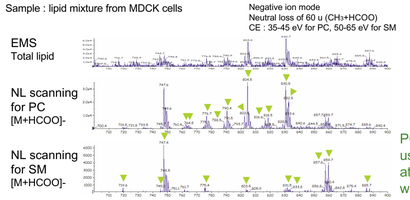
This systematic analysis of individual class of phospholipids by conditional neutral loss scanning, with subsequent analyses by MS^3 in the negative ion mode, appears to be a very effective method.

When using IT-TOF, highly accurate selection of the precursor ion was obtained at the very narrow peak width of monoisotopic ions. Thus obtained product ions are mostly detected as monoisotoic ions, both in MS/MS and MS^{2}experiments. Further mass accuracy of MS^1, MS^{2}and MS^{3}are obtained as less than 10ppm (Figure 6).

The combination of MS^{3}analyses for individual [M–CH$_3$]-ions as the intermediate product ions and selective identification of lipids by conditional neutral loss scanning was also effectively performed using IT-TOF.

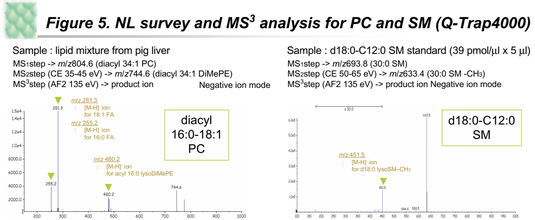
By using this combination of methods, 34 molecular species of PC(diacyland alkacyl) could be identified separately even without pre-separation by LC (Figure 7).

Figure 4. NL scanning of PC and SM (Q-Trap4000)



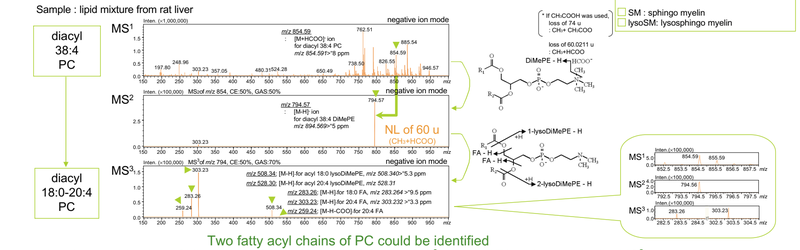
PC and SM were detected using NL scanning of 60 u at different CE without pre-separation by LC.

Figure 5. NL survey and MS^3 analysis for PC and SM (Q-Trap4000)



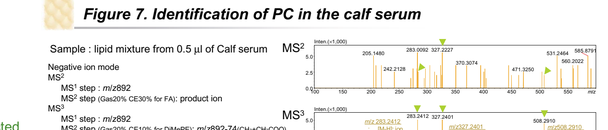
The MS^{3}analyses for PC or SM gave identification of the fatty acyl chains of PC or sphingosine derivatives and their N-acyl species of SM.

Figure 6. MS^2 for NL survey and MS^3 analysis for PC



Two fatty acyl chains of PC could be identified
using the combination of NL survey of 60 u (CH$_3$+HCOO) and MS^{3}of DiMePE(product ion in the MS^2).
Mass accuracy of MS^1, MS^{2}and MS^{3}are obtained as less than 10ppm.

Figure 7. Identification of PC in the calf serum



identification	FA detection	MS^2	MS^3
approximate	2 (diacyl)-[1]	11	15
	1 (alkacyl)-[2]	10	19
eight out of ten	1 (diacyl)	6	22
error	none	72	2

[1]Two FA product ions from diacyl lipid
[2]One FA product ion from alkacyl lipid

MS^3 analysis (NL survey + MS^3) gave high-accurate information for identification of phospholipid compared to MS^2 analysis.

Discussion and Conclusions

- By selecting the proper conditions for scanning for neutral loss of 60u (HCOO+CH$_3$), SM species were identified separately from PCs (Figure 4).
- New systematic analysis of individual class of phospholipids by conditional NL survey (MS^1+MS^2), with subsequent analyses by MS^3, appears to be a very effective method (Figures 5-7). This method will be useful for lipidome (lipid metabolome) analysis.
- When using IT-TOF, mass accuracy of MS^1, MS^2 and MS^3 are obtained as less than 10ppm (Figure 5). This indicated that NL survey + MS^3 method gave high-accurate identification of set of two FA of phospholipid.

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