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Biochemistry of inositol lipids

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1. ABSTRACT

Nature has created an immense combinatorial and structural heterogeneity among lipids. It is becoming increasingly accepted that the vast range of unique chemical entities encodes for distinct functions within biological systems. A unique group of lipids which stands out in terms of diversity as well as biological activity are inositol-containing lipids. The most well characterized inositol lipids are the phosphoinositides, phosphorylated derivatives of glycerophosphoinositol, which play a wide variety of cellular roles in many eukaryotic cells. Less well understood are ceramides containing inositol in fungi, and inositol glycolipids in pathogens. Here we review biochemical aspects of inositol-containing lipids with a focus on novel analytical procedures for their characterization.

2. INTRODUCTION

Inositol is a polycyclic alcohol which was first described as a component in lipids in the early 1930's (1). The simplest forms of inositol lipids contain either a glycerol or a sphingoid backbone, giving rise to glycerophosphoinositol (GPIns) and inositol phosphorylceramide (IPC, Figure 1B), respectively. Unique features of inositol lipids are the variable modifications on the inositol ring. It is amenable to phosphorylation, glycosylation, and acylation, resulting in lipids with complex chemistries which may form unique entities in specific organisms (Table 1).

Inositol lipids function as (i) integral building blocks of biological membranes, (ii) precursors for second messengers, and (iii) as signaling molecules which interact

spatially with proteins. Inositol lipids represent a great challenge to analysts due to their complex chemistry and often low abundance.

Detailed structural and quantitative analysis of lipids has made a great leap in recent years driven by rapid technological advancements in analytical chemistry, most predominantly mass spectrometry (MS). Here we will review some of the recent advances in analysis of inositol lipids and provide an outlook on current and future developments.

3. DIVERSITY OF INOSITOL LIPIDS IN DIFFERENT ORGANISMS

Inositol is unique among the building blocks of lipids. Contrary to glycerol or serine, it is not metabolized further but instead, is turned over in more or less dynamic ways within cells. We do not attempt exhaustive descriptions of inositol lipids across living organisms. Nevertheless, three important classes of inositol lipids, namely inositol-containing sphingolipids (Figure 1B), glycosylphosphatidylinositol (GPI, Figure 1C), and phosphatidylinositol mannosides (PIMs, Figure 1D), in addition to the more commonly reported GPIns and phosphoinositides (PIs, Figure 1A) will be described.

3.1. Phosphoinositides

PIs (Figure 1A), phosphorylated derivatives of GPIns, are a class of phospholipids originally described in connection to cellular signaling in the mid-1950s (2). An important feature of these lipids is the inositol headgroup which can be reversibly phosphorylated and dephosphorylated by kinases and phosphatases (3). Seven naturally occurring PIs are known to date in eukaryotes. It is not known whether additional forms exist, e.g. 2-PIs or pyro-phosphate isomers, the latter of which are known to exist in the case of inositol polyphosphates.

PIs have distinct roles in regulating many cellular events, including membrane trafficking, cell motility, apoptosis, vesicle transport and calcium mobilization (4-7). There is now accumulating evidence that a distinct pool of nuclear PIs exists, with implied roles in cell cycle progression, chromatin remodeling, transcriptional regulation and mRNA processing (8-10).

The functions of PIs are mediated by (i) interactions between the phosphorylated headgroups and effector molecules bearing specific PI-binding domains (e.g. PH, PX, FYVE, ENTH, etc) (11), (ii) soluble metabolites (inositol phosphates and diacylglycerols) which are generated through the action of phospholipases, and (iii) fatty acyl derivatives which originate from the membrane bound portion of the lipid molecule. Thus, PIs can be considered “high-power” signaling entities. Indeed, aberrant metabolism of PIs has been implicated in diverse human pathologies, including cancer and neurodegeneration. There are many excellent and recent review articles and book chapters on biological aspects of PIs (5,10,12-19).

3.2. Inositol-containing sphingolipids

Sphingolipids are structural components of eukaryotic cell membranes, often described in context with sterols and specialized microdomains (20,21). They also play important regulatory functions during differentiation, migration and apoptosis (22,23). The backbone of sphingolipids comprise of a long-chain amino alcohol to which a fatty acid can be connected to form ceramide. Further modifications, such as glycosylation, of the hydroxyl at the C1 position of ceramide gives rise to hundreds of sphingolipids. Two groups of glycosphingolipids exist, distinguished by the relation of their carbohydrate to the ceramide moiety. The sugar groups can be either directly linked to ceramide or coupled via an intermittent inositol phosphate (24). Inositol-phosphate is transferred from GPIns to ceramides (25), generating the simplest form of inositol sphingolipid, inositol phosphorylceramide (IPC, Figure 1B). Inositol-containing sphingolipids have been described in yeast, fungi, plants and eukaryotic parasites such as *Toxoplasma gondii* and *Leishmania* (table 1).

The yeast *Saccharomyces cerevisiae* has served as a unique model to uncover sphingolipid metabolic pathways and elucidation of sphingolipid functions (26,27). In *S. cerevisiae*, IPC is further modified (in the Golgi) with additional mannosyl and phosphoinositol moieties to form mannosyl inositol phosphorylceramide (MIPC, Figure 1B) and mannosyl di(inositol phosphoryl) ceramide (M(IP)2C) respectively. These lipids constitute up to ~40% of the total inositol-containing lipids and are mostly found in the plasma membrane (28).

The interest in inositol sphingolipids is sparked by the essential roles of sphingolipids in diverse biological functions, including growth and development (29-31). The metabolic pathways, which are distinct from mammals, represent promising targets for anti-fungal and anti-parasitic drug development (32-34).

3.3. Glycosylphosphatidylinositol

GPI (Figure 1C) is a glycolipid unique to eukaryotes (35), although archaea contain a distinct phosphoglycolipid with an identical head group core (36). GPI exists in three major forms: (i) a protein-anchored form, (ii) the free GPI lipid, termed, glycosylated inositol phospholipid (GIPL), and, (iii) a more complex glycoconjugated form known as lipophosphoglycan (LPG) (37-41). In mammals, GPI is formed by sequential addition of sugar residues to GPIns, which is initiated on the cytoplasmic face of the endoplasmic reticulum (ER) and completed in the ER lumen. It is important to note that the core glycan (mannose-alpha-1-4-glucosamine) and various substituents are introduced to the moiety of mannosides or inositol in an organism-specific and tissue-specific manner. Mature GPI is then exported to the cell surface, free or covalently linked to a protein. Details of the biosynthetic pathway and trafficking of GPI have been well covered in recent reviews (37,42-46).

Here we highlight a few characteristics of the lipid portion of GPI. This part of the molecule is generally

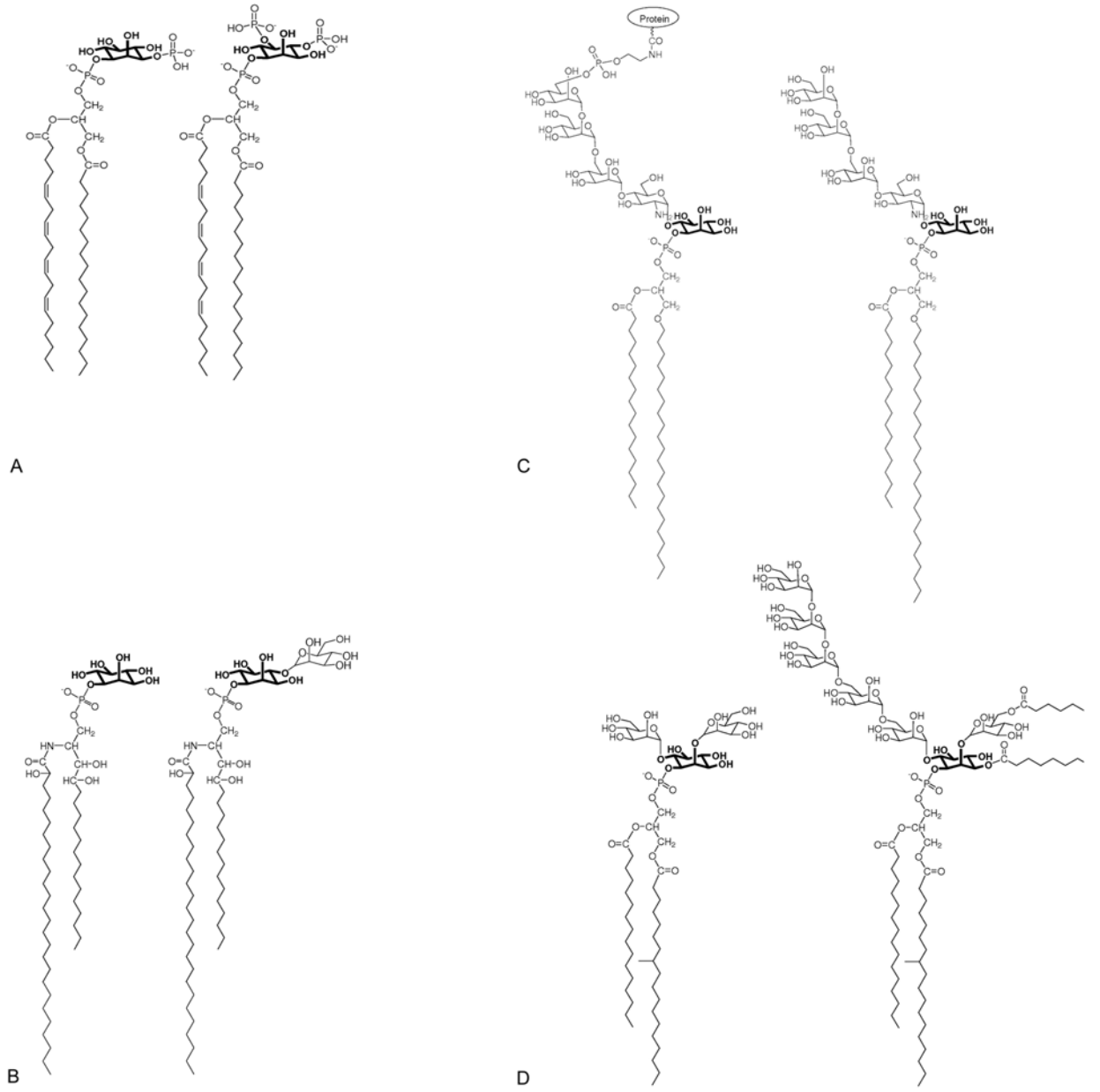

Figure 1. Structures of inositol lipids. (A) Phosphoinositides (PIs), 38:4 phosphatidylinositol-3-phosphate (PI-3-P, left) and 38:4 phosphatidylinositol-4,5-bisphosphate (PI-4,5-P2, right) [Note: 38:4 denotes total fatty acyls composition of 38 carbon atoms and 4 double bonds]. (B) Inositol sphingolipids, a t18:0/26:0 monohydroxylated inositol phosphorylceramide (IPC-C, left) and mannosyl inositol phosphorylceramide (MIPC-C, right) [Note: t18:0/26:0 denotes a trihydroxy sphinogoid base containing 18 carbon atoms with no double bond (t18:0) and fatty acyl chain containing 26 carbon atoms with no double bond (26:0)]. (C) Glycosylphosphatidylinositol (GPI). The glycan core of GPI anchored to proteins typically consists of mannose-alpha1-2-mannose-alpha1-6-mannose-alpha1-4-glucosamine (left). Additional substituents may be found on the core structure, for instance, palmitoylation at 2-hydroxyl of inositol (not shown). Free forms of GPI, known as glycosylated inositol phospholipid (GIPL, right) vary in the number and position of mannosyl groups on the mannose-alpha1-4-glucosamine core, which may be distinct from protein-anchored forms. (D) Phosphatidylinositol mannosides (PIMs). PIMs are a heterogeneous class of lipids, which vary in the number of mannosyl and acyl groups. Shown in this figure are the structures of PIM2 (left) and Ac2PIM6 (right), which denote a di-acylated PIM with 2 mannose residues and a tetra-acylated PIM with 6 mannose residues respectively. Note the alpha1-2 linkage of the two terminal mannose residues on AC2PIM6.

quite heterogeneous in structure. A variety of lipids such as diacylglycerol, ether linked core structures (e.g. 1-alkyl-2-acylglycerol), as well as monoacylglycerol and ceramide backbones are utilized as a membrane anchored moiety. An

Table 1. Inositol lipids of different organisms, including humans, various experimental model organisms and human pathogens.

Organism	LIPID				
	GPIns[1]	PIs[2]	IPC[3]	GPI[4]	PIM[5]
Homo sapiens	+	+		+	
Rattus norvegicus	+	+		+	
Drosophila melanogaster	+	+		+	
Caenorhabditis elegans	+	+		+	
Saccharomyces cerevisiae	+	+	+	+	
Mycobacterium tuberculosis	+				+
Leishmania mexicana	+	+	+	+	

Abbreviations: Glycerophosphoinositol[1], Phosphoinositides[2], Inositol phosphorylceramide[3]; Glycosylphosphatidylinositol[4], Phosphatidylinositol mannoside[5].

interesting, yet not overly well understood, characteristic of GPI lipids is 'remodeling'. Often, fatty acyls which are incorporated into the anchor during the early stages of biosynthesis are later re-placed during GPI maturation (45-48). The precise mechanisms remain poorly understood, and detection and characterization of metabolic intermediates in the pathway will definitely shed light into this process (49).

GPIs play important structural, signaling and regulatory roles. Several proteins are anchored to membranes via the GPI moiety and many GPI-anchored proteins localize to highly specialized microdomains known as lipid rafts, where they form signal transducing complexes (50-52). Enzymatic activities are altered (regulated) when GPI anchored proteins are released from the membrane (53,54). In addition, GPIs are precursors of inositolphosphogylcans, which are able to mimic some actions of pharmacologically active hormones and cytokines and these act as diffusible second messengers (55-58).

The (patho)physiological importance of GPI lipids (and their anchored proteins) is reflected by a spectrum of human diseases, including paroxysmal nocturnal hemoglobinuria (59) and degenerative presentations associated with prion proteins which contain a GPI anchor (60,61). Furthermore, the surface of eukaryotic parasites, such as *Plasmodium* and *Leishmania* (Table 1), is almost entirely covered with GPIs. Not only is the lipid an anchor for most proteins associated with the plasma membrane in these organisms, in the *Trypanosmatidae* family, GIPL and LPG are the major glycolipids and macromolecules lining the cell surface (40,62,63). Parasites such as *Plasmodium* and *Leishmania* (Table 1) have evolved diverse GPIs to regulate host cell function (e.g. in macrophages and lymphocytes) to their advantage and these lipids have been implicated in parasite survival and virulence (64-66). The parasitic GPI entities are structurally distinct from host GPIs and are potentially amenable to specific therapeutic inhibition and vaccination (63,67-69) (see section 5).

3.4. Phosphatidylinositol Mannoside

Structurally related to GPI are mannosylated forms of GPIns, the PIMs. This is another heterogeneous family of lipids, differing in the number of mannosyl and acyl residues in their structures. The core PIM structure consists of a GPIns anchor with a mannosyl unit attached to the C2 position of myo-inositol. The C6 position of myo-inositol can be substituted with up to three mannosyl units. Mannosyl units can be further transferred to the C2 position of the terminal mannose of a tetramannosylated PIM. Very often PIMs carry fatty acyl moieties attached to the carbohydrate portion (*myo*-inositol and 2-mannosyl unit attached to *myo*-inositol) (Figure 1D), which is not very common in the case of GPI (Figure 1C).

While GPIns is uncommon in bacteria, GPIns and the mannosides are found in mycobacteria and other members of *Actinomycetes* (70). In mycobacteria, PIMs are components of the cell membrane and bacterial capsule, either in a free form, or are hyperglycosylated to form other cell-wall components such as lipomannan (LM) and lipoarabinomannan (LAM) (71,72). PIMs and the hyperglycosylated forms have gained substantial interest over the past decade for their role during interaction of mycobacteria with their hosts (73). However, knowledge on their biosynthesis, transport and turnover is still rather fragmented.

4. BIOCHEMICAL ANALYSIS OF INOSITOL LIPIDS

4.1. Isolation and purification

The structural diversity found in inositol lipids challenges common textbook definitions of lipids as molecules that are highly soluble in organic solvents. Polarity differs depending on the lipid backbone as well as the headgroup modification. Sugar modification and phosphorylation make some of these lipids highly polar, and thus they may escape into the aqueous milieu during isolation. Acylation (of PIM and GPI, see above) on the other hand may render the molecule more non-polar, resulting in a mixture of molecules of different polarity within the same class of lipids.

4.1.1. Phosphoinositides

PIs, due to their polar nature and low abundance, are poorly recovered using conventional Bligh and Dyer or Folch methods of extraction. Acidification, and therefore protonation of the phosphate groups, increases solubility in organic solvents and thus improves recovery. To minimize interference of other phospholipids during analysis, a two-step extraction approach with a neutral solvent followed by acidification has been proposed (74,75). However, partial hydrolysis of ester linkages is a limitation of extraction under acidic conditions. Recently, Pettitt *et al.* described an

Table 2. Enzymatic and chemical treatments for the analysis of the lipid moiety of glycosylphosphatidylinositol (GPI).

Treatment		Lipid Analyte
Enzyme	Phospholipase A2	Fatty acid and sn-2 lyso-GPI
	Glycerophosphoinositol-specific phospholipase C	Diacylglycerol or Monoacylglycerol
	Glycosylphosphatidylinositol phospholipase D	Phosphatidic acid
Chemical	Acid hydrolysis	Fatty acid
	Alkaline hydrolysis	Fatty acid
	Nitrous deamination	Glycerophosphoinositol
	Hydrofluoric acid	Diacylglycerol

alternative method using citric acid to overcome the problem of acid hydrolysis (76). Additional back extraction of the aqueous phase with water-saturated butanol was used to recover PIs more efficiently. Furthermore, inclusion of tetrabutylammonium hydrogen sulfate and potassium chloride as ion pair agents aid in solubility and therefore recovery of PIs into the organic phase.

4.1.2. Inositol-containing sphingolipids

Inositol-containing sphingolipids in yeast are poorly soluble in anhydrous solvents. Instead, water-rich, slightly alkaline solvents appear to be required for extraction (77). Removal of interferences from other lipids can be achieved by capitalizing on the chemistry of these lipids. Sphingolipids, in contrast to glycerophospholipids, are more resistant to mild alkaline hydrolysis (78). Such chemical removal of the bulk of cellular glycerolipids may aid substantially in the analysis of inositol sphingolipids, in particular when mass spectrometry based methods are employed (79,80).

4.1.3. Glycosylphosphatidylinositol

So far biochemistry has to a large extent been limited to analysis of the glycan, lipid and/or protein parts of GPI as separate entities (41,81-83). This typically involves isolation of the free GPI or the anchored protein, for instance by detergent partitioning and affinity chromatography, followed by procedures (Table 2) to generate anchor fragments. MacRae and Ferguson reported the use of nitrous acid deamination and sodium borodeuteride reduction of the glucosamine residue to yield [1-^{2}H]-2,5-anhydromannitol to provide a quantitative readout of GPI levels in highly purified as well as crude preparations (84). However, information on the lipid composition is lost using this method.

Extractability of free GPI and LPG remains a challenge and there is at present no commonly accepted standard method available. Monophasic extraction with chloroform, methanol and water followed by biphasic separation using butanol has been described in several studies (85-87). More complex LPG can be purified by chromatographic methods (40).

4.1.4. Phosphatidylinositol Mannoside

Similarly, there is no commonly accepted method available for extraction of PIMs from biological samples. Since PIMs can be apolar or polar depending on their degree of acylation and/or mannosylation, optimal recovery of this heterogeneous class of lipids is a challenge. Generally, a multiple-step extraction method with different ratios of chloroform-methanol and chloroform-methanol-water mixtures is used to maximize recovery (88-90). Furthermore, acetone precipitation, butanol or phenol washes are employed to eliminate interference such as neutral and nonpolar lipids, salt or proteins.

4.2. Detection, separation and quantification

Biochemical analytics for detection, characterization and quantification of inositol lipids has made enormous progress in the past 10-15 years. We limit our discussion to biochemical approaches but it is important to acknowledge the powerful methods which are now available for monitoring inositol lipids (mainly PIs) in living cells using optical probes.

4.2.1. Metabolic labeling

Early studies relied on incorporation of radioactive metabolic precursors to label macromolecules in tissues and cells, followed by subsequent isolation and analysis. This well-established approach has already become textbook knowledge also for inositol lipids and we will thus not discuss it in detail here.

Common substrates for labeling of inositol-containing lipids include phosphate and fatty acyl groups (non-selective), and inositol (specific). Furthermore, sugar groups of glycosylated inositol lipids, including GPI, MIPC and PIMs, can also be readily labeled. Additional substrates for metabolic labels include ethanolamine for GPI and serine, which is incorporated into the sphingoid base of inositol-containing sphinoglipids. Generally, such approaches yield mass levels if the label is exposed to the cell or tissue long enough to ensure steady-state incorporation (and assuming equilibration into all metabolic pools). Such labeling regimes are typically followed by chromatographic separation and for a long time have been used to measure and quantify lipids.

4.2.2. Chromatography

4.2.2.1. Thin layer chromatography

Thin layer chromatography (TLC) offers a rapid method for separation of complex lipid mixtures. Separation of PIs using TLC requires pretreatment of the silica plates with potassium oxalate to chelate positive ions, which form salts with lipids, thereby interfering with migration (91,92). Visualization of the separated lipids can be achieved with various staining reactions. Orcinol is selective for glycolipids such as MIPC, GPI and PIMs. Alternatively, lipids can be more specifically detected by overlay with affinity probes (see section 4.2.4). Lipid spots can be easily recovered by elution and studied by other analytical procedures. TLC is particularly common in

metabolic labeling experiments to separate lipids, which can then be detected by autoradiography or fluorometry for radio- and fluorescent-labeled lipids respectively.

4.2.2.2. Liquid chromatography

Analysis of complex inositol lipids using liquid chromatography (LC) is still rather in its infancy. LC using anion exchange has been an excellent tool for separation of poly-phosphorylated inositols. For analysis of PIs, lipid headgroups are first chemically de-acylated, re-isolated and next separated by strong anion ion exchange (SAX) chromatography, followed by radiometric or suppressed conductivity detection. The latter procedure combines high sensitivity without a need for metabolic labeling. It has been successfully used for analysis of PIs in complex cell and tissue extracts. Since lipids are de-acylated prior to analysis, all information on fatty acyl compositions is lost. More recently, LC has been used in combination with mass spectrometry to measure intact PIs (76). This has helped to resolve some stereoisomers (see also Section 4.2.3). Shui *et al.* have recently described an liquid chromatography-mass spectrometry (LC-MS)-based method which allows separation of a wide variety of different lipid structures including PIs and PIMs (93).

4.2.3. Mass spectrometry

MS-based detection of inositol lipids has made enormous progress in recent years. A number of different ionization modes allow qualitative and quantitative analysis of GPIns, PIs (75,76,94-97), inositol sphingolipids (80,98), GPIs (46,47,49,99) and PIMs (93,100-103). MS, due to its high sensitivity and resolution, is thus a method of choice in particular for low abundant and complex inositol lipids. We will limit our discussion mainly to PIs and inositol sphingolipids. It should be noted however that tandem mass spectrometry (MS/MS) has contributed significantly to the characterization of GPIs and PIMs, in particular some of their detailed chemical compositions (49,82,100,101,103).

Single stage MS provides a snapshot and often many (hundreds of) ions are detected in a complex lipid mixture. Figure 2A shows typical negative ion mode electrospray ionization (ESI) mass spectra of lipid extracts obtained from mammalian brain (left panel) and yeast cells (right panel). Various classes of sphingo- and glycerophospho-lipids, including GPIns and IPC are detected. Note the low signals for PIs and more complex sphingolipids in both extracts. The limit of detection for a class of lipids is affected by the presence of other lipids, and the chemical property of the lipid (e.g. charge, size). For instance, the limit of detection for PIs can be enhanced by the removal of glycerophosphocholine (GPCho) (94,97). Enrichment of inositol lipids (and de-enrichment of "contaminant" lipids) can be achieved by specific extraction protocols or prior LC separation (see above sections). Some PIs and M(IP)2C form both singly $[M-H]^-$ and doubly deprotonated $[M-2H]^{2-}$ ions, further complicating detection and quantification. Optimization of instrument parameters is thus required to improve detection of ions of a particular ionization state (76,98).

Another problem associated with single stage MS analysis are isobaric complications, i.e. different lipids may share the same mass. This is obviously a particular problem in the case of PIs where seven naturally occurring isomers are known. Detailed studies of fragmentation pathways have helped to discriminate stereoisomers *in vitro* (75,96,104). Although MS-based methods alone may not achieve complete resolution of isomers, if coupled with LC, it is technically possible to separate and selectively detect individual isomers (76,93). In addition, ultra high resolution MS (e.g. Fourier Transform Ion Cyclotron MS) also helps in the separation of molecules with very similar masses.

Fragmentation patterns obtained from MS/MS aid in characterization and identification of lipids. For instance, collision-induced dissociation (CID) of GPIns and IPC yields a common fragment of m/z 241 (Figure 2B), originating from the inositol phosphate headgroup (80,95,98). It is noteworthy that although the two classes of inositol lipids discussed here (i.e. glycerophosphoinositols (GPIns), left panel, and inositol phosphorylceramides (IPC), right panel) share common diagnostic fragments, GPIns will liberate fatty acid carboxylate ions and dehydrated glycerophosphate ions. These distinct features allow differentiation of inositol glycerophospho- and sphingo-lipids in MS/MS experiments. In addition to the fragmentation pattern, inositol sphingolipids can be discriminated from inositol phospholipids based on the nitrogen rule (105,106), since deprotonated molecular ions of inositol sphingolipids have even nominal masses due to the presence of a nitrogen atom, while ions of inositol phospholipids have odd nominal masses. The common diagnostic ion of the inositol phosphate headgroup has indeed been used in various studies to selectively measure GPIns and IPC in complex lipid mixtures by precursor ion scanning in the negative ion mode (Figure 2C). Addition of a phosphate moiety to the inositol headgroup of GPIns increases the molecular weight by 80 mass units and precursor ion scans for m/z 321 and m/z 401 can be used to generate profiles of phosphatidylinositol monophosphate (PIP) and bisphosphate (PIP2) respectively (96). Similarly, addition of a mannosyl moiety to IPC increases the molecular weight by 180 and precursor ion scans for m/z 421 can be used to generate profiles for MIPC. Such focused analysis of inositol-containing lipids by precursor ion scanning and/or neutral loss scanning improves the sensitivity of detection compared to single stage analysis (107).

As an alternative to the precursor scanning mode, selected lipid species of interest can be specifically monitored using multiple reaction monitoring (MRM). In this mode, parent/daughter ion transition pairs are followed in tandem mass operation. Both precursor ion scanning and MRM methods require prior knowledge of the fragmentation pattern in order to selectively monitor the specific class of lipids. In the case of GPIns, PIs and inositol sphingolipids, such quantitative analysis of these lipids have been established. However, this is not the case for the structurally more complex inositol lipids, GPIs and PIMs, LMs and LAMs. Nevertheless, the availability of LC and MS has enabled the development of methods designed to analyze many lipids of diverse chemistry from a biological source rapidly and with high sensitivity (93,106).

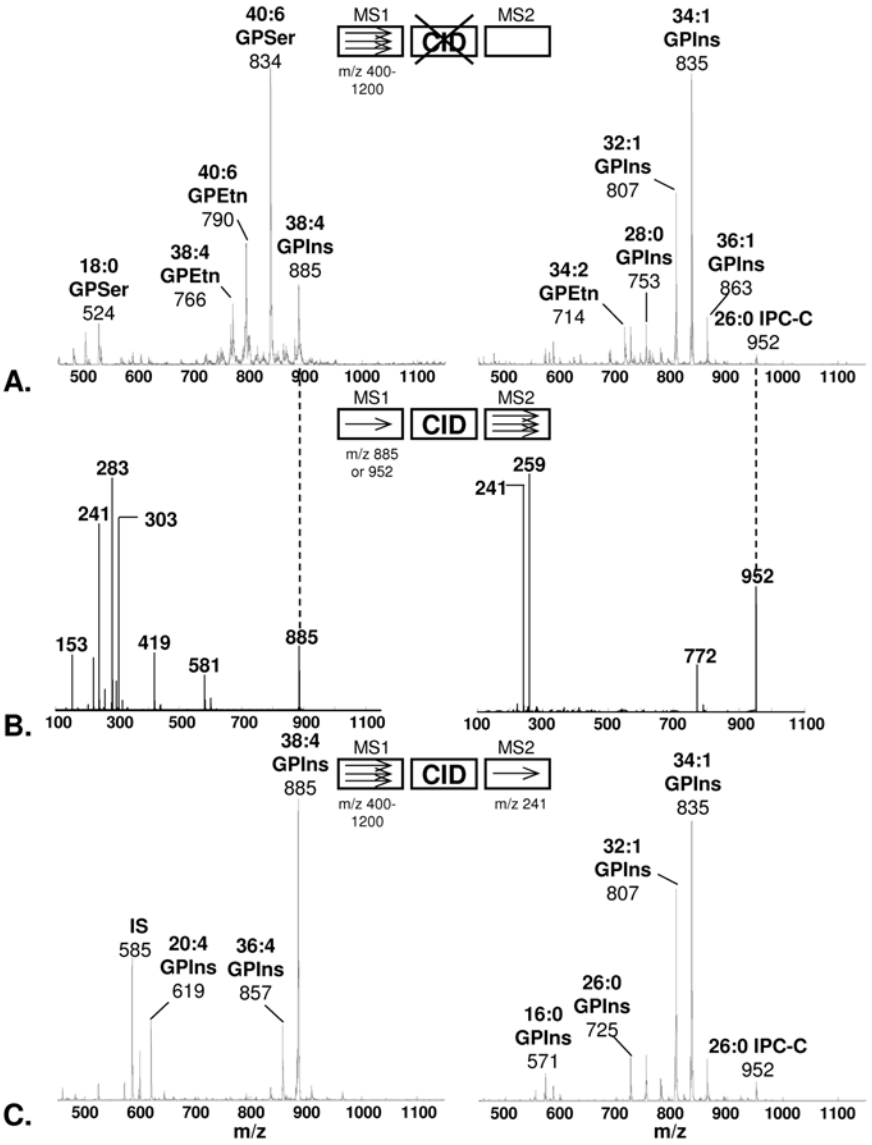

Figure 2. Mass spectrometry analysis of inositol lipids. Left panels represent spectra of lipids extracted from mammalian brain tissue using acidified Bligh and Dyer method and right panels represent spectra of lipids extracted from *Saccharomyces cerevisiae* using water-rich alkaline solvents. (A) Single stage electrospray ionization mass spectrum (ESI-MS) in the negative ion mode. Majority of phospho- and sphingo-lipids are detected in the mass range of 400-1200. The ions can be tentatively assigned by their mass-to-charge (m/z) ratio. Characterization of ions can be achieved by collision-induced dissociation (CID) and tandem mass spectrometry (MS/MS). (B) MS/MS spectra of ions with m/z 885 (left panel) and 952 (right panel). An ion of interest can be selected in the first mass analyzer (MS1) and after CID, the fragment ions are analyzed in the second mass analyzer (MS2). The product ions of the parent with m/z 885 (38:4 GPIns) includes m/z 153, 241, 283 and 303, which correspond to ions arising from the glycerol phosphate backbone, inositol phosphate headgroup and two fatty acyls, respectively. For t18:0/26:0 IPC-C (m/z 952), the fragmentation pattern includes product ions with m/z 241, 259 and 772, which correspond to ions arising from inositol phosphate for the former two ions, and ceramide phosphate, respectively. Note both classes of inositol lipid shares the common product ions 241 and 259 (the latter is less pronounced for GPIns), which is distinctive of the inositol phosphate headgroup. Such information on a common fragment ion that is characteristic and specific for a class of lipids can be used for other MS experiments, such as multiple reaction monitoring (MRM) and precursor ion scans. (C) Precursor ion scans for lipids containing inositol phosphate headgroup (m/z 241). The second mass analyzer is fixed at m/z 241 and the first analyzer scans the mass range of interest. Consequently, ions with the propensity to form fragment ions with m/z 241 are selectively detected. Samples can be spiked with internal standards (IS) to allow for semi-quantitative profiling. In this case, the internal standard with m/z 585 is a dioctyl GPIns (left panel), which is typically not found in samples derived from mammalian tissues and yeast.

One of the major limitations in the precise characterization and quantification of inositol lipids by LC and MS is the lack of pure standards (with the exception of PIs, see also section 5). An alternative approach is to use knockout organisms which accumulate lipids that normally occur in low abundance. Houjou *et al.*, for instance, employed an LC-ESI-MS/MS method to analyze mutants of the GPI biosynthetic machinery and identified various metabolic intermediates of GPI during early stages of biosynthesis (49).

4.2.4. Affinity probes for inositol lipids

The use of affinity probes, which are increasingly available, has made important contributions to inositol lipid detection. Affinity probes not only allow detection of lipids, but are tools for functional assays (e.g. protein-lipid interactions, enzymatic assays).

Analogous to arrays in genomics and proteomics, which capitalize on specific capture of nucleic acids and proteins/peptides by an affinity probe, it is possible to generate arrays to capture lipids. An attractive potential of these probes is the provision of a visual readout of lipids in living cells. Clearly, the specificity of lipid functions is not only restricted to their structures per se, but the precise localization and set of interacting partners. This is best exemplified by the spatial and temporal regulation of PIs (14).

Affinity probes for inositol lipid detection exist in various forms – headgroup specific protein modules, antibodies, toxins and proteins/peptides – which can be from a naturally occurring source or genetically and/or chemically synthesized. For instance, anti-phospholipid antibodies were discovered in the early 80's as a cause of autoimmune diseases in humans. Furthermore, the presence of anti-inositol lipid(s) antibodies in patient sera has been reported in various diseases such as cancer (108), pulmonary TB and malarial infection (109,110). While serum antibodies are a potential source of affinity probes, anti-lipid antibodies have been produced for various classes of inositol lipids (111-113) and have found widespread application in their analyses (113,114). However, the spectrum of anti-lipid antibodies is still limited and specificity is often poor.

For detection of GPI-anchored proteins, toxins such as aerolysin from *Aeromonas hydrophila* and alpha-toxin from *Clostridium septicum* have been employed in protein overlay and immunofluorescence studies (115,116). The binding determinant for aerolysin is more stringent than alpha-toxin as it is dependent on the N-glycan of the proteins in addition to the GPI anchor (117). Recombinant probes can be prepared based on the fact that some lipids bind/interact with proteins. For instance, effector proteins bind their particular PIs via specific domains and fusions of PI-binding domains to fluorescence or small peptide tags are useful probes in fluorescence microscopy of live as well as fixed cells (118-120).

5. ORGANIC SYNTHESIS OF INOSITOL LIPIDS

The important roles of inositol lipids in cellular processes combined with their often organism-specific biosynthetic pathways make this class of compounds attractive for drug and vaccine development. Sphingolipids, for example, are attractive potential targets in anti-fungal and anti-parasitic drug and biomarker development.

Pure and synthetic forms of PIs have helped tremendously in research (e.g. for construction of affinity matrices) and in the development of novel analytical tools (e.g. as standards for LC and MS). Efforts in organic chemical synthesis of PIs are now also focusing on analogues for inhibition of lipid kinases and phosphatases (121).

There are also a few encouraging reports on total synthesis of the more complicated structures found in GPI and PIM lipids (122,123,124). It can be expected that, once more established and available, such compounds will drive research in the area of infectious diseases and immunology in a way the first synthetic phospholipids and PIs did in cell biology and signaling. These new tools will be used in vaccine development and antibody production. They will obviously also help to establish more quantitative biochemical methods for GPI and PIM detection in complex mixtures based on mass spectrometry.

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Abbreviations: CID, collision induced dissociation; ER, endoplasmic reticulum; ESI, electrospray ionization; GIPL, glycosylated inositol phospholipid; GPCho, glycerophosphocholine; GPEtn, glycerophosphoethanolamine; GPI, glycosylphosphatidylinositol; GPIns, glycerophosphoinositol; GPSer, glycerophosphoserine; IPC, inositol phosphorylceramide; IPC-C, monohydroxylated IPC; LAM, lipoarabinomannan; LC, liquid chromatography; LM, lipomannan; LPG, lipophosphoglycan; MIPC, mannosyl inositol phosphorylceramide; M(IP)2C, mannosyl di(inositol phosphoryl) ceramide; MRM, multiple reaction monitoring; MS, mass spectrometry; MS/MS, tandem mass spectrometry; m/z, mass-to-charge ratio; PIs, phosphoinositides; PIM, phosphatidylinositol mannoside; PIP, phosphatidylinositol monophosphate; PIP2, phosphatidylinositol bisphosphate; SAX, strong anion ion exchange; TLC, thin layer chromatography

Key Words: Phosphoinositides; Sphingolipids; Glycosylated Inositol Lipids; Chromatography; Mass Spectrometry; Synthetic Probes, Review

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Chylomicrons are an exogenous pathway of lipid metabolism because they are where dietary fats go directly. Chylomicrons appear in the blood about 2 hours after a meal and disappear from the blood about 16 hours after a meal (having been taken up by the liver). They are large and concentrated enough to visibly cloud your plasma. VLDLs are an endogenous pathway because thatâ€™s where fats synthesized de novo in the liver or derived from adipose tissue go. CMs and VLDLs are distinguished by which APOB is on their surface. HDL serves as a reservoir for different apolipoproteins and can exchange them Inositol Phospholipid Metabolism and Phosphatidyl Inositol Kinases, Volume 30 (Laboratory Techniques in Biochemistry and Molecular Biology) [A. Kuksis] on Amazon.com. *FREE* shipping on qualifying offers. This book offers a wide ranging and review of cutting edge developments along with tried and tested methods for isolation.Â "In this excellent new book, Kuksis shows how our knowledge of the remarkable activities of these compounds has only been possible because of the continuous development of novel analytical methodologies...In short, it is hard to conceive of a more comprehensive and thorough guide to the subject of analysis and biochemistry of phosphatidylinositol and its metabolites.