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Annotation of phospholipids in mass spectrometry-based metabolomics

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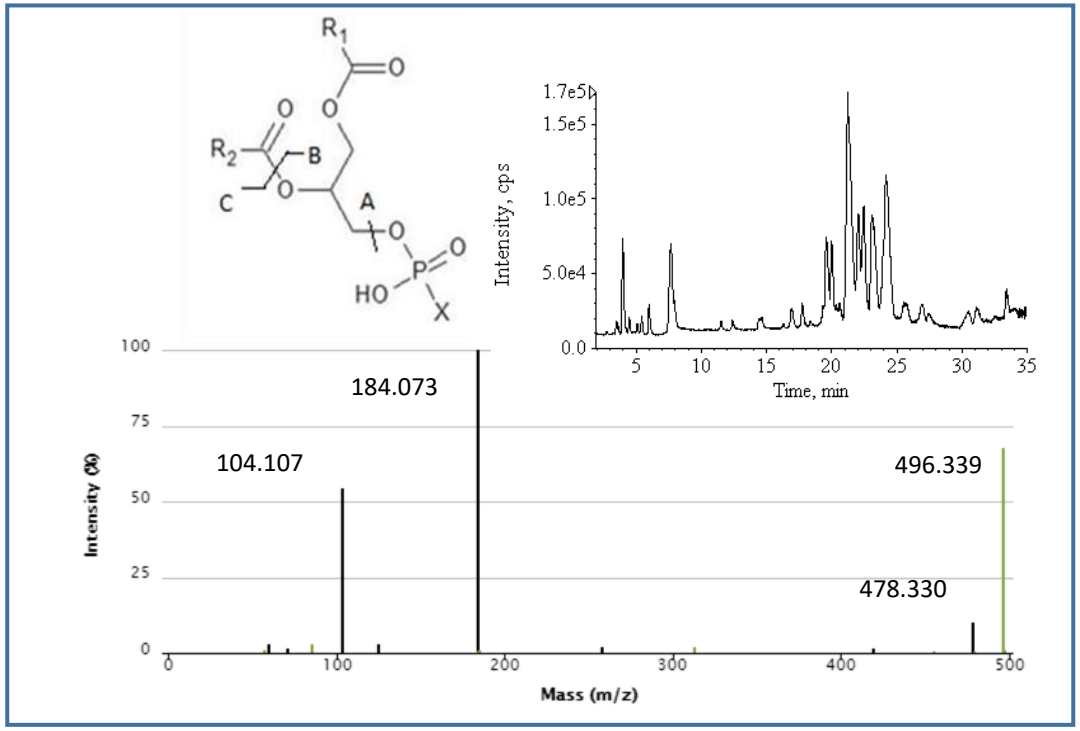


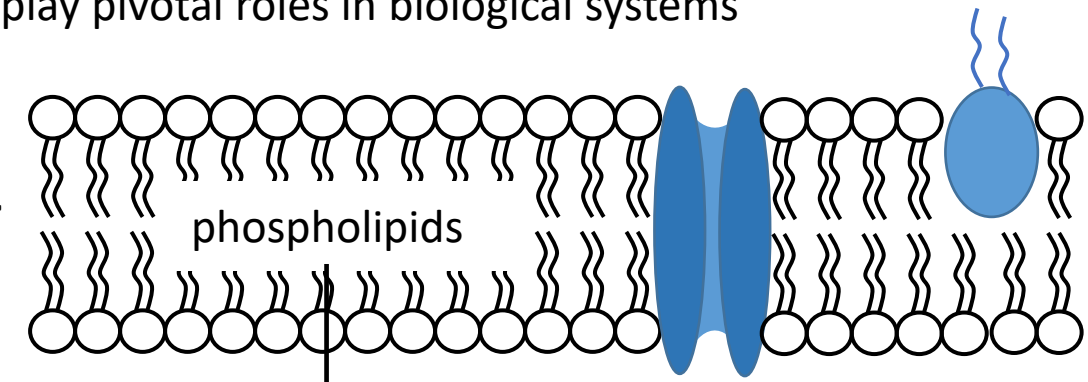
Abstract:
Phospholipids play numerous roles in biological systems, including the formation of membrane lipid bilayers and the signaling of multiple biological pathways, so that their dyshomeostasis have been associated with the development of multiple diseases, such as Alzheimer's disease and cancer. Metabolomics based on mass spectrometry has been largely employed to investigate these disease-related perturbations in the phospholipidome. However, the annotation of discriminant features still remains as a major bottleneck in the metabolomic pipeline. Chemical standards of individual phospholipid species are normally not commercially available due to the large number of isomers, so the knowledge of their characteristic fragmentation patterns upon tandem mass spectrometry is of great utility for their annotation. In this work, we provide a simplified guideline for the MS/MS-based identification of the most important phospholipid classes and their fatty acid composition.

Keywords: phospholipids; mass spectrometry; annotation

Introduction

Phospholipids play pivotal roles in biological systems

Formation of cellular membranes



Anchorage of integral membrane proteins

phospholipases

→ lysophospholipids

→ platelet activating factors

→ diacylglycerides

→ fatty acids → eicosanoids

Precursor of lipid mediators (neural cell homeostasis, immune responsiveness, oxidative stress, neuroinflammation)

Introduction

Numerous diseases elicit abnormal phospholipid homeostasis

- Alzheimer's disease
- Parkinson's disease
- Cancer

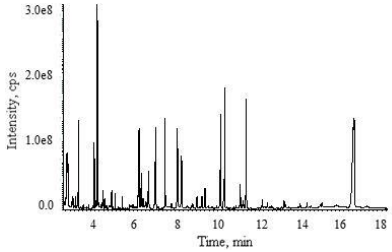
Phospholipids and related metabolites have a great potential to elucidate **pathological hallmarks** associated with diseases and to discover candidate **diagnostic biomarkers**

Metabolomics and **Lipidomics**

Introduction

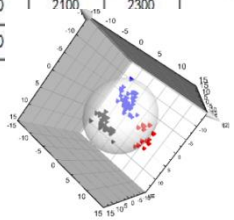
1) Sample preparation

2) Analysis

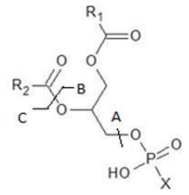


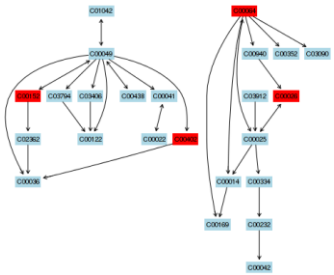
3) Data processing & statistical analysis

	Sample 1	Sample 2	Sample 3	Sample 4	...
Variable 1	1000	1100	1050	9900	
Variable 2	2200	1950	2100	2300	
Variable 3	1050	1000			
...					



4) Annotation





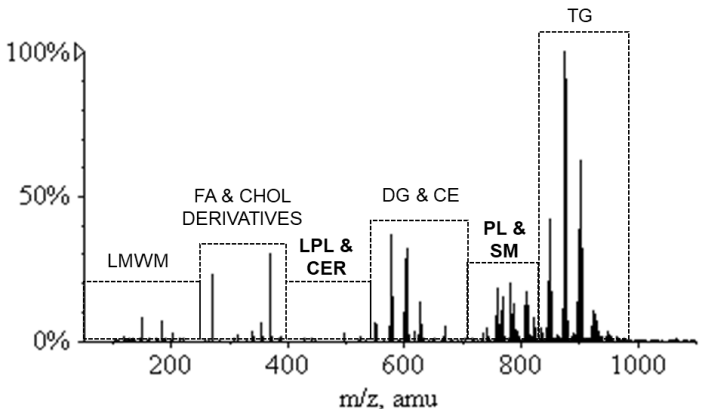
5) Biological interpretation

- Various analytical platforms can be employed to characterize the phospholipidome
- Annotation of phospholipids is a major bottleneck in the metabolomic pipeline

Results and Discussion

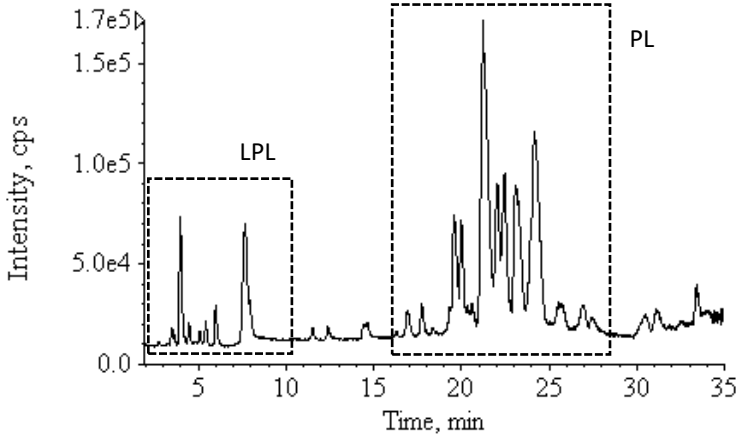
MS-based characterization of the phospholipidome

Direct Mass Spectrometry



- ✓ Short analysis time
- ✓ Wide coverage

Liquid chromatography Mass Spectrometry



- ✓ Reduced matrix effects
- ✓ Separation of isomers

Results and Discussion

MS-based characterization of the phospholipidome

	ESI+	ESI-
Phosphatidylcholines (PC)	$[M+H]^+$, $[M+Na]^+$, $[M+K]^+$	$[M-H]^-$, $[M-CH_3]^-$, $[M+Cl]^-$, $[M+FA]^-$
Phosphatidylethanolamines (PE)	$[M+H]^+$, $[M+Na]^+$	$[M-H]^-$
Phosphatidylinositols (PI)	-	$[M-H]^-$
Phosphatidylserines (PS)	$[M+H]^+$	$[M-H]^-$
Phosphatidylglycerols (PG)	$[M+H]^+$	$[M-H]^-$
Phosphatidic acids (PA)	-	$[M-H]^-$

Table 1. Major adducts detected upon electrospray ionization

Results and Discussion

Annotation of phospholipids

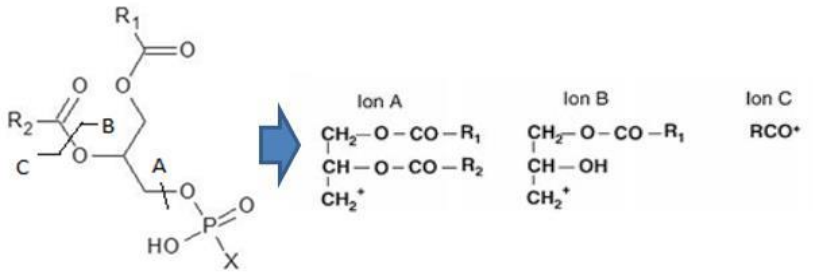
	ESI+	ESI-
Phosphatidylcholines (PC)	184.07, 104.11, 86.09 $[m/z-59]^+$ $[M+H-183]^+$, $[M+Na-205]^+$, $[M+K-221]^+$	168.04 $[m/z-60]^-$ for [M+FA] $[m/z-50]^-$ for [M+Cl]
Phosphatidylethanolamines (PE)	$[M+H-141]^+$, $[M+Na-163]^+$	196.04
Phosphatidylinositols (PI)	-	241.02
Phosphatidylserines (PS)	$[M+H-185]^+$	$[M-H-87]^-$
Phosphatidylglycerols (PG)	$[M+H-171]^+$	171.03
Phosphatidic acids (PA)	-	153

Table 2. Characteristic ions upon MS/MS fragmentation for each phospholipid class

Results and Discussion

Annotation of phospholipids

MS/MS fragmentation in the positive ionization mode

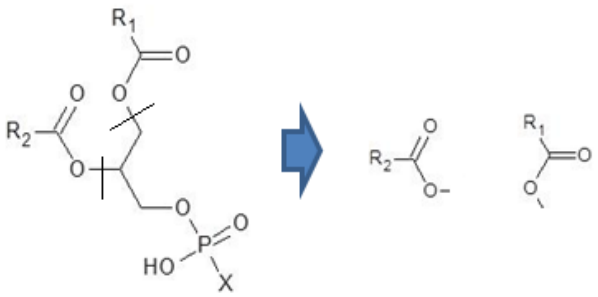


Fatty acid	m/z	
	ion B	ion C
Lauric acid	257.212	183.175
Myristic acid	285.243	211.206
Palmitoleic acid	311.259	237.222
Palmitic acid	313.274	239.237
Linolenic acid	335.259	261.222
Linoleic acid	337.274	263.237
Oleic acid	339.290	265.253
Stearic acid	341.306	267.269
Araquidic acid	369.337	295.300
Eicosapentaenoic acid	359.259	285.222
Araquidonic acid	361.274	287.237
Docosahexaenoic acid	385.274	311.237

Results and Discussion

Annotation of phospholipids

MS/MS fragmentation in the negative ionization mode



Fatty acid	m/z $RCOO^-$
Lauric acid	199.170
Myristic acid	227.201
Palmitoleic acid	253.217
Palmitic acid	255.232
Linolenic acid	277.217
Linoleic acid	279.232
Oleic acid	281.248
Stearic acid	283.264
Araquidic acid	311.295
Eicosapentaenoic acid	301.217
Araquidonic acid	303.232
Docosahexaenoic acid	327.232

Conclusions

- ✓ MS-based metabolomics provides wide coverage of the phospholipidome
- ✓ Phospholipids show characteristic fragmentation patterns upon ESI-MS analysis, thus facilitating their annotation
- ✓ Depending on the phospholipid class, characteristic daughter ions are detected in the positive and negative ion modes
- ✓ MS/MS breakage of ester bonds between fatty acids and the glycerol backbone allows identifying the fatty acid composition of phospholipids