SPHINGOSINE-1-PHOSPHATE
STRUCTURE, OCCURRENCE, BIOSYNTHESIS AND ANALYSIS

1. Occurrence and Biosynthesis

Sphingosine-1-phosphate, a zwitterionic lysophospholipid, is an important cellular metabolite, derived from ceramide, that is synthesized de novo or as part of the sphingomyelin cycle in animal cells. It has also been found in insects, yeasts and plants. It is an intermediate in the irreversible degradation of sphingolipids and has important biological properties, although it is a minor lipid in quantitative terms. For example, sphingosine-1-phosphate has vital roles in health and disease in that it affects cardiac function, vascular development, immune cell function, inflammation, cancer and Alzheimer’s disease. Unlike other lysophospholipids it is primarily a single molecular species.

The primary precursor is sphingomyelin, which is hydrolysed by sphingomyelinases to produce ceramides. The enzyme ceramidase acts upon ceramides to release sphingosine, and this is phosphorylated by sphingosine kinase, a ubiquitous enzyme in the cytosol and endoplasmic reticulum, to form sphingosine-1-phosphate. The reverse reaction can occur also by the action of sphingosine phosphatases, and the enzymes act in concert to control the cellular concentrations of the metabolite, which are always low.

There are in fact two sphingosine kinases, designated Types 1 and 2 (SphK1 and SphK2). They are part of a super-family of enzymes that includes ceramide kinase and diacylglycerol kinase. They are distributed ubiquitously in tissues, but are especially abundant in erythrocytes and...
epithelial cells. Although the enzymes differ substantially in size, they have a high degree of polypeptide sequence similarity, but with different developmental expression, and tissue and subcellular distributions, suggesting that each has distinct physiological functions. The type 1 sphingosine kinase is predominantly cytosolic and pro-survival, probably by inhibiting ceramide biosynthesis. It regulates the cytosolic and extracellular levels of sphingosine-1-phosphate. The Type 2 enzyme is present in several intracellular compartments, depending on cell type, and it is able to translocate into the nucleus, where it can activate apoptosis by increasing the levels of enzymes that synthesise ceramide. In mammalian cells, there is an unusual pathway for the salvage of sphingosine that requires its phosphorylation by SphK2 (but not SphK1) and then dephosphorylation by a specific phosphatase for re-acylation to ceramide by ceramide synthase.

Sphingosine-1-phosphate may also be synthesised on the inner leaflet of the plasma membrane, and it is able to cross this via the action of at least two transporter proteins (from the ATP-binding cassette (ABC) family) to interact with specific receptors on the surface of the same cell or on nearby cells, or it can be transported in plasma to more distant tissues. In addition, it can be produced in plasma by the hydrolysis of sphingosyl-phosphorylcholine by the enzyme autotaxin.

In cells, sphingosine-1-phosphate occurs at concentrations in the low nanomolar range. However, in plasma, it can reach a concentration of 0.2 to 0.9 µM, and it is found in intimate association with the lipoproteins, especially the high-density lipoproteins (HDL), where it is bound to apolipoprotein M, a member of the lipocalin protein superfamily, which has a lipophilic binding pocket within the lipocalin structure. It is produced continuously and stored in relatively high concentrations in human platelets and erythrocytes. The latter especially have highly active sphingosine kinases and lack the appropriate degradative enzymes, and they are now believed to be the primary source of sphingosine-1-phosphate in plasma, although there is also a contribution from vascular endothelial cells. This lipid is released from platelets upon stimulation by thrombin, a product of the coagulation process. Levels in lymph fluid are four to five fold lower than plasma, while those in interstitial fluid are roughly 1000 fold lower, a gradient that is of great importance for directing immune cells to lymphoid organs and regulating their egress into blood and lymph. Mast cells and monocytes also secrete sphingosine-1-phosphate.

In humans, platelets contain dihydrosphingosine-1-phosphate in addition to sphingosine-1-phosphate, but in mice only the dihydrosphingosine derivative is found. There is some evidence that the two lipids may have different and even opposing functions.

### 2. Biological Functions

Like its precursors, sphingosine-1-phosphate is a potent messenger molecule that perhaps uniquely operates both intra- and inter-cellularly, i.e. it is both an autocrine and a paracrine agent, but with very different functions from ceramides, ceramide 1-phosphate and sphingosine. The balance between these various sphingolipid metabolites is important for health and has sometimes been termed the ‘sphingolipid rheostat’, although the readiness with which each of them can be interconverted does make it difficult to determine the true function of each.
A general hypothesis is that this mechanism evolved early in the development of life to regulate cell survival under environmental stress. For example, within the cell in contrast to ceramides and sphingosine, sphingosine-1-phosphate promotes cellular division (mitosis) as opposed to cell death (apoptosis), which it inhibits in fact. However, it can also enhance apoptosis in some circumstances. Intracellularly, sphingosine-1-phosphate also functions to regulate calcium mobilization and cell growth in response to a variety of extracellular stimuli. Unlike most other sphingolipids, it is not believed to participate in raft formation in membranes.

As with the lysophospholipids, especially lysophosphatidic acid (see the appropriate web page) with which it has some structural similarities, sphingosine-1-phosphate exerts many of its extracellular effects through acting as a ligand for specific receptors, in this instance for five G protein-coupled receptors on cell surfaces (designated S1P1 to S1P5). Both sphingosine-1-phosphate and its dihydro analogue bind to them with a high affinity. In mammals, S1P1, S1P2, and S1P3 are found in all tissues, whereas S1P4 is restricted to lymphoid tissues and lung, and S1P5 to brain and skin. The ligand-receptor interactions are important for the growth of new blood vessels, vascular maturation, cardiac development and immunity, and for directed cell movement. In addition, sphingosine-1-phosphate and its receptors influence inflammatory processes, and they regulate corticosteroid-hormone biosynthesis and function.

Sphingosine-1-phosphate is released into the blood stream upon activation by physiological stimuli, such as growth factors, cytokines, and receptor agonists and antigens. Conversely, sphingosine-1-phosphate may stimulate the growth factor and cytokine signalling cascades. It may have a critical role in platelet aggregation and thrombosis and could potentially aggravate cardiovascular disease. On the other hand, the relatively high concentration of the metabolite in HDL is now believed to have beneficial implications for atherogenesis. Sphingosine-1-phosphate activates the receptor S1P1 when bound to apolipoprotein M, and their combined interactions may be of relevance to atherosclerosis. There is accumulating evidence that sphingosine-1-phosphate and its receptors regulate heart rate, blood flow in the coronary artery and blood pressure by mechanisms that have yet to be identified. It has been suggested that sphingosine-1-phosphate, together with other lysolipids such as sphingosylphosphorylcholine and lysosulfatide, are responsible for the beneficial clinical effects of HDL by stimulating the production of the potent anti-atherogenic and anti-inflammatory signalling molecule nitric oxide by the vascular endothelium.

In contrast, sphingosine-1-phosphate has a pro-inflammatory function in that in response to certain cytokines and bacterial lipopolysaccharides it induces up-regulation of the enzyme cyclooxygenase-2 (COX-2) and thence production of the prostaglandin PGE2.

It appears that the cellular location of sphingosine-1-phosphate production may dictate its functions, even to the extent of producing opposing biological effects, although the two biosynthetic enzymes may compensate for each other if one is inhibited. Thus, there is evidence that cytosolic sphingosine-1-phosphate formed by the action of SPHK1 stimulates cell proliferation and inhibits synthesis of ceramide de novo, while the SPHK2 isoform located in the endoplasmic reticulum promotes ceramide synthesis through the sphingosine salvage pathway. Loss of both enzymes is embryonically fatal.

Sphingosine-1-phosphate has a key role in the immune system by signalling newly made B and T lymphocytes to migrate from the bone marrow and thymus, respectively, to secondary lymphoid tissues including the spleen and lymph nodes where they may encounter foreign antigens. If they are not activated by an antigen, sphingosine-1-phosphate directs their circulation via other lymphoid tissues back into lymph and then into blood.

Like lysophosphatidic acid (with which it has much in common), sphingosine-1-phosphate is a marker for certain types of cancer, and there is increasing evidence that its role in cell division or proliferation has an influence on the development of cancers. For example, in contrast to ceramide, it stimulates the growth, survival and migration of tumor cells and it is abundant in malignant tissue,
especially breast cancer. In a glioblastoma, the sphingosine-1-phosphate concentration was nine fold higher than in normal brain tissue, and there was a corresponding reduction in ceramide levels. This is currently a topic that is attracting great interest amongst medical researchers, and the potential for therapeutic intervention in sphingosine-1-phosphate metabolism, for example by inhibiting its biosynthesis from ceramide, is under active investigation. Similarly, drugs that antagonize sphingosine-1-phosphate and its receptors are being tested clinically as immuno-suppressants to prevent rejection of kidney grafts and to reduce inflammatory and allergic responses. They are also being tested against some forms of multiple sclerosis. On the other hand, sphingosine-1-phosphate is believed to have beneficial effects on wound healing by stimulating the proliferation of new cells that close the wound.

Sphingosine-1-phosphate and lysophosphatidic acid are involved similarly in the regulation of the proliferation, survival, differentiation and migration of many types of stem cells, but especially in the development of the vascular and nervous systems. It also regulates keratinocyte differentiation and epidermal homeostasis.

Most of these functions have been attributed to sphingosine-1-phosphate generated by the action of SphK1, with much less known of the function of SphK2. However, it is now believed that the latter has a role in the cell nucleus, where the sphingosine-1-phosphate produced increases the acetylation of lysine residues on histones, an essential process regulating gene transcription. SphK2 present in mitochondria is believed to be necessary for correct assembly of the cytochrome oxidase complex, and it may bind to phosphatidylinositol monophosphates, targeting it to intracellular membranes.

3. Long-chain Base-1-Phosphates in Yeasts and Plants

In yeast and plants, sphinganine, sphingosine, phytosphingosine and other long-chain bases are phosphorylated by kinases in a similar way to form the appropriate 1-phosphate derivatives. While it was initially thought that sphingosine-1-phosphate per se was the active biological agent, it is now apparent that this is not the case, as sphingosine is rarely present in detectable amounts. Rather it has been suggested that the term ‘long-chain base-1-phosphates’ should be used in discussing the metabolism of these compounds in plants. Less is known of their function in comparison to animal tissues and no receptors appear to have been found, but there is evidence that they are involved in such diverse processes as defence mechanisms, pathogenesis, calcium mobilization, membrane stability, and the response to drought or heat stress. They are certainly required for abscisic acid-mediated stomatal closure via a mechanism that involves activation of sphingosine kinase by phosphatidic acid. In yeasts, phytosphingosine-1-phosphate also has a role in the regulation of genes required for mitochondrial respiration.

4. Catabolism of Sphingosine-1-Phosphate and Long-Chain Bases

Long-chain bases can be regenerated from sphingosine-1-phosphate by the action of specific phosphatases (SPP1 and SPP2), located in the endoplasmic reticulum, and other lipid phosphate phosphohydrolases. The balance between these catabolic activities and those of the sphingosine kinases is tightly regulated. However, in animals and plants, production of sphingosine-1-phosphate (and homologues, etc) is also a key step in the catabolism of long-chain bases.

The molecule is cleaved irreversibly by the enzyme sphingosine-1-phosphate lyase, which is also located in the endoplasmic reticulum, to yield trans-2-hexadecenal, which can be further catabolized or reduced to the long-chain alcohol and incorporated into ether lipids. The reaction with sphingosine-1-phosphate lyase, which is found in many different organs, especially lymphoid tissues, but not in platelets or erythrocytes, reduces the cellular levels of sphingosine and ceramide and is ultimately the means by which all sphingolipids are removed from cells.
Sphingosine-1-phosphate lyase will only interact with the naturally occurring D-erythro-isomer of a long-chain base, but shows little specificity towards the structure of the chain. It requires pyridoxal 5'-phosphate as a cofactor. As this is a key enzyme in regulating the intracellular and circulating levels of sphingosine-1-phosphate, it is now seen as a potential target for pharmacological intervention.

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\text{sphingosine-1-phosphate lyase} \quad \text{sphingosine-1-phosphate} \\
\text{trans-2-hexadecenal} \quad \text{ethanolamine phosphate}
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The ethanolamine phosphate that is the other product of the reaction can be utilized for biosynthesis of phosphatidylethanolamine. This reaction is a further important link between sphingolipid metabolism and that of the glycerophospholipids. It may be especially relevant to the metabolism of dietary sphingolipids, since the activities of all the required enzymes are high in the intestines.

5. Analysis

Analysis of sphingosine-1-phosphate does present problems because of its high polarity and relatively low hydrophobicity. However, methods are available for quantitative extraction from tissues, and modern electrospray-ionization mass spectrometry techniques for detection and quantification afford high sensitivity and specificity.

Recommended Reading


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