1

LIPIDS AND LIPIDOMICS

1.1 LIPIDS

1.1.1 Definition

It is well known that lipids play many essential roles in life [1]. They possess functions to

- Constitute cellular membranes in biological organisms that provide hydrophobic barriers to separate cellular compartments.
- Serve as an optimal matrix to facilitate transmembrane protein function.
- Facilitate as a source of precursors for lipid second messengers during signal transduction.
- Provide the storage and/or supplement of fuel for biological processes.

More and more lines of evidence support a rationale that lipids are associated with many human diseases (e.g., diabetes and obesity, atherosclerosis and stroke, cancer, psychiatric disorders, neurodegenerative diseases and neurological disorders, and infectious diseases) (see Chapter 17). Therefore, the research on lipids has become a unique new discipline called "lipidomics" nowadays.

The majority of lipids are composed of two components. One part is largely hydrophobic ("water-fearing"), meaning that it is not suitably soluble in polar solvents (e.g., water), while the other part is often polar or hydrophilic ("water-loving")

Lipidomics: Comprehensive Mass Spectrometry of Lipids, First Edition. Xianlin Han.

and is readily soluble in polar solvents. Therefore, lipids are amphiphilic molecules (having both hydrophobic and hydrophilic portions). However, prominent exceptions are also present, including waxes, triacylglycerol (TAG), cholesterol, cholesteryl esters, all of which are predominantly hydrophobic except for their hydroxyl or carbonyl groups.

In general, lipids are defined as a group of organic compounds in living organisms, most of which are insoluble in water but soluble in nonpolar solvents. Based on this definition, any petroleum products obtained from fossil materials or synthetic organic compounds are excluded in the category of lipids. Indeed, lipids are one of the main constituents of biological cells and the major components of lipoproteins in serum. Lipids are often conjugated with carbohydrates, which are known as lipopolysaccharides.

The historical origins of the term "lipid" and its early definitions can be found elsewhere if the readers are interested [2]. The precise definition of lipids is difficult to give, as no satisfactory or widely accepted definition exists. Thus, many varying definitions about lipids can be found. For example, Merriam-Webster dictionary defines lipids as "any of various substances that are soluble in nonpolar organic solvents (such as hexane, chloroform, and ether), that with proteins and carbohydrates constitute the principal structural components of living cells, and that include fats, waxes, phospholipids, cerebrosides, and related and derived compounds." Wikipedia (http://en.wikipedia.org/wiki/Lipid) describes it as "Lipids may be broadly defined as hydrophobic or amphiphilic small molecules; the amphiphilic nature of some lipids allows them to form structures such as vesicles, liposomes, or membranes in an aqueous environment." General textbooks describe lipids as a group of naturally occurring compounds, which have in common a ready solubility in organic solvents such as chloroform, benzene, ethers, and alcohols. Unfortunately, such a definition is misleading because there are many compounds that are now widely accepted as lipids, which may be more soluble in water than in organic solvents (e.g., lysoglycerophospholipids, acyl CoA, gangliosides).

The most recent definition of lipids was provided by a group of lipid chemists who formed the consortium of lipid metabolites and pathways strategy (Lipid MAPS). They defined lipids based on the origin of the lipid structures as hydrophobic or amphipathic small molecules that may originate entirely or, in part, by carbanion-based condensations of thioesters (fatty acids, polyketides, etc.) and/or by carbocation-based condensations of isoprene units (prenols, sterols, etc.). In this book, this definition, its classification (see the following), and its recommended nomenclature are largely accepted.

1.1.2 Classification

With the different definitions, different kinds of lipid classification are frequently used in the field. For example, many lipid chemists simply classify lipids into polar and nonpolar lipids based on the overall hydrophobicity of the lipids. The nonpolar lipids include fatty acids and their derivatives (e.g., long-chain alcohols and waxes), glycerol-derived lipids (e.g., monoacylglycerols (MAG), diacylglycerols (DAG),

TAG (i.e., fats or oils)), and steroids. These nonpolar lipids are generally soluble in very nonpolar solvents such as hexane, ether, and ester. The polar lipids usually contain a polar head group, such as phosphocholine in choline glycerophospholipids (PC) (see the following), and are usually soluble in relatively polar solvents, such as alcohol, and even water.

Based on the features of chromatographic separation, lipids are classified into simple and complex molecules [2]. "Simple lipids" are those that yield mostly two types of primary products per molecule upon hydrolysis (e.g., fatty acids and their derivatives, MAG); "complex lipids" yield three or more primary hydrolysis products per molecule (e.g., PC, TAG, DAG). These hydrolysis products include fatty acids, phosphoric acid, organic bases, carbohydrates, glycerol, and many more components.

According to the functions of cellular lipids, many biochemists also refer lipids to

- Membrane lipids, which largely constitute the cellular membrane and are usually present in relatively high contents.
- Energy lipids, which are usually involved in energy storage and metabolism.
- Bioactive lipids, which serve as lipid second messengers and are generally present in low or very low abundance.

A more detailed classification is achieved by grouping lipids based on their chemical properties. **Individual lipid molecular species** (each of which has a unique molecular structure) are commonly categorized into small groups, that is, **lipid classes**, based on their chemical structural similarities. For example, individual lipid molecular species that possess an identical polar head group (e.g., phosphocholine, phosphoethanolamine, or phosphoserine) linked to a common glycerol backbone are categorized into a specific lipid class (e.g., PC, ethanolamine glycerophospholipid (PE), serine glycerophospholipid (PS), respectively) (Figure 1.1).

Among each individual lipid class, due to the presence of a unique linkage or another unique feature, these species are further classified into smaller groups, that is, the **subclasses** of the lipid class (Figure 1.2). For example, the oxygen atom of glycerol at *sn*-1 position (here *sn* means stereospecific numbering) is connected to a fatty acyl chain through an ester, ether, or vinyl ether bond in both glycerophospholipids (GPL) and glycerolipids. These different linkages define the subclasses of a GPL class (Figure 1.2a), which are called phosphatidyl-, plasmanyl-, and plasmenyl- according to the recommended nomenclature by International Union of Pure and Applied Chemistry (IUPAC), corresponding to the ester, alkyl ether, and vinyl ether linkage, respectively [3]. These subclasses are abbreviated as prefix "d," "a," and "p," respectively, throughout this book. To date, the plasmanyl and plasmenyl subclasses have only been identified in mammalian lipidomes for the classes of choline, ethanolamine, and serine glycerophospholipids (PC, PE, and PS, respectively) and may be present in the class of phosphatidic acid (PA) and cardiolipin (CL). However, these subclasses have been found in other lipid classes in other species [4]. These different linkages have also been found in DAG and TAG [5, 6]. The presence or absence of a double bond between C4 and C5 of sphingoid base (see the following)

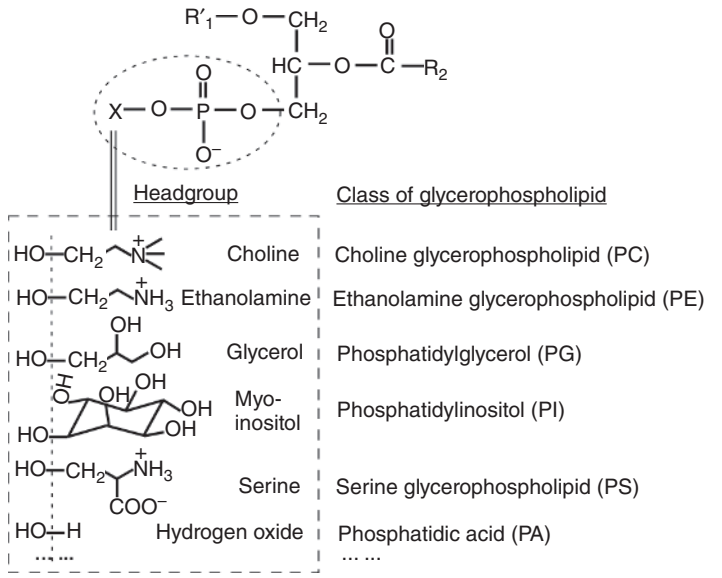

Figure 1.1 Examples of glycerophospholipid classes. Different structures of the moiety X, which are connected to the phosphate and exemplified in the box, determine the individual classes of GPL as indicated with abbreviations that are commonly used in the literature and adapted by the Lipid MAPS consortium.

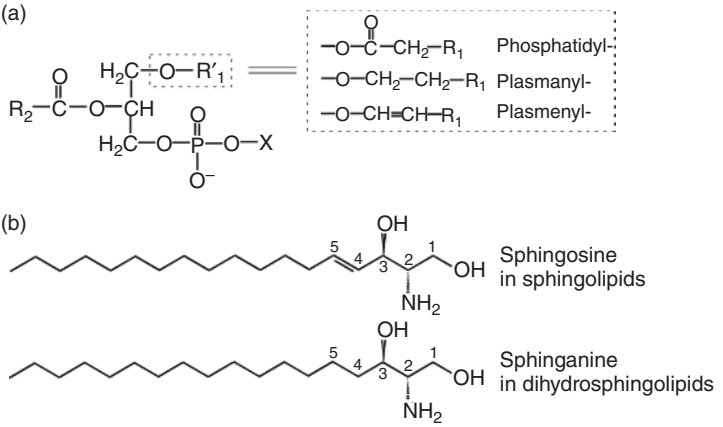

Figure 1.2 Example of lipid subclasses, which are classified based on the different linkages at a certain position or a unique structural feature of a lipid class. (a) The subclasses of phosphatidyl-, plasmanyl-, and plasmenyl- are present in GPL as a result of the different linkages (i.e., ester, ether, and vinyl ether) of a fatty acyl chain to the hydroxyl group at *sn*-1 position of glycerol. (b) The different core structures of sphingoid bases in the presence or absence of a double bond between C4 and C5 carbon atoms lead to the common subclasses of sphingolipids and dihydrosphingolipids. Other less common subclasses of sphingolipids are also present due to other structures of the sphingoid bases (see Figure 1.6).

leads to the classification of the individual sphingolipid class into sphingolipid and dihydrosphingolipid subclasses (Figure 1.2b).

Following are the two major classification systems defined based on chemical properties of lipids that are largely used in the book.

1.1.2.1 Lipid MAPS Approach Based on their mission, the Lipid MAPS consortium has classified lipids into eight categories, including fatty acyls, glycerolipids, GPL, sphingolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides [7]. Importantly, individual lipid molecular species in this comprehensive classification bears a unique 12-digit identifier, which facilitates the systematization of lipid biology and enables the cataloging of lipids and their properties in a way that is compatible with other macromolecular databases.

The **fatty acyls** are a diverse group of molecules synthesized by chain elongation of an acetyl coenzyme A (acetyl-CoA) primer with malonyl-CoA (or methylmalonyl-CoA) groups that may contain a cyclic functionality and/or are substituted with heteroatoms. Fatty acyls are characterized by a repeating series of methylene groups and are structurally the simplest lipids. This category includes various classes of fatty acids, eicosanoids, docosanoids, fatty alcohols, fatty aldehydes, fatty esters, fatty amides, fatty nitriles, fatty ethers, and hydrocarbons. Fatty acyls, in general, and fatty acids, in particular, are the basic building blocks of more complex lipids such as GPL, (glyco)sphingolipids, glycerolipids, and glycolipids. The presence of modified fatty acyls in complex lipids has been well documented [8–10].

The **glycerolipids** are the lipid species that can only be hydrolyzed into glycerol, a sugar group, fatty acid(s), and/or alkyl variants. Glycerolipids include the species of MAG, DAG, TAG, and glycolipids. The MAG, DAG, and TAG species typically have a glycerol backbone with fatty acid chains linked to the hydroxyl groups of glycerol. However, fatty alcohols linked by an ether bond are also found in these neutral lipids in low abundance [5, 6]. Glycolipid is defined by the IUPAC as a lipid in which the fatty acyl portion of the molecule contains a glycosidic linkage [3].

The **glycerophospholipids** are defined by the presence of at least one phosphate (or phosphonate) group esterified to one of the glycerol hydroxyl groups. GPL species are ubiquitous in nature, are key components of cellular membranes, and are also involved in metabolism and signaling. The complexity of GPL species is illustrated with the presence of different classes, subclasses, and individual molecular species (different fatty acyl chain structures) (Figures 1.1 and 1.2). As illustrated by its name, individual molecular species in this category of lipids contain three components: “glycero-” (i.e., at least one glycerol molecule is centered in each individual species); “phospho-” (i.e., at least one phosphate or phosphodiester is linked to a hydroxyl group of glycerol at the *sn*-3 position); and one or two aliphatic chains that are connected to the *sn*-1, *sn*-2, or both hydroxyl groups of glycerol. There are over 10 varieties of the moieties esterified with the phosphate (i.e., over 10 different classes) (Figure 1.1) and over 30 kinds of possible fatty acyl chains containing different numbers of carbon atoms (i.e., chain length), different degree of unsaturation, and different locations of these double bonds. In addition, there exist three different linkages of the fatty acyl chain with the hydroxyl group

of glycerol at the *sn*-1 position (i.e., three different subclasses). Accordingly, we can easily estimate that the possible number of individual molecular species in the category of GPL should be approximately 30,000 ($10 \times 30 \times 30 \times 3$). In practice, mass spectrometric (MS) analysis has detected the presence of a large number of individual lipid species (e.g., plasmalogen, CL, TAG) [11–13].

Sphingolipids are another category of complex cellular lipids. The sphingolipid species contain common long-chain sphingoid bases (Figure 1.2b) as their core structures. These sphingoid bases are first synthesized *de novo* from serine and a long-chain fatty acyl CoA to yield sphinganine and then dihydroceramides, which convert into ceramides, phosphosphingolipids, glycosphingolipids, and other species (Figure 1.3). The polar moieties (which also appear in GPL classes and glycolipids (see above)) that are linked to the hydroxyl group of sphingoid base at position C1 represent the individual sphingolipid classes.

The **sterol lipids** are a group of compounds that carry a core signature of four fused rings (Figure 1.4a) and are subdivided into cholesterol and derivatives, steroids, secosteroids, bile acids and their derivatives, and others (Figure 1.4) [7]. Cholesterol and its derivatives that are the most widely studied sterol lipids in mammalian systems constitute an important component of membrane lipids, along with the GPL and SM [14]. Unique sterols are present in plant, fungal, and marine sources [7]. The steroids, which also contain the same fused four-ring core structure as

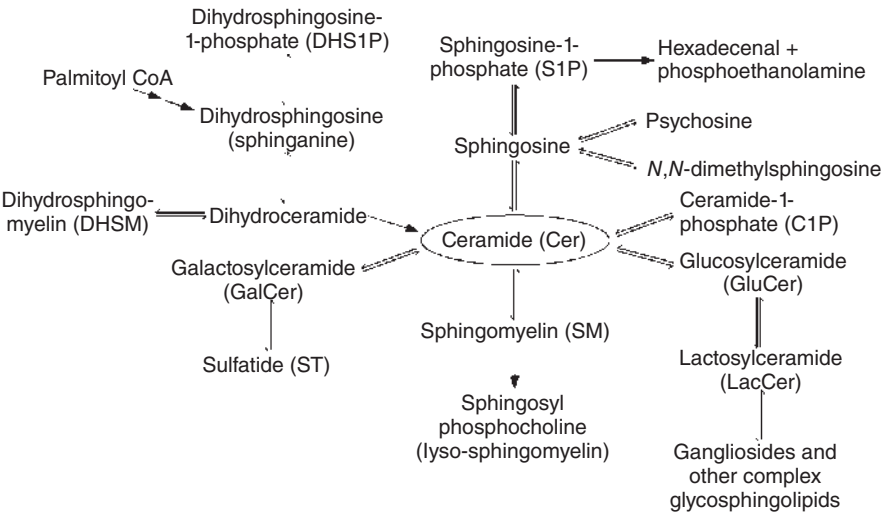

Figure 1.3 Simplified pathways and network of the common sphingolipid classes and other related lipids. The network and the pathways are derived from the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway databases. The pathways indicate the origins of sphingoid base core structures and their derivatives. Other sphingoid bases can be biosynthesized from other fatty acyl CoA by the replacement of palmitoyl CoA or some other amino acids replacing serine. The structures of individual lipid classes and their abbreviations used in the book are indicated in Figure 1.6.

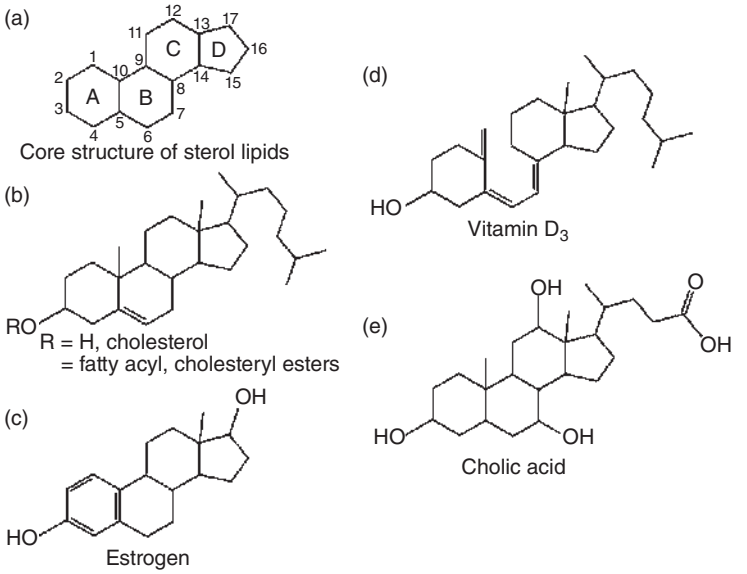

Figure 1.4 The core structure and representatives of sterol lipids. (a) The core structure of the majority of the sterol species or from which the sterol species are resulted. (b) The representative structures of cholesterol (R = H) and cholesteryl esters (R = a fatty acyl). (c) A representative structure of the steroid subgroup species (i.e., estrogen). (d) A representative structure of the secosteroid subgroup (i.e., vitamin D_3). (e) A representative structure of the species among the bile acid subgroup of sterol (i.e., cholic acid).

cholesterol, have different biological roles and function as hormones and signaling molecules [15]. The secosteroids, comprising various forms of vitamin D, are a group of molecules similar to steroids but with a "broken" B ring, hence the "seco" prefix [16]. Bile acids are primarily derivatives of cholan-24-oic acid synthesized from cholesterol in the liver and their conjugates (sulfuric acid, taurine, glycine, glucuronic acid, and others) [17].

In addition to the above-mentioned five categories of lipids, there are three more categories, including prenols, saccharolipids, and polyketides, that are relatively less studied at the current stage of lipidomics. The **prenol lipids** are synthesized from the five carbon precursors isopentenyl diphosphate and dimethylallyl diphosphate that are produced mainly via the mevalonic acid pathway [18]. Prenols are subdivided into isoprenoids, quinones and hydroquinones (e.g., unibiquinones, vitamins E, K), polprenoils, and others [7]. The category of **saccharolipids** accounts for lipids in which fatty acids are linked directly to a sugar backbone. In the saccharolipids, a sugar substitutes for the glycerol backbone that is present in glycerolipids and GPL. Saccharolipids can occur as glycan or as phosphorylated derivatives. The most familiar saccharolipids are the acylated glucosamine precursors of the lipid A component of the lipopolysaccharides in Gram-negative bacteria [19]. Typical lipid A molecules are disaccharides of glucosamine, which are derivatized with as many as seven fatty

acyl chains [19]. The **polyketides** are a diverse group of metabolites from plant and microbial sources and contain a much greater diversity of natural product structures, many of which have the character of lipids [7].

Some key features of this lipid classification, which are adapted in this book, include the following:

- The use of stereospecific numbering (*sn*) method for the glycerol-based lipids (e.g., glycerolipids and glycerophospholipids) [3]. Acyl or alkyl chains are typically linked to the *sn*-1 and/or *sn*-2 positions of glycerol, with the exception of some lipids that contain three acyl or alkyl chains or contain more than one glycerol group and archaebacterial lipids in which *sn*-2 and/or *sn*-3 modification occurs.
- The use of sphinganine and sphing-4-enine (i.e., sphingosine) as core structures for the category of sphingolipid species, where the d-*erythro* or 2*S*, 3*R* configuration and 4*E* geometry (in the case of sphing-4-enine) are implied.
- The use of "*d*" and "*t*" designations as the shorthand notation of sphingolipids, which refer to 1,3-dihydroxy and 1,3,4-trihydroxy long-chain bases, respectively.
- The use of *E*/*Z* designations to define double-bond geometry.
- The use of *R*/*S* designations (as opposed to α/β or D/L) to define stereochemistries. The exceptions are those describing substituents on glycerol (*sn*) and sterol core structures and anomeric carbons on sugar residues. In these latter special cases, the α/β format is firmly established.
- The use of the common term "lyso" denoting the position lacking a radyl group in glycerolipids and GPL.

1.1.2.2 Building Block Approach

1.1.2.2.1 Building Block Concept and Classification In this classification method, the majority of biologically occurring lipids are the combinations of some building blocks, which represent some kinds of hydrolysis products or their analogs. The commonly recognized building blocks include fatty acyls as categorized by Lipid MAPS classification (see above), a variety of polar head groups (e.g., phosphoesters (including phosphate, phosphocholine, phosphoethanolamine, phosphoglycerol, phosphoserine, and phosphoinositol) and sugar molecules (e.g., glucose, galactose, lactose)), as well as a few backbones (such as glycerol, sphingoid base, and cholesterol) as core structures. With this concept, the molecular species of an entire lipid class or a category of lipid classes could be represented by a common chemical structure.

For example, molecular species of all glycerol-centered lipid classes (e.g., GPL and glycerolipids (see Lipid MAPS classification)) are the combination of three different building blocks connected to three hydroxyl groups of glycerol backbone (Figure 1.5). In this general structure, the building blocks I and II can be a hydrogen or a fatty acyl connected to *sn*-1 and 2 positions of glycerol with an ester, ether, or vinyl ether linkage. Building block III at the *sn*-3 position of glycerol can be a hydrogen atom, a fatty acyl, or one of the various sugar ring(s) and their derivatives

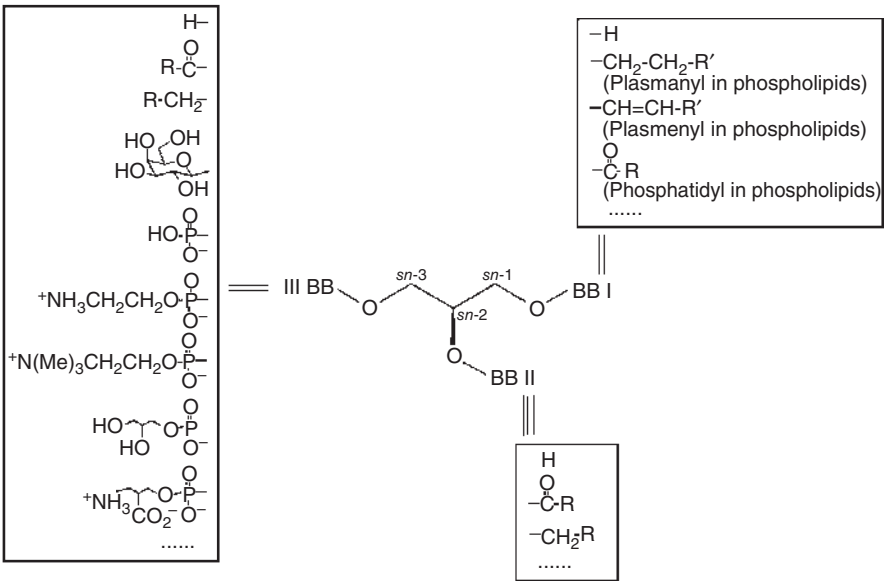

Figure 1.5 General structure of glycerolipids and glycerophospholipids. Both glycerolipids and GPL classes are centered with a glycerol molecule. Three building blocks (BB), which are separately exemplified in the boxes, are connected to the hydroxyl groups of glycerol. Building block (BB) I represents a hydrogen or a fatty acyl moiety connected to *sn*-1 position of glycerol with an ester, ether, or vinyl ether linkage, which defines the subclass as phosphatidyl-, plasmanyl-, or plasmenyl-, respectively, in glycerophospholipids. Building block (BB) II represents a hydrogen or a fatty acyl moiety connected to *sn*-2 positions of glycerol with an ester, ether, or vinyl ether linkage. Building block (BB) III represents a hydrogen atom, a fatty acyl, or one of the various sugar ring(s) and their derivatives in glycerolipids, or phosphoesters in glycerophospholipids and lysoglycerophospholipids. R′ and R are usually unbranched saturated or unsaturated aliphatic chain containing 12–20 and 13–21 carbon atoms, respectively.

in glycerolipids, or phosphoesters in GPL and lysoGPL. Here, the fatty acyl chain typically contains 12–24 carbon atoms with variable degrees of unsaturation or modifications.

Similar to glycerol-centered lipids, the majority of the sphingolipid species can be represented with a general structure composed of three building blocks (Figure 1.6). Building block I represents a different polar moiety that links to the oxygen at the C1 position of a sphingoid base. These polar moieties include hydrogen, phosphoethanolamine, phosphocholine, galactose, glucose, lactose, sulfated galactose/lactose, and other complex sugar groups, which correspond to ceramide, ceramide phosphoethanolamine, sphingomyelin (SM), galactosylceramide (GalCer), glucosylceramide (GluCer), lactosylceramide (LacCer), sulfatide (ST), and other glycosphingolipids such as gangliosides, respectively (Figure 1.6). These polar moieties can readily make over 20 sphingolipid classes. Building block II represents a fatty acyl moiety, which is acylated to the primary amine at the C2 position of

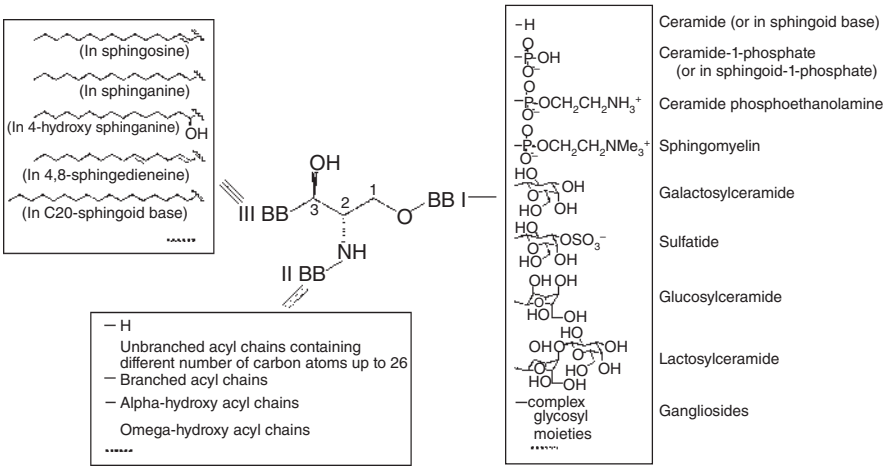

Figure 1.6 General structure of sphingoid-based lipids with three building blocks. Building block (BB) I represents a different polar moiety (linked to the oxygen at the C1 position of sphingoid backbone). These moieties determine the poplar head groups of sphingolipid classes as indicated. Building block II represents fatty acyl chains (acylated to the primary amine at the C2 position of sphingoid backbone) with or without the presence of a hydroxyl group, which is usually located at the alpha or omega position. Building block III represents the fatty acyl chains in all of possible sphingoid backbones, which are carbon–carbon linked to the C3 position of sphingoid backbones and vary with the aliphatic chain length, degree of unsaturation, location of double bonds, presence of branching, and presence of an additional hydroxyl group.

the sphingoid backbone. A variety of fatty acyl chains, including those that contain a hydroxyl group (usually located at the alpha or omega position) (Figure 1.6), are linked to this position. Building block III represents the aliphatic chain present in all sphingoid bases. This building block is connected through a carbon–carbon bond to the C3 position. This aliphatic chain varies in alkyl chain length and branching, the number and positions of double bonds, the presence of additional hydroxyl groups, and other features (Figure 1.6). Over 100 types of this aliphatic chain, after considering the hydroxyl-containing varieties, can also be readily counted. Therefore, a combination of these three factors would yield at least 200,000 ($20 \times 100 \times 100$) sphingolipid molecular species, whereas thousands of possible sphingolipid species can be theoretically constructed from the combination of these three building blocks by using only common aliphatic chains [20]. At the current stage, tens to hundreds of sphingolipid molecular species are readily analyzed by using different lipidomics approaches [21–23].

Sterols are a class of lipids containing a common steroid core of a fused four-ring structure with a hydrocarbon side chain and an alcohol group. Cholesterol is the primary sterol lipid in mammals and is an important constituent of cellular membranes. Oxidization and/or metabolism of cholesterol yield numerous oxysterols, steroids, bile acids, etc., many of which are important signaling molecules in biological systems. Cholesteryl esters esterified with a variety of fatty acyls are enriched in

lipoprotein particles, such as low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL).

1.1.2.2.2 The Significance of Building Block Classification The significance of this classification method is twofold: (1) ready to construct theoretical lipid databases that are expandable; and (2) effectively identify a large number of individual lipid species through identification of the relatively smaller number of building blocks. Luckily, these building blocks can be identified with their corresponding characteristic fragments by using two powerful tandem MS techniques (i.e., neutral loss scan (NLS) and precursor-ion scan (PIS)) [24]. These techniques and their applications for identification of lipid species are described in Chapters 2 and 6 in details.

1.2 LIPIDOMICS

1.2.1 Definition

The entire collection of chemically distinct lipid species in a cell, an organ, or a biological system has been referred to as a lipidome [25]. By analogy to other "omics" disciplines, lipidomics is an analytical chemistry-based research field studying lipidomes in a large scale and at the levels of intact molecular species. The research in lipidomics involves the following:

- Precisely identifying the structures of cellular lipid species including the number of atoms, the number and location of double bonds, the core structures and head groups, individual fatty acyl chains, and the regiospecificity of each isomer, etc.
- Accurately quantifying individual identified lipid species for pathway analysis, comparably profiling the lipid samples for biomarker discovery.
- Determining the interactions of individual lipid species with other lipids, proteins, and metabolites *in vivo.*
- Disclosing the nutritional or therapeutic status for prevention or therapeutic intervention of diseases.

Owing to the different utilities of lipidomics in its research, some subcategories of lipidomics are also frequently named in the literature as molecular/structural lipidomics [26–28], functional lipidomics [29, 30], nutritional lipidomics [31], dynamic lipidomics [32], oxidized lipidomics [33, 34], mediator lipidomics [35], neurolipidomics [36], sphingolipidomics [23, 37, 38], fatty acidomics [39], etc., to reflect their particular focus on the lipidomic studies. The analysis of lipid structures, mass levels, cell functions, and interactions in a spatial and temporal manner provides the dynamic changes of lipids during physiological (e.g., nutritional) or pathological perturbations or cell growth. Accordingly, lipidomics plays an essential role in defining the biochemical mechanisms underlying lipid-related disease processes through identification of alterations in cellular lipid signaling, metabolism, trafficking, and homeostasis.

Overall, lipids are considered as biological metabolites. Hence, lipidomics is covered under the umbrella of the general field of "metabolomics." However, lipidomics

is a distinct discipline because of the uniqueness and functional specificity of lipids relative to other metabolites. For example, most components in the cellular lipidome are extractable with organic solvents, so they are readily recovered and separated from other water-soluble metabolites. Lipids form aggregates (i.e., dimers, oligomers, micelles, bilayers, or other aggregated states) in all solvents essentially as their concentrations increase [1]. This unique property results in substantial difficulties for the quantitative analysis of individual lipid species in their intact forms by mass spectrometry (MS). This topic is addressed in detail in Chapters 15 and 16.

Cellular lipidomes are variable and highly complex. Tens of thousands of possible lipid molecular species are predictably present in the cellular lipidome at the level of attomole to nanomole of lipids per milligram of protein [20, 38, 40] (see above). These individual molecular species belong to a variety of different lipid classes and subclasses and comprise different lengths, degrees of unsaturation, different locations of double bonds, and potential branching in aliphatic chains. Moreover, additional factors make the study of this already complex and diverse system even more difficult. These include the following facts: (1) cellular lipid molecular species and composition are quite different among different species, cell types, cellular organelles, membranes, and membrane microdomains (e.g., caveola and/or rafts); and (2) the cellular lipidome is dynamic, depending on nutritional status, hormonal concentrations, health conditions, and many others [41].

Recent studies in lipidomics have largely focused on the following areas [42]:

- Identification of novel lipid classes and molecular species.
- Development of quantitative methods for the analysis of attomole to femtomole levels of lipids in cells, tissues, or biological fluids.
- Network analysis that clarifies metabolic adaptation in health and disease and biomarker analysis that facilitates diagnosis of disease states and determination of treatment efficacy.
- Tissue mapping of altered lipid distribution present in complex organs.
- Bioinformatics approaches for the automated high-throughput processing and molecular modeling with lipidomics data.

1.2.2 History of Lipidomics

Although the terms lipidome and lipidomics did not appear in the literature until the early 2000, researchers have initiated the study of cellular lipids on a large scale and at the intact molecular levels at much earlier times [43–52]. These pioneering studies truly demonstrated the possibilities of lipidomic analysis by using a variety of tools. Most importantly, these studies also provided initial insight into the utility of identifying alterations in membrane structure and function that mediate biological responses to cellular adaptation in health and maladaptive alterations during disease, thereby providing the foundation for development of the new discipline, lipidomics. The role of MS in characterization and analysis of lipids can be found in the classical book written by Dr Robert Murphy in 1993 [53].

Most early studies focused on one species, one lipid class, or one enzyme-catalyzed pathway. During these studies, investigators have clearly recognized that the metabolism of individual lipid molecular species or individual lipid classes is interwoven. To conduct research on lipid metabolism only from an isolated system, or only being focused on one molecular species, or one lipid class, has substantial limitations. The metabolism of the entire lipidome of the organelle, the cell type, the organ, the system, or the species should be investigated in a systems biology approach. Therefore, the need for such a comprehensive approach for studies of lipid metabolism greatly catalyzes the emerging of lipidomics and accelerates its development.

Investigators in lipidomics examine the structures, functions, interactions, and dynamics of a vast majority of cellular lipids and identify their cellular organization (i.e., subcellular membrane compartments and domains). The number of lipids in a cellular lipidome is estimated to be in the tens of thousands to millions [20, 38, 40]. Thus, in lipidomic research, a vast amount of information describing the spatial and temporal alterations in the content and composition of different lipid species in a selected system is accrued after perturbation of a cell through changes in its physiological (e.g., nutritional status, hormonal influences, health condition, metabolic levels) or pathological (diabetes, ischemia, neurodegeneration, etc.) state. The information obtained is processed by bioinformatics, which provides mechanistic insights into changes in cellular function. Therefore, lipidomic studies play an essential role in defining the biochemical mechanisms of lipid-related physiological/pathological processes through identifying alterations in cellular lipid metabolism, trafficking, and homeostasis in the selected system.

The term "lipidome" first appeared in the literature in 2001 [25]. In 2002, Rilfors and Lindblom [54] coined the term "functional lipidomics" as "the study of the role played by membrane lipids." In 2003, the field bloomed with different definitions [41, 55], demonstrations of technologies [41, 56], and biological applications [41, 57, 58]. Han and Gross first defined the field of lipidomics through integrating the specific chemical properties inherent in lipid species with a comprehensive mass spectrometric approach [41]. Since then, all areas of the field have been greatly accelerated.

Many modern technologies (including mass spectrometry (MS), nuclear magnetic resonance (NMR), fluorescence spectroscopy, high-performance liquid chromatography (HPLC), and microfluidic devices) have been used in lipidomic research. An edited book using these technologies for lipidomics is available [30]. MS, in part due to the development of new types of instruments and techniques (see Chapter 2), has greatly accelerated the progress of lipidomics. The website http://lipidlibrary.aocs.org/ constantly updates the publications including review papers that utilize modern MS methods for lipidomics. Several special issues on lipidomics have been published including the following:

- *Frontiers in Bioscience*, Volume 12, January 2007.
- *Methods in Enzymology*, Volumes 432 and 434, November 2007.

- *European Journal of Lipid Science and Technology*, Volume 111(1), January 2009.
- *Journal of Chromatography B*, Volume 877(26), September 2009.
- *Methods in Molecular Biology* (Springer Protocols), Volume 579–580, September 2011.
- *Biochimica et Biophysica Acta*, Volume 1811(11), November 2011.
- *Analytical Chemistry*, *Virtual Issue: Lipidomics*, http://pubs.acs.org/page/vi/2014/Lipidomics.html.
- *Analytical and Bioanalytical Chemistry*, Volume 407 (17), July 2015.

A few edited books on the areas of lipid analysis and lipidomics written by the experts and/or pioneers in the field have also been published [30, 59–61]. The current book provides a comprehensive description of the lipidomics discipline by using MS, from the fundamental, theory, and methods for identification and quantification, to applications.

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