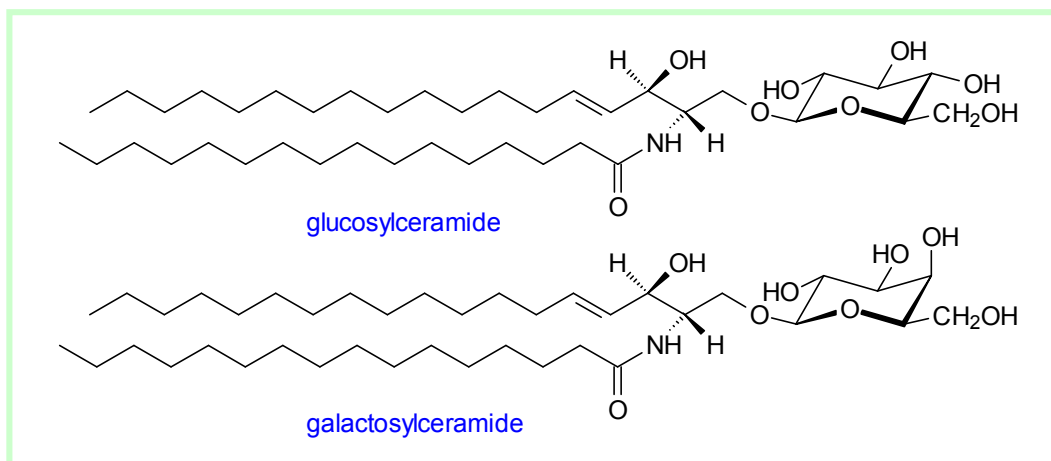


# MONOGLYCOSYLCERAMIDES (CEREBROSIDES)

## STRUCTURE, OCCURRENCE, BIOCHEMISTRY AND ANALYSIS

### 1. Structures and Occurrence

**Galactosylceramide** (Gal $\beta$ 1-1'Cer) is the principal glycosphingolipid in brain tissue, hence the trivial name "cerebroside", which was first conferred on it in 1874, although it was much later before it was properly characterized. In fact, galactosylceramides are found in all nervous tissues, but they can amount to 2% of the dry weight of gray matter and 12% of white matter. They are major constituents of oligodendrocytes.



**Glucosylceramide** (Glc $\beta$ 1-1'Cer) is also found at low levels in animal tissues, such as spleen and erythrocytes, as well as in nervous tissues, especially in the neurons. The d18:1/16:0 molecular species are illustrated above. Glucosylceramide is a major constituent of skin lipids, where it is essential for lamellar body formation in the stratum corneum and to maintain the water permeability barrier of the skin. In addition, the epidermal glucosylceramides (together with sphingomyelin) are the source of the unusual complex **ceramides** that are found in the stratum corneum (described on the appropriate web page). Similarly, higher than normal concentrations of this glycosphingolipid have been reported for the apical plasma membrane domain of epithelial cells from the intestines (especially the absorptive villous cells) and urinary bladder.

However, of greater importance than the natural occurrence of glucosylceramide *per se* is its role as the biosynthetic precursor of lactosylceramide, and thence of most of the complex oligoglycolipids and gangliosides. In contrast, galactosylceramide can be sulfated to form a sulfatide or sialylated to form ganglioside GM<sub>4</sub>, but only a small proportion is subjected to further galactosylation to form Gal<sub>2</sub>Cer as the precursor for the limited gala-series of oligoglycosphingolipids.

Interestingly, the proportion of galactosylceramides relative to glucosylceramides in myelin glycolipids increases greatly in the ascending phylogenetic tree. Similarly, the ratio of hydroxy- to nonhydroxy-fatty acids in cerebroside increases with the complexity of the central nervous system. There is also an interesting sex difference in kidney, where it has been shown that galactosylceramide (as opposed to glucosylceramide) only occurs in male mice (or androgen-treated adult females).



Glucosylceramide is a common component of the lipids of yeast and other fungi, including most fungal pathogens. However, it does not occur in the yeast *Saccharomyces cerevisiae*, which is widely used as an experimental model, although trace levels of galactosylceramides have been detected. 9-Methyl-4,8-sphingadienine is the characteristic fungal sphingoid base.

The fatty acid and long-chain base compositions of cerebrosides from two plant sources are listed in Table 2. Perhaps surprisingly, the fatty acid components are not very different in nature from those in animal tissues, comprising mainly longer-chain saturated and monoenoic acids, with a high proportion being saturated and having a hydroxyl group in position 2. Interestingly, long-chain monounsaturated hydroxy fatty acids are found in cerebrosides of leaves of plant species that are resistant to chilling, but apparently not otherwise. In the examples selected for the table here, both di- and tri-hydroxy long-chain bases were found, mainly diunsaturated (*Z/Z* and *E/Z*) and almost entirely C<sub>18</sub> in chain-length. While saturated 2-hydroxy acids predominate in most plants, some cereal glucosylceramides contain high proportions of *n*-9 mono-unsaturated very-long-chain fatty acids.

**Table 2. Composition of fatty acids and long-chain bases (wt % of the total) in cerebrosides of seeds from scarlet runner beans and kidney beans.**

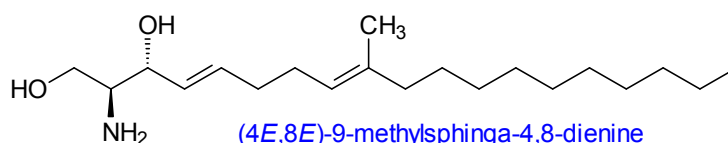
Fatty acids <sup>a</sup>	Long-chain bases <sup>b</sup>	
	Runner beans	Kidney beans
16:0	4	5
Other non-hydroxy (C <sub>16</sub> -C <sub>26</sub> )	1	2
14:0-OH	1	1
15:0-OH	1	1
16:0-OH	58	58
18:0-OH	trace	trace
20:0-OH	trace	trace
22:0-OH	7	6
23:0-OH	2	1
24:0-OH	23	23
25:0-OH	1	1
26:0-OH	1	1
t18:0	trace	trace
t18:1-8t	13	11
t18:1-8c	10	9
d18:0	trace	trace
d18:1-8c/t	1	3
d18:1-4t	trace	trace
d18:2-4t,8t	45	60
d18:2-4t,8c	31	17

From Kojima, M., Ohnishi, M. and Ito, S., *J. Agric. Food. Chem.*, **39**, 1709-1714 (1991).

<sup>a</sup> including 2-hydroxy acids

<sup>b</sup> di- and tri-hydroxy bases with *cis* or *trans* double bonds in the positions indicated.

In fungi, ceramide monohexosides are highly conserved molecules, with the ceramide moiety containing the distinctive sphingoid base, (4*E*,8*E*)-9-methyl-4,8-sphingadienine (or rarely phytosphingosine), linked to 2-hydroxy-octadecanoic or hexadecanoic acids (occasionally these with a *trans*-double bond in position 3), and with the carbohydrate portion consisting of one residue of either glucose or less often galactose (in contrast to plants). However, the nature of these can vary dramatically during different stages of growth (yeast versus mycelial forms).



Other monoglycosylceramides found in nature include fucosylceramide, which has been isolated from a colon carcinoma, a xylose-containing cerebroside identified in an avian salt gland, and glycosylceramides containing mannose from certain microorganisms. Cerebrosides linked to  $\alpha$ -D-galactose are only known to occur in a marine sponge; they are potent stimulators of mammalian immune systems. The genus *Sphingomonas* is unique among gram-negative bacteria in that it lacks lipopolysaccharides in its outer membrane, and instead has two sphingolipids, a tetraglycosyl ceramide and a cerebroside analogue,  $\alpha$ -galacturonosyl-ceramide, i.e. with a galacturonic acid moiety with an  $\alpha$ - rather than a  $\beta$ -linkage to the ceramide unit. The latter contains 2-hydroxy-myristic acid as the predominant fatty acid with sphinganine, (13Z)-erythro-2-amino-13-eicosene-1,3-diol and (13Z)-erythro-2-amino-13,14-methylene-1,3-eicosanediol as the long-chain bases. A few other bacterial species contain a similar lipid, while the phototrophic green sulfur bacterium, *Chlorobium limicola*, contains neuraminic acid linked to ceramide.

## 2. Biosynthesis and Function of Monoglycosylceramides

The biosynthesis of monoglycosylceramides in animal tissues resembles that discussed elsewhere for **glycosyldiacylglycerols**, i.e. there is a direct transfer of the carbohydrate moiety from a sugar-nucleotide, e.g. uridine 5-diphosphate(UDP)-galactose, UDP-glucose, etc, to the ceramide unit. During the transfer, which is catalysed by specific glycosyl-transferases, inversion of the glycosidic bond occurs (from *alpha* to *beta*). Synthesis of galactosylceramide takes place on the luminal surface of the endoplasmic reticulum, although it has free access to the cytosolic surface by an energy-independent flip-flop process, while glucosylceramide is produced on cytosolic side of the early Golgi membranes, with the possible exception of neuronal tissues. The latter must be translocated to the lumen of the Golgi, if it is to be converted to more complex glycolipids, while both must be transported to and then across the plasma membrane for their function in protein interactions and signalling. However, the nature of these transport processes is poorly understood.

In certain animal cells, studied *in vitro*, ceramides with 2-hydroxy acids are converted to galactosylceramide, whereas those with normal fatty acids are used for glycosylceramides, but this is not a universal rule. It is apparent that both ceramides synthesised *de novo* and those produced by catabolism of sphingomyelin are used for synthesis of glucosylceramide. In yeasts, the nature of the ceramide unit appears to determine whether it is utilized for glucosylceramide or **ceramide phosphorylinositol** synthesis.

In contrast, there is evidence that an important mechanism for glucosylceramide formation in plants involves sterol glucoside as the immediate glucose donor, although a pathway that uses UDP-glucose exists also in some plant species. It is possible that the former mechanism occurs on the cytosolic side and the latter on the luminal side of the plasma membrane. There is also evidence for a requirement for ceramides containing  $\Delta^4$  *trans*-double bonds for synthesis of glucosylceramides but not other sphingolipids in some plant and fungal tissues.

A remarkable property of cerebrosides is that their 'melting point' is well above physiological body temperature, so that glycolipids have a para-crystalline structure at this temperature. Each cerebroside molecule may form up to eight inter- or intramolecular hydrogen bonds by lateral interaction between the polar hydrogens of the sugar and the hydroxy and amide groups of the sphingosine base of the ceramide moiety. This dense network of hydrogen bonds is believed to contribute to the high transition temperature and the compact alignment of the cerebrosides. As with **sphingomyelin**, monoglycosylceramides tend to be concentrated in the outer leaflet of the plasma membrane together with cholesterol in the specific membrane domains termed '**rafts**'. Indeed, the latter appear to facilitate segregation to a greater extent than sphingomyelin via the combination of hydrogen bonds and hydrophobic interactions. These forces are also of great importance for binding to the wide range of proteins, including enzymes and receptors, which are found in rafts. It is evident that the same physical properties of cerebrosides are essential for

myelin formation in nervous tissues. In plant membranes, similar sphingolipid-rich domains are formed in association with the plant sterols stigmasterol and sitosterol.

The evidence for the function is glycosylceramides in animals has been derived mainly from cell lines of animals in which synthesis of the lipid has been suppressed by various means. It appears that glucosylceramide is not essential for the viability of certain cell lines in culture, but disruption of the synthase gene results in the death of embryos. Many different functions for glucosylceramides have been suggested, but the experimental data have often been contradictory. However, a report that glucosylceramide is essential for the activity of tyrosinase, a key enzyme in melanin biosynthesis, may be significant.

In plants, glucosylceramides with a 9-methyl group within the sphingosine backbone elicit defense responses in rice. Similarly, cerebrosides with double bonds in positions 4 and/or 8 of the long-chain base appear to be involved in the defense of some plant species against fungal attack. There is recent evidence that glycosylceramides (but not glycosyldiacylglycerols) with sterols are located in 'rafts' in plant membranes, in an analogous manner to sphingolipids in animal tissues, and that they are associated with specific proteins. Correlative studies suggest that glucosylceramides help the plasma membrane in plants to withstand stresses brought about by cold and drought. For example, glycosylceramides containing 2-hydroxy monounsaturated very-long-chain fatty acids and long-chain bases with 4-*cis* double bonds appear to be present in higher concentrations in plants that are more tolerant of chilling and freezing.

Less is known of the function of glucosylceramide in fungi, but they may be involved in such processes as cell wall assembly, cell division and differentiation, and signalling, and in the case of fungal pathogens recognition by the immune system and the regulation of virulence. Some molecular species of this lipid from plants (a  $\Delta 8$  double bond in the long-chain base is essential) show fruiting-inducing activity in the fungus *Schizophyllum commune*.

Small but significant amounts of plant glucosylceramides are ingested as part of the human diet, and they are broken down to ceramides and then to long-chain bases in the intestines before being absorbed. There is some preliminary evidence that they may have anticancer properties.

### 3. Catabolism of Monoglycosylceramides

In animals, the main sites for the degradation of glycosphingolipids are the lysosomes. These are membrane-bound organelles that comprise a limiting, external membrane and internal lysosomal vesicles, which contain digestive enzymes that are active at the acidic pH of this organelle. Most of these enzymes are soluble and localized in the lysosomal lumen. All membrane components are actively transported to the lysosomes to be broken down into their various primary components. In the case of glycosphingolipids, this means to fatty acids, sphingoid bases and monosaccharides, which can be recovered for re-use or further degraded. Thus, sections of the plasma membrane enter the cell by a process of endocytosis, and they are then transported through the endosomal compartment to the lysosomes. As the degradative enzymes are soluble but the substrates are membrane-bound in vesicular structures, the process requires the presence of specific activator proteins and of negatively charged lipids. The compositional and physical arrangement of the lysosomal membranes is such that they are themselves resistant to digestion.

Degradation of oligoglycosylceramides and gangliosides occurs by sequential removal of monosaccharide units via the action of specific exohydrolases from the non-reducing end until a monoglycosylceramide unit is reached. Then glucosylceramide  $\beta$ -glucosidase or an analogous  $\beta$ -galactosidase removes the final carbohydrate moiety to yield ceramides, which are in turn hydrolysed by an acid ceramidase to fatty acids and sphingoid bases. In addition, a non-lysosomal degrading enzyme for glucosylceramide has been found in the endoplasmic reticulum.

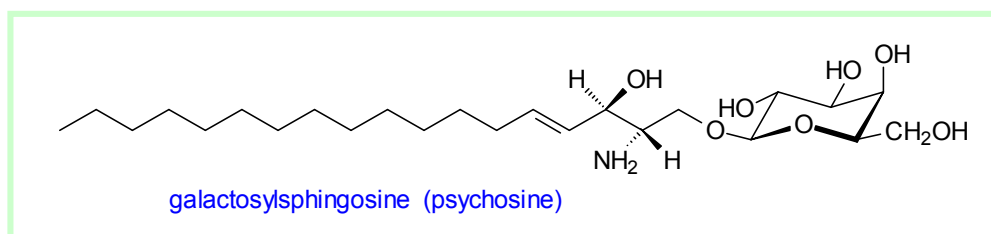
*In vivo*, the process requires the presence of specific activator proteins, which are glycoproteins of low molecular weight. These are not themselves active catalytically but are required as cofactors either by directing the enzyme to the substrate or by activating the enzyme by binding to it in some manner. Five such proteins are known, the GM<sub>2</sub>-activator protein (specific for gangliosides) and saposins A, B, C and D. The four saposins are derived by proteolytic processing from a single precursor protein, prosaposin, which is synthesised in the endoplasmic reticulum, transported to the Golgi for glycosylation and then to the lysosomes. Saposin A is essential for the degradation of galactosylceramide, saposin B for that of sulfatide and globotriaosylceramide, and saposin C for that of glucosylceramide. Although there are suggestions that it may activate the acid ceramidase, the function of saposin D is less clear.

Harmful quantities of glucosylceramide accumulate in the spleen, liver, lungs, bone marrow, and, in rare cases, the brain of patients with **Gaucher's disease**, the most common of the inherited metabolic disorders involving storage of excessive amounts of complex sphingolipids. Three clinical forms (phenotypes) of the disease are commonly recognized of which by far the most dangerous (Types 2 and 3) are those affecting the brain. All of the patients exhibit a deficiency of the enzyme glucocerebrosidase that catalyses the first step in the catabolism of glucosylceramide (the enzyme may be present, but a mutation prevents it assuming its correct conformation). Other than in the brain, the excess glucosylceramide arises mainly from the biodegradation of old red and white blood cells. The result is that the glucosylceramide remains stored within the lysosomes of macrophages, i.e. the specialized cells that remove worn-out cells by degrading them to simple molecules for recycling, thus preventing them from functioning normally. Enlarged macrophages containing undigested glucosylceramide are termed Gaucher cells. In the brain, glucosylceramide accumulates when complex lipids turn over during brain development and the formation of the myelin sheath of nerves. Deficiency of saposin C can also lead to similar symptoms.

Fortunately, there is now a highly effective enzyme replacement therapy for patients with the milder (non-neurological or Type 1) form of Gaucher's disease. This successfully reverses most manifestations of the disorder, including decreasing liver and spleen size and reducing skeletal abnormalities.

#### 4. Psychosine

**Psychosine** is the trivial name for a monoglycosylsphingoid, which is the non-acylated or lyso form of a cerebroside, normally galactosylsphingosine. It is a minor intermediate in the catabolism of monoglycosylceramides, and is normally present in tissues at very low concentrations. However, it may have some specific function in animal cells, for example in pathophysiology or in signalling since specific receptors have been found. It is unusual in being a basic (cationic) lipid, so it may have binding properties that differ from those of other lipids.



A deficiency of the enzyme  $\beta$ -galactosylceramidase, responsible for catabolism of galactosylceramide, is usually noted when psychosine accumulates in significant amounts. Deacylation of the galactosylceramide would then lead to formation of psychosine, although addition of galactose to sphingosine cannot be ruled out.

Psychosine accumulates in tissues in the genetic disorder, **Krabbe's disease** (globoid cell leukodystrophy), and to a certain extent also in Gaucher's disease. It is believed to inhibit

cytokinesis, i.e. the last stage in the process by which a single cell divides to produce two daughter cells, with production of multinucleate cells instead.

O-Acyl and plasmalogen forms of psychosine with hexadecanal or octadecanal linked to the carbohydrate moiety through 4,6- or 3,4-cyclic acetal bonds, termed 'plasmalopsychosines', have been detected in brain tissues of certain species. In particular, 4,6-plasmalopsychosine displays distinctive neurological effects. Two stereoisomers can exist in theory, but only the *endo* form appears to occur naturally. A glyceroplasmalopsychosine has also been isolated from brain tissue and characterized.

## 5. Analysis

Methods involving high-resolution thin-layer chromatography and high-performance liquid chromatography (HPLC) are now well established for the separation and analysis of monoglycosylceramides. HPLC in the reversed-phase mode is now the standard method for separation of molecular species, often after benzylation so that the lipids can be detected by sensitive UV spectrophotometry. However, mass spectrometric methods are now being used increasingly for characterization purposes.

## Recommended Reading

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