Identification of positional isomers of linoleic acid containing phospholipids involved in pancreatic ductal adenocarcinoma

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Overview

- To enhance lipid identification, Oxygen Attachment Dissociation (OAD) MS/MS has been applied to provide double bond specific dissociation, enabling C=C position assignments.
- High resolution OAD MS/MS was applied in positive and negative ion mode to provide further confidence in lipid identification in a pancreatic adenocarcinoma biomarker study.

1. Introduction

Oxygen Attachment Dissociation (OAD) MS/MS is a radical induced dissociation where atomic oxygen (O) and hydroxyl radicals (OH) are generated in a radical source and introduced to a collision cell instead of a collision gas effectively generating C=C specific fragmentation. Given the high diversity of positional isomers in lipids and the impact on enzyme selectivity, lipid mediator functions, tissue-specific membrane composition, and physical properties there is a need to identify lipids with greater certainty.

In this study, the novel radical induced MS/MS technique of Oxygen Attachment Dissociation (OAD) has been applied to provide double bond specific dissociation in lipids previously identified as potential biomarkers in Pancreatic ductal adenocarcinoma (PDAC).

2. Materials and Methods

2.1 LC-MS/MS method

High resolution QTOF LC-MS/MS analysis with CID and OAD MS/MS (LCMS-9050, Shimadzu Corporation, Japan) was used to structurally characterize lipids found to be significantly diminished in serum samples from PDAC patients relative to healthy controls.

- **Reverse phase LC separation**
  - Acquity C18 BEH (2.1x100mm 1.7µm); 50ºC, flow rate 0.4 mL/min.
  - Binary gradient; water + 0.1% formic acid, and acetonitrile + 0.1% formic acid.
  - Cycle time 35 minutes.
- **LC-MS/MS Mass Spectrometry detection with simultaneous CID and OAD**
  - Untargeted metabolite profiling method used a TOF MS mass scan followed by DDA-MS/MS mass scans in ESI+ and ESI- mode.
  - TOF MS mass scan m/z 60-1250; 100 msecs.
  - ESI+ mode: 5 DDA-MS/MS mass scans, m/z 40-1250, 200 msecs per precursor.
  - ESI- mode: 3 DDA-MS/MS mass scans m/z 40-1250; 300 msec per precursor.
  - Collision gas pressure was set to 17kPa and a collision energy spread of 6-30V was applied.
  - External mass calibration for positive and negative mode ESI.
- **Data processing and lipid identification**
  - LabSolutions Insight™ (Shimadzu Corporation) was used to process data.
  - DDA spectra acquired in ESI+ and ESI- mode for each lipid were analyzed to assign C=C double bond positions.
  - Lipids were identified according to the omega and delta nomenclature, counting carbons from the methyl end or the carboxylic end of the fatty acyl, respectively.

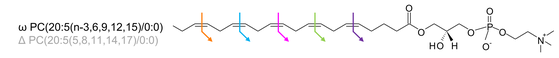
3. Results

OAD MS/MS is an innovative technology, providing additional fragmentation to CID in positive and negative ion mode on the LCMS-9050 QTOF Mass Spectrometer.

- The OAD Radical Source generates neutral radicals such as O/OH/H radicals through microwave discharge of raw material gases (water vapor and hydrogen gas) under vacuum.
- The neutral radicals are introduced through a quartz tube into the OAD cell where they react with precursor ions and specifically oxidize/dissociate double bonds between carbons. This results in fragment ions that cannot be obtained by conventional CID fragmentation.

Application of OAD to enhance identification of potential lipid biomarkers of PDAC.

- In this study, potential biomarkers of PDAC identified in a previous metabolomic profiling study using LC-DIA-MS/MS have been analyzed in greater depth using a combination of OAD to enable C=C position assignments.
- Figure 1 shows an example of how spectra with simultaneous OAD and CID MS/MS were annotated to structurally characterize PC 20:5/0:0, one of the lipids found to be significantly reduced in the serum of PDAC patients compared to healthy controls.



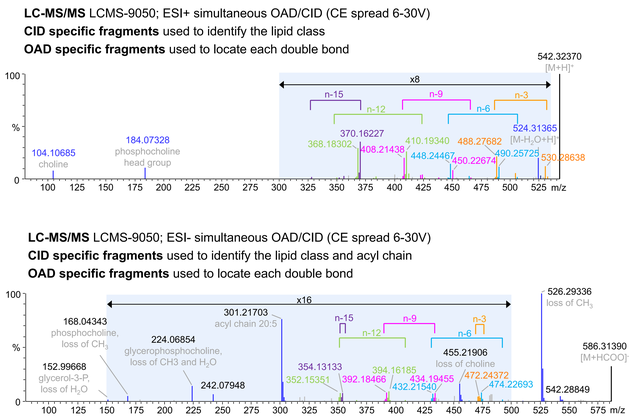


Figure 1. Structural characterization of PC 20:5/0:0 using OAD and CID MS/MS. DDA-MS/MS acquired with simultaneous OAD and CID in ESI+ and ESI- on the precursor for this lipid. CID specific fragments are highlighted in blue and OAD specific fragments are highlighted in different colors to show the ions corresponding to each C=C double bond in the structure. The omega and delta nomenclature are given for each identification.

Following the process described in Figure 1, other significant lipids including PC(18:2(n-6,9)/0:0), PE(18:1(n9)/0:0), PE(18:2(n6,9)/0:0), PC(18:1(n9)_18:2(n6,9)), PC(18:2(n6,9)/18:2(n6,9)) and PC(20:4(5,8,11,14)_18:2(9,12)) were identified to the structural level. Examples of OAD spectral annotation are shown below in Figure 2.

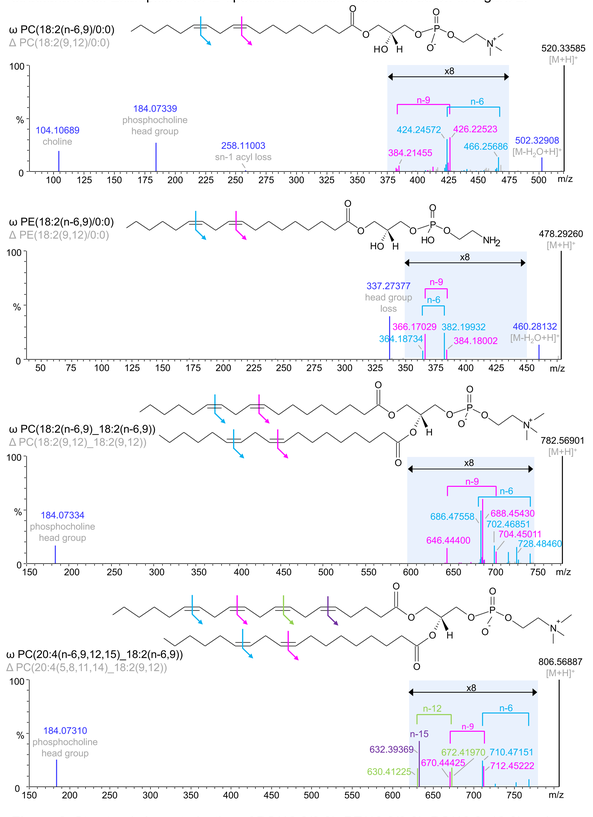


Figure 2. Structural characterization of PC(18:2/0:0), PE(18:2/0:0), PC(18:2_18:2) and PC(20:4_18:2) using DDA-MS/MS with simultaneous OAD and CID MS/MS in ESI+. CID specific fragments are highlighted in blue and OAD specific fragments are highlighted in different colors to show the ions corresponding to each C=C double bond in the structure. The omega and delta nomenclature are given for each identification.

4. Discussion

In high resolution metabolomics analysis by LC-CID-MS/MS and DPiMS™, patient PDAC serum samples were found to exhibit significantly different lipid profiles compared to healthy serum controls. OAD-MS/MS has been applied to increase the certainty of lipid biomarker identification by locating the position of the C=C double bonds that may relate to the biological mechanisms involved in pancreatic ductal adenocarcinoma.

- **Serum phospholipids containing omega-6 linoleic acid (18:2(n-6,9)) are reduced in PDAC patients compared to healthy controls.**
  - Consistent with previously published LC-MS/MS literature, phospholipids containing linoleic acid were significantly reduced.
  - Lipids included PC(18:2/0:0), PE(18:2/0:0), PC(18:1_18:2), PC(18:2_18:2) and PC(18:2_20:4).
  - OAD-MS/MS revealed that all contained linoleic acid, with double bonds in the n-6 and n-9 positions corresponding to omega-6 linoleic acid (18:2(n-6,9)).
- **LPC 20:5 and LPE 18:1 were also highlighted as potential biomarkers for PDAC**
  - OAD has now enabled the structural characterization of PC(20:5/0:0) and PE(18:1/0:0) that were also found to be significantly lower in the serum profiles of PDAC patients compared to healthy controls. OAD revealed the double bond positions at n-9 for PE(18:1/0:0) and n-3,6,9,12 and 15 for PC(20:5/0:0).
- **OAD MS/MS is a unique technology useful for expanding lipidomics workflows**
  - The higher specificity of OAD simplifies the process of structural elucidation by generating spectra that are easy to interpret.
  - The enhanced level of structural identification provided by OAD-MS/MS allows the potential to improve our understanding of the biological roles of lipids.
  - OAD is less hazardous than other methods and provides higher efficiency fragmentation, especially for singly charged positive and negative ion precursors.
  - Simultaneous fragmentation with CID-MS/MS and OAD-MS/MS enables greater reporting confidence in lipid identification.

5. Conclusions

- OAD-MS/MS is a high specificity fragmentation technique to enable C=C positional assignments in lipids. Analysis is easy to perform alongside CID-MS/MS and spectra are easy to interpret.
- OAD-MS/MS has been applied to structurally characterize lipids found to be potential markers of PDAC in a previous study using high resolution metabolomics analysis by LC-CID-MS/MS and DPiMS™.
- The use of this new technology aims to enhance pancreatic cancer research by providing more comprehensive identification needed to understand the biological roles of the lipids identified as potential markers of PDAC.