thematic review

Global analyses of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry: a bridge to lipidomics

Xianlin Han[1,*,†] **and Richard W. Gross**[*,†,§,**]

Division of Bioorganic Chemistry and Molecular Pharmacology,* Departments of Medicine,† Pharmacology & Molecular Biology,§ and Chemistry,** Washington University School of Medicine, St. Louis, MO 63110

Abstract **Lipidomics is a rapidly expanding research field in which multiple techniques are utilized to quantitate the hundreds of chemically distinct lipids in cells and determine the molecular mechanisms through which they facilitate cellular function. Recent developments in electrospray ionization mass spectrometry (ESI/MS) have made possible, for the first time, the precise identification and quantification of alterations in a cell's lipidome after cellular perturbations. This review provides an overview of the essential role of ESI/MS in lipidomics, presents a broad strategy applicable for the generation of lipidomes directly from cellular extracts of biological samples by ESI/MS, and summarizes salient examples of strategies utilized to conquer the lipidome in physiologic signaling as well as pathophysiologically relevant disease states. Because of its unparalleled sensitivity, specificity, and efficiency, ESI/MS has provided a critical bridge to generate highly accurate data that fingerprint cellular lipidomes to facilitate insight into the functional role of subcellular membrane compartments and microdomains in mammalian cells. We believe that ESI/MS-facilitated lipidomics has now opened a critical door that will greatly increase our understanding of human disease.**—Han, X., and R. W. Gross. **Global analyses of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry: a bridge to lipidomics.** ***J. Lipid Res.*** **2003.** 44: **1071–1079.**

Supplementary key words Alzheimer's disease • diabetes • electrospray ionization mass spectrometry • phospholipid • plasmalogen • platelets • tandem mass spectrometry • triacylglycerol

Lipidomics is a rapidly expanding research field fueled by recent advances in, and novel applications of, electrospray ionization mass spectrometry (ESI/MS). Lipidomics is focused on identifying alterations in lipid metabolism and lipid-mediated signaling processes that regulate cellular homeostasis during health and disease. Research in lipidomics incorporates multiple techniques to quantify the precise chemical constituents in a cell's lipidome, identify their cellular organization (subcellular membrane compartments and domains), delineate the biochemical mechanisms through which lipids interact with each other and with crucial membrane-associated proteins, determine lipid-lipid and lipid-protein conformational space and dynamics, and quantify alterations in lipid constituents after cellular perturbations. Through the detailed quantification of a cell's lipidome (e.g., lipid classes, subclasses, and individual molecular species), the kinetics of lipid metabolism, and the interactions of lipids with cellular proteins, lipidomics has already provided new insights into health and disease. The true power and promise of lipidomics, however, is only beginning to be realized.

Decades of painstaking research in the 1970s and 1980s developed a straight and reversed-phase HPLC system that could "rapidly" (30 min) separate phospholipid classes, molecular species, and regioisomers into individual chemical constituents (1–6). These techniques, however, were labor intensive and required sensitive separation methods for which quantitation was difficult, because the $\pi \rightarrow \pi^*$ transition during UV detection was not strictly proportional to mass content (7). Moreover, these procedures could not meet the needs for the application of lipidomics to the study of human disease because they were plagued by cumulative errors from multistep chromatographic procedures. Finally, these early efforts required a substantial amount of mass for adequate signal-to-noise ratios and suffered from an inability to accurately quantify

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Abbreviations: AA, arachidonic acid; AD, Alzheimer's disease; ESI, electrospray ionization; FFA, free fatty acid; GalC, galactocerebroside; MS, mass spectrometry; NL, neutral loss; PC, choline glycerophospholipid; PE, ethanolamine glycerophospholipid; PlsEtn, plasmenylethanolamine; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdGro, phosphatidylglycerol; PtdIns, phosphatidylinositol; PtdSer, phosphatidylserine; SM, sphingomyelin; TAG, triacylglycerol.

[1] To whom correspondence should be addressed.
e-mail: xianlin@pcg.wustl.edu

minor mass constituents, which are of substantial importance in lipid-mediated signaling processes. These facts notwithstanding, substantial insights were made through HPLC, fast atom bombardment/MS, and gas chromatography/MS approaches in the 1980s (7, 8–11), and the stage was set for the growth of lipidomics in the 1990s.

Because the extraordinary sensitivity of ESI/MS for lipid analyses was first identified in the initial analyses of platelet activating factor by Weintraub et al. (12) and diacylglycerols by Duffin et al. (13), multiple ESI/MS techniques have been developed and extensively used for the analyses of various classes, subclasses, and individual molecular species of lipids from biological sources. An excellent and extensive review covering these developments and applications has recently been published (14). Herein, we will provide an overview of the essential role of ESI/MS in the development of lipidomics and present a strategy we have utilized to obviate chromatographic separation of lipids resulting in the unparalleled precision, accuracy, and speed in the determination of individual cellular lipidomes.

PRINCIPLES OF ESI

ESI is an ionization technique used for the mass spectrometric analysis of polar compounds that was initially developed by Fenn and colleagues (15). The technique has been extensively explored, and many theories of the physical processes involved in ion production have been proposed and validated in some detail (16–19). ESI is effected by applying a strong electric field ($\sim 10^6$ V/m with a potential difference of 3–6 kV) under atmospheric pressure to a liquid passing through a capillary tube with a slow flow (normally 1–10 μl/min). The field induces charge accumulation at the liquid surface located at the end of the capillary, and mechanical forces are utilized to spray the mobile phase into highly charged droplets. A sheath gas flowing coaxially to the infused spray forces dispersion to be limited in space and minimizes the effects of mobile phase differences during online ESI/MS. These droplets then pass either through a curtain of heated inert gases (most often nitrogen) or through a heated capillary, or both, for subsequent desolvation, leaving the generated ions ready for MS analysis.

The evaporation of the solvent in droplets causes them to shrink to the point where the repulsive Coulombic forces approach the magnitude of the forces of surface tension, as predicted from the formula of Lord Rayleigh over 100 years ago:

$$q^2 = 8\pi^2\varepsilon_0\gamma D^3 \qquad (Eq.\ 1)$$

where q represents charge, ε_0 is the permittivity of the environment, γ is the surface tension, and D is the diameter of a spherical droplet. When the Rayleigh stability limit is exceeded, the droplets "explode," emitting a number of smaller droplets. These droplets then undergo a cascade of iterative ruptures, yielding consecutively smaller and smaller droplets. After the solvent is sufficiently evaporated from the droplet, the analyte has been effectively ionized (17).

ESI can also be used for molecules that do not possess any intrinsic ionizable site through formation of adduct ions, as illustrated in **Fig. 1**. Thus, as long as a sufficient dipole potential is present in a molecule to interact with either a small anion or cation, it can be ionized during the ESI process if appropriate conditions are utilized. For example, although triacylglycerols (TAGs) containing long-chain fatty acids are nonpolar lipids, TAG can be ionized and quantitated with a sensitivity in the low picomole range through formation of lithiated adducts formed from chelated lithium ions noncovalently associated with the carbonyl in the infused solution (20, 21).

GENERAL STRATEGY OF LIPIDOME ANALYSES BY ESI/MS

The utility of selective ESI ionization is based on the differential propensity of each lipid class to acquire either positive or negative charges under the source high voltage. This was exploited to allow the resolution of lipid classes directly from chloroform extracts without prior chromatographic separation (22, 23). In essence, lipid classes can be separated through their endogenous electric potential, thereby obviating multiple sequential chromatographic procedures. Through judicious use of sample preparation, each class of lipid can be resolved in the ionization source and individual molecular species can be further resolved by MS and/or tandem MS. During the last decade, studies in our group and those of our colleagues have demonstrated that ESI/MS of lipids represents one of the most sensitive, discriminating, and direct methods to assess alterations in the cellular lipidome (21–30).

Through appropriate sample preparation ESI/MS allows *1*) the complete quantitative analysis of lipid classes, subclasses, and individual molecular species in minutes without prior chromatographic separation or derivatization; *2*) a higher signal-to-noise ratio in comparison to other mass spectrometric approaches; *3*) a nearly linear relationship between the relative intensities of molecular ions and the mass of individual lipids over a 10,000-fold

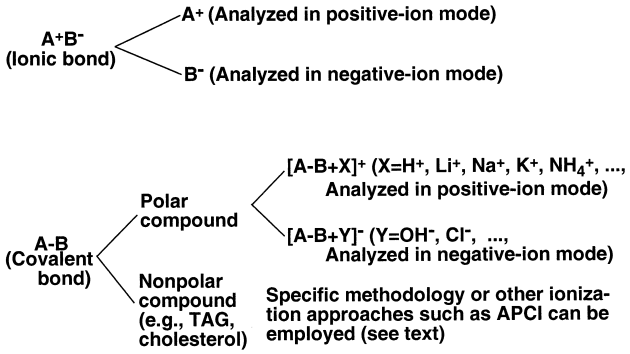

Fig. 1. Diagram of the ionization of ionic or covalent-linked compounds by electrospray.

dynamic range; *4*) independence of ion intensity [within experimental error (<5%)] on the nature of the polar lipid subclass or the individual molecular species; and *5*) excellent reproducibility of sample measurements (<5% of experimental error). Through implementation of these techniques, a high throughput platform for the detailed study of lipid alterations has been developed at a time when lipid-induced disease states are epidemic in industrialized nations (e.g., diabetes, obesity, and atherosclerosis).

We have stressed that this approach is valid only at low concentrations of lipid in the infusion solution, in which lipid-lipid interactions and ion suppression are rare. The concentrations we have utilized range from fmol to pmol of total lipid per μl, as we have demonstrated (22). The linearity within these analyte concentrations has recently been independently verified (31). When the concentration of lipids in the infusion solution increases to the point where lipid-lipid interactions predominate, the effects of acyl chain length and unsaturation on lipid quantitation become apparent and must be avoided (31, 32). This is largely due to lipid aggregation in the sprayed droplets during ionization under these high concentration conditions since lipid packing (or aggregation) is highly dependent on the physical properties of lipids (33, 34).

A commonly used strategy for lipidome analyses from different biological samples without the need for prior chromatographic separation of lipidomes is illustrated in **Fig. 2**. Anionic lipids, including cardiolipin, phosphatidylglycerols (PtdGros), phosphatidylinositols (PtdInss), phosphatidylserines (PtdSers), phosphatidic acids, and sulfatides in the diluted chloroform extracts of biological samples can be analyzed by negative-ion ESI-MS and quantitated by comparisons of the individual ion peak intensity with an internal standard (e.g., 14:0–14:0 PtdGro for anionic phospholipids or N16:0 sulfatide for sulfatides) after

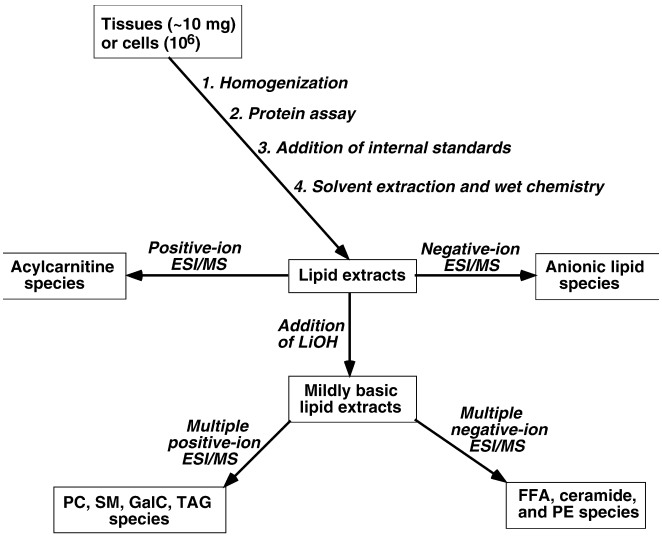

Fig. 2. Schematic diagram of the experimental strategy used for global analyses of cellular lipidomes directly from crude extracts of biological samples.

correction for ^{13}C isotope effects relative to the internal standards as described (23, 35) (see ref. 21 for considerations for ^{13}C isotope effects). Previously, it has been demonstrated that different molecular species of anionic phospholipids have nearly identical ionization efficiencies after corrections for ^{13}C isotope effects (±10%) for molecular species containing acyl chains with 14 to 20 carbons and different numbers of double bonds (22). A typical negative-ion ESI/MS mass spectrum of a mouse myocardial lipid extract (**Fig. 3A**) demonstrates multiple abundant anionic phospholipid molecular species that have been identified by tandem MS (25, 36). It should be noted that ESI/MS was utilized instead of ESI tandem MS for quantitation of lipidome because the latter technique results in differential fragmentation rates for individual molecular species containing different acyl constituents that are highly sensitive to the collisional activation energy employed (21, 25, 28, 37). However, since many peaks are comprised of isobaric molecular species, tandem mass spectrometry should be performed to estimate the molar ratio of isobaric molecular species as we previously described (36).

Prior to the analyses of galactocerebrosides (GalCs), choline glycerophospholipids (PCs), ethanolamine glycerophospholipids (PEs), and sphingomyelins (SMs) in the diluted lipid extracts, LiOH in methanol (50 nmol/mg of protein) is usually added to each individual cellular extract of biological samples to supply counter ions for GalC, PC, and SM analysis, and to turn PE molecular species into anionic phospholipids, thereby achieving the separation of lipid classes in the electrospray ion source (Fig. 2). PE molecular species can then be directly quantitated by comparison with an internal standard (e.g., 15:0–15:0 PtdEtn) after correction for ^{13}C isotope effects relative to the internal standard by ESI/MS in negative-ion mode (Fig. 2). A typical negative-ion ESI/MS mass spectrum of the mouse myocardial lipid extract (the identical extract used for the acquisition of Fig. 3A) after addition of a small amount of LiOH displays multiple abundant PE molecular species (Fig. 3B). Identification of ion peaks containing phosphoethanolamine can be achieved utilizing precursor ion analysis as described previously (25). Acyl chain(s) of each individual PE molecular species can be identified either by utilizing product ion ESI tandem MS as previously described (36) or by employing an efficient two-dimensional fingerprinting technique by ESI tandem mass scanning of all potential acyl carboxylate ions in the precursor ion mode. A typical two-dimensional precursor ion fingerprint (**Fig. 4**) demonstrates different acyl chain constituents of PE molecular species of a mouse myocardial lipid extract (the identical extract used for the acquisition of Fig. 3B). Quantification of ion peaks corresponding to multiple individual molecular ions can be substantiated utilizing product ion ESI tandem mass spectrometric analyses as described previously (36). Plasmenylethanolamine (PlsEtn) molecular species can be distinguished from alkyl-acyl phospholipid molecular species by treating lipid extracts with acidic vapors prior to mass spectrometric analyses as described previously (38).

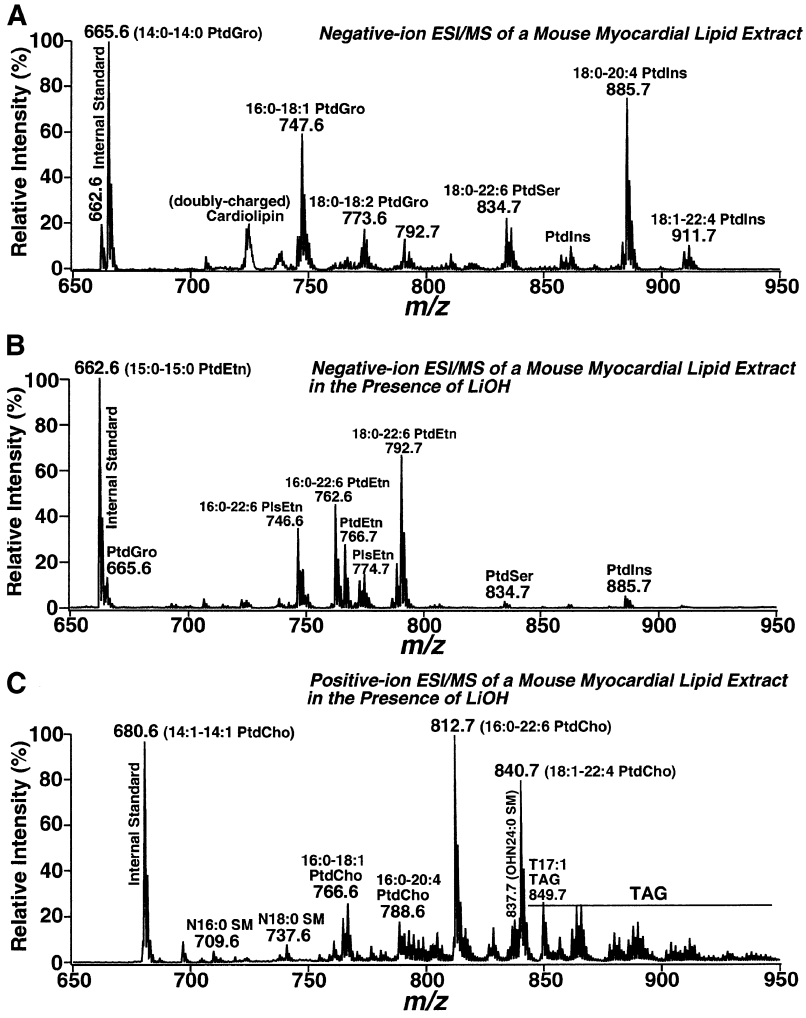

Fig. 3. Electrospray ionization (ESI) mass spectra of a mouse myocardial lipid extract. A: Shows a negative-ion ESI mass spectrum in the absence of LiOH in the lipid extract. B and C: Negative- and positive-ion ESI mass spectra of the lipid extract after addition of LiOH, respectively. Mouse myocardial lipids were extracted by a modified Bligh and Dyer method. The identities of all indicated molecular species have been confirmed by ESI tandem mass spectrometry (MS).

PC and SM molecular species in the diluted tissue extracts can be directly quantitated as their lithium adducts by comparison with an internal standard [e.g., lithiated 14:1–14:1 phosphatidylcholine (PtdCho)] after correction for ^{13}C isotope effects relative to the internal standard in the positive-ion mode (Fig. 2). GalC molecular species in the diluted tissue extracts can also be directly quantitated as their lithium adducts by comparison with an internal standard (e.g., lithiated d35-N18:0 GalC) after correction for ^{13}C isotope effects relative to the internal standard in the positive-ion mode (Fig. 2). Individual molecular species can be identified by tandem MS (25, 26, 36). A typical positive-ion ESI/MS mass spectrum of a mouse myocardial lipid extract (the identical extract used for the acquisition of Fig. 3B) demonstrates multiple abundant choline-containing phospholipid molecular species (Fig. 3C).

Because of the low abundance of some lipid metabolites (less than a few percents of total lipids) in lipid extracts of biological samples, either special sample preparations or separate ESI/MS analyses typically need to be performed. For example, after rendering the lipid extract solution basic by addition of a small amount of LiOH in methanol, free fatty acids (FFAs) in solution will be converted to their carboxylate anion and can be easily quantified by ESI/MS in negative-ion mode scanning through the mass range from 200 to 400. Therefore, quantification is easily accomplished utilizing an internal standard [e.g., deuterated FFA, FFA with odd carbon numbers, or FFA as only a minor component in the lipid extracts (e.g., 20:0 FFA). However, precautions should be taken to ensure there is no significant contribution from endogenous components]. A typical negative-ion ESI mass spectrum of a mouse myocardial lipid extract in the presence of a small amount of LiOH (the identical solution used for the acquisition of Figs. 3B and 3C) displays very abundant FFA molecular species (**Fig. 5A**).

It has been demonstrated that nonhydroxy and 2-hydroxy subclasses of ceramide molecular species displayed distinct fragmentation patterns in product ion ESI tan-

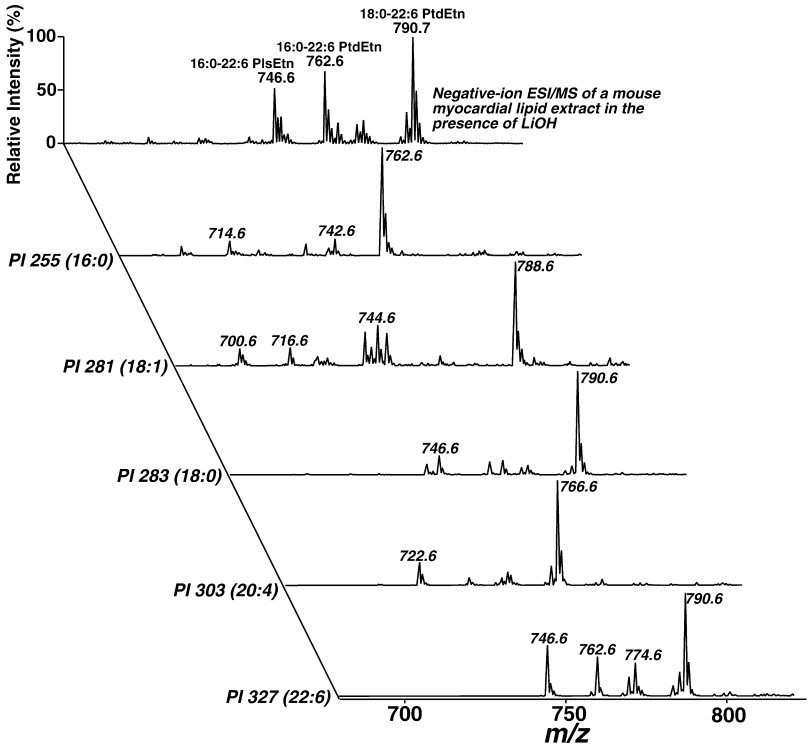

Fig. 4. The two-dimensional fingerprint of ethanolamine glycerophospholipid molecular species in a mouse myocardial lipid extract by negative-ion ESI tandem MS in the precursor ion mode. The lipid extract is identical to the one described in the legend of Fig. 3 obtained in the presence of LiOH. All precursor ion (PI) mass spectra displayed are normalized to the base peak in the individual mass spectrum.

dem mass spectra (28). A unique abundant product ion corresponding to the neutral loss (NL) of mass 256.2 or 327.3 units is present in the fragmentation pattern of nonhydroxy or 2-hydroxy ceramide molecular species, respectively. Thus, negative-ion NL scannings of mass 256.2 and 327.3 units during direct infusion of crude lipid extracts in the presence of LiOH can be performed to identify nonhydroxy and 2-hydroxy ceramide molecular species in the lipid extracts (28). In addition, a common product ion with identical abundance corresponding to the NL of mass 240.2 units for both subclasses of ceramide is also present in all product ion mass spectra of ceramides containing N-acyl sphingosine with an 18-carbon homolog. Thus, quantitation of ceramide molecular species from crude extracts of biological samples in comparison with an internal standard after correction for ^{13}C isotope effects can be achieved by NL scanning of mass 240.2 units (28). For example, negative-ion ESI tandem MS with NL scanning of mass 240.2 units of a mouse myocardial lipid extract in the presence of a small amount of LiOH (the identical solution used for the acquisition of Figs. 3 and 5A) demonstrates over 10 ceramide molecular species (Fig. 5B), which can be quantified in comparisons to an internal standard [N17:0 ceramide (m/z 550.6)].

Intriguingly, TAG (a class of nonpolar lipids, Fig. 1) also shows abundant lithiated ions in the positive-ion ESI mass spectra of lipid extracts under the ionization conditions employed (Fig. 3C). Direct TAG quantitation as their lithiated adducts by positive-ion ESI/MS is still confounded, however, by the presence of overlapping peaks from PCs and the presence of multiple isobaric molecular species in the majority of TAG pseudomolecular ion peaks (Fig. 3C). Accordingly, we have recently exploited the rapid loss of phosphocholine from PCs in conjunction with NL scanning of individual fatty acids of TAG to directly quantitate TAG from biological extracts, as previously described in detail (21). Deconvolution of overlapping and isobaric peaks in the positive-ion ESI mass spectra of lipid extracts by two-dimensional fatty acyl group analyses is accomplished by iterative processing resulting in a detailed molecular species fingerprint of individual TAG molecular species directly from chloroform extracts of biological samples. A typical two-dimensional fingerprint of TAG molecular species of a mouse myocardial lipid extract (as shown in Fig. 3C) demonstrates the lipid constituents (**Fig. 6**). In this figure, the importance of the rapid loss of phosphocholine from PC and its impact on leaving the TAG molecular species behind for NL analyses of fatty acyl chains has been well demonstrated. For example, the spectrum acquired from NL scanning of 328.3 units (corresponding to 22:6 FFA) displays very low-abundant ion peaks corresponding to PC molecular species containing 22:6, while the abundant displayed peaks in the NL spectrum are those from low-abundant TAG ion peaks in the MS spectrum. This method readily detects as little as 0.1 pmol of each TAG molecular species from crude lipid extracts and is linear over a 1,000-fold dynamic range (21). Therefore, fingerprinting and quantitation of individual

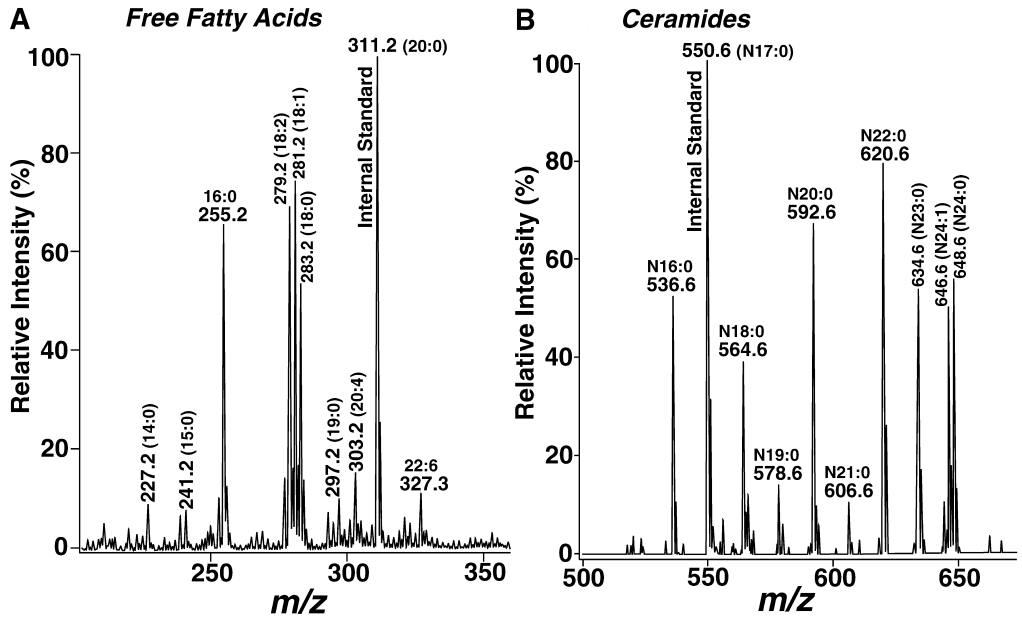

Fig. 5. Quantitative analyses of free fatty acid (FFA) and ceramide molecular species by ESI/MS. Negative-ion ESI mass spectrum (A) of the identical extract after addition of LiOH used in Fig. 3 demonstrates multiple FFA molecular species in the extract. Negative-ion ESI neutral loss (NL) scanning of 240.2 units (B) of the identical lipid extract after addition of LiOH demonstrates multiple ceramide molecular species.

TAG molecular species directly from chloroform extracts of biological samples can be achieved with an error of ~10%, which has been routinely attained in our laboratories (21, 39–41).

For each class of other nonpolar lipids (Fig. 1), specific methodology may need to be developed. For example, cholesterol and its esters can be quantitated by ESI/MS after one-step derivatization, as described previously (42). Alternately, other soft ionization techniques of MS, such as atmospheric pressure chemical ionization, which has recently been reviewed (43), may be employed to analyze these nonpolar lipids.

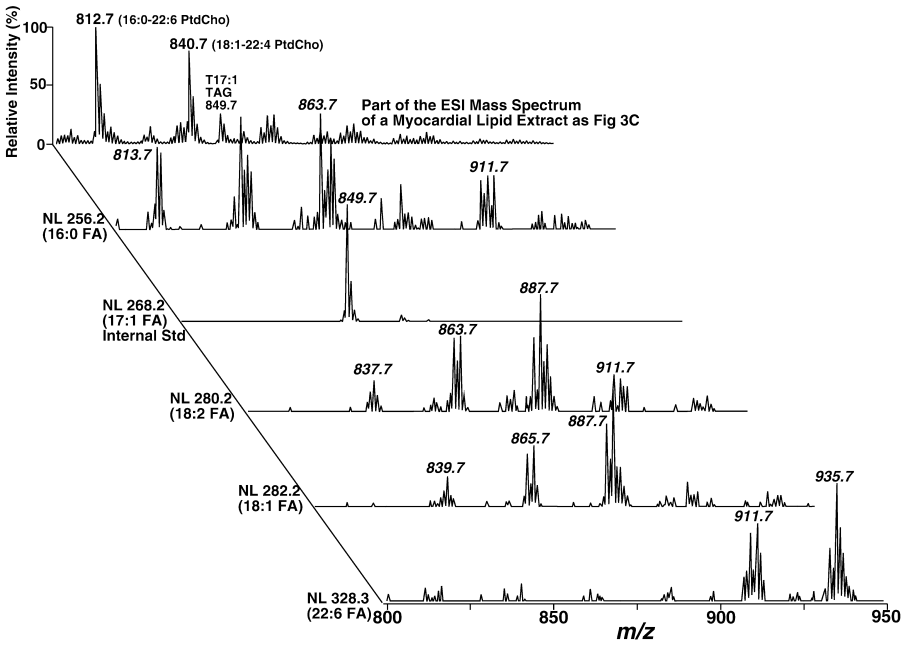

Fig. 6. Two-dimensional fingerprint of triacylglycerol molecular species of a mouse myocardial lipid extract by positive-ion ESI tandem MS. The lipid extract is identical to the one described in the legend of Fig. 3 in the presence of LiOH. All NL mass spectra displayed are normalized to the base peak in the individual mass spectrum.

EXAMPLES OF LIPIDOMIC STUDIES BY ESI/MS

Alterations in phospholipids during human platelet activation

The first lipidome analysis determined directly from crude lipid extracts of biological samples was performed over 7 years ago (23). In that study, the amounts of arachidonic acid (AA) mobilized in human platelets during thrombin stimulation were quantified, and the individual molecular species from which it was released were identified. Through analytical quantitation of released AA and changes in pool sources, several long-standing questions regarding the amount of AA mass mobilized and the enzymatic mechanisms responsible for the released AA were answered. In that study, it was found that specific AA-containing molecular species were present in human platelets, accounting for 48 mol% of PE species, 27 mol% of PC species, 52 mol% of PtdSer, and 80 mol% of PtdIns. Plasmalogens were highly enriched in the PE pool, representing 52 mol% of total PE species or 20 mol% of total phospholipids. Moreover, 53 mol% of these PlsEtn species contained AA, accounting for 58 mol% of AA-containing PE species and 26 mol% of the entire AA-containing phospholipid pool. After 90 s of thrombin stimulation, a total of 60 pmol of AA-containing phospholipids/10^6 platelets was hydrolyzed. This included the loss of 32 pmol/10^6 platelets from PE, but only 11 pmol/10^6 platelets from PC, while PtdSer and PtdIns contributed equal amounts of AA-containing mass ($\sim$8.5 pmol/10^6 platelets). Intriguingly, 63 mol% of the mass lost from the PE pool was from the hydrolysis of PlsEtn. These results set a lower boundary of $>$60 pmol/10^6 platelets for the rate of thrombin-induced AA mobilization in human platelets. Quantitation of individual molecular species identifies specific kinetic constraints and substrate selectivities of the phospholipase(s) activated during platelet stimulation. This study underscored the importance of PlsEtn as the major storage depot of AA in resting platelets and as the major source of AA mobilized after thrombin stimulation of human platelets.

In this first application of ESI/MS to lipidome determination, ESI/MS results were compared with those obtained using conventional HPLC methods (2–4). Two advantages of ESI/MS over HPLC method were obvious from these early experiments. First, individual molecular species of low-abundance phospholipid classes such as PtdIns and PtdSer could be easily analyzed [see Fig. 3 in ref. (23)] by ESI/MS, but the limited sensitivity present with traditional HPLC approaches previously made this quite difficult. Second, although the total alterations in PC and PE pools in human platelets induced by thrombin stimulation determined by ESI/MS and HPLC were similar, ESI/MS could resolve many more individual molecular species than HPLC could achieve enhancing its mechanistic utility to dissect compartmentalized lipid flux [see Tables 1 and 2 in ref. (23)].

Diabetes-induced changes in specific lipid molecular species in rat myocardium

Changes in myocardial lipid metabolism have been linked with intrinsic cardiac dysfunction during the diabetic state. Therefore, ESI/MS techniques were exploited to perform a detailed analysis of the lipidome in diabetic myocardium (20). The ability of insulin to prevent alterations in lipid constituents in diabetic myocardium was studied, and the enzymatic mechanisms through which they are mediated were identified (20). Four specific alterations in lipids from rat myocardium rendered diabetic by streptozotocin treatment (a well-accepted model of Type-I diabetes) were identified. First, a massive remodeling of TAG molecular species occurred in diabetic rat myocardium, including an over 5-fold increase in tripalmitin mass and a 60% decrease in TAG molecular species containing polyunsaturated fatty acids. Second, the myocardial PtdIns mass increased 46% in diabetic rats in comparison to normal controls. Third, there was a 44% elevation in myocardial PlsEtn content in streptozotocin-treated rats relative to untreated ones. Finally, a 22% decrease in 18:0–20:4 PtdEtn occurred in the absence of a reduction in nonesterified AA or total myocardial AA mass. Insulin treatment allowed the complete recovery of each of the diabetes-induced alterations in phospholipid classes, subclasses, and individual molecular species. In sharp contrast, the dramatic alterations in TAG molecular species were not prevented by peripheral insulin treatment after induction of diabetic state. These results segregate alterations in myocardial lipid metabolism in the diabetic state into changes that either can be remedied easily by routine peripheral insulin treatment or are difficult (or perhaps impossible) to recover by routine insulin administration alone. Accordingly, the study of lipidomics has already led to new insights into the diabetic state and the importance (and potential lipotoxicity) of saturated versus nonsaturated fatty acids and their differential metabolic fates. Through the detailed study of the lipidome, new insights into lipotoxicity and the differential effects of saturated and unsaturated acyl moieties in cellular metabolism and TAG storage pools were first demonstrated.

Specific lipid changes at the very early stages of Alzheimer's disease

Very recently, alterations in the lipidome of subjects with dementia in Alzheimer's disease (AD) were explored by using ESI/MS techniques (44, 45). A systematic examination of alterations in lipidome of postmortem gray and white matter from different brain regions including superior frontal cortex, superior temporal cortex, inferior parietal cortex, and cerebellum of human subjects with different degrees of AD dementia was performed. In those studies, three specific changes of lipids at the earliest clinically-recognizable stage of AD were demonstrated. Specifically, a dramatic reduction of PlsEtn mass (up to 40 mol% of total plasmalogens) in white matter occurred in early AD subjects in comparison to those age-matched cognitively normal controls (44). A correlation of the deficiency in PlsEtn mass levels with the AD severity is present (i.e., $\sim$10 mol% of depletion at early stage to $\sim$30 mol% of reduction at advanced stage) in gray matter of all cerebral regions (44). Second, sulfatides, a specialized component of the myelin sheath, were depleted by 93 and 58 mol% in

gray and white matter, respectively, in all examined brain regions from AD subjects with very mild dementia (45). Third, specific alterations in the lipidome of AD subjects relative to controls was the dramatic elevation (>3-fold) of ceramide content in the white matter of all examined regions from early AD subjects (45). No alterations in other lipid classes, including PtdEtn, PtdCho, SM, GalC, PtdIns, PtdGro, and PtdSer, were present at early stages, and a modest reduction (~15 mol%) of these lipidomes occurred in very severe cases (44, 45). These results suggest that alterations in specific lipidomes may play an important role in the pathogenesis of AD and may be linked with early events in the pathological process of AD, including neurodegeneration, synapse loss, and synaptic dysfunction in AD.

SUMMARY

Because of its unparalleled sensitivity, high selectivity, and high efficiency, ESI/MS has demonstrated itself as a powerful research tool in lipidomics, extending our knowledge of the role of lipid alterations in several disease states. By exploiting the advantages inherent in ESI/MS techniques, alterations in the lipidome can be obtained directly from chloroform extracts of biological samples. Through analysis of lipid mass changes, the biochemical mechanisms underlying disease states are now beginning to be dissected with high precision. Thus, during the last 3 years, our understanding of the biochemical mechanisms underlying several important disease states has been rapidly expanding. It is now clear that the power of lipidomics is just beginning to be realized.

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