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Direct Tissue Imaging and Characterization of Phospholipids Using MALDI SYNAPT HDMS System

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Abstract

In this aplication note, the MALDI imaging mode of acquisition was used to obtain information on the spatial distribution of phospholipids directly from thin tissue sections.

Benefits

The phosphatidylcholines were characterised using exact mass MS/MS data acquired with a precursor ion window of 1 Da directly from tissue

Introduction

The last decade has seen a significant rise in the interest in the analysis of lipids.[1] Lipid biology has become a major research area, and as a result the initiation of large-scale comprehensive lipid profiling experiments has

begun. Lipids play an important role in many biological processes, including the formation of cell membranes. Lipid families consist of highly similar structures, with differences in the aliphatic tails. This results in structures with similar *m/z* values. Analyzing these by tandem mass spectrometry, with a wide precursor ion selection window could easily result in the misidentification of the lipid structures. Here we show that a Waters MALDI SYNAPT HDMS System can confidently characterise phospholipids directly from tissue using exact mass MS/MS. Despite the complexity of the sample, it was possible to select the phospholipids of interest by using a 1 Da precursor ion window on the quadrupole.

The MALDI imaging mode of acquisition was used to obtain information on the spatial distribution of phospholipids directly from thin tissue sections.

Experimental

a-cyano-4-hydroxycinnamic acid matrix was used at a concentration of 20 mg/mL in 95% EtOH, 5% water.

- The kidney sections were coated with matrix using an airbrush (Iwata, Portland, OR). The matrix coating was built up over 50 spray cycles, consisting of five passes each
- The sample was analyzed using a MALDI SYNAPT HDMS System
- The instrument was operated in V-mode over a *m/z* range of 50–1000
- The instrument was externally calibrated using a mixture of standard poly(ethylene glycols)
- The imaging resolution used was 250 µm

Results and Discussion

Distribution of phosphatidylcholines

The spatial distribution of three phosphatidylcholines, PC (16:0, 18:1), PC (16:0, 18:2), and PC (16:0, 16:0) have been determined. The structures of these three phospholipids are shown in Figure 1.

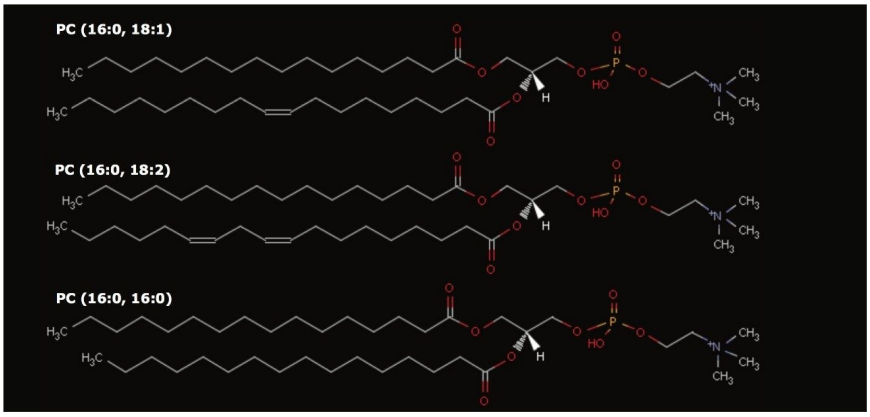

Figure 1. Structures of three of the phosphatidylcholines observed (m/z 756.55, 758.57, and 760.58).

Data were combined across the kidney section to produce a representative mass spectrum, shown in Figure 2. The three peaks of interest are marked (756.5524, 758.5696, and 760.5848), the mass spectral resolution observed for these peaks was ca 12,000 (V-mode) and mass accuracy of 0.78 ppm (RMS) was observed (Lockmass on 734.5700 $[M+H]^+$ of PC (16:0, 16:0)).

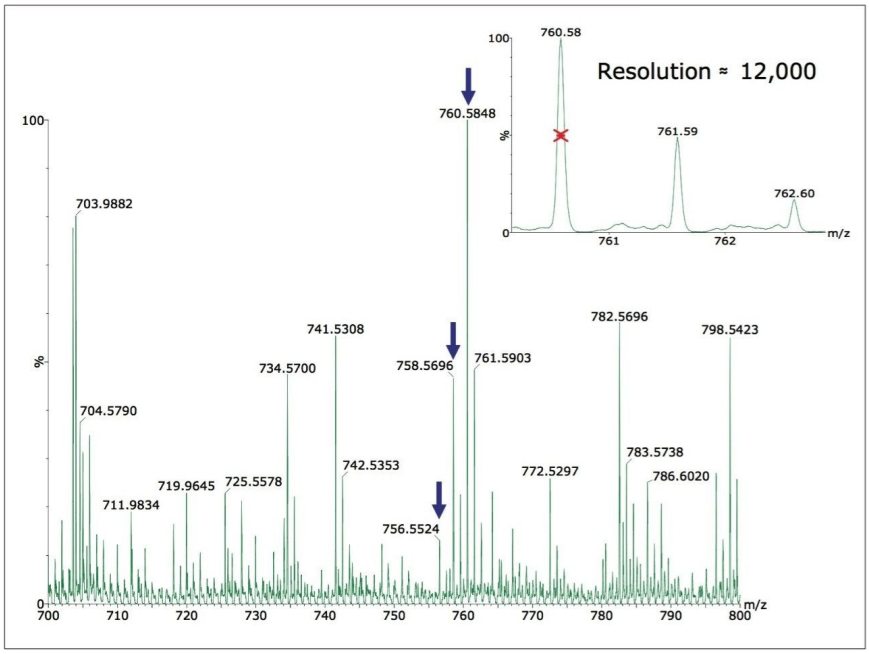

Figure 2. TOF mass spectrum obtained directly from kidney section, showing the [m+H]+ ions of PC (16:0, 18:1), PC (16:0, 18:2) and the [m+Na]+ ion of PC (16:0, 16:0).

The identities of the ions of mass 756.5524, 758.5696, and 760.5848 were confirmed using MS/MS. All MS/MS data were acquired directly from the tissue. In order to isolate these species the quadrupole transmission window was set to 1 Da for precursor ion selection. With a larger precursor ion selection window, unambiguous identification of the phospholipids would have been problematic. A representative MS/MS spectrum is shown in Figure 3. It can be seen that many fragments related to the head-group are observed. To assign a more precise structure, the low intensity fragment ions, 478.34 Da and 500.31 Da, resulting from the neutral loss of the fatty acid groups were used.

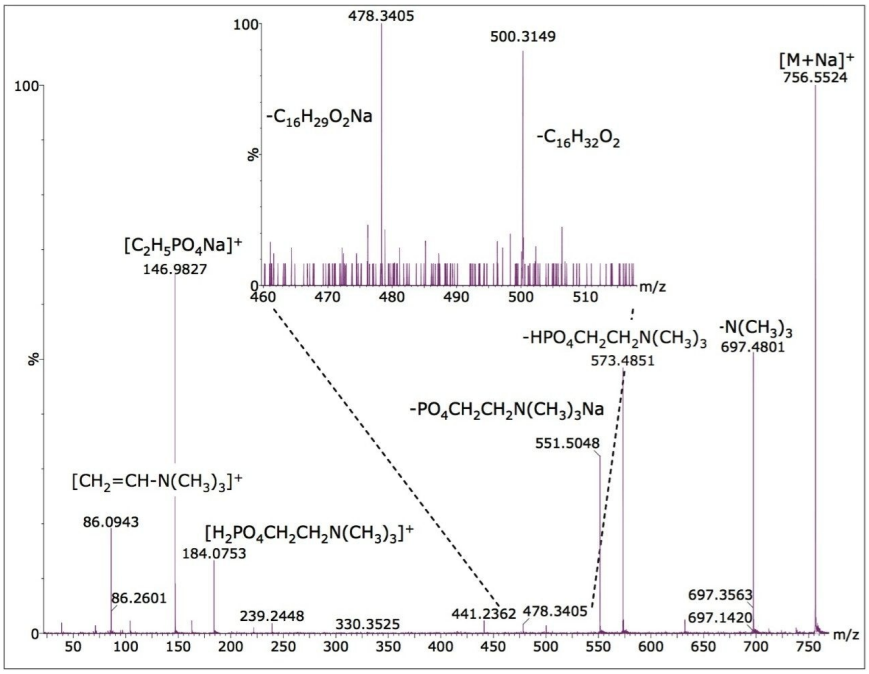

Figure 3. MS/MS spectrum of m/z 756.5524. The inset shows an enlargement of the m/z region 450–550 containing diagnostic fragment ions of the neutral loss of the fatty acid chain, allowing assignment of the structure as PC (16:0, 16:0).

Using the spatial information contained within the tissue imaging data set, ion intensity maps could be produced for the three phosphatidylcholines identified. The three images generated are shown in Figure 4. It can clearly be seen that the phospholipids localize differently, indicating that they have different biological functions.

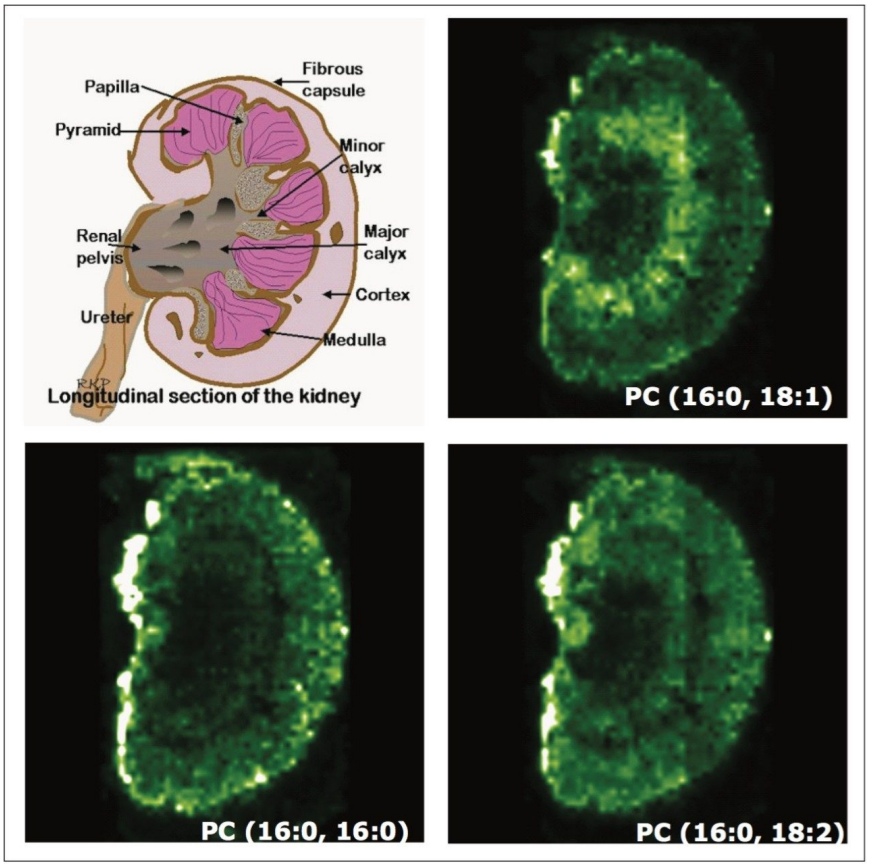

Figure 4. Ion intensity maps for the $[M+H]^+$ ions of PC (16:0, 18:1), PC (16:0, 18:2), and the $[M+Na]^+$ ion of PC (16:0, 16:0).

Conclusion

- Using MALDI orthogonal acceleration Tof Mass Spectrometry on a MALDI SYNAPT HDMS System high resolution exact mass MS and MS/MS data were obtained directly from tissue
- The phosphatidylcholines were characterised using exact mass MS/MS data acquired with a precursor ion window of 1 Da directly from tissue
- The imaging capabilities of the MALDI SYNAPT System were demonstrated, showing the distribution of three phosphatidylcholines (*m/z* 756.5524, 758.5696, and 760.5848) in kidney tissue

References

1. Schiller, J. *et al.*, *Prog. in Lipid Res.*, 2004, 43, 449–488.

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