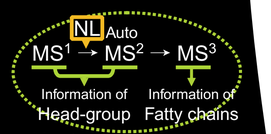
Precise Identification of Molecular Species of Phosphatidylethanolamine and Phosphatidylserine by Neutral Loss Survey with MS^3 and Accurate Mass Measurement

Mayuko Ishida[1, 2], Shinichi Yamaguchi[3], Junichi Taniguchi[3], ○Junko Iida[3], Kozo Miseki[3], Osamu Nishimura[1], Takao Shimizu[2], Ryo Taguchi[2, 4]
[1]Life Science Laboratory, Life Science Business Unit, Analytical and Measuring Instruments Division, Shimadzu Corporation, Japan, [2]Graduate School of Medicine, The University of Tokyo,
[3]MS/GC Business Unit, Analytical and Measuring Instruments Division, Shimadzu Corporation, Japan, [4]Crest JST

Overview

Introduction:
We established the system for analyses of molecular species of phosphatidylcholine with neutral loss (NL) survey and succeeding MS^2 analyses. We next tried to establish a system for an analysis of other phospholipids in a mixture of lipid.



Methods:
The ESI-MS analyses were performed using a LCMS-IT-TOF, time-of-flight-ion trap mass spectrometer. The extracted phospholipids from rat liver were subjected directly to ESI MS^2 and MS^3 analysis with Si60 column.

Results:
The identities of the fatty acyl chains of PE and PS species could be effectively obtained by NL survey of 141 (phosphoryl-ethanolamine, Fig. 2) and 87 (serine, Fig.3), respectively.
LCMS-IT-TOF gave good mass accuracy for MS^3 product ions to assist reliable identification of two fatty acyl chains (Table 2).
By using this new method added to MS^2 method, about 132 of molecular species of various phospholipids, could be identified (Table 3).
This new method is expected to be useful for comprehensive lipid metabolome analysis.

Introduction

To elucidate the function of phospholipids, it is important to analyze not only their classes and subclasses but also molecular species.
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 (NL) 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 (Fig.1). By using this new methods, 34 molecular species of phosphatidylcholine (PC) could be identified separately even without pre-separation by LC. We next tried to establish a system for an analysis of phosphatidylethanolamine (PE) and phosphatidylserine (PS) in a mixture of lipid.

Methods

The total phospholipids were extracted from rat brain (about 2g), liver (about 5g), and calf serum (100µl) by Bligh and Dyer's method. The ESI-MS analyses were performed using a LCMS-IT-TOF, time-of-flight-ion trap mass spectrometer (Shimadzu, Kyoto, Japan) with a LC-10AD; HPLC system combined with a SIL-10AD autosampler (Shimadzu, Kyoto, Japan). The extracted phospholipids were subjected directly to ESI MS^2 and MS^3 analysis with Si60 column (1×100 mm, Nomura Chem., Japan). The mobile phase composition was acetonitrile /methanol (plus 0.1% ammonium and 0.3% acetate).

Results

MS^2 of $[M+H]^+$ ion for PE gave an $[M\text{-phosphorylethanolamine (Pi-EthN)}]^+$ ion, corresponding $[\text{diglyceride-OH}]^+$ ion, as product ion in the positive ion mode (Fig.2). By selection of this $[M\text{-(Pi-EthN)}]^+$ ion as the second precursor ion, the identities of the fatty acyl chains of PE species could be effectively obtained by MS^3 analysis causing $[\text{fatty acid (FA)-OH}]^+$ ions by neutral loss of monoglyceride (MG) moieties, or $[\text{MG-H}]^+$ ion by neutral loss of FA related moieties. MS^2 of $[M\text{-H}]^-$ ion for PS gave an $[M\text{-serine}]^-$ ion, corresponding $[\text{phosphatidic acid-H}]^-$ ion, as product ion in the negative ion mode (Fig.3). By selection of this $[M\text{-serine}]^-$ ion as the second precursor ion, the identities of the fatty acyl chains of PS species could be effectively obtained by MS^3 analysis causing corresponding $[\text{FA-H}]^-$ ion.
By using this new method added to MS^2 method in an rapid analysis (Figs.4-5), 7 of molecular species of PS could be identified (Table 1, Fig.6). This NL survey with MS^3 method gave high-precision identification of set of two fatty acyl chains of phospholipids. In addition to NL survey information with MS^2, LCMS-IT-TOF gave good mass accuracy for MS^3 product ions to assist reliable identification of two fatty acyl chains (Table 2).
Identification summary of phospholipids was 132 (PC, sphingomyelins, lysophosphatidylcholine, PE, lysoPE, PS, phosphatidylinositol, phosphatidylglycerol, and triglyceride) in lipid mixture from the rat liver (Table 3).
A possibility of quantitative analysis for metabolome was tested using this method. It is possible to detect a 1.3-fold increase or a 20% decrease (Fig.7).
This new method is expected to be useful for comprehensive lipid metabolome analysis.

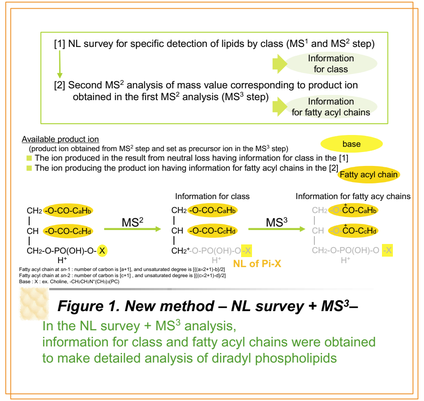


Figure 1. New method – NL survey + MS^3–
In the NL survey + MS^3 analysis, information for class and fatty acyl chains were obtained to make detailed analysis of diradyl phospholipids

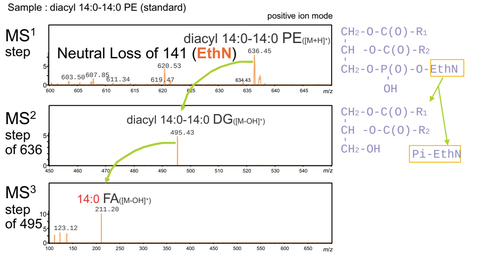


Figure 2. MS^2 for NL survey and MS^3 analysis for PE
Two fatty acyl chains of PE could be identified using the combination of NL survey of 141u ($C_2H_3NH_2$+H) and MS^3 of DG (product ion in the MS^2).

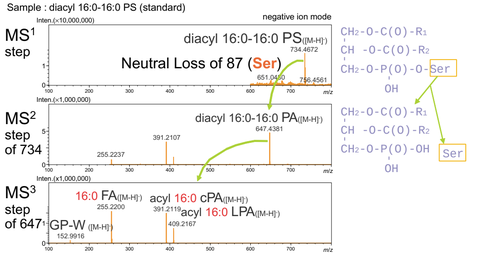


Figure 3. MS^2 for NL survey and MS^3 analysis for PS
Two fatty acyl chains of PS could be identified using the combination of NL survey of 87u ($C_2H_3(NH_2)COOH$+H) and MS^3 of PA (product ion in the MS^2).

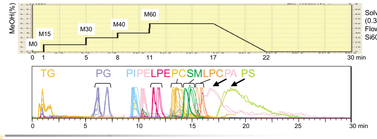


Figure 4. Retention time of each class of lipid using Si column

rapid measurement (30 min)
easy handling (i.d. 1mm column)
simple analysis (NL of 74 -> PC)
high-precision identification (good mass accuracy)

LCMS-IT-TOF, LC-10AD, SIL-10AD autosampler
m/z 300-1000
Negative ion mode (main) + Positive ion mode
Si60 column (i.d. 1x100 mm), 0.1 ml/min, 30 min
AcN : MeOH stepwise (0.3% CH3COOH, 0.1% NH3)
Combination of auto MS^2 and NL survey

Figure 5. Simple and rapid analysis system

	molecular species	theoretical *m/z*	difference (Da)	mass accuracy (ppm)
1	diacyl 38 : 4 PS (18 : 0 - 20 :4)	810.5285	0.0017	2.1
2	diacyl 40 : 6 PS (18 : 0 - 22 : 6)	834.5285	0.0020	2.4
3	diacyl 40 : 5 PS (18 : 0 - 22 : 5)	836.5442	0.0034	4.0
4	diacyl 36 : 1 PS (18 : 0 - 18 : 1)	788.5442	0.0019	2.4
5	diacyl 36 : 2 PS (18 : 0 - 18 : 2)	786.5285	0.0069	8.8
6	diacyl 36 : 4 PS (16 : 0 - 20 :4)	782.4972	0.0037	4.7
7	diacyl 38 : 6 PS (16 : 0 - 22 :6)	806.4972	0.0015	1.8

Table 1. Identification of PS in the rat liver

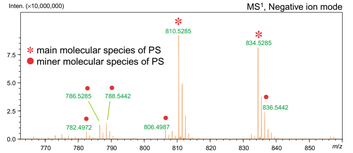


Figure 6. Mass spectra of PS in the rat liver

class of phospholipid	Identification summary	class of phospholipid	Identification summary	class of phospholipid	Identification summary
PC	10	LPE	9	PG	11
SM	3	PS	7	LPG	5
LPC	8	PI	2	TG	67
PE	7	LPI	3	Total	132

Table 3. Identification summary of phospholipids in lipid mixture from the rat liver

- NL : Neutral loss
- PE : phosphatidylethanolamine
- EthN : ethanolamine
- DG : diglyceride
- FA : fatty acid
- alk : alkylacyl and/or alkenylacyl
- PS : phosphatidylserine
- PA : phosphatidic acid
- GP : glycerophosphate
- TG : triglyceride
- PG : phosphatidylglycerol
- PI : phosphatidylinositol
- LPE : lysophosphatidylethanolamine
- PC : phosphatidylcholine
- SM : sphingomyelin
- LPC : lysophosphatidylcholine

A factor of identification : *m/z* of MS^1

	a possibility of a peak at *m/z* 810		theoretical *m/z*	difference (Da)	mass accuracy (ppm)	a reduction of the number of the possibility
1	diacyl 38 : 4 PS	-H	810.5285	0.0017	2.1 10ppm	10ppm : 1
2	alkacyl 41 : 10 DiMePE	-H	810.5438	0.0170	20.9	20ppm : 3
3	alkacyl 43 : 10 PE	-H	810.5438	0.0170	20.9 20ppm	130ppm : 10
4	diacyl 40 : 10 DiMePE	-H	810.5074	0.0194	24.0	
5	diacyl 42 : 10 PE	-H	810.5074	0.0194	24.0	
6	alkacyl 39 : 4 PS	-H	810.5649	0.0381	47.0	
7	alkacyl 40 : 11 PS	-H	810.4710	0.0558	68.9	
8	diacyl 39 : 3 DiMePE	-H	810.6013	0.0745	91.9	A percentage of reduction
9	diacyl 41 : 3 PE	-H	810.6013	0.0745	91.9	10ppm : 100% (=1/1*100)
10	diacyl 39 : 11 PS	-H	810.4346	0.0922	113.8 130ppm	20ppm : 33% (=1/3*100)
11	alkacyl 40 : 3 DiMePE	-H	810.6377	0.1109	136.8	130ppm : 10% (=1/10*100)
12	alkacyl 42 : 3 PE	-H	810.6377	0.1109	136.8	

A factor of identification : *m/z* of MS^3

	a possibility of a peak at *m/z* 283		theoretical *m/z*	difference (Da)	mass accuracy (ppm)	a reduction of the number of the possibility
1	acyl 18 : 0 FA	-H	283.2637	0.0000	0.0 10ppm	10ppm : 1
2	alk 19 : 0 FA	-H	283.3001	0.0364	128.5	
3	alk 20 : 7 FA	-H	283.2062	0.0575	203.1	
4	acyl 19 : 7 FA	-H	283.1698	0.0939	331.6	

	a possibility of a peak at *m/z* 303		theoretical *m/z*	difference (Da)	mass accuracy (ppm)	a reduction of the number of the possibility
1	acyl 20 : 4 FA	-H	303.2324	0.0028	9.3 10ppm	10ppm : 1
2	alk 21 : 4 FA	-H	303.2688	0.0392	129.2	

Table 2. List of a possibility of a peak at MS^1 and MS^3
Mass accuracy of MS^1 and MS^3 were obtained as less than 10ppm. This NL survey with MS^3 high-precision identification of set of two fatty acyl chains of phospholipids.

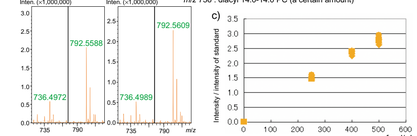


Figure 7. A possibility of quantitative analysis for metabolome
It is possible to detect a 1.3-fold increase or a 20% decrease.

Discussion and Conclusions

- By selecting the proper conditions for scanning for neutral loss of 141u or 87u, PE or PS species were identified separately from other phospholipids (Figures 2-3).
- New systematic analysis of individual class of phospholipids by conditional NL survey (MS^1 + MS^2), with subsequent analyses by MS^3, appeared to be a very effective method (Figure 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 were obtained as less than 10ppm without internal calibrants (Table 3). This indicates that NL survey + MS^3 method could give high-precision identification of set of two FA of phospholipid.