DIRECT PROFILING OF LIPID DISTRIBUTION IN BRAIN TISSUE USING MALDI-TOFMS

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INTRODUCTION

Lipids comprise a major portion of brain tissue accounting for almost one half of the brain dry weight. They perform numerous tasks in the body such as storage of energy, major building blocks of biological membranes, and aid in signal transduction across biomembranes. Brain lipids consist mainly of three major categories: cholesterol, sphingolipids (sphingomyelin, cerebrosides, sulfatides, gangliosides), and glycerophospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositols). Altered levels of lipids in brain tissue are associated with several diseases such as Alzheimer's disease, neuronal ceroid-lipfuscinosis, and Niemann-Pick disease.

Recent developments in mass spectrometry have permitted direct analysis of biomolecules in tissue. However, most studies have focused on proteins with emphasis on biomarker discovery. In the present work, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) was used for the direct analysis of lipids in rat cerebellum. The lipid bilayer role as a storage depot for small organic molecules such as therapeutic drugs, and pollutants such as DDT, as well as the ability to compare lipid profiles in healthy and diseased animal models are a few of the many reasons why the direct probing of tissue for determining the qualitative and possibly quantitative lipid content could be a very useful tool. Molecular ions corresponding to phosphatidylcholines, sphingomyelins, and phosphatidylethanolamines were recorded in positive ion mode, while ones corresponding to phosphatidylinositols, phosphoserines, sulfatides, and gangliosides were recorded in negative ion mode. MALDI-MS/MS was also employed for direct tissue analysis in order to provide structural data on lipid species.

EXPERIMENTAL

Tissue Sectioning: Male Sprague-Dawley rats were euthanized with a sodium pentobarbital intraperitoneal injection (> 65 mg/kg) and were decapitated upon cessation of respiration. The brains were quickly removed from the skull and frozen in dry ice-chilled isopentane for 15 seconds, prior to storage at –80°C. Frozen brain tissue was cut into thin sections (14 µm thickness) in a cryostat. The tissue samples were attached to the cryostat sample stages using ice slush made from distilled water. Serial brain sections were alternately collected onto a MALDI sample target and poly-L-lysine coated microscopic slide.

Mass Spectrometer: A MALDI-TOF/TOF (4700 Proteomics Analyzer, Applied Biosystems, Foster City, CA) was used in this study for mass analysis in both positive and negative ion mode. A Nd:YAG laser (355 nm) at a repetition rate of 200 Hz was employed for ionization. For MS analysis, mass spectra were the sum of 400 laser shots and acquired in reflectron mode. For MS/MS analysis, mass spectra were the sum of 1000 laser shots and air was used as the CID gas to induce fragmentation.

Sample Preparation: The MALDI matrix used in this study was 2,6-dihydroxyacetophenone (DHA) and was dissolved in 50% ethanol at a concentration of 30 mg/mL. In order to produce lithium adduct formation in tissue sections DHA was dissolved in 100mM lithium chloride (50% ethanol). 0.1 µL of matrix solution was deposited directly on the tissue section and allowed to air-dry prior to insertion into the mass spectrometer. Porcine brain polar lipid extract (Avanti Polar Lipids, Alabaster, AL) and bovine brain ganglioside mixture (CalBiochem, La Jolla, CA) were used as lipid standards.

RESULTS

Detection of phosphatidylcholine, sphingomyelin, phosphatidylethanolamine

Phospholipids, one of the most common classes of lipids found in the brain, are amphipathic and thus found in all membranes. The presence of a positively charged quaternary amine in some makes their ionization in positive ion mode a fait accompli. Figure 1 contains MALDI mass spectra of porcine brain extract and rat brain tissue in positive ion reflectron mode with DHA matrix. Table 1 lists mass peak assignments for lipid species from positive ion mass spectra of the brain extract and two brain regions. In total thirteen phospholipid species, consisting of ten phosphatidylcholines (PC), five sphingomyelin (SM), and three phosphatidylethanolamines (PE), were assigned to recorded mass peaks. For each assigned phospholipid specie, three molecular ions, $[M+H]^+$, $[M+Na]^+$, and $[M+K]^+$, were observed. Although PC and PE have similar concentrations in brain tissue, the relative abundance of PE species are weak compared to PC species in the mass spectral profiles, due to the presence of the positively charged quaternary ammonium group in PC, which greatly facilitates molecular ion formation in positive ion mode.

MALDI-MS/MS was conducted on rat brain tissue in order to provide structural information on PC and SM species. In order to obtain more structural data, salt adducts of PC and SM species were probed. The most complete structural data for PC and SM species were acquired from MS/MS of lithium adducts. Lithium adducts were produced by depositing DHA matrix dissolved in 100 mM LiCl directly onto tissue sections. Figure 2 contains MALDI-MS/MS spectra of $[PC\ 34:1+H]^+$, $[PC\ 34:1+K]^+$, and $[PC\ 34:1+Li]^+$.

Figure 1. Positive Ion Mode MALDI-MS

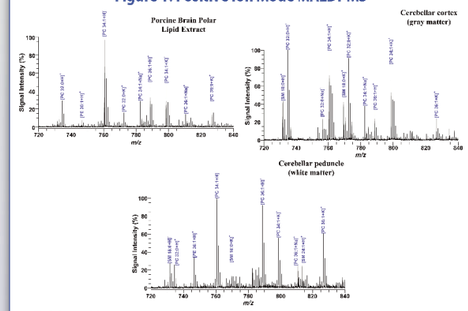


Table 1. Mass peak assignments for lipid species in positive ion mode.

Mass Peak[a]	m/z (theoretical)[b]	Brain Polar Lipid Extract	Cerebellar cortex (gray matter)	Cerebellar peduncle (white matter)
PC 30:0+H	706.538	706.522	----	----
PC 30:0+Na	728.520	728.561	728.537	728.556
PC 30:0+K	744.494	744.593	744.528	744.587
PC 32:0+H	734.569	734.569	734.569	734.566
PC 32:0+Na	756.551	756.555	756.553	756.566
PC 32:0+K	772.525	772.526	772.524	772.574
PC 34:0+H	762.601	762.591	762.596	762.599
PC 34:0+Na	784.583	784.565	784.577	784.560
PC 34:0+K	800.557	800.548	800.549	800.562
PC 34:1+H	760.585	760.585	760.585	760.586
PC 34:1+Na	782.567	782.565	782.564	782.568
PC 34:1+K	798.541	798.542	798.543	798.540
PC 36:1+H	788.616	788.615	788.609	788.615
PC 36:1+Na	810.598	810.602	810.596	810.599
PC 36:1+K	826.572	826.573	826.563	826.567
PC 36:2+H	786.601	786.600	786.598	786.601
PC 36:2+Na	808.583	808.582	808.569	808.601
PC 36:2+K	824.557	824.567	824.552	824.556
PC 36:4+H	782.569	782.565	782.564	782.568
PC 36:4+Na	804.551	804.566	804.540	804.471
PC 36:4+K	820.525	820.532	820.525	820.545
PC 38:4+H	810.601	810.602	810.596	810.599
PC 38:4+Na	832.583	832.575	832.566	832.661
PC 38:4+K	848.557	848.611	848.554	848.633
PC 38:6+H	806.569	806.552	806.549	806.536
PC 38:6+Na	828.551	828.546	828.574	828.560
PC 38:6+K	844.525	844.521	844.513	844.667
PC 40:6+H	834.601	834.569	834.590	834.623
PC 40:6+Na	856.583	856.560	856.585	856.622
PC 40:6+K	872.557	872.568	872.546	872.548
SM 18:0+H	731.606	731.610	731.600	731.604
SM 18:0+Na	753.588	753.579	753.584	753.576
SM 18:0+K	769.562	769.559	769.561	769.558
SM 20:0+H	759.637	759.589	759.607	759.631
SM 20:0+Na	781.619		781.617	781.586
SM 20:0+K	797.593		797.589	797.588
SM 22:0+H	787.669	787.610	787.587	787.620
SM 22:0+Na	809.651	809.602		809.622
SM 22:0+K	825.625	825.579		825.584
SM 24:0+H	815.700	815.702	815.524	815.679
SM 24:0+Na	837.682	837.647	837.661	837.635
SM 24:0+K	853.656	853.652		853.639
SM 24:1+H	813.684	813.683		813.675
SM 24:1+Na	835.666	835.645	835.606	835.633
SM 24:1+K	851.640	851.638		851.639
PE 36:1+H	746.569	746.596	746.566	746.602
PE 36:1+Na	768.551	768.543	768.551	768.550
PE 36:1+K	784.525	784.565	784.577	784.560
PE 38:4+H	768.554	768.543	768.551	768.550
PE 38:4+Na	790.536	790.612	790.582	790.513
PE 38:4+K	806.510	806.552	806.549	806.536
PE 40:6+H	792.554	792.578	792.534	792.549
PE 40:6+Na	814.536	814.524	814.530	814.657
PE 40:6+K	830.510	830.510	830.504	830.478

[a]PC and PE species number equal the total length and number of double bonds of both acyl chains. SM specie number corresponds to the length and number of double bonds of the acyl chain attached to the sphingosine base. [b]Masses are monoisotopic.

34:1 Phosphatidylcholine

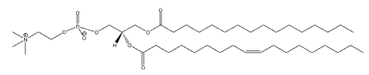
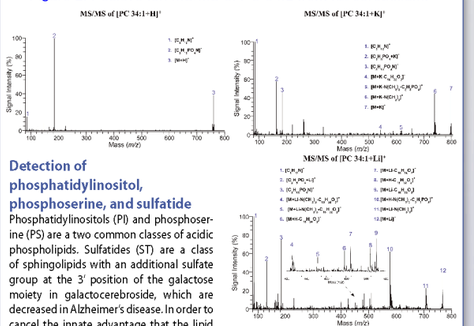


Figure 2. Positive Ion MS/MS of PC 34:1 from rat cerebellum.



Detection of phosphatidylinositol, phosphoserine, and sulfatide

Phosphatidylinositols (PI) and phosphoserine (PS) are a two common classes of acidic phospholipids. Sulfatides (ST) are a class of sphingolipids with an additional sulfate group at the 3' position of the galactose moiety in galactocerebroside, which are decreased in Alzheimer's disease. In order to cancel the innate advantage that the lipid species containing quaternary amines have, negative ion mode was used. Figure 3 shows MALDI mass spectra of brain extract and rat brain tissue acquired in negative ion reflectron mode with DHA matrix. Table 2 lists mass peak assignments for lipid species from negative ion mass spectra of the standard brain extract and the two brain regions. Mass spectra of the cerebellar cortex in negative ion mode are dominated by mass peaks attributed to PI 38:4 and PS species with less intense signals observed for peaks associated with ST and ST(OH) species, while mass spectra of the cebellar peduncle in negative ion mode is dominated by mass peaks assigned to PS, ST and ST(OH) species with a weak signal observed for the peak associated with PI 38:4. Previous distribution profiles of phosphatidylinositol species have measured PI 38:4 as the dominant specie, which is in agreement with our results.[1] Furthermore, the detection of sulfatides in negative mode is expected since they constitute approximately 6% of the total lipid in adult brain and contain a negatively charged sulfate group, which aids ionization in negative mode.[1] Additionally, sulfatides are known to be concentrated in white matter, while phospholipids, such as phosphatidylinositols, are more widely represented in gray matter than in white matter, which also agrees with our mass spectral results.[2] Direct tissue analysis using MALDI-MS/MS was conducted for PS,PI, and ST species for structural conformation. MS/MS spectra of a representative from each class are illustrated in Figure 4.

Figure 3. Negative Ion Mode MALDI-MS

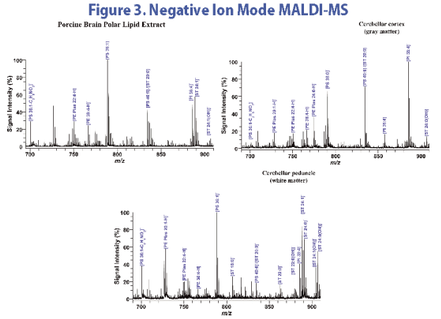
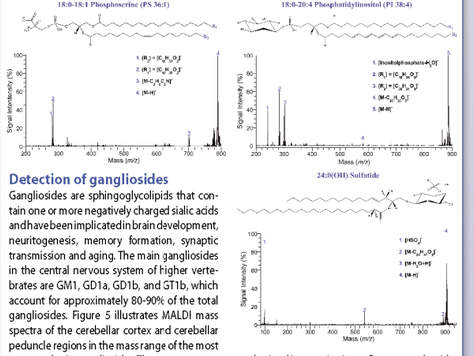


Table 2. Mass peak assignments for lipid species in negative ion mode.

Mass Peak[a]	m/z (theoretical)[b]	Brain Polar Lipid Extract	Cerebellar cortex (gray matter)	Cerebellar peduncle (white matter)
PI 36:4	857.517	857.521	857.521	857.535
PI 38:4	885.549	885.553	885.549	885.550
ST 24:0 (OH)	906.633	906.630	906.631	906.633
ST 24:1 (OH)	904.618	904.627	904.618	904.620
ST 22:0 (OH)	878.602	878.594	878.600	878.601
ST 20:0 (OH)	850.571	850.587	850.532	850.572
ST 18:0 (OH)	822.540	822.570	822.555	822.542
ST 24:0	890.639	890.636	890.635	890.633
ST 24:1	888.623	888.625	888.607	888.621
ST 22:0	862.607	862.600	862.600	862.607
ST 20:0[d]	834.576	834.532	834.529	834.560
ST 18:0	806.545	806.546	806.539	806.547
PS 36:0[c]	790.559	790.537	790.544	790.542
PS 36:0-$C_3H_9NO_2$	702.519	702.533	702.538	702.548
PS 36:1	788.544	788.542	788.546	788.545
PS 36:1-$C_3H_9NO_2$	700.504	700.518	700.537	700.534
PS 38:4	810.528	810.525	810.534	810.525
PS 38:4-$C_3H_9NO_2$	722.488	722.506	722.514	
PS 40:6[c]	834.528	834.532	834.529	834.560
PS 40:6-$C_3H_9NO_2$[c]	746.488	746.503	746.524	746.547
PE 36:0-H[c]	746.570	746.503	746.524	746.547
PE Plas 20:0-H	730.576	730.557		730.568
PE 36:1-H	744.555	744.546	744.564	744.557
PE Plas 20:1-H	728.560	728.549	728.571	728.562
PE 38:4-H	766.539	766.532	766.548	766.545
PE Plas 22:4-H	750.544	750.536	750.549	750.546
PE 40:6-H[c]	790.539	790.537	790.544	790.542
PE Plas 24:6-H	774.544	774.532	774.547	774.542

[a]PI and PS species number equal the total length and number of double bonds of both acyl chains. ST species number corresponds to the length and number of doublebonds of the acyl chain attached to the sphingosine base. [b]Masses are theoretical monoisotopic. [c]Tentative assignment.

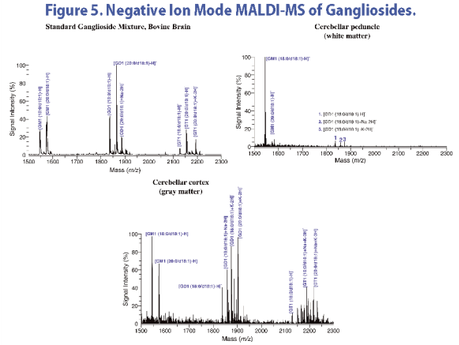
Figure 4. Negative Ion MS/MS of PI, PS, and ST species from rat cerebellum.



Detection of gangliosides

Gangliosides are sphingoglycolipids that contain one or more negatively charged sialic acids and have been implicated in brain development, neuritogenesis, memory formation, synaptic transmission and aging. The main gangliosides in the central nervous system of higher vertebrates are GM1, GD1a, GD1b, and GT1b, which account for approximately 80-90% of the total gangliosides. Figure 5 illustrates MALDI mass spectra of the cerebellar cortex and cerebellar peduncle regions in the mass range of the most common brain gangliosides. These mass spectra were obtained in negative ion reflectron mode with DHA matrix. In the cerebellar cortex, mass peaks assigned to GM1, GD1, and GT1 gangliosides were detected, while in the cerebellar pedencule only mass peaks associated with GM1 were observed. Although GD1 gangliosides were detected, we are unable to distinguish between GD1a and GD1b gangliosides because they are structural isomers differing only in the position of one sialic acid. Compared to other lipid species observed from tissue in this work, the molecular ions of gangliosides are less intense most likely due to fragmentation of gangliosides in the MALDI ionization process. Previous MALDI-MS studies of gangliosides have demonstrated their propensity for losing their sialic acid residues.[3,4] Thus, the main information derived from the mass spectra should be the presence/absence of gangliosides species while the relative abundance of gangliosides species observed should be taken with caution. One additional note is that for direct tissue analysis gangliosides with more than one sialic acid residue (GD1 has two and GT1 has three) either sodium or potassium cations bind to the ganglioside ions to bring the charge to negative one. Recent studies using immunostaining techniques have shown similar results in the rat cerebellum in which GM1 is concentrated in white matter regions while GD1a and GD1b are more prevalent in gray matter regions.[5,6]

Figure 5. Negative Ion Mode MALDI-MS of Gangliosides.



CONCLUSIONS

In this work, a method for *in situ* analysis of cholesterol, sphingolipids, and glycerophospholipids in brain tissue was developed using MALDI-MS and MALDI-MS/MS. This method was employed for the analysis of the cerebellar cortex (gray matter) and the cerebellar peduncle (white matter) in rat brain tissue. Mass spectral lipid profiles obtained from rat cerebellum were in qualitative agreement with traditional lipid analysis techniques for most lipid classes. MALDI-MS/MS was employed successfully for the structural identification of lipid species. The method developed in this study provides several advantages compared to traditional methods for lipid analysis. First, it allows for rapid analysis with little sample preparation when compared to methods involving sample extraction or immunostaining. Additionally, it provides better spatial resolution and uses less tissue sample when compared with techniques, which require sample extraction prior to analysis. Furthermore, by altering the matrix used and the mass range analyzed, this method can easily be adapted to include the additional analysis of peptides and proteins. Future studies will include expanding the mass range and the probing of lipids in additional regions of the brain and other organs. Another possible improvement for this method will be to combine laser capture microdissection (LCM) with MALDI-MS for lipid analysis, which will improve the spatial resolution down to specific cell types.

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