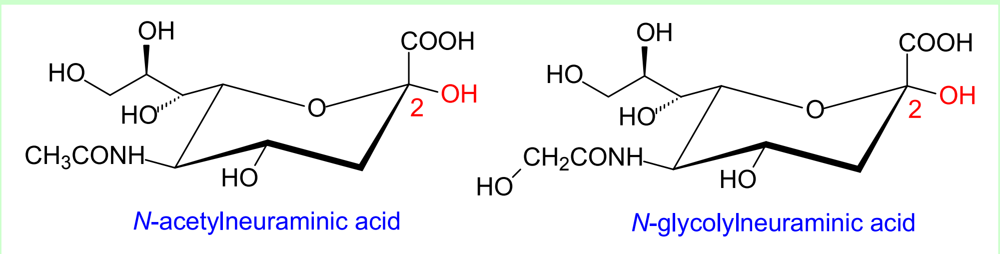
GANGLIOSIDES

STRUCTURE, OCCURRENCE, BIOLOGY AND ANALYSIS

1. Structure and Occurrence

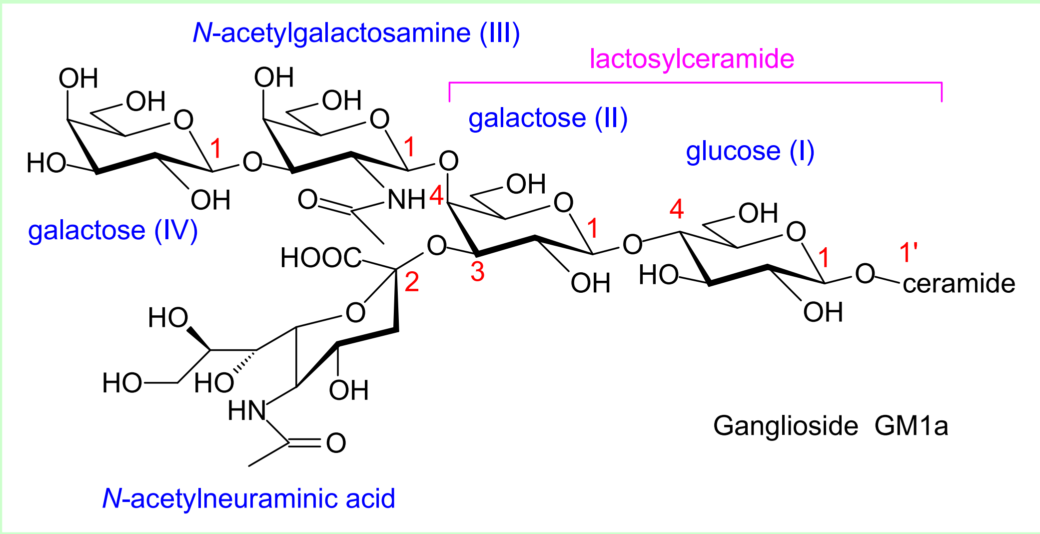
The name **ganglioside** was first applied by the German scientist Ernst Klenk in 1942 to lipids newly isolated from ganglion cells of brain. They were shown to be **oligoglycosylceramides** derived from lactosylceramide and containing a sialic acid residue such as *N*-acetylneuraminic acid (‘NANA’ or ‘SA’ or ‘Neu5Ac’ or ‘NeuAc’). Less often the sialic acid component is *N*-glycolyl-neuraminic acid (Neu5Gc), or a Neu5Ac analogue in which the amine group is replaced by OH (3-deoxy-D-glycero-D-galacto-nonulosonic acid, given the abbreviation ‘KDN’). These are joined via α-glycosidic linkages to one or more of the monosaccharide units, i.e. via the hydroxyl group on position 2, or to another sialic acid residue. The polar head groups of the lipids carry a net-negative charge at pH 7.0 and they are acidic.



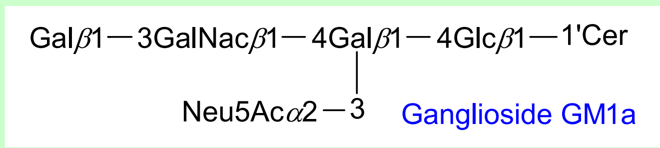
NeuAc is the biosynthetic precursor of NeuGc, which is a component of gangliosides from most animal species, including mice, horse, sheep and goats. NeuGc is not synthesised in humans, although it is present in other primates such as the great apes, and indeed anti-NeuGc antibodies are produced in humans by the injection of NeuGc-containing glycoconjugates. The absence of a number of relevant genes in humans, both for sialo-lipids and peptides, suggests that this may have been a major biochemical branch-point in human evolution. However, some NeuGc is obtained from the diet and may be incorporated into human gangliosides to a limited extent.

Most of the common range of gangliosides are derived from the ganglio- and neolacto-series of **oligoglycosphingolipids** (see the appropriate web page), and they should be named systematically in the same way with the position of the sialic acid residue(s) indicated as for branched structures. However, they are more conveniently defined by a short-hand nomenclature system proposed by Svennerholm in which M, D, T and Q refer to mono-, di-, tri- and tetrasialogangliosides, respectively, and the numbers 1, 2, 3, etc refer to the order of migration of the gangliosides on thin-layer chromatography. For example, the order of migration of monosialogangliosides is GM3 > GM2 > GM1 (sometimes defined by subscripts, e.g. G_{M1} or GM_1). To indicate variations within the basic structures, further terms are added, e.g. GM1a, GD1b, etc. Although alternatives have been proposed that are more systematic in structural terms, the Svennerholm nomenclature is that encountered most often in the literature. Ganglio-series glycosphingolipids having 0,1, 2,and 3 sialic acid residues linked to the inner galactose unit are termed asialo-, a-, b- and c-series gangliosides, respectively, while gangliosides having sialic acid residues linked to the inner *N*-galactosamine residue are classified as α-series gangliosides.

As of 2009, 188 gangliosides with variations in the carbohydrate chain had been characterized in vertebrates alone. One of the common monosialo-gangliosides (ganglioside GM1a or Neu5Ac*α*2-3(Gal*β*1-3GalNAc*β*1-4)Gal*β*1-4Glc*β*1Cer) is illustrated.



It can also be depicted as –



An alternative nomenclature uses the Ganglio (Gg) root structure (see the web page on neutral oligoglycolipids) with Roman numerals to designate each hexose unit and the location of the Neu5NAc along the carbohydrate chain and with Arabic superscripts to designate the hydroxyl group to which this is linked. By this system, GM1a is defined as II^3-*α*-Neu5NAc-Gg_4Cer.

Gangliosides can amount to 6% of the weight of lipids from brain, where they constitute 10 to 12% of the total lipid content (20-25% of the outer layer) of neuronal membranes, for example. Aside from this, they are synthesised and are present at low levels (1-2%) in all animal tissues, where like the neutral oligoglycosphingolipids they are concentrated in the outer leaflet of the plasma membrane in 'rafts'. Among the extraneural tissues, relatively high concentrations of ganglioside GD1a are present in erythrocytes, bone marrow, testis, spleen and liver, while GM4 is more abundant in kidney, GM2 in bone marrow, GM1 in erythrocytes and GM3 in intestine. SSEA-4, a globo-series ganglioside, is a specific component of human embryonic stem cells. Gangliosides in milk, which are derived from the apical plasma membrane of secretory cells of the mammary gland, may be of nutritional importance for the newborn but they are poorly characterized and quantified in foods in general. Gangliosides are not found out with the animal kingdom.

The brain contains as much as 20 to 500 times more gangliosides than most non-neural tissues, with three times as much in grey as in white matter. As the brain develops, there is an increase in the content of gangliosides and in their degree of sialylation. There are large differences between species and tissues. For example, during embryogenesis and the postnatal period in the human central nervous system, the total amount of gangliosides increases approximately threefold while that of GM1 and GD1a increases 12 to 15-fold. During the same period the hemato-series gangliosides, GM3, GD3, and 9-OAc-GD3 are the predominant ganglioside species, but they are present in much lower amounts in adults and then in some areas of the brain only. The main gangliosides of adult human brain are GM1, GD1a, GD1b and GT1, while GM3 is found mainly in the extra-neural tissues. Indeed, gangliosides GM2, and GM3 are largely absent from normal brain tissues in humans and some other species. In mouse brain, the total amount of gangliosides is almost 8-fold greater in adults than in embryos, with a similar shift in composition from simple to more complex gangliosides.

A de-*N*-acetylated form of ganglioside GD3 has been detected in human melanoma tumors. In addition, *O*-acetylation or lactonization of the sialic acid residue adds to the potential complexity.

Gangliosides containing *O*-acetylated sialic acids, such as 9-*O*Ac-GD3, occur in certain tumors and may protect them from apoptosis. This is also found in the retina and cerebellum of adult rats, but not other brain regions. It is possible that they are even more widespread, but they are missed when gangliosides are isolated after treatment with mild alkali, a common analytical practice. A further complexity is the occurrence of gangliosides with sulfate groups, and these have been isolated from human, mouse and monkey kidney cells. Gangliosides with glycosyl inositol-phosphoceramide structures have been isolated from marine invertebrates, while KDN-containing gangliosides are minor components of egg, ovarian fluid, sperm and testis of fish and of some mammalian tissues.

In general, the ceramide structures of gangliosides tend to be relatively simple. Sphingosine is usually the main sphingoid base, accompanied by the C_{20} analogue in gangliosides of the central nervous system mainly. Stearic acid (18:0) can be 80 to 90% of the fatty acid constituents, accompanied by small amounts of 16:0, 20:0 and 22:0, but with little or no polyunsaturated or 2-hydroxy acids, other than in some exceptional circumstances (e.g. some carcinomas). However, the nature and concentrations of the fatty acid and sphingoid base constituents change markedly during development, and for example, the ratio of C_{20}/C_{18}-sphingosine in ganglioside GD1a of cerebellum increases 16-fold between 8-day-old and 2-year-old rats.

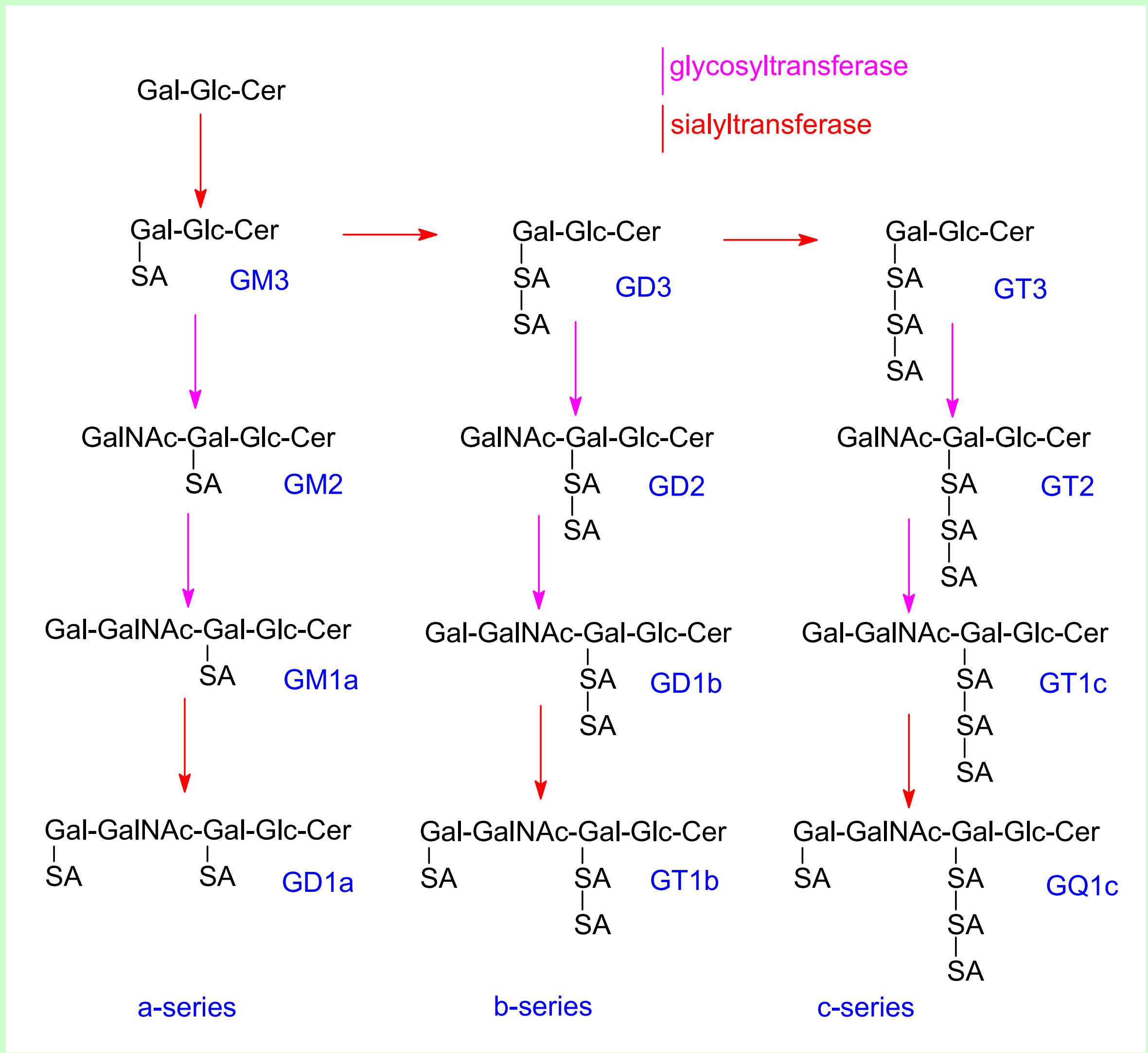
The nature of the ceramide component is relevant to the biological function of gangliosides, and changing the fatty acid component to α-linolenic acid by synthetic means alters the biological activity of gangliosides dramatically *in vitro*. However, it is the carbohydrate moiety that has the primary importance for most of their functions, and detailed discussion of these structures would take us into realms of chemistry best left to carbohydrate experts (see the reading list below). In any given cell type, the number of different gangliosides may be relatively small, but their nature and compositions may be characteristic and in some way related to the function of the cell. It is noteworthy that some terminal glycan structures of gangliosides are also present in certain glycoproteins.

2. Biosynthesis

There is evidence that the pool of glucosylceramide and thence of **lactosylceramide** that is utilized for ganglioside biosynthesis is different from that for the other **neutral oligoglycosylceramides** (GM4 is the exception in that galactosylceramide is its precursor). This may explain some of the differences in the fatty acid and sphingoid base components between the two groups. How the precursors for ganglioside biosynthesis enter the Golgi is an open question, but it appears that the regulation of intracellular sphingolipid traffic may be as important as the control of enzyme expression and activity in determining the final compositions of the various glycosphingolipid types.

Thereafter, the pathways for the biosynthesis of the common series of gangliosides of the ganglio-series, for example, involve sequential activities of sialyltransferases and glycosyltransferases as illustrated below for the three main a-, b- and c-series of gangliosides. The required enzymes are bound to the membranes of the Golgi apparatus in a sequence that corresponds to the order of addition of the various carbohydrate components. The sialyltransferase that catalyses the synthesis of the relatively simple ganglioside GM3 is located in the *cis*-region of the Golgi, while those that catalyse the terminal steps of ganglioside synthesis are located in the distal or *trans*-Golgi region. The GM3 synthase in particular, which catalyses the transfer of NeuAc from cytidine monophosphate (CMP)-NeuAc onto the terminal galactose residue of lactosylceramide, has a unique specificity.

Thus, a simple ganglioside GM3 is synthesised by addition of sialic acid to lactosylceramide by CMP:LacCer α2-3 sialyltransferase (or GM3 synthase), before GD3 and GT3 are produced in turn by the action of appropriate synthases. Subsequently, GM3, GD3 and GT3 serve as precursors of more complex gangliosides by the action of further glycosyl- and sialyltransferases. An alternative theory with some supporting evidence proposes that a multiglycosyl-transferase complex is responsible for the synthesis of each individual ganglioside rather than a series of individual enzymes. Finally, the gangliosides are transferred to the external leaflet of the plasma membrane by a transport system involving vesicle formation. Further sialization of each of the series illustrated occurs to give an increasingly complex range of products. The changes that occur in ganglioside compositions of brain and other tissues in the embryonic and post-natal stages are governed mainly by changes in the expression level and activity of the glycosyl- and sialyltransferases.



The presence of a distinctive sialidase that differs from the catabolic lysosomal enzymes (see below) in raft-like regions of the plasma membrane may bring about further changes in composition of the cell surface gangliosides, causing a shift from poly-sialylated species involving a decrease of GM3 and formation of GM2, then GM1 and eventually lactosylceramide. This may have consequences for important cellular events, such as neuronal differentiation and apoptosis. Conversely, sialylation may occur in some neuronal membranes, increasing the proportions of poly-sialylated species.

3. Ganglioside Function

The sialoglycan components of gangliosides extend out from the cell surface, where they can participate in intermolecular interactions. They function by recognizing specific molecules at the cell surface and by regulating the activities of proteins in the plasma membrane. In the latter, it is believed that gangliosides, and especially the simplest - GM3 or Neu5Acα2-3Galβ1-4Glcβ1Cer, also have a structural role and are segregated together with other sphingolipids and cholesterol into **raft** micro-domains, where the very large surface area occupied by the oligosaccharide chain imparts a strong positive curvature to the membrane. For example, molecules of GM3 and other gangliosides are present as clusters on the surface of lymphocytes of human peripheral blood. Many of the biological functions of rafts are mediated through the location of gangliosides in these domains or in a subset, the **caveolae**.

However, there are also suggestions that gangliosides and other oligoglycosyl-ceramides cluster together through hydrogen donor-acceptor (*cis*) interactions because of the presence of hydroxyl and acetamide groups to form glycosynaptic domains, which are functionally distinct from raft signalling platforms (with lower cholesterol concentrations). These glycosynaptic domains and their ganglioside components may have specialized functions in cell adhesion, growth, and motility through interactions with specific proteins and signal transduction pathways. For example, the phosphorylation state and activity of insulin receptors in caveolae and thence the insulin resistance of cells is controlled by the concentration of GM3, which is the main ganglioside in plasma and other extraneural tissues in vertebrates. This ganglioside also inhibits receptors for epidermal growth factor and regulates the integrin signalling machinery.

The techniques of molecular biology, which enable specific enzymes to be eliminated from experimental animals, are now leading to a better understanding of the function of each ganglioside. It is evident that they are essential to central myelination, to maintain the integrity of axons and myelin, and for the transmission of nervous impulses. These effects may be mediated by interactions of the negatively charged sialic acid residues of gangliosides with calcium ions, which are critical for neuronal responses. By stabilizing neuronal circuits, gangliosides may have a function in memory.

Gangliosides added to many types of cell preparations *in vitro* are rapidly taken up by the cells, while gangliosides injected into animals *in vivo* are rapidly internalized by tissues. They can cross the blood-brain barrier, and via the placenta they can enter the foetus. There is evidence also that dietary gangliosides are absorbed intact by intestinal cells and remodelled in the enterocyte prior to export and transport in plasma to other tissues.

Changes in ganglioside composition can be induced by nerve stimulation, environmental factors or drug treatments. The various interconvertible ganglioside types in the plasma membrane of neurons are particularly important for its development in that they regulate such processes as axonal determination and growth, signalling and repair. For example, the mono-sialoganglioside GM1 has been shown to promote the differentiation of various neuronal cell lines in culture. In addition, gangliosides are believed to be functional ligands for maintenance of myelin stability and the control of nerve regeneration by binding to a specific myelin-associated glycoprotein. The occurrence of gangliosides in cell nuclei suggests a possible involvement of gangliosides in the expression of genes relevant to neuronal function. Ganglioside GM1 is also important for Ca^{2+} homeostasis in the nucleus and in regulating the effects of platelet-derived growth factor.

Cell–cell interactions occur by sialoglycans on one cell binding to complementary binding proteins (lectins) on adjacent cells, bringing about cell–cell adhesion and enabling regulation of intracellular signalling pathways. Thus, in experimental systems, gangliosides have been shown to be cell-type specific antigens that control growth and differentiation of cells. In particular, they have key functions in the immune defense systems. They act as receptors of interferon, epidermal growth factor, nerve growth factor and insulin and in this way may regulate cell signalling. Intact gangliosides inhibit growth by rendering cells less sensitive to stimulation by epidermal growth factor, but removal of the *N*-acetyl group of sialic acid enhances this reaction and stimulates growth.

Ganglioside lactones have been detected as minor components in brain tissues. As the process of lactonization profoundly influences the shape and biological properties of the original ganglioside, it is possible that lactonization-delactonization in a membrane might be a further trigger for specific cellular events.

4. Catabolism

The principles of catabolism of glycolipids in general are discussed in the webpage dealing with **monoglycosylceramides**. In relation to gangliosides, sialidases and exoglycohydrolases remove individual sialic acid and sugar residues sequentially from the non-reducing terminal unit with the formation of ceramide, which is eventually split into long-chain base and fatty acids by ceramidases. This degradation occurs through the endocytosis-endosome-lysosome pathway with a requirement for an acidic pH inside the organelle. In addition to the sialidases and exoglycohydrolases, the various reactions require effector molecules, termed 'sphingolipid activator proteins', including saposins and the specific GM2-activator protein. This process constitutes a salvage mechanism that is important to the overall cellular economy since a high proportion of the various hydrolysis products are re-cycled for glycolipid biosynthesis. By generating ceramide and sphingosine, it may also be relevant to the regulatory and signalling functions of these lipids.

As with the neutral oligoglycosylceramides, a number of unpleasant lipidoses have been identified involving storage of excessive amounts of gangliosides in tissues because of failures in the catabolic mechanism. The most important of these are the GM2 gangliosidoses, i.e. Tay-Sachs disease (and the similar Sandhoff disease), a fatal genetic disorder found mainly in Jewish populations in which harmful quantities of ganglioside GM2 accumulate in the nerve cells in the brain and other tissues. As infants with the most common form of the disease develop, the nerve cells become distended and a relentless deterioration of mental and physical abilities occurs. The condition is caused by insufficient activity of a specific enzyme, β-*N*-acetylhexosaminidase, which catalyses the biodegradation of gangliosides. In addition, a generalized GM1 gangliosidosis has been characterized in which ganglioside GM1 accumulates in the nervous system leading to mental retardation and enlargement of the liver. The condition is a consequence of a deficiency of the lysosomal β-galactosidase enzyme, which hydrolyses the terminal β-galactosyl residues from GM1 ganglioside, glycoproteins and glycosaminoglycans.

5. Gangliosides and Disease

In addition to the lipidoses discussed above, gangliosides are involved in pathological states such as cancer, as certain distinctive gangliosides are found in tumors but not in the normal healthy tissue. Indeed, they can be shed from the surface of tumor cells into the local environment, where they can influence interactions between cancer cells, including the transition of tumors from a dormant to a malignant state (angiogenesis). Simple monosialogangliosides like GM3 are anti-proliferative and pro-apoptotic (and may have therapeutic potential), while more complex gangliosides such as GD1a enhance the proliferation, invasion and metastasis of tumor cells. One reason for this property of GM3 is its ability to suppress tyrosine phosphorylation of growth factor receptors in membranes of tumor cells. There can be a significant accumulation of unusual gangliosides containing *N*-glycolyl sialic acid in some cancers.

The Guillain–Barré syndrome is an acute inflammatory disorder, usually triggered by a severe infection, which affects the peripheral nervous system. Antibodies to gangliosides are produced by the immune system, leading to damage of the axons. It can result in paralysis of the patient. Impaired ganglioside metabolism is also relevant to Alzheimer's disease, as complexation with ganglioside GM1 causes aggregation of the amyloid β-protein deposits that characteristically accumulate in brain. Similarly, Huntington's disease is believed to involve disruption of the metabolic pathways between glycosylceramides and gangliosides, and there is a human autosomal recessive infantile-onset epilepsy syndrome caused by a mutation to a sialyl transferase. In contrast, gangliosides are believed to have a neuroprotective role in certain types of neuronal injury, Parkinsonism, and some related diseases.

Also, gangliosides bind specifically to viruses and to various bacterial toxins, such as those from botulinum, tetanus and cholera, and they mediate interactions between microbes and host cells during infections. The best-known example is cholera toxin, which is an enterotoxin produced by *Vibrio cholerae*; its specific cell surface receptor is ganglioside GM1. Similarly, ganglioside GM2 binds to a toxin secreted by *Clostridium perfringens*. Influenza viruses have two glycoproteins in their envelope membranes, hemagglutinin, which binds to cellular receptors such as gangliosides, and sialidase (neuraminidase), which cleaves the sialic acid from the receptors. It has been proposed that toxins utilize the gangliosides to hijack an existing retrograde transport pathway from the plasma membrane to the endoplasmic reticulum. The carbohydrate moiety of gangliosides is essential for initial binding of viruses, but the lipid moiety is believed to be important for controlling their intracellular transport.

6. Analysis

Gangliosides are not the easiest of lipids to analyse, as they are most 'un-lipid-like' in many of their properties. For example, in the conventional Folch method for extraction of lipids from tissues, the gangliosides partition into the aqueous layer rather than with the conventional lipids in the chloroform layer. Nonetheless, methods have been devised for quantitative extraction, and they can then be sub-divided into the various molecular forms by high-performance thin-layer chromatography (or less often by high-performance liquid chromatography). Mass spectrometry is the main method for structural analysis, including identification and sequencing of the carbohydrate chains, with invaluable assistance from nuclear magnetic resonance spectroscopy.

Recommended Reading

- IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN). Nomenclature of glycolipids. Recommendations 1997. Eur. J. Biochem., 257, 293-298 (1998).
- Kolter, T. Glycosphingolipids. In: *Bioactive Lipids*. pp. 169-196 (edited by A. Nicolaou and G. Kokotos, The Oily Press, Bridgwater) (2004).
- Levery, S.B. Glycosphingolipid structural analysis and glycosphingolipidomics. *Methods Enzymol*., **405**, 300-369 (2005).
- Lingwood, C.A. A holistic approach to glycolipid function; is the lipid moiety important? *Trends Glycosci. Glycotechnol*., **12**, 7-16 (2000).
- Lopez, P.H.H. and Schnaar, R.L. Gangliosides in cell recognition and membrane protein regulation. *Curr. Opinion Structural Biol*., **19**, 549-557 (2009).
- McJarrow, P., Schnell, N., Jumpsen, J. and Clandinin, T. Influence of dietary gangliosides on neonatal brain development. *Nutr. Rev*., **67**, 451-463 (2009).
- Merrill, A.H. Sphingolipids. In: *Biochemistry of Lipids, Lipoproteins and Membranes (5th Edition)*. pp. 363-397 (Vance, D.E. and Vance, J. (editors), Elsevier, Amsterdam) (2008).
- Merrill, A.H. Sphingolipid and glycosphingolipid metabolic pathways in the era of sphingolipidomics. *Chem. Rev*., **111**, 6387–6422 (2011).
- Prokazova, N.V., Samovilova, N.N., Gracheva, E.V. and Golovanova, N.K. Ganglioside GM3 and its biological functions. *Biochemistry-Moscow*, **74**, 235-249 (2009).
- Sonnino, S., Mauri, L., Chigorno, V. and Prinetti, A. Gangliosides as components of lipid membrane domains. *Glycobiology*, **17**, 1R-13R (2007).
- Taube, S., Jiang, M.X. and Wobus, C.E. Glycosphingolipids as receptors for non-enveloped viruses. *Viruses-Basel*, **2**, 1011-1049 (2010).

- Tettamanti, G. Ganglioside/glycosphingolipid turnover: new concepts. *Glycoconjugate J.*, **20**, 301-317 (2004).
- Todeschini, A.R. and Hakomori, S.I. Functional role of glycosphingolipids and gangliosides in control of cell adhesion, motility, and growth, through glycosynaptic microdomains. *Biochim. Biophys. Acta*, **1780**, 421-433 (2008).
- Xu, Y.H., Barnes, S., Sun, Y. and Grabowski, G.A. Multi-system disorders of glycosphingolipid and ganglioside metabolism. *J. Lipid Res.*, **51**, 1643-1675 (2010).
- Yu, R.K., Nakatani, Y. and Yanagisawa, M.J. The role of glycosphingolipid metabolism in the developing brain. *J. Lipid Res.*, **50**, S440-S445 (2009).
- Yu, R.K., Tsai, Y.-T., Ariga, T. and Yanagisawa, M. Structures, biosynthesis, and functions of gangliosides - an overview. *J. Oleo Sci.*, **60**, 537-544 (2011).
- Yu, R.K., Yanagisawa, M. and Ariga, T. Glycosphingolipid structures. In: *Comprehensive Glycoscience*. pp. 73-122 (ed. J. P. Kamerling, Elsevier, Oxford, UK) (2007).

William W. Christie

James Hutton Institute (and Mylnefield Lipid Analysis), Invergowrie, Dundee (DD2 5DA), Scotland

Last updated: January 20th, 2012

