衝突断面積に基づくMALDIイメージング及びMS / MS フラグメントデータの関連付けによる脂質の同定と局在化解析

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INTRODUCTION

Mass Spectrometry Imaging（MSI）は、リピドミクスの研究において確立された技術になりつつある。MSIを用いることにより幅広い範囲と数の脂質を組織切片上に可視化することができる。しかしながら多くの数の同質量もしくは近似した質量の脂質が存在するため同定は容易ではない。

脂質は同じもしくは連続した組織切片から抽出し、MS/MS測定を行うことにより同定することも可能である。しかしながら、抽出し同定されたイオンをイメージングのデータと関連付ける際に、信頼性を確保することは簡単ではなく、特に質量のみに同定を依存することは難しい。

本研究では、イオンモビリティーによりイオンを分離し、さらに衝突断面積（CCS）を測定した。この情報を精密質量と共に用いることにより、MALDIイメージングデータと抽出した脂質のデータおよびMS/MSによる同定に利用し、関連付けの信頼性向上させることを試みた。

METHODS

Sample preparation
A thin section of a rat brain section was produced using a cryotome and deposited on a non-conductive glass slide.
9-Aminoacridine (9-AA) matrix was applied evenly to the sample in several coats using a Suncollect™ sprayer- 20 coats at 20ul/min of 5mg/ml 9-AA in 4:1 ethanol : water.

Lipids were extracted from consecutive tissue sections using either 2:1 chloroform : ethanol or 4:1 ethanol : water. Up to 10x1µL droplets were placed on to the tissue section using a pipette then drawn off and pooled prior to spotting on a target plate. 1µLof the same 9-AA matrix solution was mixed on target with the extract samples.

Mass Spectrometry
Data were acquired using a SYNAPT HDMS G2-Si mass spectrometer (Figure 1) in MS mode with tri-wave ion guide optics to separate ions according to their ionic mobility in the gas phase. The mass range of acquisition was m/z 50-1,150 where lipid species can be detected, as well as matrix species and small endogenous metabolites.

The mobility cell was calibrated using poly-alanine to allow for CCS area calculations. 1µL of 10mg/ml poly-alanine in methanol, was deposited on a target plate followed by 1ul of the 9-AA solution. The CCS area calibration was performed using Intellistart™ , which automatically generates the constants for the CSS area calculation.

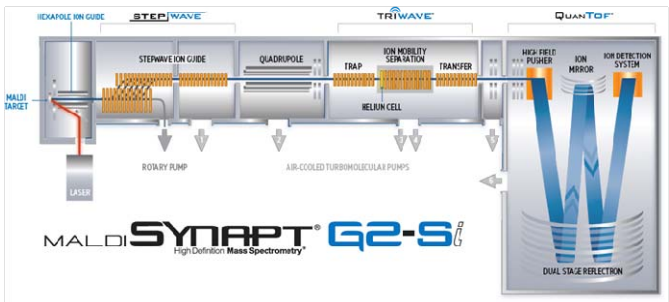

Figure 1. Schematic of the MALDI SYNAPT G2-Si HDMS.

MALDI-MS

Laser	:Solid state Nd:YAG laser (λ = 355nm)
Pulse rate	:2500 Hz
Polarity	:Negative
Spatial resolution	:45 µm (lateral)
IMS pressure	:3.3 mBar
Wave height	:40 V
Wave velocity	:1000 to 250 m/s

Data management
Imaging data sets were subsequently processed using High Definition Imaging 1.2 (HDI) MALDI software for detailed image analysis.

Lipid assignments based on accurate mass were made using an in-house script utilising the LIPID MAPS Structure Database (www.lipidmaps.org).

Lipid MS/MS searches were performed using the online LIPID Maps database

RESULTS

After peak picking the imaging data set with an ion intensity cut off of 50, 7,598 peaks were detected in the range of m/z 400-1150 (figure 2A). Matching the peaks using accurate mass against a subset of the lipid maps structural database containing Fatty Acyls, Glycerolipids, Glycerolphospholipids and Sphingolipids, resulted in 195 matching peaks (+/- 3ppm).

Plotting the matching peaks as m/z vs CCS area, a general lipid trend line can be observed as well as several CCS outliers (figure 2B). By estimating the CCS area trend line, a broad based filter can be applied to remove the outliers. In future with additional information on the lipid CCS area, it will be possible to refine this CCS area based filter. After CCS filtering, 165 lipid candidates remained (figure 2C).

Each lipid candidate could be represented by multiple isobaric lipid species, containing also several isomers. In addition without the orthogonal dimensionality of ion mobility there is the potential that a given m/z feature may be comprised of more than one lipid.

To confirm the identity of the peaks, MS/MS needs to be performed. This could be achieved directly on the tissue after imaging, but this limits the number of peaks that can be identified due to limited tissue surface area.

It is therefore desirable to extract the lipids directly from the same tissue section or a consecutive tissue section and cross reference the datasets by accurate mass.

This however poses a question, given that a peak may represent multiple lipid species and distribution information has been lost, are you sure that the lipid that has been extracted is the same lipid that was seen in the imaging dataset?
Here we propose that the CCS area of the peak can be used as an additional qualifying factor to ensure that the peaks in the two data sets represent the same species.

Two extractions were performed, one using a typical chloroform based extraction solution (extract 1) and one with the same composition as the Matrix solvent (extract 2).

The extract IMS-MS data were peak picked by Apex 3D and run through the same database as the image dataset. The MS spectra can be seen in figure 3.

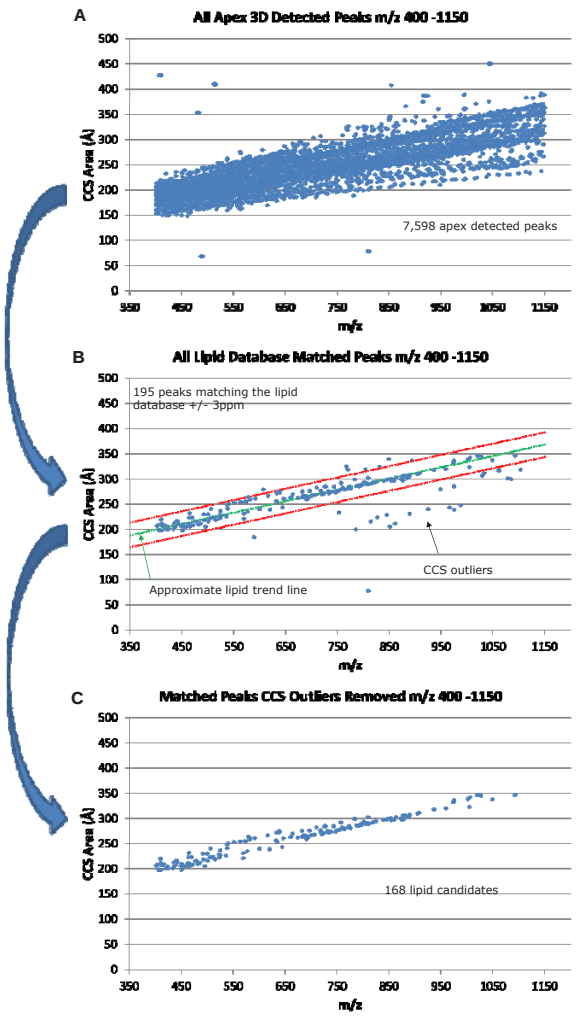

Figure 2. Screening of Apex picked peaks for lipid candidates. A— CCS and m/z of all Apex picked peaks detected with an intensity threshold of 50 within m/z 400 to 1150. B— Peaks shown to match lipid database (green line indicates approximate lipid trend line, red lines show CCS / m/z filter used to remove CCS outliers). C—Lipid candidates with CCS outliers removed.

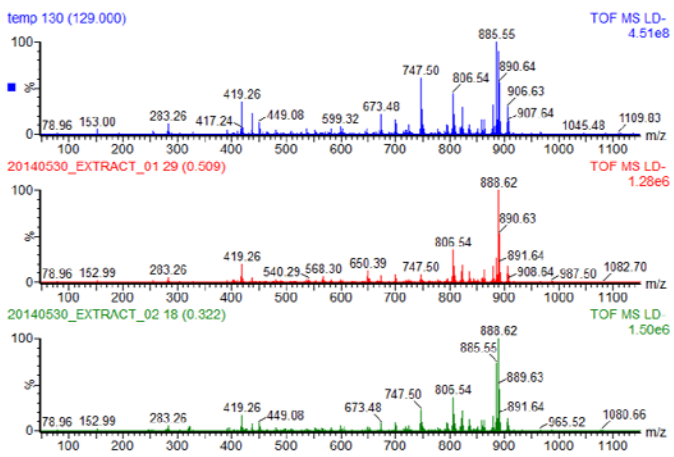

Figure 3. MS spectra of combined image (top), chloroform: ethanol based extract (middle) and ethanol: water based extract (bottom)

The resulting lipid candidate lists were then compared to the list of 168 lipid candidates from the MALDI imaging data set. The CCS areas of the peaks were cross validated (+/-0.5%). The average CCS difference for the matches was found to be +/- 0.11%.

After comparing the 2 lists, 50 candidate lipid peaks were identified as being common to both the MALDI imaging data set and lipid extracts MS data set (figure 4). Those lipid peaks that were indicated to be possible glycerophospholipids, were selected for MS/MS measurement. Where possible the MS/MS spectra were acquired from the ethanol water based extraction as this displayed superior crystallization. The results of the MS/MS experiments can be seen in table 1, MS/MS peak lists were matched against the online lipid maps database. The corresponding ion images can be seen in figure 5.

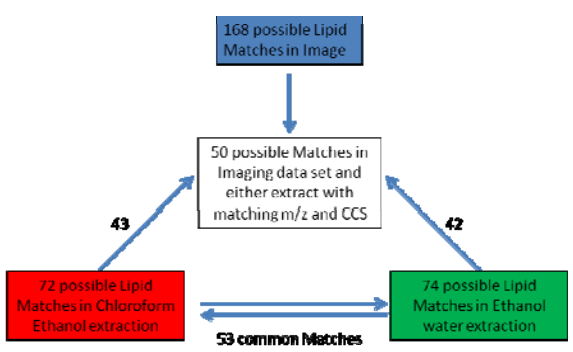

Figure 4. Results of database search on the MALDI imaging and extracted peak lists showing the number of peaks common to both data sets.

Table 1. Results of MS/MS experiments on peaks putatively assigned as glycerophospholipids

#	Matched Mass [M-H}-	Obs. m/z	Obs.CCS (Å)	m/z error (ppm) (1 dp)	Possible Lipid Species	Top Matching Lipid Isomer(s)
1	409.236	409.2352	197.59	-2.0	LPA(16:0)	PA(16:0/0:0)
2	435.2517	435.2504	202.97	-3.0	LPA(18:1) PA(P-18:0)	LPA(18:1)
3	437.2673	437.2664	205.61	-2.1	LPA(18:0) PA(O-18:0)	PA(18:0/0:0)
4	457.236	457.2349	204.70	-2.4	LPA(20:4)	PA(20:4/0:0)
5	461.2673	461.2671	208.82	-0.4	LPA(20:2)	LPA(20:2)
6	463.283	463.2821	211.12	-1.9	LPA(20:1) PA(P-20)	LPA(20:1)
7	478.2939	478.2925	214.67	-2.9	LPE(18:1) LPC(15:1) PE(P-18:0)	PE(18:1/0:0)
8	481.236	481.2348	209.13	-2.5	LPA(22:6)	PA(22:6/0:0)
9	485.2673	485.2663	211.58	-2.1	LPA(22:4)	PA(22:4)
10	508.3045	508.3047	223.44	0.4	LPS(P-18:0)	PE(4:0/15:0) PE(15:0/4:0)
11	599.3202	599.3187	239.52	-2.5	LPI(18:0)	PI(18:0/0:0)
12	619.2889	619.2875	239.09	-2.3	LPI(20:4)	PI(20:4/0:0)
13	673.4814	673.4797	262.05	-2.5	PA(34:1)	PA(16:0/18:1) PA (18:1/16:0)
14	687.497	687.4951	264.08	-2.8	PA(35:1)	PA(17:0/18:1) PA(18:1/17:0)
15	718.5392	718.5373	271.76	-2.6	PE(34:0) PC(31:0)	PE(18:0/16:0) PE(16:0/18:0)
16	719.4657	719.4637	268.20	-2.8	PA(38:6)	PA(22:6/16:0) PA(16:0/22:6)
17	721.4814	721.4801	269.66	-1.8	PA(38:5)	PA(18:1/20:4) PA(20:4/18:1)
18	723.497	723.4954	271.25	-2.2	PA(38:4)	PA(18:0/20:4) PA(20:4/18:0)
19	745.4814	745.4792	273.33	-3.0	PA(40:7)	PA(22:6/18:1) PA(18:1/22:6)
20	747.497	747.4969	275.56	-0.1	PA(40:6)	PA(18:0/22:6) PA(22:6/18:0)
21	750.508	750.5079	276.24	0.1	PE(37:5)	PE(22:5/15:0) PE(15:0/22:5)
22	774.5079	774.5084	280.58	0.6	PC(36:7) PE(39:7)	PE(22:6/17:1) PE(17:1/22:6)
23	788.5236	788.5243	285.68	0.9	PE(40:7) PC(37:7)	PE(22:6/18:1) PE(18:1/22:6)
24	792.5185	792.5183	285.97	-0.3	PS(O-38:6) PS(P-38:5)	PS(O18:2/20:4)
25	794.5341	794.532	287.86	-2.6	PS(P-38:4) PS(O-38:5)	PS(P-18:0/20:4) PS(O-16:0/22:5)
26	797.5338	797.5326	287.76	-1.5	PG(38:4)	PG(18:1/20:3) PG(20:3/18:1) PG(20:4/18:0) PG(18:0/20:4)
27	817.5236	817.5242	291.33	0.7	LPI(34:3) PI(O-34:3) PI(P-34:2)	PI(O-16:2/18:1)
28	818.5341	818.5335	292.52	-0.7	PS(P-40:6) PS(O-40:7)	PS(O-18:2/22:5)
29	838.5392	838.5412	296.54	2.4	PE(44:10) PC(41:10)	PE(22:5/22:5)
30	845.5549	845.5533	297.97	-1.9	PI(O-36:3) PI(P-36-2)	PI(P-18:0/18:2)
31	847.5706	847.5723	299.45	2.0	PI(O-36:2) PI(P-36:1)	PI(O-18:0/18:2) PI(P-18:0/18:1) PI-(O-20:2/16:0)
32	857.5185	857.5173	296.73	-1.4	PI(36:4)	PI(16:0/20:4) PI(20:4/16:0)
33	883.5342	883.5325	301.42	-1.9	PI(38:5)	PI(18:1/20:4) PI(20:4/18:1)
34	885.5498	885.549	303.34	-0.9	PI(38:4)	PI(18:0/20:4) PI(20:4/18:0)

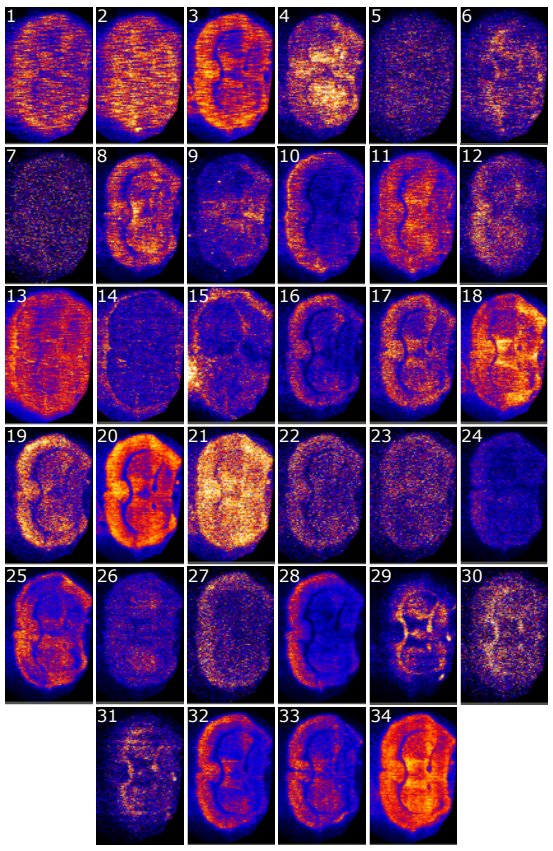

Figure 7. Ion images of glycerophospholipids identified by MS/MS. Numbers correspond to the identifications from table 1

In total 34 of 36 potential glycerophospholipid peaks were successfully identified. Although it was possible to distinguish the head group and the length of the chains it was not possible to determine the order of the chains (sn1 / sn2) due to the pattern of fragmentation in negative mode.

Further work needs to be done to improve the efficiency of the lipid extraction. Future work will be carried out in positive mode with the inclusion of lithium to aid in the determination of the chain order, and potentially using LC-MS/MS.

CONCLUSION

- スクリーニングMALDIイメージングのデータと組織切片から抽出したデータを関連させてデータ解析の複雑さを軽減した。
- CCSを疑陽性を減らすフィルターとして利用できる可能性が示された。
- 脂質を組織切片から抽出刷ることにMS/MSによる同定を迅速に行った。
- CCSは同定の信頼性を高める手段として利用できる可能性が示された。