Fine Structural Elucidation of Phospholipids with Practical Electron-Based Fragmentation on Q-TOF Instruments

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Introduction

Chemically diverse lipid species are recognized to play a widening role in biological process and disease pathology. However, it remains challenging to distinguish lipid isomers and unambiguously reveal fine structural differences. Contemporary mass spectrometry approaches have largely been based on collisional induced dissociation (CID) which provide only partial information on lipid structure. Alternative techniques such as ozone-induced dissociation MS have been developed to identify double bond location, among other structural features. In this work, we demonstrate that a practical electron-based dissociation (ExD) cell, easily retrofitted into an LC/Q-TOF instrument, provided unique fragmentation spectra that discriminated phosphatidylcholine (PC) lipid class, distinguished PC sn-1/sn-2 regioisomers, and determined PC double bond location, with sufficient sensitivity to accommodate a chromatographic timescale.

Experimental

Samples

Phosphatidylcholine (PC) standards (Avanti) were reconstituted and diluted in 1:1 methanol/isopropanol containing 5mM ammonium formate and 0.2mM ammonium fluoride for infusion experiments (Table1). Total lipids were extracted from a 10 µL aliquot of NIST SRM 1950 human plasma with a modified butanol-methanol single phase extraction procedure.

Abbreviation used in this study	Avanti Cat #	Formula	*m/z* [M+H]+	FA sn-1	FA sn-2
PC 18:0/18:1	850467	$C_{44}H_{86}N_1O_8P_1$	788.6164	18:0 (Δ9 cis)	18:1(Δ9 cis)
PC 18:1/18:0	850476	$C_{44}H_{86}N_1O_8P_1$	788.6164	18:1(Δ9 cis)	18:0(Δ9 cis)
PC 18:1(9)/18:1(9)	850375	$C_{44}H_{84}N_1O_8P_1$	786.6007	18:1(Δ9 cis)	18:1(Δ9 cis)
PC 18:1(6)/18:1(6)	850374	$C_{44}H_{84}N_1O_8P_1$	786.6007	18:1(Δ6 cis)	18:1(Δ6 cis)

Table 1. PC lipid standards used in this study.

Experimental

LC/MS Methods

Standards and plasma lipid extracts were separated with a 16-minute C18 RP-LC method. Syringe-based infusions and LC eluents were analyzed with an Agilent 6546 LC/Q-TOF equipped with a Jet Stream ionization source and an electromagnetostatic ExD cell ("ExD AQ-253 Option", e-MSion). The Q-TOF was operated in positive-ion Targeted MS/MS and Auto MS/MS modes. For comparison purposes, CID spectra were acquired on a separate LC/Q-TOF with a standard CID collision cell with 25 eV collision energy. ExD and CID spectra were analyzed with Agilent Qualitative Analysis software and NIST MS Interpreter software.

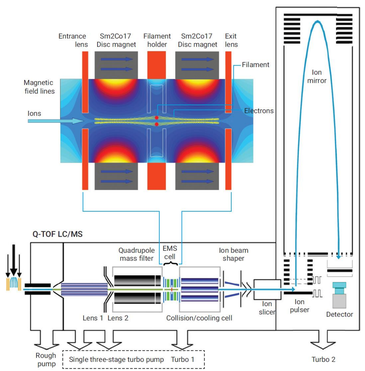

Figure 1. Schematic of an electromagnetostatic ExD cell and its position in an Agilent 6546 LC/Q-TOF

The peptide Substance P was used to initially tune the ExD cell. With these tune settings Electron Induced Dissociation (EID) spectra were observed from infusions of the regioisomer pair PC 18:0/18:1 and PC 18:1/18:0, and these tune settings were further optimized on the EID fragments.

Results and Discussion

EID produced rich PC fragmentation spectra compared to CID

Several fragment ions were shared between EID/CID, including the typical PC/SM headgroup fragment ion (*m/z* 184, not shown). However, many fragment ions were observed that were unique to EID.

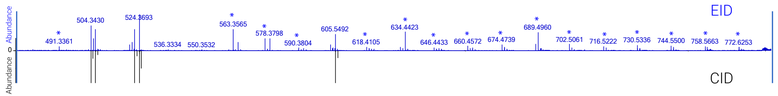

Figure 2. Zoomed MS/MS spectral mirror plot of PC 18:0/18:1 fragmentation, comparing EID spectra (blue) to CID spectra (black). Some of the important fragments uniquely produced with EID that provided structural information are marked with an asterisk.

EID spectra distinguished PC sn-1/sn-2 regioisomers

Both CID and EID produced several fragment ions corresponding to losses of the fatty acyl groups that provided the identities, but not locations, of the esterified fatty acyl (FA) groups. However, EID additionally produced a single unique fragment ion that revealed the identity+location of the esterified sn-2 acyl chain, and through deduction the identity+location of the sn-1 acyl chain could also be determined.

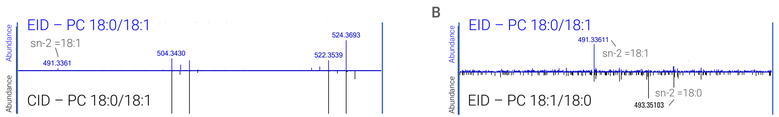

Figure 3. (**A**) Zoomed MS/MS spectral mirror plot of PC 18:0/18:1 fragmentation, comparing EID spectra (blue) to CID spectra (black). The fragments *m/z* 504.3430, 506.3591, 522.3539, and 524.3693 were common between CID/EID and indicated cleavages of the C1—O and C2—O bond in the glycerol backbone, revealing the identities of the esterified FA 18:0 and FA 18:1 groups. The unique EID fragment *m/z* 491.3361 likely resulted from C1—C2 cleavage and localized FA 18:1 to the sn-2 position. (**B**) Zoomed MS/MS spectral mirror plot comparing EID spectra of PC 18:0/18:1 (blue) versus PC 18:1/18:0 (black), where fragments *m/z* 491.3361 and *m/z* 493.35103 localized FA 18:1 and FA 18:0 to the respective sn-2 locations.

EID spectra determined PC fatty acyl double bond location

EID produced a series of ions with spacing of 14.014 Da that corresponded to fragmentation of the PC acyl chain substituents (ΔCH_2 units). As seen with other alternate fragmentation techniques the signal intensity was diminished at the double bond location and increased at neighboring single bond positions, creating a characteristic "V pattern" that was indicative of double bond position.

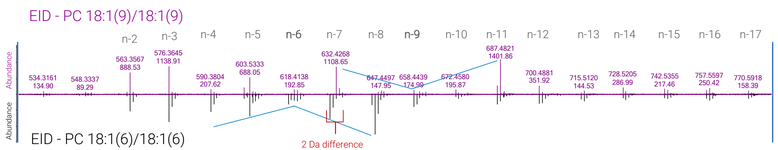

Figure 4. (A) Zoomed MS/MS spectral mirror plot comparing EID spectra of PC 18:1(9)/18:1(9) (purple) versus PC 18:1(6)/18:1(6) (black). The characteristic V patterns (blue) revealed the double bond location in each lipid. Additionally, the expected 2-Da offset (2H atoms) was observed between the n-6 and n-9 fragmentation in the comparison of the two series.

Results and Discussion

EID spectra distinguished PC/SM lipid class

The PC/SM headgroup fragment ion (m/z 184) typically observed with CID was also observed with EID (not shown). However, EID spectra contained additional ions corresponding to fragmentation of the glycerol backbone that provided specificity for PC and ruled out SM, demonstrating the ability of ExD to distinguish lipid classes.

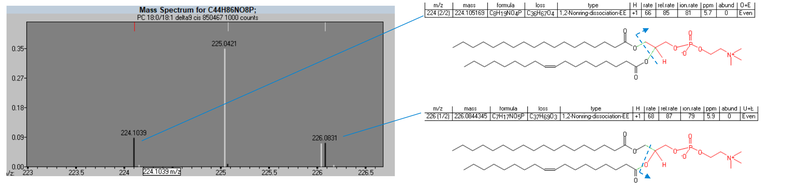

Figure 5. View of NIST MS Interpreter software loaded with PC 18:0/18:1 EID spectra and corresponding lipid structure (imported as .sdf). MS/MS peaks with explained fragmentation appear in black and unexplained are white. Selection of the spectral peaks *m/z* 224.0139 and 226.0831 were each explained with two fragmentations in or near the PC glycerol backbone. The software colored the proposed fragmented bonds green and structural portions representing the selected MS/MS peaks red. Blue arrows were manually added for clarity.

EID provided PC structural information from a typical sample introduced with LC

Compared to CID, EID fragmentation efficiency is low. To determine if the ExD cell implementation on the Agilent 6546 LC/Q-TOF provides sufficient sensitivity on an LC timescale, a complex plasma lipid extract was injected at a typical concentration and analyzed with data-dependent MS/MS acquisition parameters. For abundant PC molecules, EID fragmentation spectra were observed that provided information on lipid class specificity, sn-1/sn-2 fatty acyl group location, and double bond position.

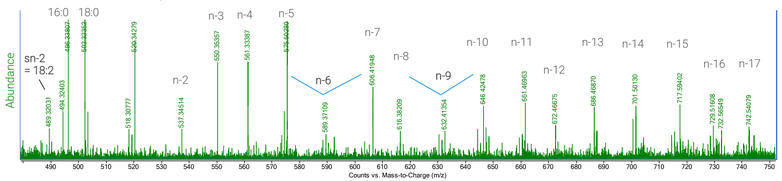

Figure 6. Zoomed view of data-dependent EID MS/MS spectra (5 scans averaged) for precursor *m/z* 758.6, RT 6.3-6.5, corresponding to the endogenous lipid PC 34:2. EID spectra that provided evidence for sn-1/sn-2 fatty acyl group location and double bond position are labeled (bold). The information extended the putative annotation to PC 16:0/18:2(6,9), although a mixture of isomers is possible.

Conclusions

ExD-6546 LC/QTOF produced EID spectra that enabled:

- Determination of sn-1/sn-2 acyl chain location
- Localization of double-bond position
- Discrimination of lipid class
- Structural information on an LC timescale

References

1. Beckman, J.S., et al. *JASMS.* 2021Apr; 32: 2081-91
2. Voinov, V.G., et al. *IJMS.* 2001 Apr; 205: 57-64
3. Blanksby, S.J. et al. *Anal Bioanal Chem.* 2015 Mar; 407: 5053-64
4. Campbel, L.J and Baba, T. *Anal Chem.* 2015 May; 87: 5837-45
5. Baba, T., et al. *J Lipid Res.* 2018 Mar; 59: 910-19

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