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Discovering Novel Brain Lipids by Liquid Chromatography/ Tandem Mass Spectrometry

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Abstract

Discovery and structural elucidation of novel brain lipids hold great promise in revealing new lipid functions in the brain and in understanding the biochemical mechanisms underlying brain physiology and pathology. The revived interests in searching for novel brain lipids have been stimulated by the expanding knowledge of the roles of lipids in brain functions, lipids acting as signaling molecules, and the advent of lipidomics enabled by the advances in mass spectrometry (MS) and liquid chromatography (LC). The identification and characterization of two classes of novel lipids from the brain are reviewed here: *N*-acyl phosphatidylserine (*N*-acyl-PS) and dolichoic acid (dol-CA). The identification of these lipids benefited from the use of efficient lipid fractionation and separation techniques and highly sensitive, high-resolution tandem MS.

Keywords

N-acyl phosphatidylserine; dolichoic acid; mass spectrometry; liquid chromatography

1. Introduction

Lipids are present at unusually high concentrations in the brain, making up more than half of its dry weight. In addition to serving as structural components of cell membranes and providing energy storage, lipids have been increasingly shown to be involved in communication and signaling within and between cells. Aberrant lipid metabolism has been associated with neurodegenerative diseases such as Alzheimer's, Parkinson's, and Niemann-Pick diseases, as well as neurological disorders, including bipolar disorders and schizophrenia [1-8]. A comprehensive qualitative and quantitative characterization of brain lipids is essential to a thorough understanding of the biochemical mechanisms underlying brain physiology and pathology. Arguably, discovering novel lipids represents one of the most exciting and important endeavors in brain lipidomics. The discovery and structural characterization of novel brain lipids may lead to the elucidation of their biosynthetic and metabolic pathways as well as their functions.

Over the last decade remarkable progress has been made in lipid analysis, particularly by using mass spectrometry (MS) [9-17]. This is largely attributed to the introduction of "soft ionization" techniques: electrospray ionization (ESI) [18], atmospheric pressure chemical ionization

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(APCI) [19,20], and matrix-assisted laser desorption/ionization (MALDI) [21]. These soft ionization techniques, together with the remarkable improvements in high-resolution tandem MS and chromatographic separation techniques, have allowed intact, labile lipid molecules to be directly analyzed by MS with exceedingly high sensitivity and specificity [9,12,14,22]. These MS-based techniques have greatly improved the capability to detect, characterize and quantify novel lipids, including those from the brain. To be reviewed are recently identified novel brain lipids: *N*-acyl phosphatidylserine (*N*-acyl-PS) [23] and dolichoic acid (dol-CA) [24] (Figure 1). The identification, possible biosynthetic and metabolic pathways, as well as potential functions of these lipids will be described and discussed.

2. Extraction, fractionation, separation and identification of novel brain lipids

The procedures for the extraction, chromatographic separation and MS characterization of brain lipids are illustrated in Figure 2.

2a. Lipid Extraction

a) *N*-acyl-PS: Mouse brain lipid extraction was performed using the Bligh-Dyer method [25]. Specifically, half of a frozen mouse brain (~0.5 g) was homogenized in 12.5 mL of ice-cold chloroform/methanol/phosphate-buffered saline (PBS, pH 7.4) (1:2:0.8, v/v) using a glass tissue grinder. After centrifugation, the supernatants were then converted to a two-phase Bligh-Dyer system of chloroform/methanol/PBS (~2:2:1.8, v/v) by adding 3.3 mL of chloroform and 3 mL of PBS. After mixing, the phases were separated by centrifugation at 3,000g at 4 °C for 10 min. The lower phase, containing most of the brain lipids, was dried under a stream of nitrogen, and stored in a freezer (-20 °C) for further analysis.

b) Dolichoic acid: The detailed procedures for isolation and lipid extraction of neuromelanin (NM) granules of human *substantia nigra* (SN) were described by Dr. Luigi Zecca and coworkers [24,26,27]. Specifically, NM was isolated from SN pars compacta region of the human midbrain of neurologically normal adult individuals within 48 h after death and immediately frozen at -80 °C. The dissection of the SN from the frozen human midbrain was performed on a cold plate at -10 °C, homogenized in distilled water (0.03 g/mL) in a glass-Teflon homogenizer, followed by centrifugation at 12,000g for 10 min. The pellets were washed twice with 30 ml of phosphate buffer (0.05 M, pH 7.4)/g of tissue. The SN pellets were next incubated at 37 °C for 3 h with 20 mL Tris buffer (0.050 M, pH 7.5) containing SDS (5 mg/mL)/g of tissue, as standard protocol for the isolation of NM granules. The suspension was centrifuged at 18,000g for 20 min at 20 °C. The supernatant was removed, and 20 mL/g of tissue of the incubating solution (as described above) containing 4 mg of proteinase K/g tissue was added. The sample was incubated for 3 h at 37 °C. The NM pigment was separated by centrifugation at 18,000g, and washed/centrifuged (12,000g) twice with 5 mL of NaCl (9 mg/mL). The NM was then washed/centrifuged (12,000g) with 5 mL of water. After dialysis against water (to remove low molecular weight compounds and remaining salts), the NM was suspended in 1 mL of methanol, sonicated for 5 min, and centrifuged (12,000g, 30 min, 20 °C). The supernatant containing the lipid component was aspirated. The pellet was re-suspended in 1 mL of hexane and centrifuged identically to the methanol fraction. The solvent extracts were combined and evaporated under nitrogen.

2b. Anion-exchange fractionation of lipids

Pre-fractionation of biological lipids prior to analysis by ESI/MS or LC/MS reduces signal suppression of the less abundant ions by the major components. Anionic lipids can be separated using a diethylaminoethyl (DEAE)-cellulose column [28-30]. While standardized DEAE columns are commercially available, the column can also be custom-prepared in the lab [31, 32]. The column capacity is typically 1-10 mg of lipid per ml of resin, and can be scaled from

the μg to the g range. Among the total lipids extracted from the brain, the uncharged lipids (triacylglycerols, diacylglycerols, ceramides, dolichols, and sterols) and the zwitterionic lipids (predominantly phosphatidylethanolamines, phosphatidylcholines, and sphingomyelins) emerge in the run-through fractions. These species account for about three quarters of the total brain lipids. The anionic lipids, including the phosphatidylserines, phosphatidylinositols, cardiolipins, sulfatides, phosphatidylglycerols, and phosphatidic acids, bind to the DEAE-cellulose column. They are step-eluted with increasing concentrations (e.g., 30, 60, 120, 240, and 480 mM) of ammonium acetate as the aqueous component of chloroform/methanol/water (2:3:1, v/v). The singly charged phosphatidylinositols, phosphatidylglycerols and phosphatidylserines are eluted with 30 to 60 mM ammonium acetate, whereas the more acidic species, such as phosphatidic acids, sulfatides, cardiolipins and *N*-acyl-PS emerge with 60 to 120 mM ammonium acetate. Residual cardiolipins, *N*-acyl-PS and minor acidic lipids, such as cytidine diphosphate (CDP)-diacylglycerols and the phosphatidylinositol phosphates, emerge with 240 to 480 mM ammonium acetate. Each fraction contains additional minor lipids, many of which are poorly characterized.

2c. Reverse phase liquid chromatography (LC)

A narrow-bore C8 column (Zorbax C8 RP column, 5 μm , and 2.1×50 mm) was used for reverse phase LC-MS/MS analysis. Samples were loaded via a PAL auto-sampler (Leap Technologies, Carrboro, NC) that maintained the samples at 4 °C. LC was performed at a flow rate of 200 $\mu\text{L}/\text{min}$ with a linear gradient as follows: 100% mobile phase A was held isocratically for 2 min and then linearly increased to 100% mobile phase B over 14 min and held at 100% B for 4 min. Mobile phase A consisted of methanol/acetonitrile/aqueous 1 mM ammonium acetate (60:20:20, v/v/v); Mobile phase B consisted of 100% ethanol containing 1 mM ammonium acetate. A post-column splitter diverted ~10% of the LC flow to the ESI source of the quadrupole time-of-flight (QqTOF) tandem mass spectrometer (Q-Star XL, Applied Biosystems, Foster City, CA).

2d. Mass spectrometry

The structural identification and characterization of unknown lipids were carried out by accurate mass measurement and MS/MS using a QqTOF tandem mass spectrometer (Q-Star XL, as above). The high resolution (10,000-15,000) of the instrument was important for assigning and confirming the molecular formulae for most lipids. The negative and positive electrospray voltages were set at -4200 V and +5500 V, respectively. Other MS settings were as follows: CUR = 20 psi (pressure), GS1 = 20 psi, DP = -55 V, and FP = -265 V. For MS/MS, collision-induced dissociation (CID) was performed in Q2 (which was offset from Q1 by 40-70 V) with nitrogen as the collision gas. Data acquisition and analysis were performed using the Analyst QS software. The quantitation of *N*-acyl-PS in pig brain total lipid extract (Avanti) was performed with reverse phase LC-coupled multiple reaction monitoring (MRM) on a 4000 Q-Trap hybrid triple quadrupole linear ion-trap mass spectrometer equipped with a Turbo V ion source (Applied Biosystems, Foster City, CA). LC-MRM analysis was performed in the negative ion mode with MS settings as follows: CUR = 20 psi, GS1 = 20 psi, GS2 = 30 psi, IS = -4500 V, TEM = 350 °C, ihe = ON, DP = -70 V, EP = -10 V and CXP = -5 V. The voltage used for collision-induced dissociation was -50 V. To estimate the level of brain *N*-acyl-PS, a known quantity of synthetic *N*-acyl-PS standard (Avanti) was added to a defined amount of the total pig brain lipids dissolved in 1 mL of $\text{CH}_3\text{OH}/\text{DMSO}$ (1:1, v/v), with the final concentrations of 0.1 $\text{ng}/\mu\text{L}$ for the synthetic *N*-acyl-PS and 0.1 $\mu\text{g}/\mu\text{L}$ for the pig brain total lipids. Ten μL of the sample solution was then injected onto a C8 column for each LC-MRM analysis, using the LC conditions as described above, but without flow splitting.

3. *N*-acyl-PS

3a. Identification

The question of whether *N*-acyl-PS molecules exist naturally has remained unanswered for almost four decades. Nelson first reported the presence of *N*-acyl-PS in sheep erythrocytes [33], but the analysis was based only on infrared spectroscopy (IR), thin-layer chromatography (TLC) and elemental analysis, and it was not subjected to MS or NMR characterization. In 1982, Donohue *et al* reported the identification of *N*-acyl-PS as a major phospholipid in *Rhodopseudomonas sphaeroides* [34]. However, this claim was challenged by Schmid and coworkers who demonstrated conclusively that the phospholipid isolated by Donohue *et al.* was actually phosphatidyl-Tris, unexpectedly generated by microbial incorporation of exogenous Tris buffer into a glycerolphospholipid [35].

The definitive identification of *N*-acyl-PS was reported only recently. Through the application of anion-exchange chromatography and high-resolution tandem MS, a family of *N*-acyl-PS molecular species were identified, first from the mouse brain, and then from other tissues or cells [23]. The anion-exchange chromatography with a DEAE-cellulose column was important in separating the *N*-acyl-PS species from the major brain phospholipids, thus reducing signal suppression effects during MS analysis. The structural characterization and verification of *N*-acyl-PS were accomplished by accurate mass measurements, tandem MS, LC/MS, and by comparing with a synthetic analog (Avanti) [23].

Figure 3A shows the presence of multiple *N*-acyl-PS ion species (m/z 1000-1100) in the negative ion ESI mass spectrum of mouse brain lipids eluting from a DEAE-cellulose column with 120 mM ammonium acetate as the aqueous component of chloroform/methanol/water (2:3:1, v/v). The *N*-acyl-PS structure was proposed based on MS/MS and exact mass measurement. Figure 3B shows the MS/MS spectrum of the most abundant *N*-acyl-PS $[M - H]^-$ ion at m/z 1026.78. The product ions at m/z 78.96 (PO_3^-), 96.97 ($H_2PO_4^-$) and 152.99 ($C_3H_6O_5P^-$) are characteristic of a glycerophospholipid. The two acyl chains esterified to the glycerol backbone were identified as stearic (C18:0) and oleic (C18:1) acids, whose carboxylic anions are observed at m/z 283.25 and 281.25, respectively. This structural assignment is consistent with the product ion at m/z 701.49, corresponding to a phosphatidic acid anion with C18:0 and C18:1 fatty acids attached to its glycerol backbone. The m/z 419.24 and 437.24 ions are derived from the phosphatidic acid anion by neutral loss of the C18:1 moiety as a fatty acid (RCO_2H) or as a ketene ($RCH=CO$), respectively. Similarly, the m/z 417.22 and m/z 435.22 ions are derived by neutral loss of the C18:0 chain as a fatty acid and as a ketene, respectively. The assignment of two acyl chain positions, with the C18:0 chain being attached predominantly to the *sn*-1 position and the C18:1 chain to the *sn*-2 position of the glycerol backbone, was according to the observation by Hsu and Turk [36].

The phosphatidic acid anion at m/z 701.49 (Figure 3B) was produced by MS/MS of the $[M - H]^-$ ion at m/z 1026.73 via a neutral loss of 325.24 amu. The odd nominal mass number of the neutral loss suggested that it contained an odd-number of nitrogen atoms (the "nitrogen rule"). The exact mass of the neutral loss moiety, 325.261 amu, was determined by the mass difference between the accurately measured $[M - H]^-$ ion mass (1026.773) (Figure 1A) and the theoretical mass (701.512) of the 18:0/18:1 phosphatidic acid anion. The exact mass of the neutral loss suggested an elemental composition of $C_{19}H_{35}NO_3$ (calculated exact mass: 325.262 amu). This elemental composition, combined with the MS/MS data, suggested *N*-palmitoyl phosphatidylserine (18:0/18:1) as the structure that gives rise to the m/z 1026.78 ion (Figures 3A and 3C). By its chemical structure, *N*-acyl-PS has a net charge of minus two, which is consistent with its elution order from the DEAE-cellulose column together with cardiolipin. The structure of *N*-acyl-PS was further confirmed by comparing its MS/MS spectrum with that

of a synthetic analog (1,2-dioleoyl-*sn*-glycerol-3-phospho-*N*-nonadecanoyl-serine) from Avanti [23].

Brain *N*-acyl-PS consists of a large number of molecular species. The mass spectrum in Figure 4A (m/z 980-1200) is an expansion of Figure 3A, revealing the presence of a series of *N*-acyl-PS ions in addition to the most prominent species at m/z 1026.80 (*N*-palmitoyl-PS). When subjected to further MS/MS analysis, these *N*-acyl-PS species were found to be highly complex because of the heterogeneity of both their *O*- and *N*-linked acyl chains. As demonstrated in Figure 3B with the MS/MS of the m/z 1026.80 ion, the various *O*-linked fatty acyl groups could be assigned based on the masses of the released fatty acid anions. The identities of *N*-linked acyl chains were deduced from the neutral losses that yield the phosphatidic acidic anions, indicating the presence of at least thirty different *N*-linked acyl chains (with chain length ranging from C14 to C30) [23].

Of particular interest is the detection of *N*-arachidonoyl-PS. As illustrated in Figure 4B, *N*-arachidonoyl-PS was one of the species with m/z 1074.77. The product ions at m/z 255.23, 281.25, 283.27, 303.24, 309.28 are the anions of C16:0, C18:1, C18:0, C20:4 and C20:1 fatty acids, respectively, indicating that these are the possible fatty acyl moieties esterified to the glycerol backbones. The masses of the neutral loss yielding the phosphatidic acidic anions in MS/MS of the isobaric ions at m/z 1074.77 were used to identify the *N*-acyl chains: 325.24 amu for *N*-palmitoyl-PS, and 351.25 amu for *N*-oleoyl-PS, and 373.25 amu for *N*-arachidonoyl-PS. As discussed below, *N*-arachidonoyl-PS might be a biosynthetic precursor of the recently discovered signaling lipid, *N*-arachidonoyl serine [37].

3b. Quantitation

To quantify the *N*-acyl-PS level in the total brain lipids, a *N*-acyl-PS analog synthesized by Avanti, 1,2-dioleoyl-*sn*-glycero-3-phospho-*N*-nonadecanoyl-L-serine, was added as an internal reference to a known quantity of total pig brain lipids (Avanti). The ion signal ratio of the endogenous and synthetic *N*-acyl-PS species was determined by using reverse phase LC-coupled multiple reaction monitoring (MRM) on an ABI 4000 Q-Trap hybrid triple quadrupole linear ion-trap mass spectrometer. The MRM pairs for the most abundant endogenous *N*-acyl-PS species and the synthetic *N*-acyl-PS analog are 1026.8/701.6 and 1066.8/699.6, respectively. In aggregate, the *N*-acyl-PS species constitute about 0.1 % by weight of the total pig brain lipids. A similar level of *N*-acyl-PS was detected in the lipid extract of the mouse spinal cord (unpublished data), suggesting that *N*-acyl-PS may play a special role in the central nervous system. In comparison, *N*-acyl-PS is much less abundant in several other tissues or cells surveyed. For example, *N*-acyl-PS accounts for only about 0.001% of the total lipids extracted from the RAW cells [23].

3c. Biosynthesis and metabolism

A functional characterization of *N*-acyl-PS in the brain will benefit from the elucidation of the biosynthetic and degradative pathways. *N*-acyl-PS is structurally similar to *N*-acyl phosphatidylethanolamine (*N*-acyl-PE) [38] whose biosynthesis and metabolism have been subjected to extensive investigation. Initially found to accumulate in myocardial infarcts [39], *N*-acyl-PEs are formed by an enzyme that transfers the *sn*-1 acyl chain of a glycerophospholipid to the primary amine of PE in a Ca^{2+} -dependent manner [40]. The identity of the structural gene(s) encoding the relevant Ca^{2+} -dependent transacylase is still unknown [38]. Functionally, *N*-acyl-PEs serve as the biosynthetic precursors of a class of lipophilic signaling molecules, the *N*-acyl ethanolamines [38] [41], which include the endocannabinoid anandamide (*N*-arachidonoyl ethanolamine) [42], the anti-inflammatory agent *N*-palmitoyl ethanolamine [43], the satiety-inducing factor *N*-oleoyl ethanolamine [44], and the pro-apoptotic lipid *N*-stearoyl ethanolamine [45].

Analogous to those for *N*-acyl-PE, the proposed pathways for the formation and degradation of *N*-acyl-PS are shown in Figure 5. Similar to *N*-acyl-PE, *N*-acyl-PS could be formed by a transacylation reaction. Experimentally, according to Figure 5, *in vitro* incubation of PS with an appropriate phospholipid co-substrate, such as PC (16:0/18:1), should result in the formation of *N*-palmitoyl-PS, the major *N*-acyl-PS species detected in the brain and RAW cells. The turnover of *N*-acyl-PS by a phospholipase D is proposed in analogy to the formation of *N*-acyl serine from *N*-acyl-PE [46]. However, *N*-acyl-PS might first be deacylated by a phospholipase A [41,47] and then converted to *N*-acyl serine by a phosphodiesterase (not shown). Recently, *N*-arachidonoyl-L-serine, a novel signaling lipid, was isolated from bovine brain in trace amounts [37]. The biological and physiological functions of *N*-arachidonoyl-L-serine include vasodilation of rat isolated mesenteric arteries and abdominal aorta, and the suppression of lipopolysacchride (LPS)-induced TNF- α production by RAW macrophage tumor cells [37].

4. Dol-CA

4a. Identification

Dol-CA was recently identified by LC/MS/MS in the lipid extract of neuromelanin granules from human *substantia nigra* (SN) [24]. While this is the first identification of dol-CA as a natural product from any tissue, the *in vitro* production of this class molecules has been reported [48]. In particular, NAD-dependent enzymatic conversion of dolichol to dol-CA in bovine thyroid extracts was demonstrated [48].

Figure 6 illustrates the LC/MS/MS identification of dol-CA species in the lipid extract of human NM. The major lipid species, as identified through MS/MS, include phospholipids, sulfatides, and sphingolipids. The dolichols and their related species are eluted off the column (reverse phase C8) in the later part of the gradient. Figure 6B is the averaged mass spectrum showing the NM lipids with m/z 1180-1440 that elute from 15.5-17.0 min. The series of peaks near m/z 1236, 1304, 1372, and 1440 are the acetate adduct $[M + Ac]^-$ ions of dolichol species with chain lengths of $n = 17, 18, 19$, and 20 , respectively (where n denotes the number of isoprene units). The peaks near m/z 1258, 1326 and 1394 represent an unknown series of polyisoprenoid derivatives, not reported in previous studies [49].

Figure 6C is the collision-induced dissociation MS/MS spectrum of the m/z 1258.1 ion. The 68 amu mass difference between several of the product ions suggested that the m/z 1258 ion is derived from a polyisoprenoid compound. Based on the MS/MS and accurate mass measurement ($[M - H]^-$ at m/z 1258.129, Fig. 6B), the structure of dol-CA ($n = 18$, Figure 6C inset) was proposed for the species at m/z 1258 (calculated exact mass of the $[M - H]^-$ ion is 1258.125). The structure of dol-CA was further confirmed by comparing its MS/MS spectrum with that of nor-dolichoic acid, a synthetic analog from Avanti [24]. Dol-CA molecular species were also detected in the mouse and pig brains, but their relative concentrations are much lower than those in the human NM. For this reason, the detection of dol-CA in the mouse and pig brains required pre-fractionation using anion-exchange chromatography [24]. Dol-CA molecular species were detected in the lipid fraction eluted from DEAE-cellulose with 30 mM ammonium acetate as the aqueous component. The presence of dol-CA molecules in the 30 mM fraction is consistent with the one net negative (-1) charge associated with dol-CA.

4b. Quantitation

Mammalian dolichol is a mixture of molecular species, usually consisting of 16–22 isoprene units [50]. Species with 18–20 units are the most abundant, reportedly accounting for 14% of the dry weight of NM pigment [51]. While being the predominant prenol lipid present in animal cells, free dolichol is initially biosynthesized as the diphosphate derivative [52]. The latter is then cleaved to dol-phosphate and free dolichol [52]. However, kinases exist that can recycle

dolichol back to dolichol-phosphate [52]. Dolichol-phosphate is much less abundant in animal cells than free dolichol. Furthermore, dolichol-phosphate is converted to several important dolichol-phosphate sugar derivatives. These include dolichol-phosphate mannose and the dolichol-diphosphate oligosaccharide (GlcNAc₂Man₉Glc₃) used for protein *N*-glycosylation [52,53]. Like dolichol-phosphate itself, these dolichol-phosphate sugar derivatives are much less abundant than free dolichol.

To estimate the dol-CA level in the human NM, a known quantity of nor-dol-CA (from Avanti) was spiked into the lipid extract of human NM as an internal reference. Remarkably, the concentration of dol-CA is quite high (30.7 µg of dol-CA per mg of NM) [24] and is about a quarter of that for free dolichol in the NM. The distribution of dol-CA chain lengths (14-20 isoprene units) in NM also differed from that of dolichol, suggesting that the enzyme(s) responsible for the conversion of dolichol to dol-CA prefers a dolichol substrate containing 19 isoprene units [24].

4c. Biosynthesis and metabolism

NM is a complex entity with melanic components bound to metals, peptides, and lipids, the structures of which are largely unknown [54-56]. The potential interplay between NM and the loss of pigmented neurons in Parkinson's disease has prompted significant interest in understanding its structure and interactions [57,58]. Elucidation of how dol-CA is made and metabolized will facilitate the study of its function within the NM.

Based on the catabolism of phytol (a plant isoprenoid) [59], dol-CA could be formed from dolichol by the successive action of an alcohol dehydrogenase and a fatty aldehyde dehydrogenase. There are two possible pathways for the degradation of dol-CA. The first is the β-oxidation of dol-CA through the peroxisomal system, yielding propionyl-CoA [60]. The second is degradation via α-oxidation, producing formic acid, which is further oxidized to CO₂, as demonstrated by the incubation studies of MDCK cells (canine distal tubulus cells) with synthetic dol-CA [60].

The identification of biosynthetic and metabolic enzymes for dol-CA may be accomplished through the expression cloning strategy, for which the development of a quantitative *in vitro* assay (e.g., using LC/MS) for following the formation and degradation of dol-CA will be a necessary first step.

5. Conclusions

Although the brain lipidome has been explored for decades, unknown brain lipid species continue to be discovered, due in large part to the development of powerful analytical techniques. The identification of *N*-acyl-PS and dol-CA benefited from the application of 1) effective fractionation and separation techniques, which allow minor lipid species to be separated from the major ones; 2) highly sensitive, high-resolution tandem MS which permits the detection and structural characterization of the minor, unknown lipids. Furthermore, searchable lipid structure databases (a subject not discussed in this review) can facilitate the quick identification of known lipids, avoiding the time-consuming, *de novo* structural characterization. While still being developed, the lipid databases available at www.lipidmaps.org and www.lipidbank.jp are informative and user-friendly.

Discovery of novel brain lipids holds great promise in expanding the knowledge of lipid function in the brain. The structures of the novel lipids provide clues for their biosynthetic and metabolic pathways [61], which will ultimately aid in the investigation of their roles in brain physiology and pathology.

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***N*-acyl-PS:** **Dol-CA:**

Figure 1.
Example structures of *N*-acyl phosphatidylserine (*N*-acyl-PS) and dolichoic acid (dol-CA).

Figure 2.
Flow chart of procedures for the extraction, separation and MS characterization of brain lipids.

Figure 3.

ESI-MS/MS identification and chemical structure of *N*-acyl-PS. (A) Negative ion ESI/MS of mouse brain lipids eluting from a DEAE-cellulose column in chloroform:methanol:120 mM aqueous ammonium acetate (2:3:1, v/v). (B) MS/MS of *N*-acyl-PS with [M-H]⁻ at m/z 1026.78. (C) The proposed brain *N*-acyl-PS structure (1-stearoyl-2-oleoyl-*sn*-glycero-3-phospho-*N*-palmitoyl-serine) for the species with [M-H]⁻ at m/z 1026.78. Abbreviations: PA, phosphatidic acid; CL, cardiolipin; PS, phosphatidylserine; ST, sulfatide.

Figure 4.

A family of *N*-acyl-PS molecular species in mouse brain. (A) Expanded negative ion mass spectrum (m/z 980-1200) from Figure 1A, showing a family of *N*-acyl-PS $[M - H]^-$ ions. These *N*-acyl-PS species were identified by the exact mass measurements and MS/MS. (B) Complex *N*-acyl chain compositions, as revealed by MS/MS analysis of the peak at m/z 1074.77. The neutral losses in the MS/MS were used to identify the *N*-acyl chains: 373.25 amu for *N*-arachidonoyl-PS, 351.25 amu for *N*-oleoyl-PS, and 325.24 amu for *N*-palmitoyl-PS.

Figure 5.

Proposed pathway for the biosynthesis of *N*-acyl-PS and its conversion to *N*-acyl serine. These reactions are analogous to those involved in the formation of *N*-acyl-PE and anandamide. Although PC is shown, other glycerophospholipids may serve as acyl donors. The predominance of the *N*-palmitoyl moiety in the *N*-acyl-PS series suggests that the *N*-acyl chain arises mainly from the *sn*-1 position of donor glycerophospholipids. PC, phosphatidylcholine; PS, phosphatidylserine; PA, phosphatidic acid.

Figure 6.

Reverse phase LC/MS/MS identification of dol-CA from human neuromelanin (NM). (A) The total ion chromatogram of LC/MS (in the negative ion mode) of the lipid extract of NM isolated from the human SN. (B) Averaged mass spectrum (m/z 1180 - 1440) of the NM lipid species eluting from 15.5-17.0 min [shaded region in (A)]. The singly charged ions at m/z 1236, 1304, 1372, and 1440 are the acetate adduct [M + Ac]⁻ ions of dolichol species with chain lengths of $n = 17$, 18, 19, and 20, respectively. The starred peaks at m/z 1258, 1326, and 1394 are unknown species, later identified as the [M - H]⁻ ions of dol-CA species with $n = 18$, 19, and 20, respectively. (C) MS/MS spectrum and the proposed fragmentation scheme of the [M - H]⁻ ion at m/z 1258 for $n = 18$ dol-CA. (Figure adapted from Ref. [24])