SPHINGOMYELIN AND RELATED LIPIDS

STRUCTURE, OCCURRENCE, BIOSYNTHESIS AND ANALYSIS

1. Structure and Occurrence of Sphingomyelin

Sphingomyelin (or ceramide phosphorylcholine) consists of a ceramide unit with a phosphorylcholine moiety attached to position 1. It is thus the sphingolipid analogue of phosphatidylcholine (see the web-page on this lipid). The d18:1/16:0 molecular species is illustrated as an example.

It is a ubiquitous component of animal cell membranes, from mammals to nematodes to protozoa, where it is by far the most abundant sphingolipid. Indeed, it can comprise as much as 50% of the lipids in certain tissues, though it is usually lower in concentration than phosphatidylcholine. For example, it makes up about 10% of the lipids of brain. It is the single most abundant lipid in erythrocytes of most ruminant animals, where it replaces phosphatidylcholine entirely. In this instance, there is known to be a highly active phospholipase A that breaks down the glycerophospholipids, but not sphingomyelin. Like phosphatidylcholine, sphingomyelin tends to be in greatest concentration in the plasma membrane, and especially in the outer leaflet, of cells.

Sphingomyelin does not appear to occur in plants or fungi, which produce ceramide phosphorylinositol instead, or in bacteria with rare exceptions, and its evolutionary significance is a matter for speculation.

Sphingosine is usually the most abundant long-chain base constituent, together with sphinganine and C20 homologues, although other bases can be present, especially in ruminant animals. In contrast, sphinganine is the major sphingoid base in the sphingomyelin of human lens membranes, where the relevant molecular species can comprise more than half of the total phospholipids. Typically, the fatty acids are very-long-chain saturated and monounsaturated, including odd-numbered components. In contrast to glycosphingolipids, 2-hydroxy acids are present only rarely, but they are found in testes, spermatozoa, kidney and skin sphingomyelin, for example. The absolute proportions of each fatty acid and sphingoid base can vary markedly between tissues and species, and some of the variability in compositions can be seen from the data in Tables 1 and 2.

Table 1. Fatty acid compositions of sphingomyelin (wt % of the total) in some animal tissues.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>20:0</th>
<th>22:0</th>
<th>22:1</th>
<th>23:0</th>
<th>23:1</th>
<th>24:0</th>
<th>24:1</th>
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<tbody>
<tr>
<td>Egg</td>
<td>66</td>
<td>10</td>
<td>1</td>
<td>4</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Bovine brain</td>
<td>3</td>
<td>42</td>
<td>-</td>
<td>6</td>
<td>7</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>27</td>
</tr>
<tr>
<td>Cow’s milk</td>
<td>14</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>22</td>
<td>-</td>
<td>32</td>
<td>-</td>
<td>19</td>
<td>5</td>
</tr>
</tbody>
</table>

Sphingomyelin and related lipids: structure, occurrence, biosynthesis and analysis.

Table 2. Long-chain base compositions of sphingomyelin (wt % of the total) in some animal tissues.

<table>
<thead>
<tr>
<th>Sphingoid base</th>
<th>d16:0*</th>
<th>d17:0</th>
<th>d17:1</th>
<th>d17:1-methyl</th>
<th>d18:0</th>
<th>d18:1</th>
<th>d19:0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>7</td>
<td>93</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bovine brain</td>
<td>19</td>
<td>81</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cow’s milk</td>
<td>9</td>
<td>15</td>
<td>8</td>
<td>11</td>
<td>10</td>
<td>44</td>
<td>3</td>
</tr>
</tbody>
</table>


* d = dihydroxy base

Palmitic acid (16:0) is the most common fatty acid component of sphingomyelin in peripheral cells of mammals, while stearic acid (18:0) is more common in that of neural tissue, but this only hints at the potential complexity as there can be variability within tissues. For example, about 60% of the fatty acids of the sphingomyelin of the grey matter of human brain consist of stearic acid (18:0), while lignoceric (24:0) and nervonic (24:1) acids make up 60% of the corresponding lipid of white matter. Approximately 100 molecular species of sphingomyelin have been detected in human plasma. Polyunsaturated fatty acids such as arachidonic acid are rarely present, although they have sometimes been mistakenly identified in the literature. Exceptions are the sphingomyelins of testes and spermatozoa, which contain very-long-chain polyunsaturated fatty acids (up to 34 carbon atoms), the major components being 28:4(n-6) and 30:5(n-6), a proportion of which have hydroxyl groups in position 2.

2. Biosynthesis, Metabolism and Function of Sphingomyelin

The biosynthesis of sphingomyelin is distinct from that of phosphatidylcholine. Indeed, it involves transfer of phosphocholine from phosphatidylcholine to ceramide, liberating diacylglycerols, and catalysed by a ceramide choline-phosphotransferase (sphingomyelin synthase). The reaction takes place primarily in the Golgi but also in the plasma membrane, with distinct integral enzymes in each organelle, i.e. sphingomyelin synthases 1 and 2, respectively. A specific ceramide transport molecule (CERT) is important to the reaction (see our web page on ceramides) in that it transfers ceramide from the endoplasmic reticulum to the trans-Golgi in an ATP-dependent and non-vesicular manner. Much of the sphingomyelin produced in the Golgi is delivered to the apical plasma membrane by vesicular transport.
Sphingomyelin synthase 2 in the plasma membrane is not dependent on CERT-mediated ceramide delivery, but is believed to convert ceramide produced locally by a sphingomyelinase back to sphingomyelin. The location of the enzymes explains the enrichment of sphingomyelin in specific membranes and the sidedness, i.e. luminal trans-Golgi and outer leaflet of the plasma membrane. It is also present in the membranes nuclei from rat liver cells. Ceramide reaching the cis-Golgi is utilized for synthesis of glucosylceramide.

The nature of the molecular species of sphingomyelins produced differs appreciably from that of the ceramide precursors, suggesting considerable substrate specificity for the sphingomyelin synthases. The reaction can be reversible, using sphingomyelin to generating ceramide for specific signalling functions. It is evident that it forms a link between the sphingolipid signalling pathway (pro-apoptotic - see below) and that of glycerolipids via the mitogenic diacylglycerol by-products.

An alternative pathway of sphingomyelin synthesis has been demonstrated in the endoplasmic reticulum in which ceramide is first converted to ceramide phosphorylethanolamine (see below) via transfer of the head group from phosphatidylethanolamine, followed by stepwise methylation of the ethanolamine moiety. However, the physiological significance of this pathway has yet to be established.

It was long thought that the only function of sphingomyelin was to serve as a substitute for phosphatidylcholine as a building block of membranes, i.e. by forming a stable and chemically resistant outer leaflet of the plasma membrane lipid bilayer. This is one of its functions certainly, but the apparent similarity between phosphatidylcholine and sphingomyelin is superficial, and there are great differences in the hydrogen bonding capacities and physical properties of the two lipids. For example, sphingomyelin has an amide bond at position 2 and a hydroxyl on position 3 of the sphingoid base that can both participate in hydrogen bonding, while the trans double bond also appears to assist intermolecular interactions in membranes. With phosphatidylcholine, in contrast, the two ester carbonyl groups can only act as hydrogen acceptors. The degree of unsaturation of the alkyl moieties in each lipid is very different, and this gives them dissimilar packing properties in membranes.

It is now recognized that sphingomyelin and cholesterol have a high affinity for each other via strong van der Waals interactions, and they are usually located together in specific sub-domains or ‘rafts’ of membranes (discussed elsewhere) and on the surface of lipoprotein particles. Indeed, evidence has accumulated to suggest that sphingomyelin and cholesterol metabolism are closely integrated, and in particular that sphingomyelin may control the distribution of cholesterol in cells. They are most abundant in the same membranes, i.e. plasma membrane and Golgi as opposed to intracellular organelle membranes such as mitochondria (cancer cells may be an exception), although sphingomyelin can be transported through the cytosol. Other sphingolipids, such as the neutral glycosphingolipids, also promote raft formation but do not co-localize with cholesterol.

Sphingomyelin per se is generally considered to be a relatively inert molecule, although modern molecular biology methods are uncovering potential regulatory functions via interactions with specific proteins. In addition to its role in membranes, it serves as a precursor for ceramides, long-chain bases and sphingosine-1-phosphate, together with many other biologically important sphingolipids, as part of the ‘sphingomyelin’ cycle (also termed the ‘sphingolipid’ or ‘ceramide’ cycles depending on the context). Some of these metabolites have functions as intra- and intercellular messengers, and others are essential membrane constituents. These molecular relationships are illustrated only briefly below, as most are discussed in detail here on other pages on this site dealing with the relevant lipids.

In particular in animals, sphingomyelin is a major source of the ceramides (and its subsequent metabolites), via the action of sphingomyelinases (see below), which are required to trigger apoptosis and other metabolic changes. It performs this function in most cellular organelles, including the nucleus and even mitochondria, where it is a rather minor component. As ceramides
do not mix well with glycerophospholipids and cholesterol, this conversion results in the formation of new membrane domains enriched in ceramide that exclude cholesterol and so differ in composition from other sphingolipid rafts. This has profound effects on membrane function, especially of the plasma membrane, in that different proteins may be recruited or excluded depending on their relative affinities for cholesterol and ceramides.

The sphingomyelin cycle extends to other sphingolipids via the action of sphingomyelinases and other enzymes, such as glycosylhydrolases and glycosyltransferases, in the plasma membrane and operating at the cell surface leading in effect to exchange of head groups to produce innumerable new complex sphingolipids.

Sphingomyelin in the plasma membrane may be essential for the internalization of transferrin and thence of iron into cells, and it appears to be required for the activity of a number of membrane-bound proteins, including those of certain ion channels and receptors. As the most abundant sphingolipid in the nucleus, it is intimately involved in chromatin assembly and dynamics as well as being an integral component of the nuclear matrix. In addition, sphingomyelin is selectively recognized and acts as a receptor for the pore-forming toxins actinoporins, which are produced by sea anemones.

Although there is no known nutritional requirement for sphingolipids such as sphingomyelin, they are a component of any diet containing egg, meat or dairy products. Thus, it has been estimated that per capita sphingolipid consumption in the United States, for example, is of the order of 0.3-0.4 g/d. As sphingolipids are the main polar lipid constituents of milk, they may be especially important as minor but significant nutrients for infants. From animal experiments, there is evidence that feeding sphingolipids inhibits colon carcinogenesis and may alleviate some of the symptoms of inflammatory bowel disease. On the other hand, plasma sphingomyelin levels are considered to be an independent risk factor for atherosclerosis. Sphingomyelin in the plasma membrane serves as a receptor for certain pore-forming toxins.

### 3. Sphingomyelin Catabolism

The key enzymes for the degradation of sphingomyelin in most tissues are sphingomyelinases, which are similar in function to phospholipase C and generate ceramides as the end products. There are at least five such enzymes with different pH optima that operate in different regions of the cell with potentially distinct biochemical roles. For example, there is an acid sphingomyelinase (pH optimum ~5) in the endo-lysosomes, and different neutral sphingomyelinases in the plasma membrane, endoplasmic reticulum, Golgi and mitochondria. The lysosomal acid sphingomyelinase is involved in recycling of sphingolipid constituents and can migrate to the plasma membrane.
where it influences ceramide signalling. There is a related secreted acid sphingomyelinase, which can be transported to the outer membrane of the cell and is especially important in endothelial cells of the human coronary artery.

Neutral sphingomyelinases (pH optima 7.4), of which three distinct enzymes are known, are located in membranes such as the Golgi and plasma membranes, where they have signalling functions by generating ceramides and thence other biologically active sphingolipids. Neutral sphingomyelinase-2 appears to be especially important in brain and nervous tissue, where it is required for the secretion of hypothalamic releasing hormones, but is also relevant to many aspects of development in other tissues. The secreted acid sphingomyelinase can also operate at neutral pH and has multiple functions in that it is involved in many aspects of cellular signalling as well as in membrane sphingomyelin turnover.

A diverse range of factors activate the enzymes, including chemotherapeutic agents, tumor necrosis factor-alpha, 1,25-dihydroxy-vitamin D₃, endotoxin, gamma-interferon, interleukins, nerve growth factor, and most conditions known to induce cellular stress. In that they generate ceramides and other sphingolipid metabolites that have important signalling functions, sphingomyelinases are believed to function as regulators of signalling mechanisms, especially in the nucleus of the cell.

The type A and B forms of Niemann-Pick disease are lysosomal lipid storage disorders that are a consequence of a deficiency of acid sphingomyelinase, which causes an accumulation of sphingomyelin in cells and tissues. Increasing sphingomyelin levels in turn result in elevated cholesterol concentrations. A consequent lack of ceramide production may be involved in the pathology of the disease. It is noteworthy that membranes containing ceramides have a much lower binding capacity for cholesterol, so sphingomyelin degradation may play a part in cholesterol homeostasis. Type C Niemann-Pick disease is distinct and is caused by impaired transport of cholesterol from the late endosomes.

Intriguingly, there is a sphingomyelinase in the bacterium Pseudomonas aeruginosa that can also act as a sphingomyelin synthase in vitro.

In contrast to the glycerolipids, sphingolipids are not hydrolysed by pancreatic enzymes. Rather, sphingomyelin in the diet is hydrolysed in the brush border of the intestines by an alkaline sphingomyelinase to ceramide and thence by a neutral ceramidase to free fatty acids and sphingosine. The sphingosine is absorbed, some is re-esterified and the remainder is converted to palmitic acid and acylated into the triacylglycerol component of chylomicrons. In the process, some of the hydrolysis intermediates may have signalling functions in the intestines. The alkaline sphingomyelinase is unusual in that it is very different in its structure and other properties from intracellular enzymes with a related function. It is believed to have a role in the production of sphingolipid metabolites within the intestines and colon especially, which may influence a number of disease states. For example, it appears to inhibit colon cancer by generating ceramides. In addition, alkaline sphingomyelinase has phospholipase C activity towards the pro-inflammatory metabolite platelet-activating factor and towards lysophosphatidylcholine with potentially further beneficial effects. By reducing the level of endogenous sphingomyelin and increasing that of ceramides in the membranes of intestinal cells, it is believed to reduce the uptake of dietary cholesterol.
4. Sphingolipids Closely Related to Sphingomyelin

An unusual sphingolipid, \( 3\text{-O-acyl-D-erythro-sphingomyelin} \), has been found in plasma of the newborn pig and infant (but not in that of adults). In this instance, position 3 of the sphingosine residue is linked to an additional fatty acid (C\(_{16}\) or C\(_{18}\)) via an ester bond (alkali-labile).

**Sphingosine phosphorylcholine** or lyso-sphingomyelin is found at trace levels only in tissues. For example, in plasma it is present at concentrations of about 50 to 130 nM, and there is evidence that it is metabolized very rapidly. It is formed by the action of a sphingomyelin deacylase in skin (where it may have a role in atopic dermatitis), and probably by a similar route in some other tissues, including heart, blood vessels, brain and the immune system.

Sphingosine phosphorylcholine is believed to be involved in many cellular processes, including the promotion of proliferation and differentiation, although it can also inhibit the growth of cancer cells. It is produced under physiological and pathological conditions and activates various signalling cascades. In addition, it has potent anti-inflammatory properties, and reduces the level of organ dysfunction caused by lipopolysaccharides in rats *in vivo*, for example. While sphingosine phosphorylcholine has been reported to have many similar functions to **sphingosine 1-phosphate** (see the appropriate web page), the activities of the two lipids may not be easily distinguished as sphingosine phosphorylcholine can be converted to sphingosine 1-phosphate by the action of the plasma enzyme autotaxin. No specific cellular receptors for sphingosine phosphorylcholine have been positively identified.

Sphingolipids have been found in a species of earthworm with phosphorylcholine linked to the carbohydrate moiety of mono- and digalactosylceramides.

5. Ceramide Phosphorylethanolamine and Other Ceramide-Containing Phospholipids

**Ceramide phosphorylethanolamine**, the sphingolipid analogue of phosphatidylethanolamine, is a component of the lipids of insects, some fresh water invertebrates and many species of bacteria (where it is often accompanied by ceramide phosphorylglycerol), but it is present at trace levels only in mammalian cells. As an example, it is one of the main sphingolipid in *Drosophila melanogaster*, where d14:1 and d16:1 are the main long-chain bases. The insect species, *Manduca sexta*, contains tetradecasphing-4,6-dienine as a major sphingoid base component, together with 18:0, 20:0, 22:0 and 24:0 fatty acids. Ceramide phosphorylethanolamine has been fully characterized in three species of plant fungal pathogens (Oomycetes); the fatty acid and long-chain bases components vary with species, and for example one contains phytosphingosine and another an unusual branched-trienoic base. A phosphonolipid analogue is found in certain organisms (see our web pages on this topic). In addition to ceramide
phosphorylethanolamine, the protozoan parasite *Trypanosoma brucei* contains sphingomyelin and ceramide phosphorylinositol.

In insects, CDP-ethanolamine is the donor of the head group for ceramide phosphorylethanolamine synthesis (akin to phospholipid biosynthesis by the Kennedy pathway). However, in mammalian cells synthesis occurs in the endoplasmic reticulum with a distinctive synthase (SMS related protein) but by a similar mechanism to that of sphingomyelin, i.e. by transfer of phosphorylethanolamine from phosphatidylethanolamine to ceramide. Sphingomyelin synthase 2 may also produce some ceramide phosphorylethanolamine. Although the lipid never accumulates in membranes, the process appears to be important as a regulator of ceramide homeostasis.

**Ceramide phosphorylglycerol** has long been known as a constituent of the membranes of anaerobic bacteria of the genus *Bacteriodes*. More recently, an unusual form of it was identified is the most abundant lipid in the oral pathogen *Porphyromonas gingivalis* (illustrated).

In this instance, it has sphingamine or an iso-methyl-branched sphinganine as the long-chain base with an amide linkage to 3-hydroxy-iso-methylhexadecanoic acid, the hydroxyl group of which is esterified to iso-methyltetradecanoic acid. It is believed to make a significant contribution to the virulence of the organism in dental decay.

Further novel sphingolipids isolated from a cyanobacterium, *Scytonema julianum*, are ceramide phosphoglycolipids with an additional fatty acid with an ester link to position 3 of the sphingoid base. In addition, some species contain fatty acids in an estolide linkage, i.e. with an acetyl group esterified to an ω-1 hydroxyl of a long-chain fatty acid.

**Ceramide-1-phosphate** and **ceramide phosphorylinositol** are particularly important sphingophospholipids and as such have their own web pages here.

### 6. Analysis

Sphingomyelin is readily isolated from animal tissues by adsorption chromatography (TLC and HPLC), although peaks or bands can split into two or three poorly resolved fractions. This is due in part to the changes in hydrophobicity resulting from the wide range of chain lengths in the fatty acid constituents, and in part to the presence of 2-hydroxy acids. Molecular species of the intact lipid can be resolved by reversed-phase HPLC, but another useful approach is to hydrolyse to the less polar ceramides with the enzyme phospholipase C. Then, the ceramides can be analysed either by reversed-phase HPLC or by high-temperature GC. Nowadays, direct-inlet mass spectrometric methods (electrospray ionization, especially) are being used increasingly for the analysis of sphingomyelin and other sphingo-phospholipids. As with other sphingolipids, the amide bond is resistant to mild alkaline hydrolysis, so special methods are required for analysis of the fatty acid and sphingoid base components.
Suggested Reading


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