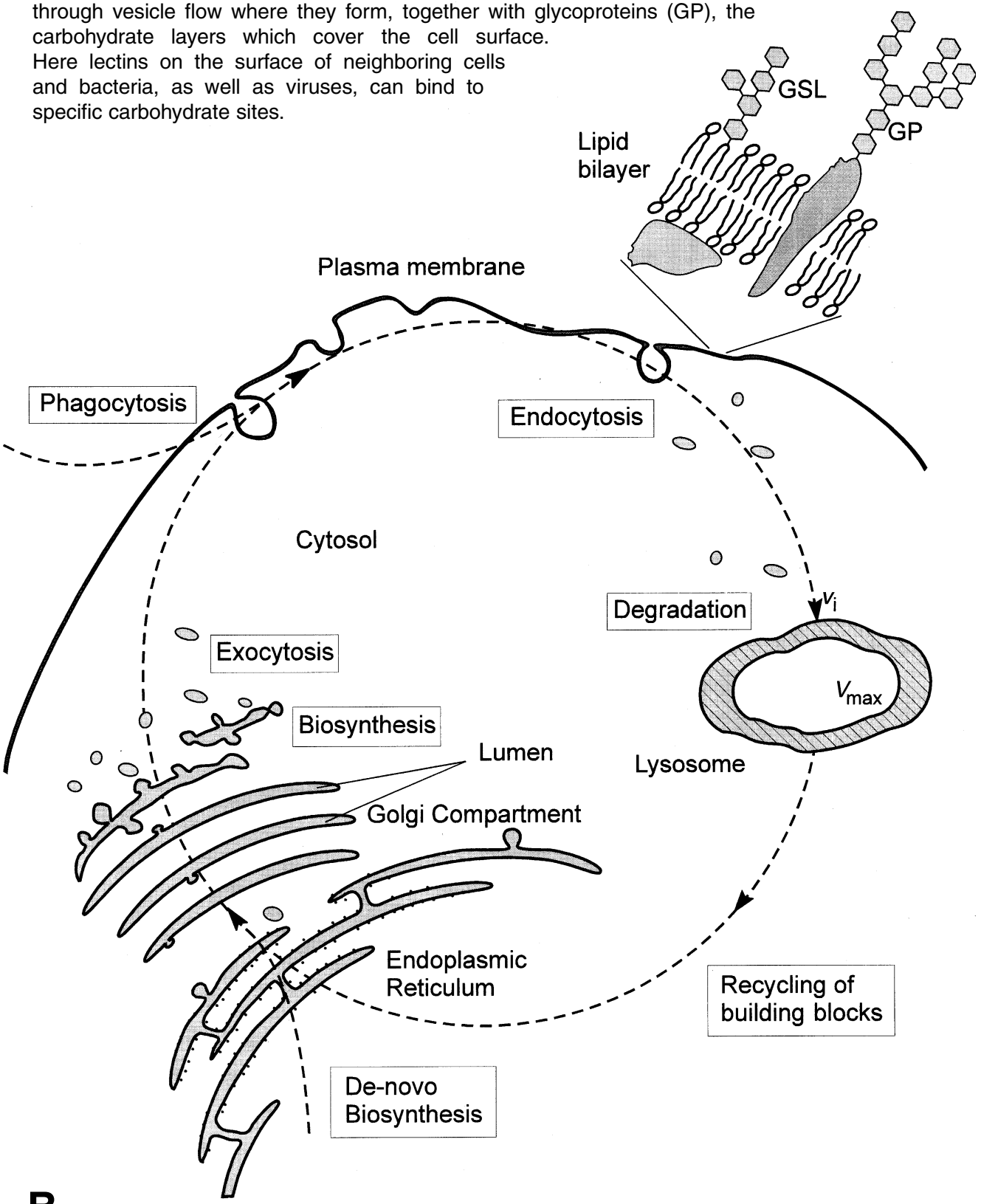
The biosynthesis of glycosphingolipids (GSL) takes place at the intracellular membranes of endoplasmic reticulum and the Golgi apparatus in eukaryonic cells. Glycolipids reach the plasma membrane through vesicle flow where they form, together with glycoproteins (GP), the carbohydrate layers which cover the cell surface. Here lectins on the surface of neighboring cells and bacteria, as well as viruses, can bind to specific carbohydrate sites.



Building blocks of the plasma membrane can reach the lysosomes by endocytosis, where they break down. The glycolipid influx rate v_i and maximum activities V_{max} of the degrading system within the lysosome are fundamental parameters in human genetic diseases that result from defects in the decomposition of sphingolipids. As well as being used in the new synthesis of glycoproteins, the building blocks, which are produced by lysosomal degradation and released into the cytosol, can be reused in a recycling process.

Sphingolipids—Their Metabolic Pathways and the Pathobiochemistry of Neurodegenerative Diseases

Thomas Kolter* and Konrad Sandhoff*

Dedicated to Professor Hans-Jürgen Quadbeck-Seeger on the occasion of his 60th birthday

Sphingolipids are building blocks of the plasma membrane of eukaryotic cells. Their function is to anchor lipid-bound carbohydrates to cell surfaces and to construct the epidermal water permeability barrier. Intermediates in sphingolipid metabolism are involved in the transduction of extracellular signals into the interior of cells. We give a review of the metabolism of these compounds in vertebrates, the intracellular topology of individual metabolic pathways, and the genetic diseases that occur as a result of defects in these pathways and which frequently lead to degeneration of the nervous system.

Keywords: enzyme inhibitors · diseases · glycolipids · glycosidases · sphingolipids

1. Historical Introduction

Glycosphingolipids (GSLs), a class of natural products, were first described in the second half of the last century. The German physician J. L. W. Tudichum was able to isolate an organic base that he called sphingosine in addition to sugar and fatty acids by fractional crystallization of alcoholic brain extracts.[1] The structure was elucidated by Carter in 1947.[2] The isolation and naming of further brain lipids such as ceramide, sphingomyelin, and cerebroside are also attributed to Tudichum.[3] At the end of the 1930s the Cologne biochemist Ernst Klenk discovered a new subgroup of acid sphingolipids in the brain tissue of patients who were suffering from amaurotic idiocy, a rare inherited disease. He called these new compounds gangliosides, and their acid component neuraminic acid.[4] The structure of *N*-acetylneuraminic acid, also known as sialic acid[5] or lactaminic acid, was elucidated by Richard Kuhn in 1962.[6] The first structure elucidation of a ganglioside, GM1 (Figure 1) was made by Kuhn and Wiegandt in 1963.[7]

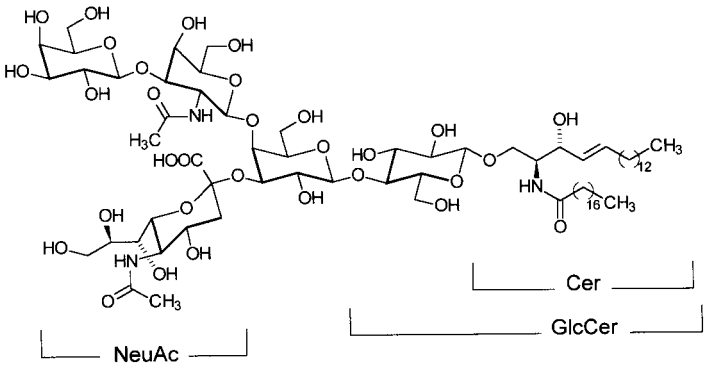


Figure 1. Structure of ganglioside GM1, an abundant glycosphingolipid bearing sialic acid in the adult human brain, which is also the intestinal cholera toxin receptor. The names of partial structures are indicated; heterogeneity within the lipid moiety is not indicated. NeuAc: *N*-acetylneuraminic acid (sialic acid), Cer: ceramide (*N*-acylsphingosine), GlcCer: glucosylceramide.

The structural identification of GSLs and the investigation of its metabolism were both facilitated by autopsy material from patients who had suffered from rare metabolic defects. Sphingolipids accumulate in the course of these storage diseases as a consequence of mutations that lead to the deficiency of proteins that are necessary for their lysosomal degradation. In our laboratory the analysis of these genetic diseases led to the identification of the molecular defects in Tay–Sachs disease, Sandhoff disease, and other sphingolipidoses (Section 6), as well as to a new hypothesis for the topology of endocytosis and lysosomal digestion (Section 5.4). It was also possible to demonstrate the function of activator proteins that are necessary for sphingolipid catabolism by the analysis of inherited diseases. Thus, the analysis of the AB-variant of GM2-gangliosidosis led to the identification of the GM2-activator protein (Section 6.2.4), and the detection of the specificity of saposins A–D (SAP-A–D) in vivo was only

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made possible with the help of the two only known patients with SAP-precursor deficiency (Section 6.11).

2. Structure and Function of Sphingolipids

The surfaces of eukaryotic cells are covered by a carbohydrate layer. This so-called glycocalix consists of glycoproteins, glycolipids, and glycosaminoglycans. Ceramide, the component of most sphingolipids, functions essentially as the anchor of lipid-bound carbohydrates in eukaryotic cells.[8–10] Ceramide itself consists of a long-chain amino alcohol, D-*erythro*-sphingosine, the amino group of which is acylated with a fatty acid. Carbohydrate-free sphingolipids also exist, such as ceramide itself, the membrane lipid sphingomyelin, or intermediates of sphingolipid metabolism such as sphingosine, sphinganine, or sphingosine-1-phosphate.

A number of GSLs have been found in nature that differ from each other in the type, number, and coupling of the individual carbohydrate building blocks. The GSL composition of an organism is species specific. The lipids can be classified into a few series (Figure 2 and 3) that are characteristic of species that are related in their evolutionary history.[10] GSLs form cell-type specific patterns on the cell surface (Figure 4), which change with the differentiation stage and with viral or oncogenic transformations.[11] The heterogeneity within this substance class is not limited to the carbohydrate moiety, however; the lipid anchor can also vary in alkyl chain length and the degree of unsaturation and hydroxylation.

GSLs on the cell surface are involved in cell-type specific adhesion processes.[12] It is known that they can function as binding sites for toxins, viruses,[13] and bacteria.[14] These pathogens profit from the close spatial neighborhood of specific carbohydrate recognition sites on the cell surface and the plasma membrane. Cell adhesion phenomena of this type result from a binding of the carbohydrate moiety of the membrane-bound GSLs to lectins on the surface of neighboring cells. The formation of myelin layers around axons of

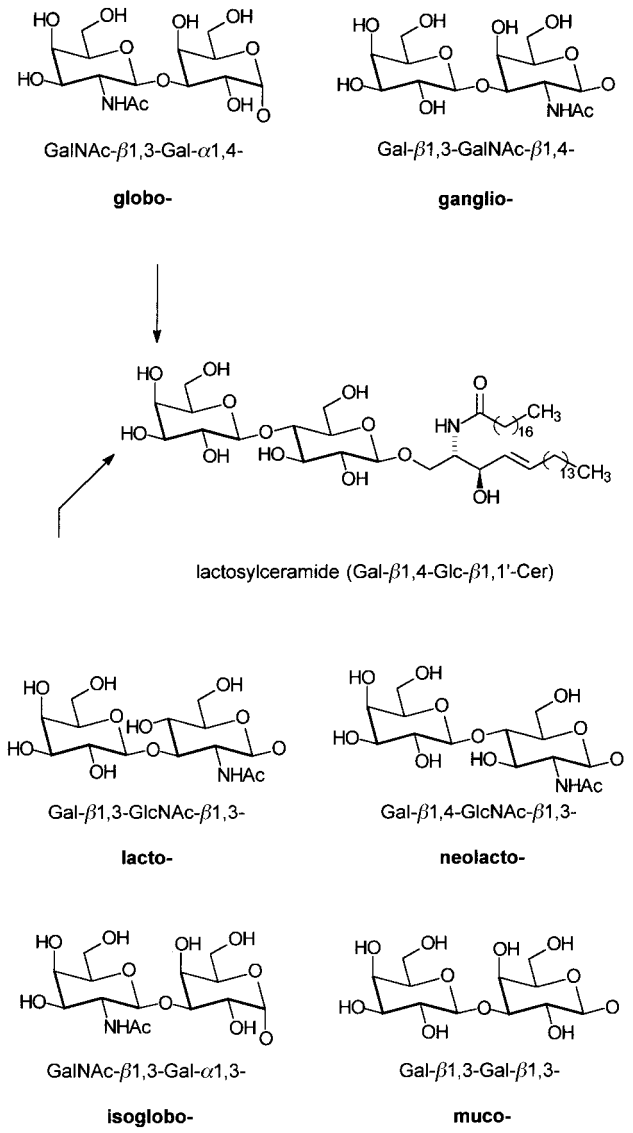

Figure 2. Structures and trivial names of the mammalian glycosphingolipid series derived from lactosylceramide.

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K. Sandhoff

Thomas Kolter was born in 1963 and studied Chemistry at the Universität of Bonn. He completed his Ph D with A Giannis on the synthesis of peptide mimetics in 1993. His work with Konrad Sandhoff is in the area of lipid biochemistry. His particular interest lies in bioorganic aspects of glycolipid metabolism.

Konrad Sandhoff was born in 1939 and studied Chemistry at the Universität München. He completed his Ph D under the guidance of H. Jatzkewitz and F. Lynen and then worked at the Max-Planck-Institut für Psychiatrie, München, the John-Hopkins University, Baltimore, and the Weizmann-Institut, Rehovot. In 1979 he became Professor of Biochemistry at the Kekulé-institut für Organische Chemie and Biochemie der Universität Bonn. The honors he has received include the naming of a human disease as "Sandhoff's disease", the award of the 1992 Richard-Kuhn Medaille der Gesellschaft Deutscher Chemiker (German Chemical Society) and the 1998 K.-J.-Zülch-Preises der Max-Planck-Gesellschaft. Since 1991 he has been the spokesman for the SFB 284 (glycoconjugate and contact structures of cell surfaces) and has been a member of the Editorial Board of Angewandte Chemie. His research interests are the analysis of lysosomal storage disorders and all aspects of the metabolism of glycolipids.

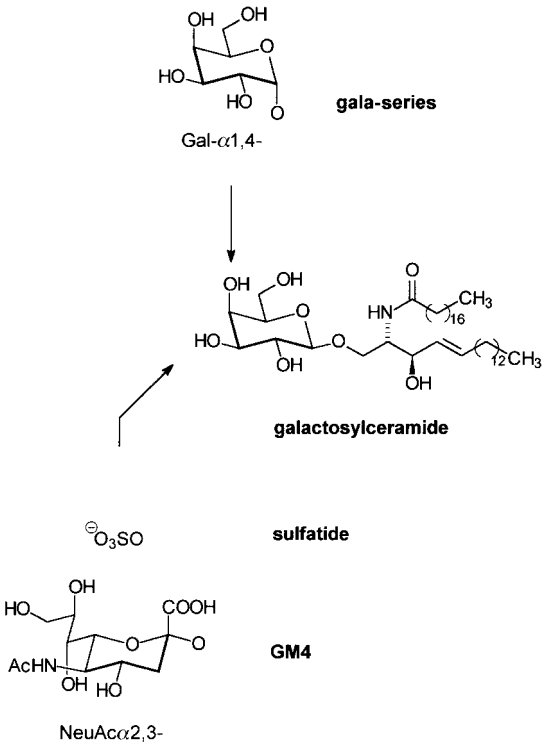


Figure 3. Structures and trivial names of the mammalian glycosphingolipid series derived from galactosylceramide.

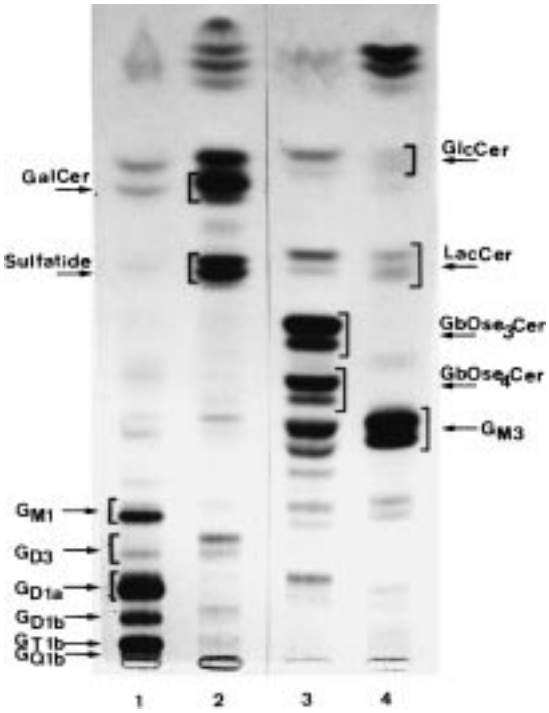


Figure 4. Biosynthetic labeling of cellular glycolipids with [^{14}C]galactose.[438] Cells were labeled by incubation with [^{14}C]galactose (2 μCi mL^{-1}) for 48 h, harvested, and extracted. The glycosphingolipids were separated by thin layer chromatography and visualized by fluorography. Lane 1: granule cells of murine cerebellum; Lane 2: oligodendrocytes; Lane 3: fibroblasts; Lane 4: neuroblastoma cells (B 104). The mobility of the standard lipids is indicated; see following figures for the abbreviations used.

neuronal cells is an example that is based upon this principle. In this case gangliosides on the surface of neuronal cells serve as ligands for the myelin-associated glycoprotein (MAG), which is located on the surface of myelinizing oligodendrocytes.[15] Interactions of GSLs with receptors and enzymes, which, like themselves, are located in the same membrane have been described and are of possible physiological relevance.[16] For example, ganglioside GM1 can activate the nerve growth factor,[17] and ganglioside GM3 inhibits tyrosine phosphorylation of the epidermal growth factor receptor.[18]

Many physiological processes can be affected by GSLs, for example embryogenesis, neuronal cell and leukocyte differentiation, cell adhesion, and signal transduction.[19] Lipophilic intermediates of GSL metabolism such as sphingosine, ceramide, and their phosphorylated derivatives have been recently identified as novel signal substances.[20, 21] Finally, complex GSLs form a layer on the extracytosolic faces of cellular membranes, which protects them from degradation and uncontrolled membrane fusion.[22, 23] However, little is known about the precise function of individual sphingolipids in vivo. Numerous observations suggest that they are involved in biological processes, but in many cases clear evidence for their function is not available. Only minor disorders have been observed in the nervous system of genetically modified mice that cannot form complex gangliosides because of inactivation of GM2- and GD2-synthase;[24] in contrast, severe functional disorders of the testes have been observed. Furthermore, inhibition of the biosynthesis of GSLs, which are derived from glucosylceramide, does not impair the development of fish[25] and mice embryos.[26] In contrast, however, the formation of glucosylceramide itself is essential for embryogenesis: genetically modified mice whose glucosylceramide synthase is missing die as early as the 19th day of embryogenesis.[27] Galactosylceramide, sulfatides, and gangliosides are essential for myelin function (Section 3.1), and ceramides for skin function (Section 2.1). Conservation of GSL structure during evolution and the absence of inherited diseases arising from disorders in their biosynthesis also indicate that they fulfill essential functions for the living organism.

2.1. Skin Sphingolipids

Sphingolipids are essential for the function of human skin where they contribute to the formation of the water permeability barrier.[28, 29] This barrier is localized in the horny layer of the epidermis and consists of a tightly stacked system of terminally differentiated keratinocytes (corneocytes) that are embedded in a highly organized multilamellar lipid matrix of free fatty acids, cholesterol, and ceramides. The structure of the ceramides involved here differ in a characteristic manner from the membrane anchors normally found in the GSLs of vertebrates (Figure 5 and 6). Thus, for example, unusually long fatty acid residues with up to 34 carbon atoms and sphingoid bases that are hydroxylated at various positions are found.[30]

A characteristic sphingoid base of keratinocytes is phytosphingosine (D-*ribo*-4-hydroxysphinganine), which is otherwise

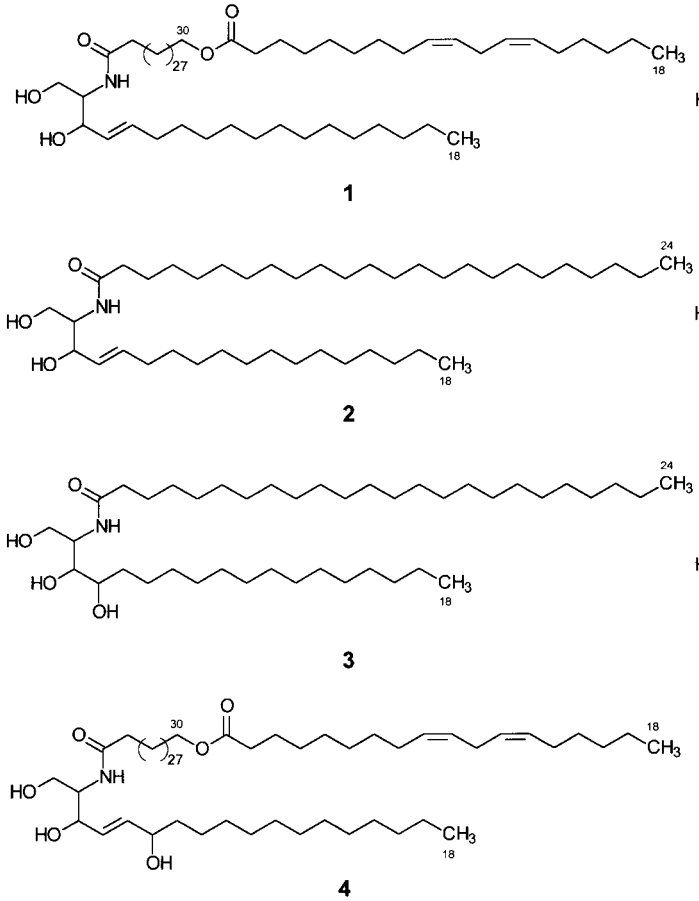


Figure 5. Sphingolipids of human skin (I): Structures of ceramides 1 – 4.[30]

found as a component of the sphingolipids of yeasts and other lower eukaryots. In humans it forms about 40 % of the sphingoid bases of epidermal ceramide.[31] The function of the additional free hydroxyl group is presumed to be to increase the rigidity of intercellular lipid aggregates through the formation of a large number of hydrogen bonds and hence reduce transepidermal water loss.[32] Covalent bonds between the ceramides and proteins such as involucrin also contribute to the stability of skin.[33] The biosynthesis of sphingolipids in human keratinocytes remains unclarified and is currently under investigation by us. In 1991 Elias and Menon hypothesized that the ceramides of intracellular lipid lamella are formed by extracellular degradation of complex GSLs. The lipid lamella originate in vivo from so-called Odland bodies that secrete their lipid content into the extracellular space.[34] Odland bodies are ellipsoidal organelles, and in addition to lipid aggregates they also contain an array of lysosomal proteins that can be used for the degradation of precursor lipids.[35]

We were able to elucidate details of this process during investigations with genetically modified mice that had no SAP precursor protein (Section 6.11) and which correspondingly exhibit a reduced capacity for degradation of glucosylceramides. A glucosyl derivative of ceramide (**1**; Figure 5) was identified in the epidermis, which is normally transferred onto the acidic amino acid side chains of proteins by transesterification followed by cleavage of the glucose residue.[445] This is a key step for the formation of extracellular lipid aggregates

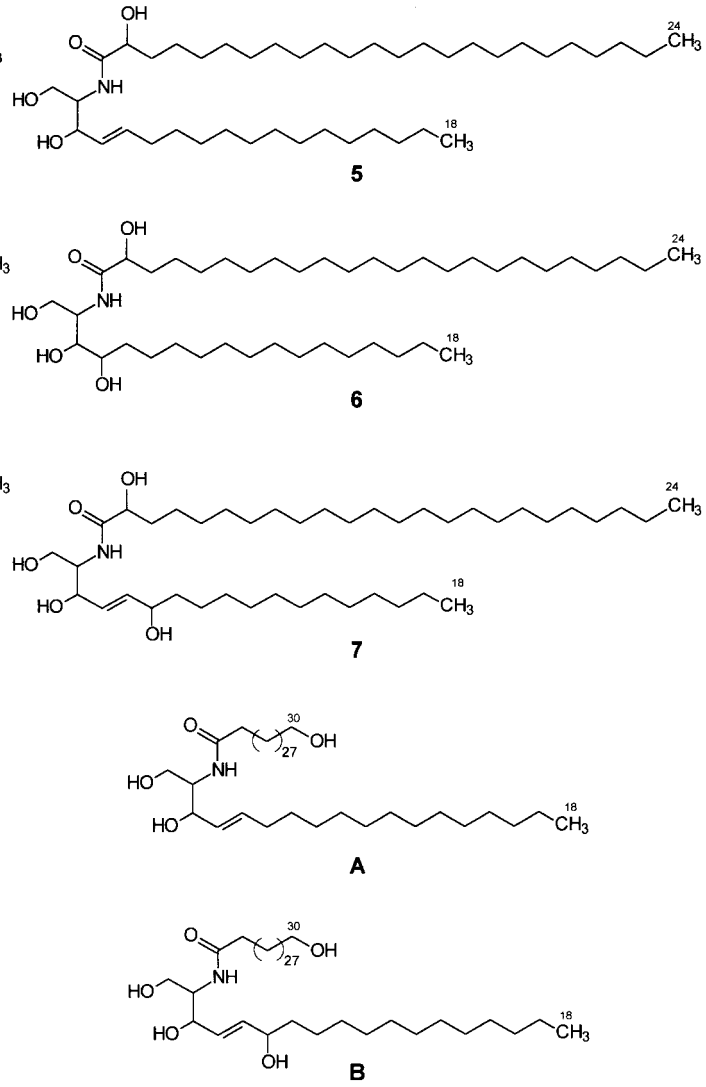


Figure 6. Sphingolipids of human skin (II): Structures of ceramides 5 – 7, A, and B.[30]

that are essential for the epidermal water permeability barrier of land animals. The significance of sphingolipids for skin function is itself confirmed by an inherited disease: in rare cases of Gaucher's disease where there is a complete failure of glucocerebrosidase, the patients die of transepidermal water loss within a few hours of birth.[36] Because of the significance of ceramides for skin function, there is interest in ceramides and ceramide replacements in the cosmetic industry.[37] In this respect reference is made to the function of ceramide as a signal molecule (Section 5.1), which makes the use of ceramide itself appear problematical for skin care.

2.2 Glycosphingolipids and the Immune System

Glycolipids can be recognized by the immune system. Historically, the identification of the Forssman antigen as a GSL is particularly significant.[38] Individual glycosphingolipids are blood group antigens; autoantibodies against gangliosides were known as the cause of peripheral neuropathies. For example, anti-GM1 and anti-GQ1b antibodies are regarded as

the cause of Guillain–Barre Syndrome. Anti-glycolipid antibodies can also be detected in other autoimmune diseases, for example, against sulfatide in insulin-dependent (type 1) diabetes.[39] A number of glycolipids play a role as tumor-associated antigens and in the immunotherapy of individual cancer forms.[40] α-Configured glycolipids have been described as potent immunostimulants.[41] On the other hand, inhibition of sphingolipid biosynthesis by the natural product myriocin (Section 4.1) is associated with severe immunosuppression.[42] Sphingolipids are also involved in signal transduction processes within the immune system.[43]

3. Biosynthesis and Intracellular Topology

3.1. Sphingolipid Biosynthesis

GSL patterns that are characteristic for a cell type in a certain stage of development reside on the cell surface. Coordinated and interrelated processes are hence involved in the biosynthesis of these compounds, their degradation, and their intracellular transport.

Biological membranes consist of lipid bilayers in which the cytosolic and anticytosolic layers can be of different composition. GSLs and sphingomyelin are most frequently located on the outer half of the plasma membrane (Figure 7). Since their biosynthesis and degradation occurs in different cellular organelles, they and their precursors are also found in intracellular membranes that are associated with the plasma membrane through membrane fusion processes. The enzymes that are involved in sphingolipid biosynthesis are membrane-bound proteins. Little is known of their structure, catalytic mechanism, biosynthesis, and regulation.

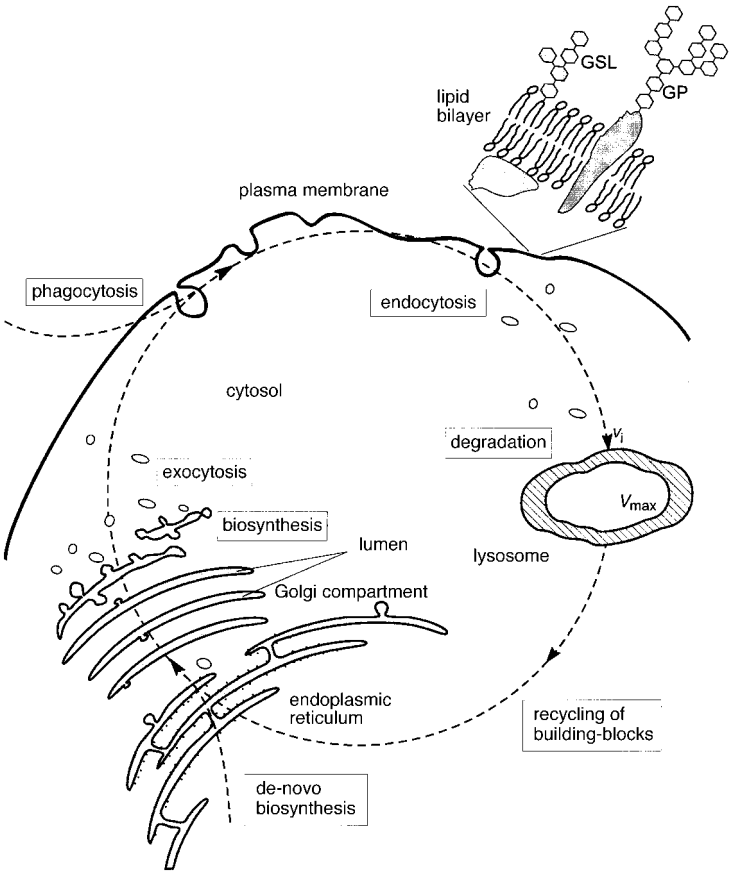


Figure 7. Intracellular metabolic flux of sphingolipids. GSL: glycosphingolipid; GP: glycoprotein; v_i = the influx rate of the substrate into the lysosome; for further information see the text.

De novo biosynthesis of GSLs[44] (Figure 8) takes place in the same compartments as glycoprotein biosynthesis. It is

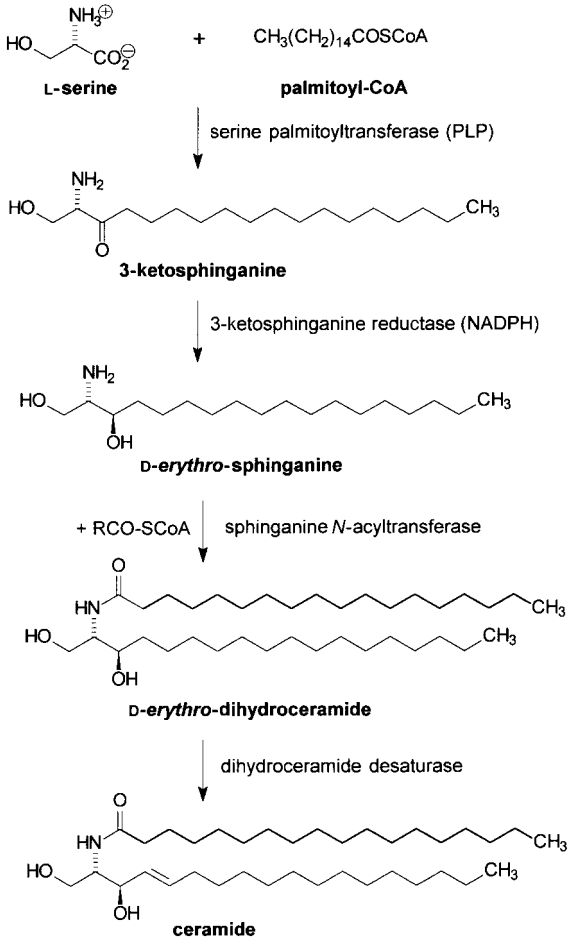


Figure 8. Ceramide biosynthesis. The formation of ceramide occurs on the cytosolic face of the membrane of the endoplasmic reticulum.[44]

coupled to the intracellular vesicular transport of the growing lipid molecule that leads to the plasma membrane through the cisternae of the Golgi apparatus. It begins with the formation of ceramide on the membranes of the endoplasmic reticulum (ER). The condensation of the amino acid L-serine with a fatty acid activated as a coenzyme A derivative to give 3-ketosphinganine is catalyzed by serine palmitoyltransferase (SPT).[45–47] SPT is a pyridoxal phosphate dependent enzyme and is mechanistically related to aminolevulinate synthase, which catalyzes the initial reaction of heme biosynthesis (see reference [100]). Serine palmitoyltransferase has a lower activity than the subsequent enzymes of ceramide biosynthesis and catalyzes the rate-determining step of this metabolic pathway. It preferentially uses fatty acid coenzyme A esters of a chain length of 16 C atoms so that a long-chain base with a C18 chain is formed. Serine palmitoyltransferase is the only enzyme of ceramide biosynthesis for which sequence

data are available from yeast[48, 49] and humans.[50] In the subsequent NADPH-dependent reaction, 3-ketosphinganine is reduced to D-*erythro*-sphinganine by 3-ketosphinganine reductase.[46] Finally, sphinganine *N*-acyltransferase acylates sphinganine to dihydroceramide.[51, 52] The enzyme[53] exhibits a selectivity towards coenzyme A activated stearic acid and is also able to acylate sphingosine formed in the salvage pathway of sphingolipid degradation.[54]

There are also reports of an enzyme activity that acetylates sphingosine after stimulation by platelet activation factor.[55] Dihydroceramide is dehydrogenated to ceramide by dihydroceramide desaturase.[56–58] The sequence of acylation and the introduction of the double bond was disputed for a considerable time, but it is now accepted that dihydroceramide is desaturated, not sphinganine. Sphingosine, the parent compound of the sphingolipids, is thus not an intermediate in sphingolipid biosynthesis, but is formed during sphingolipid degradation. The biosynthesis of ceramides containing phytosphingosine is unknown; however, the gene that is responsible for the biosynthesis of these compounds in yeast has been characterized.[59]

Ceramide is the common precursor of GSLs and sphingomyelin. In the GSLs of vertebrates a glucose or galactose residue is linked β glycosidically in the 1-position of ceramide. Glycosyltransferases, which catalyze the formation of these compounds, transfer individual nucleotide-activated sugars to ceramide. The galactosylation of ceramide,[60] which leads to the formation of glycolipids of the gala series, occurs mainly in the oligodendrocytes of the brain and in the kidney. Galactosylceramide (GalCer) and sulfatide (GalCer-3-sulfate) are present in high concentrations in the multilamellar layers of the myelin that surrounds the axons of neuronal cells.[61] Knockout mice, in which the gene of ceramide galactosyltransferase was inactivated and which consequently cannot form any galactosylceramide or sulfatide, are still able, however, to construct myelin of essentially normal structure. In place of the absent GalCer they incorporate a GlcCer hydroxylated in the fatty acid moiety into their myelin. However, the stability and function of these myelin layers are considerably impaired.[62, 63] The reaction of the 3′-OH group in the galactose residue of GalCer with sulfate activated as PAPS (3′phosphoadenosine-5′-phosphosulfate) yields sulfatide.[64]

The biosynthesis of most vertebrate GSLs does not require galactosylation, but the glucosylation of ceramide. GlcCer synthase has been isolated recently from rat liver,[65] and the cDNA of the human protein has been cloned.[66] It transfers a glucose residue from UDP-glucose to ceramide. The fatty acids that are found in GlcCer and GalCer differ in length and degree of hydroxylation, which can be attributed to the different cell types in which these structures are formed.[9] Although GlcCer and GalCer synthase catalyze similar reactions, there is no sequence homology between the cDNAs that encode for these enzymes.[67] Knockout mice, in which GlcCer synthase is absent and thus also the majority of complex GSLs, are unable to survive and die during embrogenesis.[27] This finding demonstrates the vital functions of glycolipids in morphogenesis and embryogenesis. Lactosylceramide, the common precursor of five GSL series that are found in vertebrates, is formed by the action of galactosyltransferase I, which transfers a galactose residue from UDP-galactose to glucosylceramide. The enzyme was recently purified from rat brain and cloned.[68]

Ceramide is also the precursor of sphingomyelin, a structural component of the plasma membrane. It is a 1-ceramide phosphorylcholine that is located predominantly in the plasma membrane where it occurs mainly in the externally orientated layer. The sphingomyelin content of plasma membranes depends upon the cell type and can reach 25 mol %. Sphingomyelin differs functionally from glycerolipids such as phosphatidylcholine by a higher melting temperature. The reason for this lies in the higher proportion of saturated fatty acid chains and the existence of intermolecular hydrogen bonds between the 3-OH group and the amide N atom. Sphingomyelin is formed by transfer of the phosphorylcholine headgroup from phosphatidylcholine to ceramide during which diacylglycerol, an activator of protein kinase C, is released and secretory vesicles in the Golgi apparatus are formed.[69] The mode of formation of sphingomyelin suggests a tight coupling between sphingolipid and glycerolipid metabolism. Indeed, an inverse correlation between the amounts of sphingomyelin and phosphatidylcholine is observed in many biological membranes.

3.2. Topology

Some experimental findings on the topology of sphingolipid biosynthesis are in part contradictory. The presumed most important transport pathways are described here. Thus, the first four steps of sphingolipid biosynthesis, which lead to the formation of ceramide, are catalyzed by membrane-bound enzymes at the cytosolic face of the endoplasmic reticulum (ER).[70, 71] Since the formation of glucosylceramide occurs on the cytosolic face of the Golgi apparatus or a pre-Golgi compartment,[72, 73] ceramide, or at least in part dihydroceramide too, must be transported from the ER to the Golgi apparatus. This occurs either by vesicle flow or by a protein-mediated transport process. In CHO cells (CHO = Chinese hamster ovary cells), a direct transport pathway appears to exist so that glucosylceramide can reach the plasma membrane from the cytosolic face of the Golgi apparatus.[74] Introduction of the next sugar residue that leads to the formation of lactosylceramide occurs on the luminal site of the Golgi apparatus.[75] This implicates a membrane translocation of glucosylceramide, which could be mediated by a protein, an as yet uncharacterized "flippase".[76] The biosynthesis of higher gangliosides then occurs on the luminal face of the Golgi apparatus.[77] Consequently, the glycan chains on the membrane-bound GSLs are orientated extracytosolically. This orientation is topologically equivalent to the situation in the plasma membrane, where the carbohydrate residues of complex glycolipids project into the extracellular space.

The bulk of sphingomyelin is formed on the luminal face of the Golgi compartment,[78] although other sites of synthesis have been described.[79] This requires an additional membrane translocation step at the ceramide stage. The formation of galactosylceramide with hydroxylated fatty acids in the side

chain appears to occur on the luminal face of the ER membrane, whereas non-hydroxylated GalCer species are formed on the cytosolic face of the cis-Golgi.[80] The biosynthesis of sulfatide[81] and digalactosylceramide[80] takes place in the lumen of the Golgi apparatus.

3.3. Biosynthesis of Complex Gangliosides

Gangliosides are acid glycolipids of the ganglio or lacto series[9] that contain one or more sialic acid residues. Gangliosides of the ganglio series are particularly prevalent on the surfaces of cells of the nervous system.[82] Their biosynthesis is described briefly here. In both gangliosides and glycoproteins, sialic acids are only found in α-glycosidic linkage. On the other hand, most other sugars can be present as glycoconjugates in both the α and the β configuration. In a hypothesis we have correlated this observation with the specificity of sialyltransferases that use nucleoside monophosphate activated sugars, for example CMP-NeuAc as glycosyl donor, instead of the nucleoside diphosphate activated sugars used by other enzymes.[83]

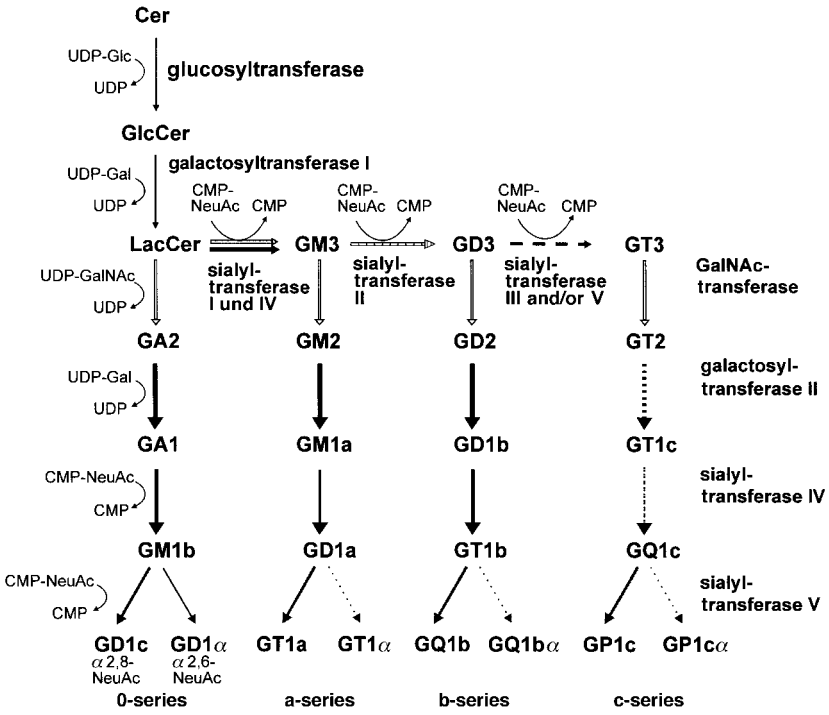


Figure 9. Biosynthetic scheme of complex gangliosides[44] with consideration of reference [439]. The reaction steps are catalyzed by membrane-bound glycosyltransferases in the lumen of the Golgi apparatus.

With the exception of GM4, gangliosides are derived structurally and biosynthetically from lactosylceramide. Further sugar residues, including sialic acid, are transferred stepwise to the growing glycan chains in the Golgi apparatus by membrane bound glycosyltransferases. Lactosylceramide and its sialylated derivatives GM3, GD3, and GT3 serve as precursors for complex gangliosides of the 0, a, b, and c series (Figure 9). Gangliosides from the c series are found only in trace amounts in human tissues. The stepwise glycosylation of these precursors is surprisingly performed by only a few nonspecific glycosyl transferases, which, as we have been able to demonstrate in enzyme kinetic experiments, transfer the respective sugar to glycosyl acceptors that differ only in the number of sialic acids bound to the inner galactose.[84–86] Experiments in vivo have demonstrated that the early sialyltransferases I and II are far more specific for their glycolipid substrates than the late sialyltransferases IV and V as well as Gal-II and GalNAc transferase. This concept is supported by the observation that the cDNAs for GD3 and GT3 synthase are identical and that both GD3 and GT3 can be biosynthesized by the same enzyme in humans.[87] The distribution of these glycosyltransferases within the cisternae of the Golgi apparatus has been investigated with the help of inhibitors of vesicular membrane flow. Monensin, a cationic ionophore, inhibits membrane flow between proximal and distal Golgi cisterna and leads to an increased biosynthetic galactose labeling of GlcCer, LacCer, GM3, GD3, and GM2, whilst the labeling of complex gangliosides is reduced. The addition of brefeldin A, which causes a fusion of the ER mainly with parts of the cis and medial Golgi, leads to a reduced labeling of the gangliosides GM1a, GD1a, GD1b, GT1b, and GQ1b as well as to a lesser extent of sphingomyelin.[88]

Although glycosyltransferase activity has been detected in many Golgi subcompartments,[89] current data suggest that the precursor molecules GM3 and GD3 are formed in early Golgi compartments, and the complex gangliosides predominantly in the trans Golgi network.[90] In addition to de novo biosynthesis, GSL can also be formed in salvage processes in which monosaccharides and especially sphingosine from GSL degradation are utilized.[91, 92]

3.4. Regulation

Maintenance of stable GSL patterns on individual cell surfaces requires a precise control of GSL biosynthesis, degradation, and intracellular transport. Since not only regulated sphingomyelin degradation but also increased ceramide biosynthesis can be held responsible for cellular responses,[93, 94] regulation of this metabolic pathway is of particular importance. Regulation of GSL metabolism and transport is not well understood, and there are only a few leads. Serine palmitoyltransferase appears to be the first control point in sphingolipid formation. The enzyme activity correlates with the relative amounts of sphingolipids that are found in different tissues.[95] Since sphingosine reduces SPT activity in cultured neuronal cells, autoregulation of the enzyme appears to be possible.[121] In addition, removal of skin lipids increases SPT activity.[96]

During ontogenesis and cell transformation a correlation between GSL expression and the activity of glycosyl trans-

ferases that leads to their synthesis has been observed. Therefore, control of glycosyltransferases, possibly at the transcription level, appears to be a significant regulation point (review: reference [44]). Since most glycosyltransferases have been cloned in the recent past,[97, 98] it is expected that information for an understanding of the control at the transcription level will be available in the near future. In addition to regulation at a genomic level, some observations also suggest epigenetic regulation mechanisms. Feedback control of several glycosyltransferases either by their respective reaction product or by an end product of the respective GSL series has been observed at least in vitro;[44] The phosphorylation status of glycosyl transferases and the pH value of their environment[99] can also affect their activity.

4. Inhibitors of Sphingolipid Biosynthesis

Low molecular weight inhibitors of sphingolipid biosynthesis[100] (Figure 10) have been isolated from natural sources

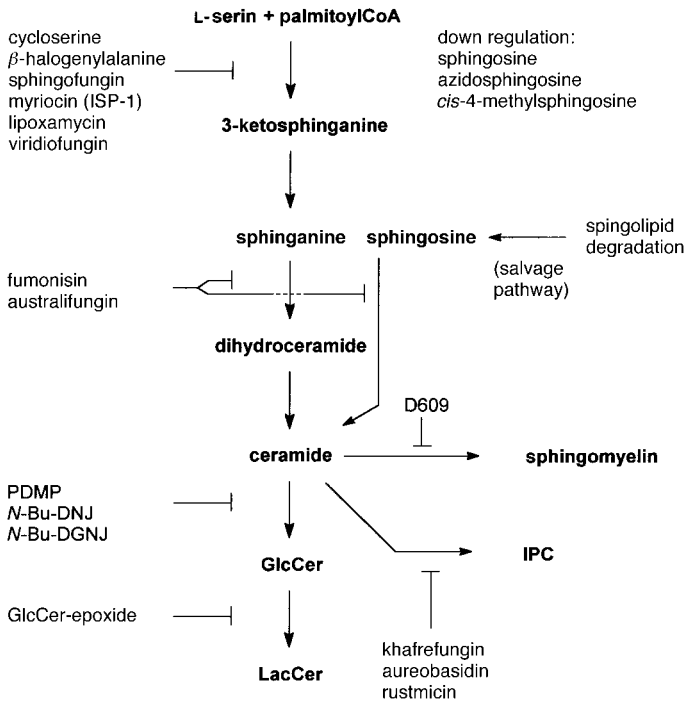


Figure 10. Flow scheme and inhibitors of sphingolipid biosynthesis (modified from reference [100]). IPC = inositphosphorylceramide; *N*-Bu-DNJ = *N*-butyldeoxyonojirimycin; *N*-Bu-DGNJ = *N*-butyldeoxygalactonojirimycin; PDMP = 1-phenyl-2-decanoylamino-3-morpholinopropan-1-ol.

or synthesized chemically. Most act at an early stage of biosynthesis and have a lipid-like structure. These inhibitors have a greater membrane permeability than those with a carbohydrate basis. Together with inhibitors of sphingolipid degradation (Section 5.2), they are valuable tools in the investigation of this metabolic pathway. For a number of reasons there is interest and a requirement for low molecular weight inhibitors that specifically inhibit a certain step in sphingolipid biosynthesis.[100] A possible application is the analysis of GSL function by switch-off experiments.[101] An interruption of sphingolipid biosynthesis at a certain stage can be achieved either with inhibitors, by the production of mutant cells, or with animals in which certain biosynthesis enzymes are absent.[102] The effect of this method is twofold: the cell or organism is depleted of metabolites downstream of the inhibited or mutated enzyme. Additionally, metabolic intermediates upstream of the blocked stage accumulate, which allows investigations of their biological function. Compounds such as myriocin or 1-phenyl-2-decanoylamino-3-morpholinylpropan-1-ol (PDMP) were also of interest as tools in the investigation of the cell cycle.[103]

Membrane permeating inhibitors of sphingolipid biosynthesis are interesting as therapeutic agents.[100] Myriocin, an inhibitor of serine palmitoyltransferase, is currently one of the most potent immunosuppressants.[42] Since carbohydrate structures of glycolipids form binding sites for bacteria, viruses, and toxins (Section 2), it is in principle possible to prevent diseases caused by these pathogens by inhibition of their synthesis. An alternative is inhibition of the biosynthesis of sialic acid, a frequent component of their recognition sites. Metastatic spread of cancer could also be favorably influenced in this way.[104] In addition to the inhibition of sphingolipid biosynthesis of the host, there is also the possibility of inhibiting that of parasites in so far as they require sphingolipids for survival or reproduction. This indication was proposed as a new principle for the treatment of fungal infections[100, 105] and malaria.[106, 107] The inhibition of biosynthesis is also of particular significance for the treatment of sphingolipidoses (Section 6.14.3), as postulated[108] by N. S. Radin and demonstrated experimentally by F. Platt[431] (Section 6.14.3). With the help of suitable inhibitors, the function of lateral aggregates of sphingolipids in cellular membranes could also be analyzed. They lead to the formation microdomains,[109] which are held responsible interalia for the transport of GPI-anchored proteins (GPI = glycosylphosphatidylinositol).[110]

4.1. Inhibitors of Serine Palmitoyltransferase

SPT is inhibited by suicide inhibitors of PLP-dependent enzymes (Figure 11) that are directed against the serine binding site. These include L-cycloserine,[111, 112] as well as β-chloro- and β-fluoroalanine.[113] Particularly high SPT activity has been found in human keratinocytes that can be inhibited by L-cycloserine and β-chloroalanine with IC_{50} values of 3.0 μM and 25 μM.[114]

Compounds with a structural similarity to sphingolipids, the sphingofungins, have been isolated from a culture of *Aspergillus fumigatus* as compounds with fungicidal but not antibacterial activity. Sphingofungin B reduced the incorporation of [^{3}H]inositol into the sphingolipids of yeast (IC_{50} = 8 nM).[115] This leads to growth inhibition and cell death. The action of sphingofungins can be abolished by the addition of phytosphingosine, but not by sphingosine. This is not unexpected, since the sphingolipids of yeast contain phytosphingosine in place of sphingosine as the most common long chain base. Sphingofungins inhibit SPT competitively with respect

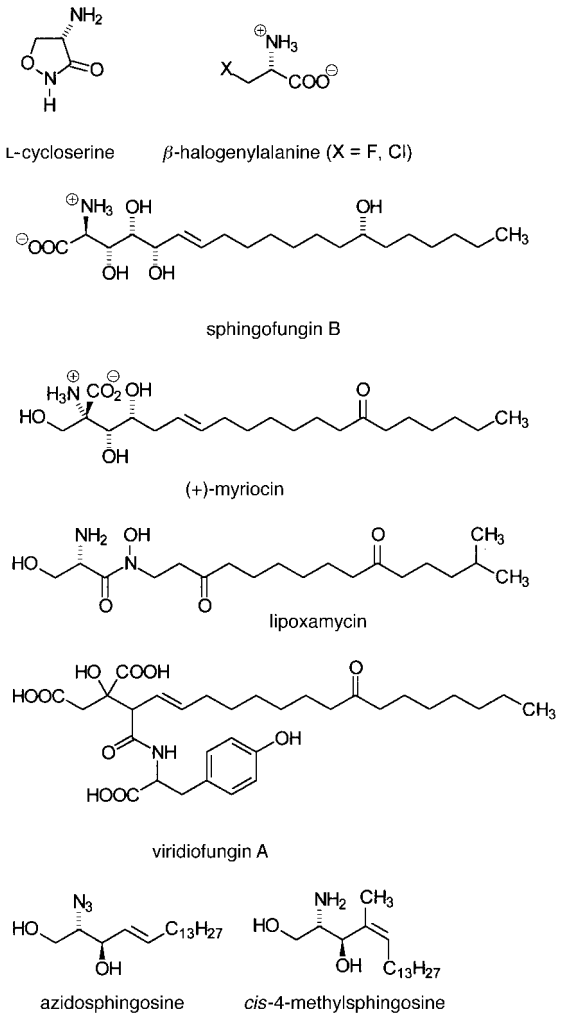


Figure 11. Inhibitors of serine palmitoyltransferase (SPT).

to serine.[115] Sphingofungins E and F have been isolated from *Paecilomyces variotii*.[116]

A further structural analogue of the sphingoid backbone is myriocin. It inhibits the biosynthesis of ceramide and the two most common GSLs in yeast, inositolphosphorylceramide (IPC) and mannosylIPC (MIPC).[117] At the same time it reduces the transport rate of GPI-anchored proteins to the Golgi apparatus and the remodeling reaction of the GPI anchor to ceramide-containing structures. The SPT inhibitor myriocin is an extremely potent immunosuppressant; it has been shown to be identical to the antibiotic ISP-1 and thermozymocidin. Unlike the frequently used immunosuppressants cyclosporin and FK-506, ISP-1 does not interfere with the formation of interleukin-2 but suppresses the IL-2 dependent growth of a cytotoxic T-lymphocyte cell line of the mouse, CTLL-2. The SPT of these cells is inhibited noncompetitively in vitro with an apparent inhibition constant of 0.28 nM.[42] The inhibition of SPT is accompanied by a suppression of T cell growth, which can be abolished again by the addition of C2 ceramide, sphinganine, or sphingosine-1-phosphate, but not, however, by sphingomyelin, GlcCer, GalCer, or GM3. It is assumed that myriocin induces apoptosis in the CTTL-2 cells.[118]

A secondary metabolite from actinomycetes, lipoxamycin, has also been described as a potent inhibitor of SPT (IC_{50} = 21 nM).[119] Noteworthy are its high toxicity towards mice and its fungicidal activity, which can be abolished by the administration of sphinganine and phytosphingosine. In addition, the antifungal agent viridiofungin, previously recognized as an inhibitor of squalene synthase in vitro, has also been proved to be an inhibitor of SPT.[120]

Other agents reduce SPT activity in cultured cells without directly inhibiting the enzyme. These include D-*erythro*-sphingosine and the metabolically stable D-*erythro*-azidosphingosine, which reduces SPT activity in primary cultured neuronal cells either with the natural *trans* or the unnatural *cis* double bond.[121] The synthetic 4-methyl derivative of *cis*-sphingosine down regulates SPT activity (IC_{50} = 10 μM), increases intracellular calcium ion concentration, and behaves as a potent mitogen in Swiss 3T3 fibroblasts. Moreover, the compound causes drastic morphological changes in cultured granule cells of the cerebellum and induces neuronal death. Both the *cis* configured double bond and the 4-methyl group are necessary for the action of the compound, since the corresponding *trans* and 5-methyl derivatives are inactive. All data suggest that a metabolite of *cis*-4-methylsphingosine is the actual active compound, namely its 1-phosphate. Sphingosine-1-phosphate is rapidly degraded by a lyase (Section 5.2); *cis*-4-methylsphingosine appears to be a prodrug for a metabolically stable analogue of sphingosine-1-phosphate.[122]

4.2. Inhibitors of Sphinganine *N*-acyltransferase

Fusarium moniliforme is a fungus that frequently infects corn and cereals. Consumption of such contaminated agricultural products leads to various, often neurological, diseases in animals and has been implicated in the occurrence of esophageal cancer in humans.[123–125] Fumonisins, mycotoxins from *F. moniliforme*, have been isolated as the active principle (Figure 12). Fumonisins B1 and B2 (which lack the 10-hydroxyl group of FB1) have been identified as inhibitors of sphinganine *N*-acyltransferase with IC_{50} values of about 0.1 μM.[126, 127] Fumonsinins are structural analogues of the sphingoid bases, which lack the 1-hydroxyl group. This contributes to the metabolic stability of these mycotoxins, since long chain bases can be cleaved after phosphorylation of the 1-position. The tricarballyl residue and the 5-OH group are important but not decisive for inhibition. Fumonisin B3, which lacks the 5-OH group, is still active, but only in concentrations above 1 μM. A similar value also applies to fumonisin B1H, which is obtained by cleavage of the tricarballyl group by treatment with mild base. Different fumonisin analogues have been synthesized and shown to be substrates and inhibitors of the acyltransferase (IC_{50} about 10 μM). The *N*-palmitoyl derivative of such an aminopentol inhibits acyltransferase (IC_{50} = 9 μM) and is cytotoxic. Unlike other 1-deoxysphinganine derivatives, fumonisin B1 is not acylated by acyltransferase in vitro.[128] Inhibition of ceramide formation by fumonisins is accompanied by an accumulation of the biosynthetic precursor sphinganine. Sphinganine may be responsible for some of the effects caused by the

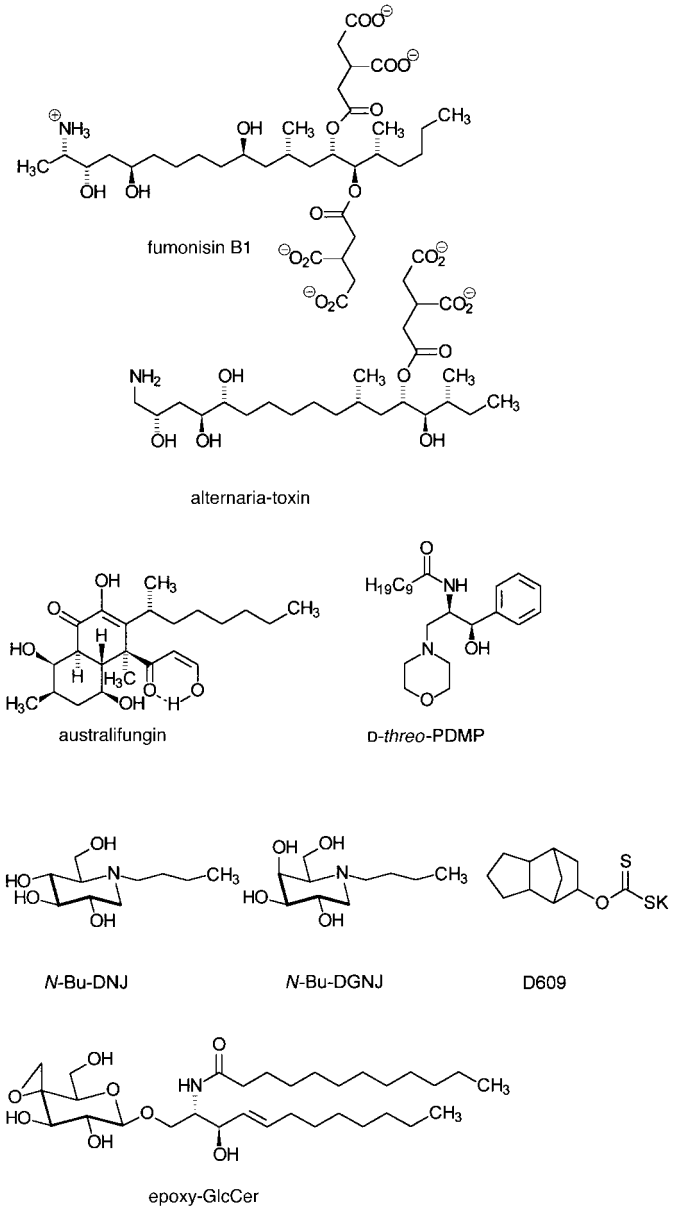


Figure 12. Further inhibitors of GSL biosynthesis. The absolute configuration of the tricarballyl residue in fumonisin B1 and alternaria toxin is not shown.

fumonisins, since it is toxic and mitogenic even in low concentrations. The ratio of sphinganine to sphingosine in the serum of animals is effective as a diagnostic indicator for the consumption of fumonisin-contaminated feed.

Alternaria toxin is a phytotoxin with a structure similar to the sphingoid backbone. It inhibits sphingolipid biosynthesis at the level of dihydroceramide formation ($IC_{50} = 1\,\mu M$), but as a result of its lower activity relative to fumonisin and its increased cytotoxicity it is of limited use for mammalian cell culture experiments.[129]

The fungal metabolite australifungin has been described as an inhibitor of sphinganine *N*-acyltransferase in vitro. Its IC_{50} is still less or equal to that of fumonisin B1, according to the cell type used as enzyme source.[130] Australifungin is a potent fungicide but shows no structural similarity to sphingoid bases.

4.3. Inhibitors of Glycosyltransferases

The introduction of carbohydrate structures into GSLs follows the formation of the membrane anchor. Unlike for most other glycosyltransferases,[131] the early glycosylation steps on ceramide belong to the few cases for which inhibitors are available. Inhibition of ceramide biosynthesis itself is unsuitable for the analysis of GSL functions since it has several consequences: a depletion of GSLs in the plasma membrane, a reduction in sphingomyelin biosynthesis, and an increase in the concentration of the metabolites upstream of the inhibited step. Potent and specific inhibitors of GlcCer biosynthesis are suitable for functional analysis. Two classes of compounds have currently been described as inhibitors of GlcCer biosynthesis.[132] D-*threo*-PDMP (D-*threo*-(1*R*,2*R*)-1-phenyl-2-decanoylamino-3-morpholino-1-propanol, Figure 12) is the most thoroughly studied representative of a series of GlcCer synthase inhibitors with a ceramide-like structure. D-*threo*-PDMP inhibits the formation of glycosylceramide in concentrations of 2.5 to 10 μM and it has already been used in functional studies.[133]

D-*threo*-PDMP exhibits a mixed inhibitor profile relative to ceramide and is noncompetitive for the glycosyl donor. The apparent K_i value is 0.7 μM. At concentrations above 25 μM the biosynthesis of sphingomyelin and the transport of secretory proteins are also inhibited.[134] Stereoisomers and analogues of PDMP have been synthesized and investigated, including D-*threo*-1-morpholino-1-deoxyceramide (73% inhibition of GlcCer synthase in Madin–Darby canine kidney (MDCK) cells at a concentration of 5 μM compared to 20% inhibition by D-*threo*-PDMP).[135] Concentrations of D-*threo*-PDMP above 100 μM or of its palmitoyl derivative PPMP above 10 μM are toxic to HL-60 cells. D-*threo*-PDMP exhibits a plethora of cellular effects, including inhibition of cell growth, which is possibly caused by ceramide accumulation and/or inhibition of sphingomyelin biosynthesis. The interruption of the cell cycle in the G1/S and G2/M phases by PDMP also appears to be attributable to cellular ceramide accumulation.[136] Various other effects of PDMP, its stereoisomers, and analogues have been reviewed.[133] It is metabolized by cytochrom P450.[137] A further effect of PDMP, which is not attributable to modulation of sphingolipid metabolism, concerns membrane transport from the Golgi apparatus to the ER induced by brefeldin A, which is blocked by PDMP.[138]

A synthetic derivative of the naturally occurring glycosidase inhibitor deoxynojirimycin, *N*-butyldeoxynojirimycin (*N*-Bu-DNJ), inhibits GlcCer formation with an IC_{50} value of 20 μM. *N*-Bu-DNJ is known to be an inhibitor of HIV replication in vitro, and clearly acts as an inhibitor of viral glycoprotein processing. Butyldeoxygalactonojirimycin (*N*-Bu-DGNJ) is a related compound with improved selectivity ($IC_{50} = 40\,\mu M$).[139] Glycosidases such as β-gluco- and β-galactocerebrosidases, and α-glucosidases I and II are not, or only poorly, inhibited by the compound. Structure–activity relationships show that an alkyl chain length of three C atoms is necessary for inhibition of GlcCer synthase, and alkyl chain lengths of C4 and C6 are optimal. Longer chain compounds do indeed show a further improvement in inhibition in vitro, but have cytotoxic properties in vivo. The corresponding

derivatives of mannose, fucose, and *N*-acetylglucosamine are inactive. A great advantage of these compounds is their low toxicity and high metabolic stability. An application of *N*-butyldesoxynojirimycin in the treatment of sphingolipid storage disease will be discussed in relation to newer therapeutic uses in Section 6.14.3.

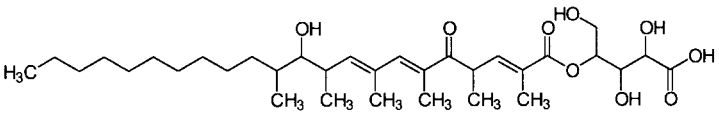
4.4. Inhibition of LacCer Synthesis

A synthetic GlcCer derivative with an additional epoxide function in the 4-position of the glucose residue causes an irreversible and concentration-dependent reduction in LacCer synthase activity in cultured cells.[140] The biosynthetic labeling of GSLs downstream from GlcCer is reduced in primary cultured neuronal cells from chick embryos, whereas the labeling in GlcCer accumulates. The derivative with gluco configuration is active, whereas the compound with the galacto configuration shows no effect. The inhibition of LacCer formation by the epoxy compound in vitro is less pronounced: a concentration of 250 μM is necessary to achieve a 30% inhibition in enzyme activity. Therefore, it cannot be excluded that the effects observed in vivo may be attributed to the inhibition of a GlcCer translocator or transcription factor.

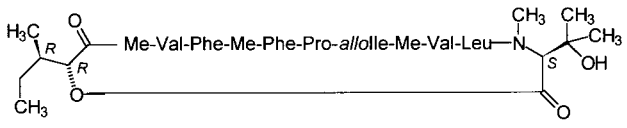
4.5. Inhibitors of Inositolphosphorylceramide (IPC) Synthase

Whereas in vertebrates a majority of sphingolipids are present in the form of GSL, in fungi compounds the binding of an inositol residue to ceramide by diphosphate diesters predominate. Inhibition of the biosynthesis of these compounds leads to a depletion in the organisms of structures that clearly fulfill essential functions for their survival.[100, 145] It is possible that in fungi sphingolipids represent the only storage form of very long chain fatty acids that are necessary for the survival of these organisms.[141] Ceramides accumulate through inhibition of IPC synthase, which at least in yeast leads to arrest of the cell cycle in the GI phase.[142] Thus, for example the fungal component khafrefungin (Figure 13) inhibits the biosynthesis of IPC in *Saccharomyces cerevisiae* and pathogenic fungi in picomolar and nanomolar concentrations and causes ceramide accumulation.[143] In vitro IPC synthase is inhibited with an IC_{50} value of 0.6 nM, and the incorporation of inositol into the sphingolipids with an IC_{50} value of 150 nM. Unlike other inhibitors that inhibit the corresponding enzyme in fungi and mammals to the same extent, sphingolipid biosynthesis in mammals is not impaired by khafrefungin.

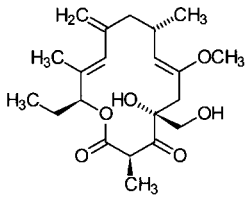
The antifungal agent aureobasidin A also inhibits the biosynthesis of IPC; its IC_{50} value is 0.2 nM.[105] Aureobasidin in micromolar concentrations also inhibits the transferase, which transfers phosphorylinositol to mannosylinositolphosphorylceramide.[144] Another, more recently described inhibitor of IPC synthase is the macrolide rustimicin,[145] also known as galbonolide A, which is also a potent fungicide. It inhibits the enzyme with an IC_{50} value of 70 pM. The suitability of the compound for the treatment of systemic fungal infections is limited by its metabolic and chemical instability as well as by resistance phenomena.[145]



khafrefungin



aureobasidin



rustmicin

Figure 13. Inhibitors of inositolphosphorylceramide formation.

4.6. Miscellaneous

D,L-α-fluoropalmitic acid also inhibits the biosynthesis of sphingolipids.[146] A recent study has shown[147] that azidothymidine (AZT), which is used as a chemotherapeutic agent in the treatment of HIV infections, inhibits the glycosylation of glycolipids and glycoproteins in clinically relevant concentrations of 1–5 μM. The primary cellular metabolite of AZT, the monophosphate, possibly inhibits the uptake of nucleotide sugars into the Golgi apparatus and in this way reduces the amount of complex acid GSLs. The toxic side effects of AZT, particularly in the maturation of blood cells, appear to be a consequence of changed glycosylation patterns and are not based on an inhibition of DNA replication.

Antisense oligodeoxynucleotides for GM2 synthase and GD3 synthase lead to a reduction of complex GSLs downstream from GM3 within the biosynthetic pathway. The treatment of human leukemia cell line HL-60 with antisense DNA results in their differentiation into monocytes and the accumulation of GM3.[148] Since suitable low molecular weight inhibitors of glycosyltransferase are currently unavailable, this approach represents a highly promising tool for the clarification of GSL function.

Sphingomyelin synthase is inhibited in vitro by a xanthogenate, D609 (Figure 12).[149] The compound has been known for some time as an inhibitor of a phosphatidylcholine-specific phospholipase C activity.[150] Concentrations in the range 40–400 μM also inhibit sphingomyelin formation in transformed fibroblasts.[149]

5. Sphingolipid Catabolism

5.1. Nonlysosomal Degradation: Sphingolipids and Signal Transduction

Constitutive sphingolipid degradation takes place in the lysosomes of the cell. An additional, highly regulated degradation pathway starting from sphingomyelin also exists, during the progression of which signal molecules are released (Figure 14).[8] Sphingomyelin can be cleaved by sphingomyelinases[151, 152] and an acid sphingomyelinase (Section 6.6) and

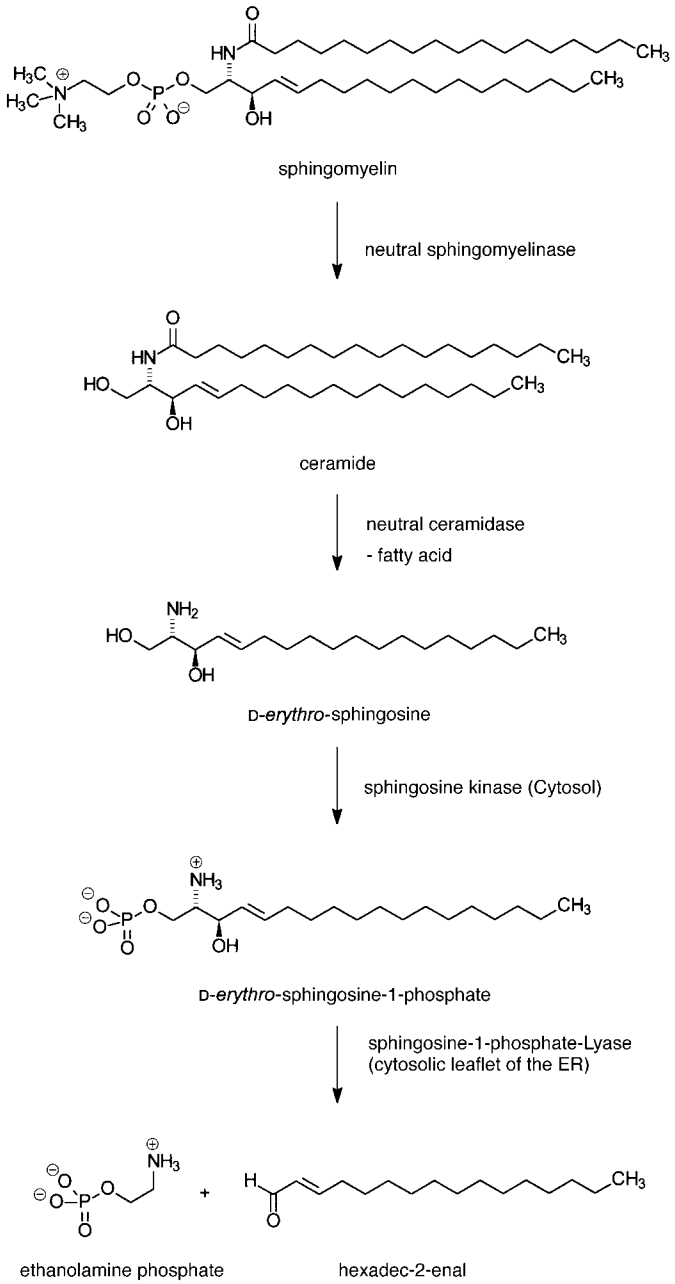


Figure 14. Nonlysosomal sphingolipid degradation.

a neutral sphingomyelinase have been cloned.[153] Ceramide is formed during the sphingomyelinase-catalyzed reaction and can be cleaved into sphingosine and a long-chain fatty acid by ceramidases of varyious subcellular localization (Section 6.10).[154] Phosphorylation to ceramide-1-phosphate is also a possibility.[155] Sphingosine itself can be phosphorylated at the 1-OH function by a cytosolic sphingosine kinase[156, 157] to form sphingosine-1-phosphate, which, like the 1-phosphates of D-*erythro*-sphinganine and D-*ribo*-phytosphingosine, is cleaved in a pyridoxalphosphate-dependent reaction by the sphingosine-1-phosphate lyase situated on the cytosolic face of the ER membrane.[158] The mechanism of the cleavage corresponds formally to a retroaldol addition. The gene of the lyase has been characterized from yeast and mouse amongst other sources.[159, 160] In addition to the degradation of sphingoid-1-phosphates, the phosphatase reaction back to sphinosine has also been characterized.[161, 162] A phosphatidic acid phosphorylase catalyzes the hydrolysis of sphingosine-1-phosphate, ceramide-1-phosphate, and lysophosphatidic acid.[163]

A few findings suggest that ceramide plays a role comparable to that of the structurally similar and functionally complementary diacylglycerol (DAG). Together with inositol-1,4,5-trisphosphate, DAG is released from phosphaditylinositol-4,5-bisphosphate as a signal molecule by phospholipase C in response to an extracellular signal.[164] The observation that sphingomyelin hydrolysis can also be induced by extracellular agents in various cell types such as lymphocytes, myelocytes, or fibroblasts has led to the discovery of the so-called sphingomyelin cycle (Figure 15).[165, 166] The first indication of this came from the observation that vitamin D3 induces the cleavage of sphingomyelin in HL-60 cells.[167] Tumor necrosis factor α, γ-interferon, or interleukin-1, which act on receptors within the plasma membrane, also lead to the release of ceramide. The cellular and molecular effects of these extracellular agents, inhibition of cell growth, induction of differentiation, modulation of protein phosphorylation, and regulation of gene transcription, can be mimicked by the addition of an exogenic membrane-permeable ceramide derivative. The actions of this C_2-ceramide (*N*-acetylsphingosine) are not observed with the corresponding saturated compound, C_2-dihydroceramide.[168] This result suggests a specific interaction between ceramide and a binding protein. The identity of the ceramide receptors and other molecules downstream within the signal flow is not unambiguously known. A ceramide-dependent kinase,[169] a phosphatase,[170] and a protein kinase C subtype[171] are currently being investigated. Protein kinase c-Raf has been identified by photoaffinity labeling as a ceramide binding protein within this signaling pathway (see Section 5.1.1).

In general, ceramide mediates antimitogenic effects such as cell differentiation, cell-cycle arrest, and cell senescence. The most spectacular of the various effects that ceramide can initiate is programmed cell death.[172] Unlike necrosis, programmed cell death (or apoptosis) is a well-defined process whose biochemical principles have only now been partially elucidated.[173–175] It is controlled by receptor-mediated mechanisms, which themselves activate intracellular signal cascades and thus influence the phosphorylation status of target proteins and finally gene expression. In the strictest sense ceramide is not a second messenger whose action is terminated within a short time. Instead, it mediates a long lasting response to stress.[20] In yeast too, sphingolipids appear

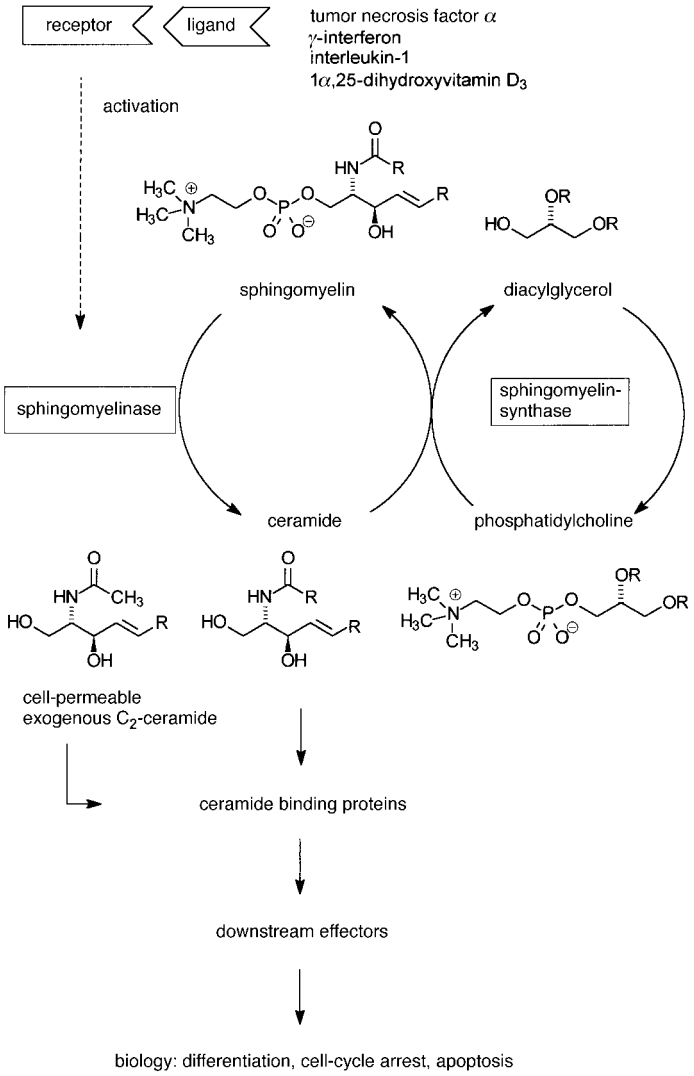


Figure 15. Sphingomyelin cycle (modified according to reference [165]). R = alkyl group.

to act as stress signals.[176] The initiation of apoptosis by ceramide can be inhibited by the overexpression of the apoptosis inhibitor Bcl-2.[177] The release of ceramide itself can also be suppressed by Bcl-2.[178] Surprisingly, the presence of ganglioside GD3 appears equally necessary for the initiation of apoptosis by ceramide in hematogenic cells[179] as the presence of acid sphingomyelinase.[180] The evaluation of many published experiments remains difficult to interpret, however, since intracellular ceramide concentrations are usually determined by the diacylglycerolkinase reaction. However, this test is not specific enough. It showed, for example, an increase in ceramide concentration in experiments on FAS-induced apoptosis of T cells, although an independent mass spectrometric method detected no change.[181]

The induction of apoptosis in U937 cells by ceramide is stereospecific: the D- and L-*threo* stereoisomers have proved to be more potent than the *erythro* compounds. In addition, experiments with *N*-octylsphingosine have shown that the amide carbonyl group is not necessary for the induction of apoptosis.[182] The *N*-acetylsphingosine (C2-ceramide) frequently used in experiments of this type is subject to metabolism;[183] it can by acylated on the 1-OH group by an enzyme with transacylase or phospholipase A2 activity.[184, 185] Ceramide can also be sulfated at the 1-position in marine organisms. This metabolic product inhibits human DNA topoisomerase I.[186]

In addition to the degradation pathway discussed previously, ceramide can be converted into bioactive metabolic products, in particular sphingosine and sphingosine-1-phosphate.[187] Unlike ceramide, sphingosine-1-phosphate mediates mitogenic effects.[188] It induces proliferation of quiescent Swiss 3T3 cells and stimulates the release of calcium ions from intracellular sources. Since ceramide, sphingosine, and sphingosine-1-phosphate are metabolically coupled, it is not clear in every case which of these molecules is responsible for a certain effect and why this pathway is mitogenic in some cells and antiproliferative in others. Unlike the unknown identity of the ceramide receptors, a binding protein (K_D = 8.1 nM) for extracellularly acting sphingosine-1-phosphate which mediates a part of its activity has been characterized on the cell surface.[189, 190] It is the Edg-1 receptor, which is coupled with an inhibitory G protein. Its expression in COS cells leads to inhibition of adenylatecyclase and to activation of mitogen-activated protein kinase (MAPK), but not to the release of calcium ions. Sphingosine and lysosphingolipids, which lack the amide-bound fatty acid, are known to be inhibitors of protein kinase C.[191] Sphingosine itself also appears to act apoptotically in some cells independent of ceramide.[192] In the case of sphingosine and ceramide, the mechanisms that lead to apoptosis are clearly different, although dependent upon the cell type they can also act cooperatively.[192]

5.1.1. Ligands of Sphingolipid Binding Protein

There is a requirement for metabolically stable ligands—agonists and antagonists—of sphingolipid binding proteins, for example of sphingosine-1-phosphate.[193] With the help of a ceramide analogue photoaffinity ligand (Figure 16) it has been possible for the first time to identify protein kinase c-Raf as a ceramide binding protein that is involved in the signal cascade of interleukin-1 binding for the activation of the mitogen-activated protein kinase (MAPK).[194] The photoaffinity ligand was radiolabeled with a specific radioactivity of >2000 Ci mmol^{-1}.[195] We have synthesized a 1-deoxy-1-ethyldihydroceramide as the first metabolically stable dihydroceramide with proapoptotic properties (Figure 16). The compound inhibits the proliferation of a human leukemic cell line with a IC_{50} value of 20 μM. It is nontoxic over an extended period and it is able to induce apoptosis in this cell line.[196]

5.2. Inhibition of Sphingolipid Degradation

The elucidation of GSL function on the cell surface is not the only motivation to influence sphingolipid metabolism. In addition to GSLs themselves,[197–199] lipophilic intermediates of GSL degradation are involved in processes of signal transduction when extracellular signals are transmitted to intra-

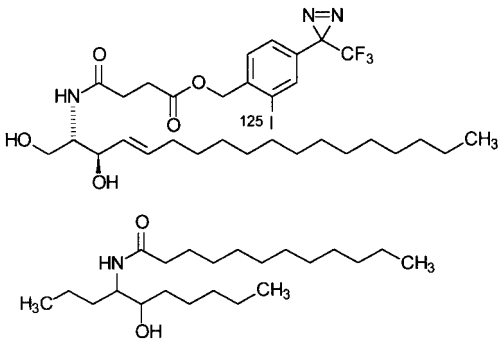


Figure 16. Ligands of ceramide binding protein ligands.

cellular regulatory systems (Section 5.1). A notable inhibitor of GSL degradation is conduritol B epoxide, which inhibits glucocerebrosidase (see Section 6.8).[200] Although it has been possible for a long time to investigate the effects of a dysfunctional sphingolipid degradation in cells of sphingolipidosis patients or genetically modified animals, a covalent inhibitor represents a valuable, independent tool for the analysis of the active site of the enzyme.

5.2.1. Inhibition of Sphingomyelinases

Inhibitors of the sphingomyelinases involved in signal transduction are interesting, for example, as potential therapeutic agents in the treatment of inflammatory and autoimmune diseases. Since the identity of the enzyme is unknown, acid and neutral sphingomyelinases are targets for inhibitory studies. The activity of acid sphingomyelinase is necessary, for example for the infection of nonphagocytizing cells by *Neisseriaq gonorrhoea*.[201] A few low molecular weight inhibitors of acid sphingomyelinase are known,[202] including phosphatidylinisotol-4′,5′-bisphosphate, adenosine-3′,5′-bisphosphate, and adenine-9-β-D-arabinofuranoside-5′-monophosphate ($IC_{50} = 1-5\,\mu M$). The calcium channel antagonist SR33557 (Figure 17) has also been described as an inhibitor of acid sphingomyelinase activity (72 % inhibition at 30 μM in a leukemia cell line of the mouse, P388/ADR).[203] Different sphingomyelin derivatives (3-deoxy-, 3-*O*-methyl-($IC_{50} = 50\,\mu M$), 3-*O*-ethyl-, 3-*O*-tetrahydropyranyl-, and *N*-demethylsphingomyelin) have been synthesized and investigated. They have been shown to be less active inhibitors of neutral and acidic sphingomyelinase.[204] The synthesis of a phosphonate analogue of sphingomyelin as an affinity ligand for the purification of sphingomyelinases has been described.[205] Ganglioside GM3 inhibits neutral sphingomyelinase with an IC_{50} value of 45 μM.[206] Glutathione is also an inhibitor of neutral sphingomyelinase (95 % inhibition at a concentration of 5 mm).[207] Scyphostatin, a constituent of *Dasyscyphus mollissimus*, has been described recently as an inhibitor of neutral ($IC_{50} = 1\,\mu M$) and acid sphingomyelinase ($IC_{50} = 49\,\mu M$).[208] An interesting lead structure is thus available for optimization.

5.2.2. Ceramidase Inhibitors

Since the signal molecules ceramide and sphingosine/sphingosine-1-phosphate are metabolically coupled by different enzymes, among them different ceramidases, ceramidase inhibitors are of interest as tools in the investigation of signal transduction mechanisms. *N*-oleoylethanolamine has been described as a ceramidase inhibitor,[209] and (1*S*,2*R*)-D-*erythro*-2-(*N*-myristoylamino)-1-phenyl-1-propanol (MAPP) is a potent inhibitor of alkaline ceramidase with an IC_{50} value in the lower micromolar range.[210]

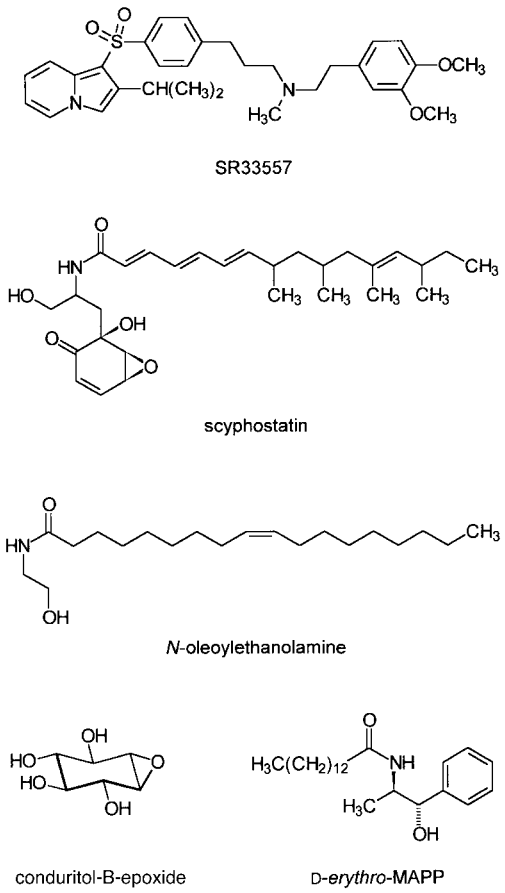


Figure 17. Inhibitors of sphingolipid degradation.

5.2.3. Inhibitors of Sphingosine Kinase

Sphingoids with a *threo* configuration are able to inhibit sphingosine kinase.[211] Since these compounds can be metabolized by acylation of the amino nitrogen atom and phosphorylation in the 1-position, they are only of limited use. *N*,*N*-dimethylsphingosine, an inhibitor of sphingosine kinase in blood platelets ($IC_{50} = 5\,\mu M$),[212] behaves differently. The compound can be phosphorylated in activated blood platelets,[213] and the resulting 1-phosphate is a functional agonist of the sphingosine-1-phosphate receptor.[214]

In addition to inhibitors of sphingolipid degradation, we have discovered a stimulator: 1-methylthiodihydroceramide at a concentration of 10 μM apparently reduces the de novo synthesis of ceramide in primary cultured neuronal cells by 90 %. Surprisingly, enzymes of ceramide biosynthesis are not affected by the compound; on the other hand, it stimulates sphingosinekinase by a factor of 2.5. The mechanism of the apparent biosynthesis inhibition is accordingly an accelerated degradation of biosynthetic intermediates such as sphinganine

and sphingosine. At the same time the compound interrupts the axonal growth of cultured hippocampus neurons in a way that is comparable to the direct inhibition of sphingolipid biosynthesis.[215] A further activator of sphingokinase is 1α,25-dihydroxyvitamine D3.[216]

5.2.4. Inhibitors of Sphingosine-1-phosphate Lyase

In order to be able to investigate the physiological functions of sphingosine-1-phosphate as a second messenger, metabolically stable analogues or inhibitors of sphingosine-1-phosphate lyase are required. Recently, *cis*-4-methylsphingosine has been identified as a prodrug of a metabolically stable sphingosine-1-phosphate analogue (Section 4.1). Stoffel and Grol[217] have prepared a deoxysphinganine-1-phosphonate, which is a competitive inhibitor of the lyase ($K_i = 5\,\mu M$, $K_M = 16\,\mu M$). This extremely toxic compound is actually cleaved by the lyase, but at a rate that is slower by a factor of 10 than the natural phosphate. A homologue, a compound which is extended in the headgroup by a single carbon atom, has been synthesized[218] and radiolabeled.[219] The synthesis of the corresponding phosphoramide[220] and the unsaturated phosphonate have also been described.[221] These compounds are potential ligands of the sphingosine-1-phosphate receptor. They possibly exhibit increased metabolic stability against cleavage by phosphatases. The synthesis of a radiolabeled 2-vinyldihydrosphingosine-1-phosphate, a potential irreversible inhibitor of the lyase, has also been described.[222]

5.3. Lysosomal Sphingolipid Degradation

Constitutive degradation of GSLs takes place in the acid compartments of the cell, the late endosomes, and, in particular, in lysosomes.[223, 224] The composition of the sphingolipids that enter the lysosomes from the cell surface is dependent upon the cell type. The neuronal plasma membrane, for example, is rich in gangliosides, whereas oligodendrocytes and Schwann cells, which form the myelin sheath around the axons of neuronal cells, are particularly rich in galactosylceramide and sulfatide. GSLs of the globo series such as globoside and globotriaosylceramide are found in different cell types of visceral organs. Finally, skin cells contain mainly ceramides and glucosylceramides with unusually long fatty acid residues. The individual sugar residues are sequentially cleaved from the nonreducing end of GSLs. Ceramide is finally formed from lower glycosylated sphingolipids, and is degraded to sphingosine and a long chain fatty acid. The building blocks thus released, namely monosaccharides, fatty acids, and sphingoid bases, are able to leave the lysosome. If because of a significant defect the function of one the proteins involved in degradation fails, nondegradable lipid substrates accumulate in the lysosome. Unlike the water soluble oligosaccharides of glycoproteins, these are poorly soluble amphiphiles that cannot be removed and form poorly soluble aggregates in the lysosomes.

5.4. Topology of Endocytosis

In order that the GSLs of the cell surface can undergo intracellular degradation they must be transported to the lysosomes. Building blocks and fragments of the plasma membrane can reach the lysosomes by endocytosis.[225] In this way, regions of the plasma membrane are budded to the intracellular vesicles as coated pits that can fuse with the early endosomes. Thus, components of the endosomal membrane are formed from previous regions of the plasma membrane. Consequently, the components of the plasma membrane reach the lysosomes as components of the lysosomal membrane by means of such a vesicular flow (Figure 18A). Finally,

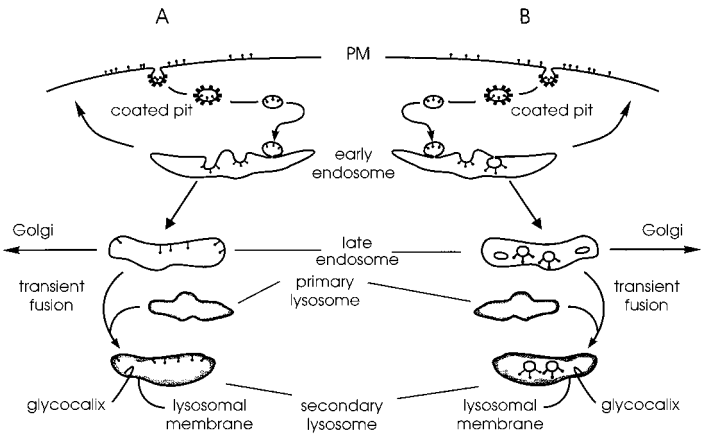


Figure 18. Models of endocytosis and lysosomal digestion of glycosphingolipids (GSLs) in the plasma membrane.[228] a) Conventional model: the degradation of the GSLs of plasma membrane occurs selectively within the lysosomal membrane. b) Our alternative model: plasma membrane GSLs are incorporated into the membrane of intraendosomal vesicles (multivesicular bodies) during endocytosis. The vesicles reach the lysosomal compartment when late endosomes are fused transiently with primary lysosomes and are degraded there. PM = plasma membrane: ● GSL.

the degradation of the lipids must occur selectively within the lysosomal membrane without impairing their integrity. There is no plausible conception how this can happen, particularly since the inner face of the lysosomal membrane is protected by a lining of carbohydrates that are composed predominantly of lactosamine structures. This glycocalix is formed from glycoproteins, the so-called "limps" (lysosomal integral membrane proteins) and "lamps" (lysosomal associated membrane proteins), which are components of the lysosomal membrane.[226]

We have proposed an alternative model (Figure 18B), according to which components of the plasma membrane can also reach the intracellular digestion apparatus as components of intra-endosomal or intra-lysosomal vesicles and membranes.[227] These could be formed by an invagination and budding off of certain regions of endosomal membrane that are particularly rich in previous components of the plasma membrane. By means of known fusion processes between the late endosomes and the lysosomes, the intra-endosomal vesicles thus formed could reach the lumen, that is the inner space of the lysosomes, where they are exposed to the digestive proteins. A number of observations support this mode of lysosomal membrane digestion.[228, 229]

5.5. Mechanisms of Lysosomal Digestion

Lysosomal degradation of individual GSLs is carried out by exohydrolases that cleave the sugar moieties from the non-reducing end of the glycoconjugates. GSL degradation thus begins at a phase frontier, presumably at the surface of intralysomal vesicles. These vesicles differ from the perimeter membrane by their curvature and their lipid composition, and consequently also by the lateral pressure operating at their surface. These factors can influence the enzymatic degradation of such structures quite considerably.[230, 231] The hydrolases that degrade the glycolipids are enzymes which are dissolved in lysosol. However, their substrates are lipids that are present in a membrane bound form. Analyses of inherited diseases of sphingolipid degradation in the laboratory of Horst Jatzkewitz and in our laboratory have shown that in the case of GSLs with short oligosaccharide chains, the lipids to be degraded are no longer accessible to the hydrolases for steric reasons. A two component system of lipid and hydrolase is then no longer sufficient for GSL degradation in vivo, but an activator protein is also required. The enzymatic degradation of the ganglioside GM2 is thus initiated by a lysosomal binding protein, the GM2-activator.[246] It acts as a weak surfactant that binds to the membrane-based ganglioside GM2 or structurally similar gangliosides to form stoichiometric, water-soluble complexes. On membrane or vesicle surfaces it clearly acts as a so-called "liftase", which recognizes, binds and releases the membrane-based ganglioside from the membrane face so that they can be transported as substrates to the water-soluble, degrading enzymes, such as hexosaminidase A (Figure 19). The activator can only penetrate those lipid bilayers with a lateral pressure less than 25 $m_N m^{-1}$.[446] Further factors that can affect glycolipid degradation in addition to lateral pressure are curvature and composition of the lipid bilayer, where negatively charged lysosomal lipids such as bis(monoacylglycero)phosphate are of importance.[230] The failure of the GM2-activator protein in a rare inherited disease, the AB-variant of GM2-gangliosidosis, leads to an accumulation of the ganglioside in neuronal cells, with fatal consequences for the patient.

There are four further sphingolipid activator proteins in addition to GM2-activator protein, called SAP A–D or saposines. As early as 1964 a protein was identified[232] which was necessary for the hydrolytic cleavage of sulfatide (galactosylceramide-3-sulfate) by lysosomal arylsulfatase A. This sulfatide activator (SAP-B) is a small lysosomal glycoprotein that consists of 80 amino acids with an *N*-glycosidically-linked oligosaccharides and is stabilized by three disulfide bridges.[233] Similar to the GM2-activator, it binds GSLs and acts as a GSL-transfer protein in vitro. Sulfatides and similar GSLs can be recognized on the surface of donor liposomes, bound in stoichiometric complexes, extracted from the membrane, and transported into the membranes of acceptor liposomes.[234, 235] It evidently acts like the GM2-activator as a liftase in the lysosomes; the sulfatide activator can bind quite different GSLs of vesicular membranes, "lift" them from the membrane face, and present them as substrates to water soluble enzymes. The inherited defect of the sulfatide activator leads accordingly to a storage disease similar to metachromatic leukodystrophy in which other GSLs accumulate in addition to sulfatide, for example globotriaosylceramide (Section 6.11). The protein-chemical and molecular biological analyses of the sulfatide activator (SAP-B) and a further activator protein, Gaucher factor (SAP-C), have shown that both proteins are formed together with two further activator proteins, SAP-A and SAP-D,[236] by proteolytic processing from a common proprotein, the SAP precursor.[237, 238] All four activator proteins, SAP A–D, are homologous to each other, have similar properties, but different, as yet, indeterminate functions.

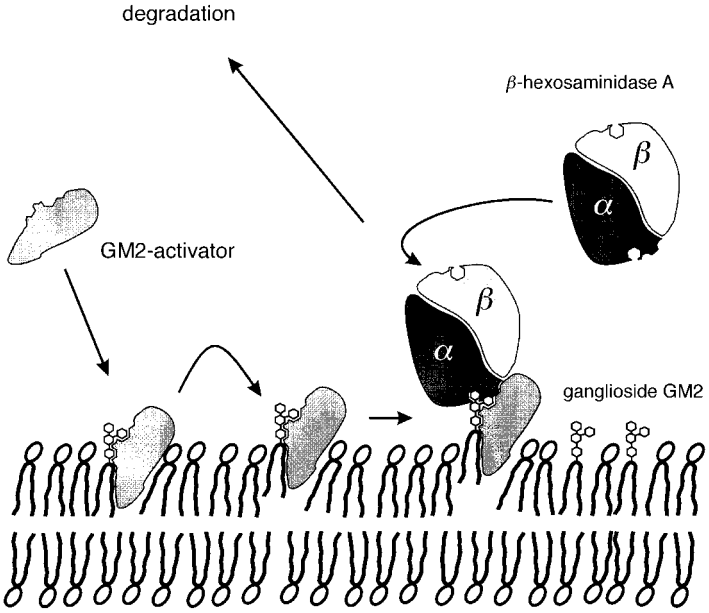


Figure 19. Model for GM2-activator stimulated hydrolysis of ganglioside GM2 by human hexosaminidase A (modified from reference [227]). The water-soluble hexosaminidase A does not attack membrane-bound ganglioside GM2 in the absence of the GM2-activator or suitable surfactants, but it cleaves ganglioside GM2 analogues that contain a short chain or no fatty acid residue (lysoganglioside GM2). These are bound less tightly to the lipid bilayer and are more water soluble than ganglioside GM2. Membrane-bound ganglioside GM2 (for example, that of intralysosomal vesicles) is only hydrolyzed in the presence of the GM2-activator. The GM2-activator binds one molecule of ganglioside GM2 and lifts it out of the membrane. The activator–ganglioside complex can then be recognized by the water-soluble hexosaminidase A and the lipid substrate is cleaved.

Membrane-active sphingolipid activator proteins act amongst other things as GSL binding proteins and thus as liftases for membrane-bound glycolipids, which enables the interaction with membrane-bound GSL substrate and the respective exohydrolase. Their function thereby need not remain limited to that of a GSL binding protein; thus, for example, a direct activation of glucosylceramide β-glucosidae by SAP-C has been detected,[239] and a specific interaction of the GM2-activator with hexosaminidase A has been demonstrated.[260] The participation of activator proteins in GSL degradation can also contribute to the protection of cellular membranes. Lysosomal enzymes or their frequently already active proenzymes appear, even if diluted, in the extracellular space because of incomplete sorting mechanisms. There, lysosomal hydrolases that do not require an activator could slowly degrade GSLs on the cell surface. This process is

normally diminished by two factors, a neutral pH value on the cell surface, at which the lysosomal hydrolases exhibit only a low activity, and also by the necessary stimulation by means of lysosomal activator proteins which occur equally in low concentrations in the extracellular space.

6. Pathobiochemistry

The lysosomal degradation of sphingolipids is associated with the occurrence of a number of inherited diseases.[240, 241] In general, the degradation of cellular components takes place mainly in the acid compartments of cells, in both lysosomes and endosomes. Here, macromolecules are degraded into their building blocks by hydrolytic enzymes. About 40 hydrolases are involved, including proteases, glycosidases, lipases, phospholipases, nucleases, phosphatases, and sulfatases. The material undergoing degradation can reach the lysosomes by way of endocytosis or autophagy. The degradation products leave the lysosomes to serve as energy sources or cellular components for further synthesis in other subcellular compartments. Their passage through the lysosomal membrane takes place by diffusion or with the help of transporter proteins.

The inherited dysfunction of one or more degradation steps leads to the accumulation of nondegradable material and to lysosomal storage diseases, which are classified according to the nature of the stored substances.[242] A differentiation is thus made between sphingolipidoses, mucopolysaccharidoses, mucolipidoses, glycoprotein storage disease, and a lysosomal glycogen storage disease, Pompe's disease. The concept that congenital metabolic dysfunction can lead to inherited diseases is attributed to Garrod.[243] In the example of Pompe's disease, Hers[244] introduced the name lysosomal disease, which is characterized by an inherited defect of an acid hydrolase with lysosomal localization and an accumulation of nondegradable enzyme substrates. Today the concepts of Garrod and Hers must be expanded to acknowledge that not only enzymatic defects can lead to storage diseases, but also defects in transport[245] and activator proteins.[246] As in Krabbe's disease, the defect of a lysosomal hydrolase is not necessarily accompanied by the lysosomal concentration of its substrate. Here the concentration of an otherwise rarely occurring toxic substance, galactosylsphingosine, is increased in the oligodendrocytes (Section 6.9). In comparison to other metabolic pathways it is conspicuous that a variety of inherited diseases is known within the GSL degradation pathway (Figure 20). This must be attributed to the strictly sequential degradation pathway of these compounds where the use of alternative pathways is not possible during metabolic blockage. Thus, mice with the enzyme defect which gives rise to Tay–Sachs disease in humans are essentially healthy because, in contrast to humans, an alternative metabolic pathway exists (Section 6.2.5).

Lysosomal glycosidases are generally specific for the cleavage of a particular terminal residue, which may be present in different substance classes, but are less so for any particular aglycon. A consequence is that in addition to lipid substrates, glycoproteins or mucopolysaccharides may be stored in a particular sphingolipidosis if the defective hydrolase is also necessary for their degradation pathway. A defect is known in humans for almost each of the individual degradation steps of sphingolipid catabolism. One exception is lactosylceramide degradation, which can be carried out by two enzymes in combination with two activator proteins.[247] Accordingly, no single enzyme defect is know that leads to the isolated storage of lactosylceramide. In contrast, lactosylceramide is stored in addition to other lipids when several activator proteins fail (Section 6.11).

In the first instance, the clinical consequences of degradation dysfunction is dependent upon the cell type that is predominantly affected by the storage. Neuronal diseases are a consequence of ganglioside storage, whereas the accumulation of ceramide and glucosylceramide affects mainly the visceral organs and skin, and the storage of galactosylceramide and sulfatide affects the white substance of the brain. With the exception of Fabry's disease, the inheritance of sphingolipidoses is an autosomal recessive. In addition, there is also a further X-chromosome-inherited lysosomal storage disease where sphingolipid metabolism is not affected. In mucopolysaccaridosis II, known as Hunter's syndrome, α-iduronate sulfatase is defective. In addition to defects in enzyme and activator proteins, incorrect posttranslational modification can lead to the expression of lysosomal storage disease. This is the case in I-cell disease (mucolipidosis II) and multiple sulfatase deficiency. A review of individual mutations that cause lysosomal storage disease is available.[248]

6.1. GM1-gangliosidosis

The inherited defect of GM1 β-galactosidase expresses itself in humans as two diseases, GM1-gangliosidosis, which is characterized predominantly by neuronal ganglioside GM1 storage,[249] and Morquio Type B syndrome, in which oligosaccharide accumulation in the viscera predominates.[250] Their causes lie in the substrate specificity of the mutated β-galactosidase. The molecular defect that leads to GM1-gangliosidosis was identified as a β-galactosidase deficiency in 1967.[251, 252] GM1-β-galactosidase is a monomeric protein with a molecular weight of about 70 kD, which together with sialidase and the so-called "protective protein" forms a ternary complex. In this form it is protected from premature proteolytic degradation.[253] Mutations in the protective protein can also cause a secondary GM1-accumulation. The cDNA[254] and the gene[255] of GM1-β-galactosidase have been cloned.

It is possible to differentiate clinically between three forms of GM1-gangliosidosis. In type 1, the infantile form, neurological symptoms appear within the first six months of life, and lead to death within two years. Cherry-red spots on the patient's ocular fundus, facial dysmorphia, liver and spleen hypertrophy, and skeletal deformation are characteristic. The late infantile or juvenile form of the disease, the so-called type 2, progresses less severely, with a life expectancy for the patient of about 10 years. Bone growth disorders and cerebral symptoms occur after the first year of life. Patients with the adult form of the disease (type 3) develop mild, slowly

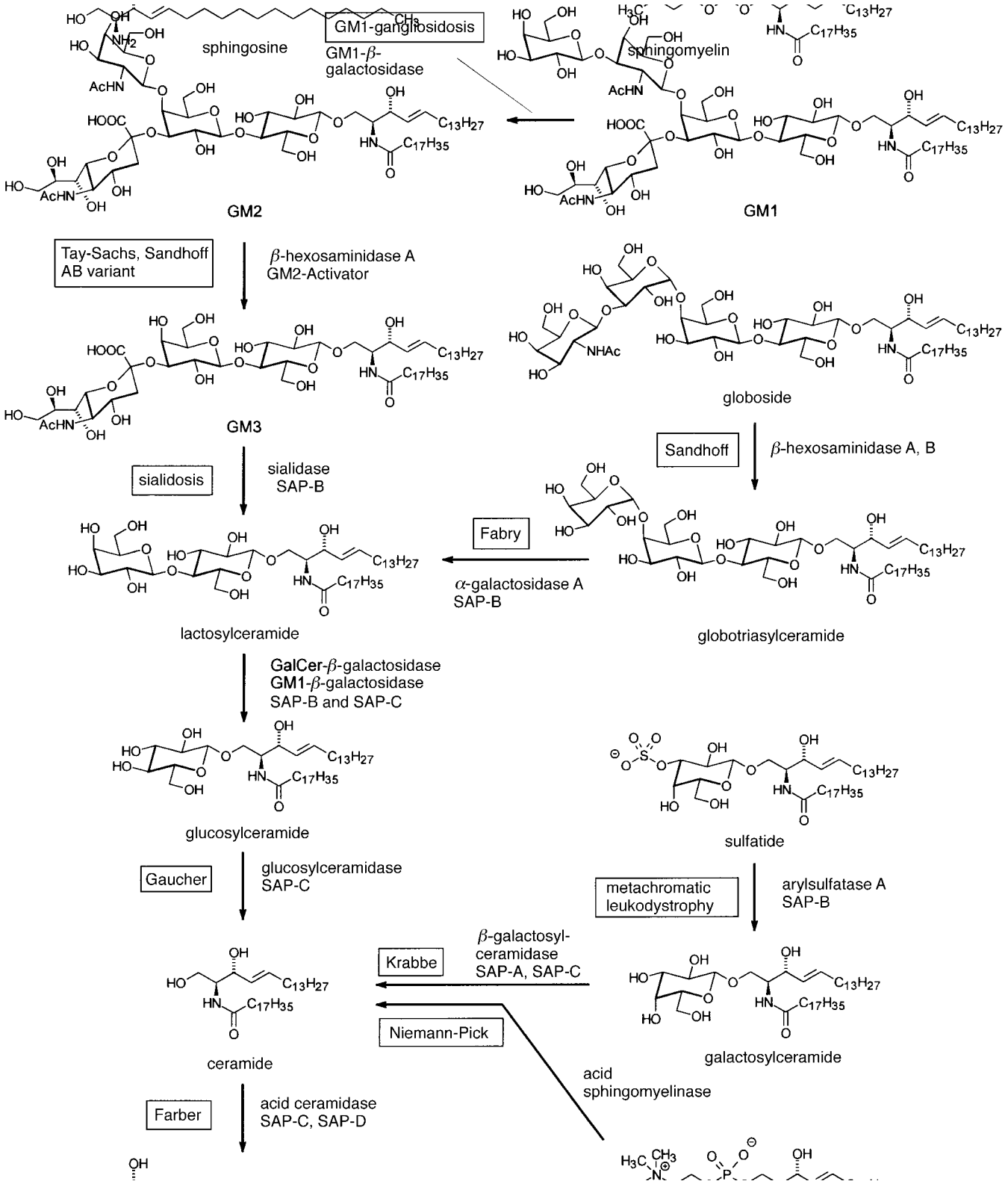


Figure 20. Lysosomal sphingolipid degradation.[223] The eponyms of known metabolic diseases and those of sphingolipid activator proteins necessary for in vivo degradation are indicated. Heterogeneity in the lipid part of the sphingolipids is not indicated. Variant AB = variant AB of GM2-gangliosidosis (deficiency of GM2-activator protein); SAP = sphingolipid activator protein.

progressive neurological disorders with little involvement of the skeleton. Transitional forms are also known.

With a β-galactosidase defect, further enzyme substrates can accumulate in addition to ganglioside-GM1, including glycolipid GA1, oligosaccharides from glycoproteins, and intermediates of keratansulfate degradation. Depending upon the site of their biosynthesis, these substances are stored in different organs. GM1-storage in neurons leads to degeneration of the nervous system. The severity and progression of the disease correlates with the residual enzymatic activity in cells and body fluids as well as with the extent of substrate storage.[256] The mutations that have been identified in infantile GM1-gangliosidosis patients (review: reference [248]) are heterogeneous, and none of the known defects occurs with special frequency. Recently, an authentic mouse model of GM1-gangliosidosis has been successfully developed.[257, 258]

6.1.1. Morquio Type B Syndrome

Morquio Type B syndrome (review: reference [250]) is clinically a mild phenotype of the nonallelic type A syndrome in which *N*-acetylgalactosamine-6-sulfatase is defective, which results in the storage of keratansulfate. As in GM1-gangliosidosis, Morquio B syndrome is attributed to a defect in GM1-β-galactosidase. It is characterized by skeletal deformation without primary involvement of the central nervous system. Oligosaccharides with terminal galactose residues accumulate in the visceral organs and are secreted in the urine.

6.2. GM2-gangliosidoses

GM2-gangliosidoses are caused by a defect in the degradation of the ganglioside GM2.[259, 263] Long term investigations have shown that three polypeptide chains are involved in the in vivo enzymatic cleavage of the terminal *N*-acetylgalactosamine residue, which are encoded by three different genes (Figure 19): the α and β chains of the β-hexosaminidases, and the GM2-activator protein. The β-hexosaminidases are dimeric proteins and cleave β-glycosidically-coupled terminal *N*-acetylglucosamine and *N*-acetylgalactosamine residues from glycoconjugates. The GM2-activator protein is required as a cofactor in the cleavage of ganglioside GM2. The α and β chains of β-hexosaminidases form three isoenzymes that differ in their subunit structures and substrate specificities. β-Hexosaminidase A has the $\alpha\beta$ subunit structure and degrades negatively charged and uncharged substrates. It has two, initially totally unexpected, active centers, one on the α chain, and one on the homologous β chain.[260]

β-Hexosaminidase B with the subunit structure β_2 cleaves mainly *N*-acetylglucosamine and *N*-acetylgalactosamine residues from uncharged substrates such as glycolipid GA2, globotetraosylceramide, and oligosaccharides. β-Hexosaminidase S is a homodimer of α chains. It is of secondary significance for GM2 degradation and far less is known about its function in vivo than for the other isoenzymes. Investigations with genetically modified mice suggest that β-hexaminidase S is involved in the degradation of glycoaminoglycans.[261] We have classified GM2-gangliosidoses according to which enzyme is still detected in the tissues of patients.[262] Mutations that affect the gene for the α-subunits of β-hexosaminidases can lead to the failure of β-hexosaminidases A and S. This defect is known as the B-variant of the GM2-gangliosidoses, and its infantile form is also known as Tay–Sachs disease. Mutations in the gene for the β subunit can lead to failure of β-hexaminidases A and B, and storage of glycolipid GA2 and globotetraosylceramide in addition to ganglioside GM2. The respective disease is the 0-variant of the GM2-gangliosidoses, or Sandhoff's disease. Defects in the gene for the GM2-activator lead to AB-variants of the GM2-gangliosidoses.

6.2.1. B-Variant of GM2-gangliosidosis

The infantile form of the B-variant is better known as Tay–Sachs disease. The symptoms of Tay–Sachs disease were first described in 1881 by the British ophthalmologist Warren Tay, who reported on a case of amaurotic idiocy.[263] Tay discovered a cherry-red spot in the retina of a one year old patient who was physically and mentally retarded. Before the turn of the century, the American neurologist Berhard Sachs coined the term amaurotic familial idiocy and described the morphological characteristics of the disease. In 1962 Lars Svennerholm identified the main storage substance in Tay–Sachs disease within the nervous system to be the ganglioside GM2, the chemical structure of which was determined in the middle of the 1960s by Makita and Yamakawa,[259] and Ledeen and Salsman.[259] The underlying metabolic defect, the deficiency of the enzyme β-hexaminidase A was identified in 1969.[264, 265]

The classical Tay–Sachs disease is characterized by the onset of neurological symptoms in early childhood. The earliest symptoms occur at the age of three to five months. Motor weakness develops: sudden noise causes abnormal startle reflexes. At six to ten months attentiveness deteriorates, and motor dexterity and sight are lost. Macrocephaly and neurological disorders worsen until death occurs. Juvenile forms of the B-variant of GM2-gangliosidoses are characterized by motor disorders at the age of two to six years and advancing dementia in early childhood. A chronic form is made conspicuous by abnormalities in gait and posture and can commence at the age of two to five years. Neurological symptoms are manifest with advancing age, and the patient can live to an age of 40 years. Adult forms of the disease show a multiplicity of symptoms; neurological disorders such as spinal muscular atrophy and psychoses can occur. However, visual capabilities and intelligence are not impaired.

Both cDNA and gene[266] of the α chain and cDNA[267] and gene[268, 269] of the homologous β chain have been characterized. The mature form of the α chain has a molecular weight of 56 kDa,[270] that of the β chain 52 kDa.[271] The β chain is cleaved proteolytically in the lysosomes of many cell types into to two smaller fragments, β_a and β_b.

Tay–Sachs disease occurs particularly frequently in populations of Ashkenasi Jews (heterozygote frequency 1:27). Three mutated alleles within the α chain are responsible for 93 % of all mutated alleles. In total over 50 mutations on the α chain have been identified (review: reference [259]). The

form and severity of the disease correlate with the residual activity of the enzyme in the lysosome.[272]

6.2.2. B1-Variant of GM2-gangliosidosis

The B1-variant of GM2-gangliosidosis[273, 274] differs enzymologically from the B-variant by a changed substrate specificity of the mutated β-hexaminidase A. Synthetic uncharged substrates, which are used for diagnosis, are cleaved, whereas no activity is detected in respect to the natural substrate and negatively charged synthetic substrates. We have found that the function of the active site of the α chain is defective, whereas subunit association, enzyme processing, and the activity of the β chain activity are unimpaired. Mutations on three positions of the α chain lead to the B1-variant. Since at the protein level Arg178 is replaced by the amino acids Cys, His, and Leu in three mutations, it was assumed that Arg178 plays a role of the catalytic amino acid in the active site. This hypothesis has been refuted,[275] but the precise role of Arg178 still remains unclear. Homozygous patients with B1-mutation present a juvenile disease progression; the late infantile form was found within compound heterozygotes with a null allele.

6.2.3. 0-Variant of GM2-gangliosidosis

The 0-variant of GM2-gangliosidoses is called Sandhoff's disease.[246] We received the post-mortal tissue of the first case to be investigated and which was diagnosed as Tay–Sachs disease. However, the isolation and analysis of the storage substance exhibited an abnormal picture.[276] Unlike the B-variant, it was noted that in addition to negatively charged glycolipids, mainly ganglioside GM2 and uncharged glycolipids were stored,[262] in particular globoside in the visceral organs, glycolipid GA2 in neuronal tissue, and, as was determined later, oligosaccharides in the urine. All these storage lipids had a terminal β-glycosidically bound *N*-acetylgalactosamine residue in common. This feature suggested a possible metabolic defect. During studies on the degradation of the radiolabeled storage components we were able to detect an almost total loss of the β-*N*-acetylgalactosaminidase activities known at that time, that is, hexosaminidases A and B, in all the tissues investigated.[276] This was the first described enzyme defect in a gangliosidosis. Later investigations showed that both hexosaminidase A and B are defective as a result of mutations on the gene of the common β chain.

The clinical and pathological picture of Sandhoff's disease corresponds essentially to Tay–Sachs disease, although hypertrophy of internal organs and bone deformation also occur. We have identified Glu355 as the catalytic amino acid in the active site of β-hexosaminidase B by photoaffinity labeling.[277, 278]

6.2.4. AB-variant of GM2-gangliosidoses

The AB-variant of GM2-gangliosidosis is attributed to a deficiency of the GM2-activator. Early investigations showed us that in spite of the presence of β-hexosaminidase A and B activities, a massive storage of the glycolipids GM2 and GA2 occurred in the brain.[224] The enzymes isolated from patient tissue were able to cleave enzymatically the stored substances isolated from the same tissue when in the presence of a surfactant. This apparent paradox prompted us to seek an endogenous, that is natural, surfactant in post-mortal human tissue. In 1978 we were able to identify the molecular defect in the AB-variant as the deficiency of a cofactor, the GM2-activator protein.[279] The AB-variant served as a prototype for the analysis of further activator deficiency diseases. Shortly thereafter we were successful in isolating the protein.[280] The mature GM2-activator is a glycoprotein containing a polypeptide thread of 162 amino acids. This forms four disulfide bridges whose mode of coupling was recently elucidated by the combination of protein-chemical and mass spectrometric techniques.[281] The cDNA and large regions of the gene are known.[282] Mutations in AB-patients have been identified.[283–285]

The clinical progression of the disease corresponds to that of Tay–Sachs disease. In comparison with Tay–Sachs disease, the appearance of symptoms is slightly delayed. Ganglioside GM2 and glycolipid GA2 are stored in AB-variant, but not, however, globoside[262] or GD1a-GalNAc, a minor storage substance in the 0- and B-variants.[286]

6.2.5. Animal Models of GM2-gangliosidoses

Different natural animal models of GM2-gangliosidoses are known (review: reference [259]). In addition, it has been possible more recently to develop mouse models for the individual forms of GM2-gangliosidosis.[287] The targeted disruption of genes for the α chain and the β chain of hexosaminidases as well as the GM2-activator protein in embryo stem cells has led to animal models of the B-variant,[288, 289] the 0-variant,[290] and the AB-variant[291] of GM2-gangliosidoses. Whereas the individual forms of GM2-gangliosidosis in humans differ phenotypically to only a slight extent, animal models show drastic differences in the progression and severity of the disease. The mouse with B-variant is phenotypically inconspicuous. In contrast, the mouse with 0-variant develops severe motor disorders, and life expectancy is greatly reduced. The cause lies in the specificity of the sialidase, which differs between mouse and humans.[290] Murine sialidase accepts GM2 as a substrate and degrades it slowly to GA2 (Figure 21). This metabolic pathway plays no significant role in humans. GA2 can be degraded by the still intact β-hexosaminidase B, so that in the Tay–Sachs mouse the metabolic block can be partially circumvented in spite a complete failure of β-hexosaminidase A. Only the failure of both isoenzymes, hexosaminidase A and B, leads to a symptomatology that corresponds to the human form of Sandhoff's disease. Murine sialidase can still convert GM2 into GA2, but GA2 can no longer be degraded since the responsible enzyme, β-hexosaminidase B, is defective.

The mouse deficient in GM2-activator expresses an intermediate phenotype, which is characterized by motor disorders and a normal life expectancy. Crossing mice of the B- and 0-variants leads to animals in which all three isoenzymes, hexosaminidases A, B, and S are defective. There is no known

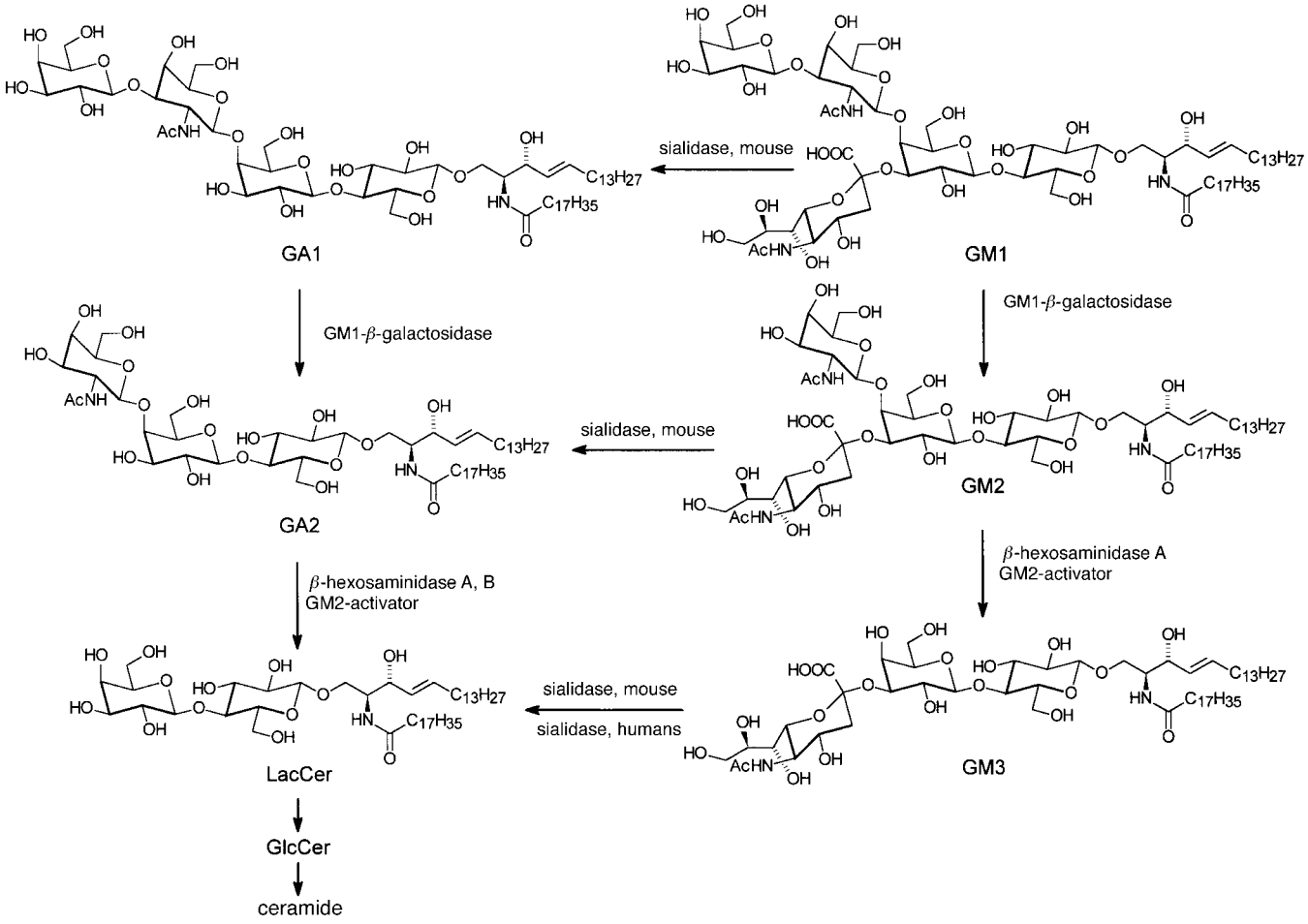


Figure 21. Degradation pathways of ganglioside GM1 in humans and mice.[257, 290]

human disease that corresponds to this defect. In addition to neuronal glycolipid storage, these animals exhibit the phenotype of a mucopolysaccharidosis and excrete large amounts of glycosaminoglycans in their urine. Accordingly, hexosaminidases are responsible for the degradation of glycoaminoglycans, whereby in human patients with B- or 0-variant the presence of the respective, intact isoenzyme is sufficient to prevent storage of glycosaminoglycans. Only the failure of all three isoenzymes leads to an accumulation of nondegradable glycosaminoglycans.[261] B-variant mice have gained in importance as a model for the therapy with low molecular weight enzyme inhibitors (Section 6.14.3).

6.3. Galactosialidosis

Galactosialidosis involves the secondary deficiency of two enzyme activities,[292] β-galactosidase and sialidase (*N*-acetylneuraminidase). The primary defect is a result of a mutation within the gene of the so-called "protective protein",[293] which associates with the two other proteins to form a stable complex of more than 600 kD.[294] The stoichiometry of the complex is still unknown; however, the presence of β-galactosidase protein is not essential for the stability of the complex. Accordingly, a deficiency of the "protective protein" leads to degradation of β-galactosidase and sialidase and thereby to the storage of sialic acid and galactose-containing substrates, including oligosaccharides and gangliosides such as GM3 and GM1.[292] Sialyloligosaccharides accumulate in the lysosomes and are finally excreted in the urine.

The "protective protein" combines different enzymatic activities. In addition to its property as a protective protein, it is a serine esterase, a carboxypeptidase (acid pH optimum), and a deamidase (neutral pH optimum). It has emerged that it is identical to a protein that is released after thrombin stimulation of blood platelets, and hydrolyzes, and thus inactivates, different peptide hormones with amidic C-termini such as oxytocin, endothelin, and substance P. On the other hand, it is identical to the lysosomal protease cathepsin A. The protease and protective functions are independent of each other, since a gene product with an inactivated catalytic site restores the degradative capacity of sialidosis fibroblasts. The "protective protein" is synthesized as a 54 kD precursor, which is processed proteolytically into two chains of 32 and 20 kD connected by disulfide bridges. In all patients so far examined, deamidase and carboxypeptidase activities of the "protective protein" are also defective. The crystal structure of the 108 kD dimer of the "protective protein" precursor has been established.[295]

Since the molecular defect of GM1-gangliosidosis was identified as a β-galactosidase deficiency,[251, 252] reduced β-galactosidase activity, which is not, however, attributable to

defective β-galactosidase, has been found in different atypical patients. In one of these patients Wenger et al. detected a combined deficiency in β-galactosidase and neuraminidase activities,[296] which was later found in other patients with atypical GM1-gangliosidosis and sialidosis. A partial restoration of sialidase activity in cells with combined enzyme defect was achieved in cell fusion experiments with neuraminidase deficient cells.[297] The nature of a postulated "corrective factor" was finally elucidated by d'Azzo et. al.[293] Galactosialidosis has been diagnosed in about 70 patients world wide (review: reference [292]). The symptoms in all patients are coarsened facial features, cherry-red spots on the ocular fundus, spinal column changes, foam cells in the spinal cord, and vacuolized lymphocytes. Three different forms of the disease are recognized phenotypically. The early infantile form leads to neurological disorders and death of the patient at an age of about eight months as a result of cardiac or renal failure. Hypertrophy of the liver and spleen, growth disorders, and cardiac damage are characteristic of the late infantile form, but there is no impairment of the nervous system. Most patients suffer from the juvenile/adult form, which is characterized amongst other things by mental retardation and neurological damage. A mouse model with defective "protective protein" corresponds biochemically essentially to the severest human form, but, in contrast, however, it exhibits unusually high β-galactosidase activity.[298]

6.4. Sialidosis

Defects in lysosomal sialidase lead above all to an accumulation and excretion of sialylated oligosaccharides and glycoconjugates. Since gangliosides play only a subordinate role in storage substances, this is not a sphingolipidosis in its narrowest sense. Sialidases that accept gangliosides as substrates are localized on the cell surface.[299–302] The infantile form of the disease, (type II sialidosis) is also called mucolipidosis I and is characterized by skeletal deformation, hypertrophy of the internal organs, and mental retardation. A juvenile form (type II sialidosis) is also known as mucolipidosis IV. The human cDNA of sialidase has been cloned.[303]

6.5. Fabry's Disease

A failure of α-galactosidase A underlies Fabry's disease. The first patients were described independently in 1898 by the dermatologists Anderson and Fabry.[304] The identity of the storage material was determined in the 1960s by Sweeley and Klionsky, and the nature of the enzymatic defect by Brady and Kint.[304] Unlike the other sphingolipidoses, Fabry's disease is inherited as an X-chromosomal disease. It leads to the accumulation of enzyme substrates with terminally α-glycosidically bound galactose. The enzyme is a homodimer of subunits of each 50 kD and was purified from liver and spleen.[305, 306] The cDNA[307] and gene[308] of α-galactosidase A have been cloned.

Fabry's disease is characterized by painful skin lesions and is manifested clinically in lipid deposition within the skin, pains in the extremities, and renal failure. The symptoms can start in infancy or adolescence, but is most frequently an adult form. Heterozygote female carriers are symptomless or show a milder disease progression. Mainly globotriaosylceramide, but also digalactosylceramide and lipids with blood group B specificity are stored. Detectable residual activities of α-galactosidase A can be attributed to the presence of α-galactosidase B (α-*N*-acetylgalactosaminidase), which exhibits a slight overlap in substrate specificity with the Fabry enzyme. This enzyme is defective in Schindler disease, of which only three patients have been currently described. The nature of the storage substance and the pathogenesis of Schindler disease are unknown.[309]

The lipids affected in Fabry patients are synthesized in nerve cells only to a small extent, so it is accordingly a systemic disease with neuronal damage only as a secondary effect. The storage sites are the epithelial cells of blood vessels, cells of the smooth musculature and the myocardium. The pathogenesis is caused by the blockage of small blood vessels by lipid deposition and manifests itself by angiokeratoma of the skin, renal failure, and cardiovascular disease. Lipid deposition in the brain of patients is attributed to the storage in blood vessels. However, the autonomic nervous system can be affected. The molecular causes that lead to the expression of Fabry's disease are multifactorial. Gene rearrangements, point mutations, and splice site mutations have been found (review: reference [248]). The detection of the enzyme defect can be carried out by the determination of the enzyme activity in different sources, but the interpretation of this is hampered in female carriers, which results from the statistical inactivation of one of their X-chromosomes in the different cells and tissues.[310] A mouse model of the disease has been recently described.[311]

6.6. Niemann – Pick Disease

In 1914 the German physician Albert Niemann reported on a young patient who suffered from hepatosplenomegaly, lymphadenopathy, and impairment of the central nervous system and who died before reaching the age of two years.[312] During subsequent histological studies, Ludwig Pick observed the occurrence of foam cells that were similar, but not identical, in appearance to those previously described in association with Gaucher's disease. Ernst Klenk identified the storage substance as sphingomyelin, and in 1965 Brady detected a reduced sphingomyelinase activity in affected cells. In 1961 Crocker classified Niemann – Pick's disease into three different types, A – C, to which further types were added later. Types A and B of Niemann – Pick disease are caused by a defect in acid sphingomyelinase (review: reference [312]) and are characterized by a lysosomal sphingomyelin storage. Type C of the disease does not involve a defect in acid sphingomyelinase.[313] The neuronopathic type A occurs during childhood and is characterized by advancing psychomotor retardation and massive hypertrophy of the internal organs. Death occurs at the age of about three years. Patients who suffer from the non-neuronopathic type B of the disease also show organ enlargement, but almost no involvement of the

central nervous system is observed. The patients can reach adulthood.

Intermediate forms are characterized by an initially mild type B-like disease picture, which worsens with advancing years with involvement of the central nervous system.[314] Patients of types A and B exhibit massive hypertrophy of the liver and spleen as well as characteristic storage cells in the bone marrow. We have purified to homogeneity and characterized the acid sphingomyelinase that is defect in types A and B of Niemann–Pick's disease in order to elucidate the molecular origin of the disease. It is a monomeric protein with a molecular weight of about 70 kD.[315] The cDNA[316] and the gene[317] of the enzyme (Figure 22) have been cloned.

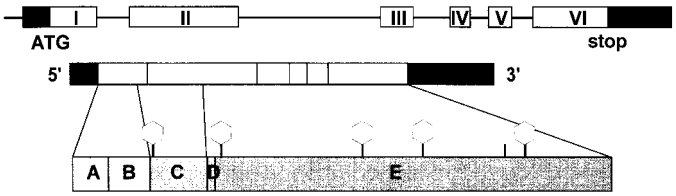


Figure 22. Genomic structure (top), cDNA (center), and protein domains (bottom) of acid sphingomyelinase.[448] I–VI: exons, A: presequence, B: prosequence, C: activator domain, D: proline-rich region, E: catalytic domain. Hexagon: utilized *N*-glycosylation sites; ATG: translation start, stop: translation stop.

Niemann–Pick's disease (review: reference [312]) is a panethnic disorder, but it occurs more frequently in Ashkenasi Jews (heterozygote frequency 1:60). The differing disease pictures of types A and B can be fundamentally attributed to the different residual enzymatic activity of the acid sphingomyelinase.[318] Unlike type A patients, patients with type B possess greater residual activity, which is still able to maintain an adequate turnover of sphingomyelin in the nervous system.[319]

Acid sphingomyelinase cleaves sphingomyelin into ceramide and phosphorylcholine. This reaction was of special interest in the recent past since ceramide has been under discussion as a signal molecule in intracellular signal transduction. The role of acid sphingomyelinase in the signal-dependent induced generation of ceramide in the so-called sphingomyelin cycle is currently disputed.[20] Unlike normal lymphoblasts, the lymphoblasts of patients with Niemann–Pick's disease and sphingomyelinase knockout mice exhibit no ceramide formation and no apoptosis in response to irradiation.[320] On the other hand, acid sphingomyelinase appears to be necessary for FAS-induced apoptosis.[180] Stimulation of cells that overexpress the magnesium-dependent neutral enzyme with tumor necrosis factor α leads to only a slight elevation of intracellular ceramide concentration.[153]

Treatment of rats with tricyclic antidepressants leads to storage of sphingomyelin in visceral organs and a disease picture which resembles Niemann–Pick's type B.[321] In cultured fibroblasts the antidepressant desipramine causes proteolytic degradation of mature lysosomal sphingomyelinase, but not of one of its precursor forms. A thiol protease is clearly responsible for this induced degradation.[322] A mouse model has also been developed for the Niemann–Pick disease, whose phenotype essentially corresponds to the human type A disease.[323, 324]

6.7. Metachromatic Leukodystrophy and Multiple Sulfate Deficiency

Metachromatic leukodystrophy (MLD) is caused by a deficiency of arylsulfatase A (ASA) and leads to the accumulation of sulfatides in different organs (review: reference [325]). The name MLD comes from the metachromatic staining of the stored substances in histological slices. Sulfatide was isolated from human brain tissue by Blix in 1933, and the structure was determined by Yamakawa in 1962. The storage of sulfatide in MLD patients was detected by Jatzkewitz (review: reference [326]), and the sulfatase deficiency independently of each other by Austin and Jatzkewitz.[3] Arylsulfatases are enzymes that can cleave artificial aryl sulfates. In MLD patients one of the different arylsulfatases is defective, namely arylsulfatase A,[327] which cleaves sulfatides in the presence of a cofactor.[232] The cofactor of arylsulfatase A, later called the SAP-B activator protein, was isolated from tissues of MLD patients.[328] The disease has been divided into late infantile, juvenile, and adult forms.[329] The adult form is rarer than the other two. The clinical symptoms arise at the end of the second or third decade. The leading symptoms are mental retardation, which leads to dementia, or behavioral abnormalities, which lead to the development of psychosis.[330]

In both Krabbe's disease and MLD the myelin-forming cells of the central nervous system are affected, but unlike Krabbe's disease a significantly higher proportion of patients with the juvenile and adult forms are found in MLD. The reduction in the number of oligodendrocytes, which supply the nerve cells with the myelin sheath, is less severe than in Krabbe's disease. The function of peripheral organs is not impaired, but the nervous system is damaged by advancing demyelination. Increased concentrations of lysosulfatide (deacylated sulfatide) have been detected in the tissue of MLD patients. This cytotoxic compound is possibly an important factor in the pathogenesis of the disease.[331] Macroscopically, a reduced volume of white substance is observed. Microscopically, a loss of myelin, a reduction in oligodendrocytes, and the occurrence of metachromatic granula have been found.

Arylsulfatase A aggregates at low pH values. The ASA gene has been cloned;[332, 333] the incidence of the disease in the white population is estimated at 1:40000, the frequency of the leukodystrophy allele is about 0.5%.[329] More than 30 different mutations that lead to MLD have been characterized in the ASA gene (review: reference [248]). The molecular cause for atypical forms of MLD lies in a deficiency of the sulfatide activator.[334] MLD can be diagnosed by activity measurement of arylsulfatase A towards the artificial substrate 4-nitrocatechol sulfate in extracts of leukocytes or cultured fibroblasts.[335] Previously, the detection of sulfatide in urine or the metachromatic staining of Schwann cells in nerve biopsies was used. Mice with artificially produced arylsulfatase A deficiency[336] show only a mild extent of neurological and

neuropathological changes, although biochemically they resemble the severe late infantile phenotype in humans. The causes of this are still unclear.

6.7.1. Multiple Sulfatase Deficiency (Austin's Disease)

Numerous natural products of different substance classes contain alcohol groups that are modified as sulfate esters. These sulfate esters are cleaved by sulfatases, of which nine different enzymes have been characterized in humans and which, with the exception of steroid sulfatase, are localized in lysosomes (reviews: reference [337, 338]). The activities of all known sulfatases are greatly reduced in multiple sulfatase deficiency. The phenotype of the patients is characterized clinically and pathologically by a combination of MLD and mucopolysaccharidosis symptoms (review: reference [325]). Dermatan sulfate and heparan sulfate are excreted in the urine, and skeletal deformation, hepatosplenomegaly, and craniofacial abnormalities occur in addition to MLD symptoms. The primary defect of multiple sulfatase deficiency is not known, however, since expression of sulfatase cDNAs in fibroblasts of MSD patients leads to enzyme proteins with reduced catalytic activity, von Figura and co-workers have concluded that a defective co- or posttranslational modification is the cause of MSD.[339] They were able to detect a defective protein modification in arylsulfatases A and B in MSD cells. This was the transformation of a cysteine into a formylglycine residue.[340] This newly discovered modification is clearly responsible for the catalytic activity of the sulfatases. A presentation of this information has appeared in this journal.[341]

6.8. Gaucher's Disease

Gaucher's disease is the most common of the sphingolipidoses and it is characterized by the deficiency of β-glucocerebrosidase and the accumulation of glucosylceramide (review: reference [342]). The disease was first described by Gaucher in 1882. The identification of the storage material was made by Aghion in 1934, and the identity of the defective enzyme was elucidated by Brady[343] and Patrick[344] in 1965. The glycoprotein glucocerebrosidase has a molecular weight of 59 to 67 kDa and consists of 497 amino acids. The complementary DNA of the enzyme was cloned in the middle of the 1980s.[345, 346] Information on the active site of glucocerebrosidase was obtained with the help of competitive covalent inhibitors of the enzyme. The amino acid residue Asp443 was labeled[348] with conduritol B epoxide (Figure 17).[347] However, an involvement of this amino acid side chain in glycoside hydrolysis was excluded by site-specific mutagenesis.[349] In contrast, Glu340 labeled with 2-deoxy-2-fluoroglucopyranose is clearly the nucleophilic residue in catalytic glycoside hydrolysis.[350]

Gaucher's disease is classified by three types, I–III. The most frequent by far nonneuropathic form (type I) is clinically heterogeneous and has an incidence of 1 in 50000 to 200000 births. It has a higher frequency amongst Ashkenasi Jews; the carrier frequency is up to 1 in 10.[351] The reticuloendothelial system is mainly affected. The nervous system is also affected in the rarer, acute neuropathic type II of the disease. An intermediate, juvenile form III is also known as the subacute neuropathic form in which the neurological symptoms develop later and progress more slowly than in form II. Gaucher's disease is panethnic; type 1 is particularly frequent amongst the Ashkenasi Jews, and type III prevails in the Swedish province of Norrbotten.

Although the deficiency in glucocerebrosidase exists in all body cells, with the exception of the very rare neuropathic form, the phenotype is only significantly manifest in macrophages, which, because of the phagocytosis of, for example, erythrocytes, have especially large amounts of sphingolipids to degrade. The lipid storage leads to a characteristic morphology of the macrophages, which are called Gaucher cells. The appearance of storage cells in the liver, lymph nodes, and spleen causes hypertrophy of these organs. Infiltration of the bone marrow by Gaucher cells results in painful bone damage and the displacement of hematogenic cells. Glucosylceramide storage is accompanied by an elevated chitotriosidase activity in the plasma, which can be used for diagnostic purposes.[352]

Four mutations are responsible for 80% of all defects so far detected (review: reference [353, 248]). Two cases of Gaucher's disease are known that are not attributable to the deficiency of glucocerebrosidase, but to an deficiency of the sphingolipid activator protein SAP-C.[354–356] The severity of the disease correlates inversely with the residual glucocerebrosidase activity that was found in cultured skin fibroblasts of Gaucher patients.[357] Particularly high levels of glucocerebrosidase activity have been found in fibroblasts and placenta, and also in the chorion villi and cultured amnion cells that are important for prenatal diagnosis. The adult form of Gaucher's disease (type I) is currently the only sphingolipid storage disease for which a causal therapy can be undertaken.[358–360] Glucocerebrosidase is used which has been obtained from human placenta or by genetic engineering and which has been equipped with targeting information for the mannose receptor on macrophages. The treatment leads to a normalization of blood parameters and a reduction in weight of the liver and spleen. Bone marrow transplantation can be carried out in patients with the severe infantile form. Animal models of infantile Gaucher's disease, type II[361] as well as II and III[362] have been described. Artificial model systems of Gaucher's disease have been generated with the help of conduritol B epoxide.[363, 364]

6.9. Krabbe's Disease

Krabbe's disease is attributed to the deficiency of lysosomal galactocerebrosidase (review: reference [365]). Together with metachromatic leukodystrophy it belongs to the classical myelin diseases, since in both cases the degradation of glycolipids, galactosylceramide, or sulfatide, which are characteristic for the myelin sheath of nerve cells, is dysfunctional. The enzyme defect that underlies globoid cell leukodystrophy was elucidated in 1970.[366]

The disease picture is manifested by neurological symptoms that usually start within the first six months of life. The patients die within two years. Adult disease forms are also known. The white substance of the central nervous system and peripheral nerves are the exclusive sites of the clinical and pathological manifestations of the disease. At the terminal stage the white substance contains almost no myelin. Oligodendroglia are replaced by astrocytes and abnormal globoid cells. Unlike all other storage diseases, there is no accumulation of the substrate of the defective enzyme in the cells particularly affected. On the contrary, the levels of galactosylceramide are unchanged or are less than normal, only the relative amount of galactosylceramide in myelin lipids is elevated. The cause lies in a rapid destruction of myelin-forming oligodendrocytes. Therefore, incomplete myelinization, accompanied by rapid demyelinization, means that galactosylceramide cannot accumulate. According to the so-called psychosine hypothesis, increased concentrations of the nondegradable galactosylsphingosine (psychosine), a lytic metabolite of sphingolipid metabolism, are responsible for the rapid depletion of the oligodendrocytes and the pathology of the disease.[367, 368]

Galactocerebrosidase is a membrane-bound protein with a molecular weight of 50 kD.[369] The cDNA[370, 371] and gene[372] of galactocerebrosidase have recently been cloned. An authentic animal model of the disease, the twitcher mouse, is known.[373] The murine galactosylceramidase cDNA has been cloned and the twitcher mutation identified as a premature stop codon in the middle of the encoding sequence.[374]

6.10. Farber's Disease

Farber's disease is caused by a deficiency in acid ceramidase and the ensuing accumulation of ceramide in various tissues (review: reference [375]). It is a rare inherited autosomal recessive disease; about 43 patients have been reported. Cell culture studies have shown that the sphingolipid activator proteins SAP-D[376] and SAP-C[447] are essential for lysosomal ceramide degradation. Farber's disease was first reported in 1947. During a symposium of the Mayo Foundation in the autumn S. Farber reported the case of a 14 month old girl with symptoms that were reminiscent of Niemann – Pick disease, but which differed from it through histological variations.[375] Because of the most obvious symptom, the occurrence of lipid-containing nodes, the disease was called a lipogranulomatosis. During childhood the patients developed a painful swelling of the joints followed by subcutaneous nodes. An advancing hoarseness and feeding difficulties as a result of laryngeal changes are also characteristic. According to the localization of the lipogranuloma, functions of organs such as lung, heart, and kidney can be impaired. Impairment of the nervous system in most protracted forms is slight. The disease leads to death within the first year, but a longer time course is possible. The storage substance was identified as ceramide in 1969,[377] and the underlying defect as a deficiency in acid ceramidase.[378, 379] We have isolated the enzyme from human urine.[380] It is a heterodimer from a α-subunit of 13 kDa and a glycosylated β-subunit of 40 kDa (Figure 23). The complementary DNA has been cloned;[381] it encodes for both subunits.

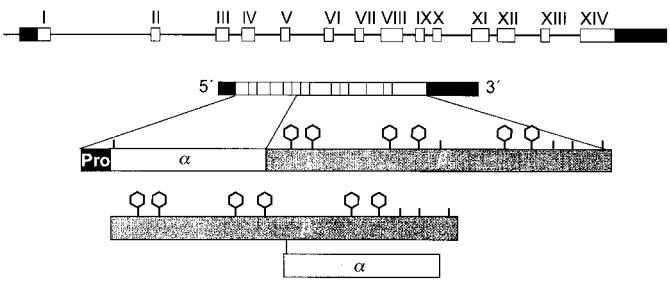


Figure 23. Gene, cDNA, and processing of acid ceramidase.[449] I – XIV: exons; hexagon: utilized N-glycosylation sites.

A number of subclasses of Farber's disease have been identified according to the clinical picture. All patients display lipid storage in the kidney, whereas the type and scope of deposition in the other tissues and organs can vary. The cause of the disease in six of the seven classes lies in a defect in lysosomal acid ceramidase. The seventh subtype is attributed to a mutation in the start codon of sphingolipid activator protein precursor.[401] As a result, SAP-C and SAP-D which are required as cofactor for acid ceramidase fail, as do two further activators. Biochemically, the storage of ceramide in tissue[377] and the increased secretion of ceramide in the urine of patients[382] is characteristic of Farber's disease. The fraction of ceramide in subcutaneous nodes can reach 20% of total lipid, and the ceramide content in the kidney is also elevated. The hydrolysis of ceramide is catalyzed by two other, non-lysosomal ceramidases, one of which achieves its optimum activity in a neutral medium,[383] the other in an alkaline medium.[379] The three ceramidases exhibit varying tissue distribution as well as different intracellular topology and substrate specificity.[375, 384] Thus, neutral and alkaline ceramidase are unable to replace the defective lysosomal acid ceramidase in Farber's disease.[378]

Results in cultured fibroblasts from Farber patients have shown that the course of Farber's disease correlates inversely with the extent of lysosomal ceramidase degradation.[385] The residual activity of the enzyme in Farber patients is higher than that of the defective enzymes of the other respective sphingolipidoses; obviously, larger amounts of ceramide from sphingolipid degradation must by converted by the enzyme, so even a slight impairment of enzyme activity leads to substrate storage.

We have identified as the first molecular defect in the gene of a patient with Farber's disease a homoallelic point mutation, which leads to a Thr222-Lys exchange in the β-subunit of acid ceramidase.[381] Diagnosis is carried out by determination of acid ceramidase activity with the help of the synthetic substrates *N*-[1-^{14}C]oleoylsphingosine and *N*-[1-^{14}C]lauroylsphingosine in the presence of surfactants,[386] or by the analysis of ceramide storage after radiolabeling in cultured fibroblasts.[387] In addition, morphological studies on biopsy and autopsy material have been carried out.

6.11. Sphingolipid Activator Protein Deficiency

The degradation of sphingolipids with short oligosaccharide chains requires the presence of small non-enzymatic proteins,

the so-called sphingolipid activator proteins (Section 5.5). Five of these small, heat-stable proteins are currently known, the GM2-activator and the four sphingolipid activator proteins SAP-A, SAP-B, SAP-C, and SAP-D, which are produced from a common precursor by proteolytic processing (Figure 24). The GM2-activator protein has already been

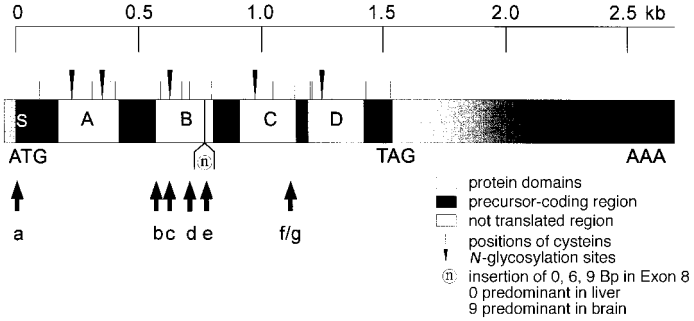


Figure 24. Structure of the SAP-precursor cDNA. The cDNA of the SAP-precursor encodes for a sequence of 524 amino acids (or 527 amino acids)[388] including a signal peptide (S) of 16 amino acids for entry into the ER.[227] The four domains of the precursor, SAP-A – D, correspond to the mature proteins found in human tissue: A = SAP-A or saposin A, B = SAP-B or SAP-1 or sulfatide activator protein or saposin B, C = SAP-C or SAP-2 or saposin C or glucosylceramidase activator protein, and D = SAP-D or saposin D or component C. ATG: translation start, TAG: translation stop, AAA: polyadenylation region. The positions of the known mutations (homoallelic) are indicated. a: A1T (Met1Leu);[401] b: g-t-transversion within the 3′-acceptor splicing site at the transition from intron e to exon 6;[440] c: C650T (Thr217Ile);[441, 442] d: G722C (Cys241Ser);[388] e: 33 BP insertion after G777 (11 additional amino acids after Met259);[443, 444] f: G1154T (Cys385Phe);[355] g: T1155G (Cys385Gly).[356]

discussed in connection with the mechanisms of lysosomal digestion and the AB-variant of GM-gangliosidosis. The number of patients in whom a sphingolipid activator protein defect could be identified is small, and the clinical picture of each of such activator deficiencies is incomplete. Amongst the activators that are produced from the SAP-precursor, only one case of SAP-precursor deficiency is currently known, as well as isolated cases of SAP-B or SAP-C defects. The structure of the SAP-precursor gene is known.[388, 389]

In addition to the already described SAP-B (Section 5.5), the activators SAP-C,[239] SAP-D,[236] and finally SAP-A have been isolated.[390] SAP-C stimulates the activity of glucosylceramidase towards natural and synthetic substrates. Unlike SAP-B, glycoprotein SAP-C binds not only to lipids but also to the enzyme and stimulates it directly.[391–393] It has a molecular weight of 20 kDa[239] and in vitro it stimulates the degradation of galactosylceramide by galactosylceramide-β-galactosidase[394] and sphingomyelin by acid sphingomyelinase.[394–396]

Insights into the physiological function of the SAPs could only be obtained after investigation on patients in whom a certain SAP is absent. Thus, SAP-B deficient patients[334, 397] show a drastic storage of sulfatide and the disease picture of a metachromatic leukodystrophy. In addition, these patients excrete increased amounts of globotriaosylceramide and digalactosylceramide in the urine.[398] The deficiency of SAP-C is expressed in a massive storage of glucosylceramide; the clinical picture of the patients corresponds to that of a juvenile variant of Gaucher's disease.[354, 399] Currently, no disease is known that is caused by the sole deficiency or defect of SAP-A or SAP-D.

The case of a patient who died at 16 weeks and his fetal brother was particularly interesting. They displayed morphologically Gaucher-like storage cells in the bone marrow and a massive lysosomal storage of glucosylceramide, lactosylceramide, and ceramide in the liver.[400] We were able to explain the molecular defect with the discovery in 1991 of a homoallelic mutation of the start codon of the SAP-precursor of ATG to TTG.[401] This causes a complete failure of the precursor protein and all four SAPs A – D. The analysis of this case gave decisive support for our hypothesis on the function of the activator proteins and for the topology of endocytosis and lysosomal digestion. As expected, the tissues of the patient and fetus stored increased amounts of all glycolipids with short oligosaccharide chains (Section 5.5): neutral glycolipids such as mono-, di-, tri-, and tetrahexosylceramide in the liver, kidney, and cultured skin fibroblasts.[402, 403] Sulfatide was stored in the kidney, and free ceramide in the liver and kidney. The amount of the gangliosides GM3 and GM2 was elevated in the liver, but not in the brain. The amount of phospholipid remained normal, as far as it has been investigated. Sphingomyelin metabolism was also not affected significantly in these patients.[402] We postulated that the accumulation of the multivesicular bodies was morphologically conspicuous (see Figure 18).[402] These could also be detected in cultured fibroblasts of the patient and as pathologically modified lysosomes under the electron microscope.[229] Feeding the patient with the missing precursor protein rectified both the storage of glycolipid and the occurrence of intralysosomal storage vesicles.[229] Genetically modified mice, which are homozygous for the inactivated gene of the SAP-precursor protein, correspond in their phenotype to the symptoms observed in humans.[404]

6.12. Pathogenesis of Sphingolipidoses

Sphingolipidoses belong to those inherited diseases where a lot of information is available about their biochemical basis and primary causes. The nature of the storage substances and the underlying defects at a protein and nucleic acid level are extensively known. This is in contrast to their pathogenesis, where correlation of genotype and phenotype is not yet possible. On the one hand, as with many other diseases, defects in quite different structural genes can lead to clinically similar disease pictures. Thus, defects in the α chains of the hexosaminidase A and S, defects in the β chains of the hexosaminidases A and B, and defects in the GM-activator lead to disease pictures that earlier were collected together under the term amaurotic idiocy.[263] On the other hand, different mutations in one and the same structural gene can lead to different disease courses that are sometimes even given different eponyms. Thus, severe forms of α-iduronidase defects are known as Hurler's disease, and the milder course as Scheie's disease.[337] A β-galactosidase deficiency can cause a GM1-gangliosidosis with neurological damage or lead to Morquio type B disease in which no neurological involvement is observed, but mainly skeletal deformation is evident.

Even patients with identical mutations within the same structural gene, for example in that of arylsulfatase A,[405] can show different clinical forms that are probably a result of a respective different genetic background. Knowledge of the primary defect at a DNA level is certainly a necessity, but in no way a sufficient condition for an understanding of the pathogenesis of these diseases. This applies especially to the adult forms of sphingolipidoses.[318] Despite their enormous heterogeneity and the only indirect correlation between genotype and phenotype of a disease form, it is possible to correlate the genotype and phenotype of the disease and to specify a number of significant factors that influence the pathogenesis. One significant factor is clearly the cell type specific expression of individual sphingolipids. This entails that during storage dysfunction, storage occurs primarily in cells and tissue in which the lipid substrates of the mutated enzyme step are predominantly synthesized (for example, complex gangliosides in neurons) or by which they are taken up during phagocytosis (for example, glucosylceramide storage in macrophages in Gaucher's disease).

A further factor is the nature of the storage material. It is assumed that sphingolipids are generally nontoxic, and their accumulation is tolerated by the cells within wide limits. Only by mechanical damage is the cell function disrupted. A degradation disorder can also lead to the accumulation of morphogenetically active compounds[406] or to a concentration of toxic lysoglycolipids.[365] A decisive factor is the residual enzyme activity of the mutated degrading system in the lysosome. Numerous examples have confirmed that the different extent of a degradation disorder results in different clinical forms of the disease. The measured residual activity of the affected enzyme correlates with the form of the disease The initial symptoms in neurolipidoses can be expected to arise when the function of the most severely affected nerve cells are impaired by storage. Accordingly, in the animal model of the AB-variant of GM2-gangliosidosis, only a defined population of nerve cells are observed in which GM2 accumulates.[291] The theory of residual activity also explains that a minimal change in residual enzyme activity can cause major changes in substrate turnover.

6.12.1. Theory of Residual Enzyme Activity

The extent of residual enzyme activity is a significant pathogenetic factor. The activity of the degrading system can be reduced to different residual levels within the lysosomal compartment by different mutations and pathobiochemical mechanisms. The level of this residual activity should have a direct effect upon the pathogenesis of clinical forms. The respective mutated protein and the interrelated maximum activities (V_{max}) of the degrading system form a decisive link between the genotype and phenotype of the respective disease form.

Starting from the Michaelis–Menten equation, we have calculated the substrate concentration $[S]_{eq}$ within the lysosome as a function of the residual activity under steady state conditions.[407] The substrate concentration $[S]_{eq}$ at the steady state is approximately dependent upon only two factors [Eq. (1)], the influx rate of the substrate into the lysosome v_i

$$[S]_{eq} = K_M/(V_{max}/v_i - 1)$$

and the enzyme kinetic parameters K_M and V_{max} of the degrading enzyme.

In normal cells the substrate concentration usually lies below the K_M value of the degrading enzyme, that is, the Michaelis–Menten constant. The K_M values of lysosomal enzymes usually are in the millimolar range. A reduction in enzyme activity to values of 20–50 % of the value of a normal cell does not affect the turnover rate v since the reduced enzyme activity is compensated by an increased substrate concentration and consequently a larger substrate saturation of the enzyme (Figure 25). The ratio v/v_i remains constant; this situation is met in heterozygous carriers of such inherited diseases. This compensation mechanism functions until the maximum degradation activity of the mutated enzyme falls below the value of substrate influx into the lysosome. At this threshold value ($v_{max}^{mut}/v_i = 1$) all enzyme molecules exist in the form of the enzyme-substrate complex; a reduction in residual enzyme activity to this theoretical threshold value, however, does not lead to an irreversible substrate accumulation. This explains why probands with pseudodeficiency with, for example a loss of up to 90 % of activity of γ-hexosaminidase A or arylsulfatase A are asymptomatic. Only a reduction in enzyme activity below the critical threshold value causes storage of the corresponding lipid substrate, since only a part of the entering substrate can be degraded.

This model was checked by substrate-flux and enzyme-activity measurements in cultured skin fibroblasts from different patients with GM2-gangliosidosis and metachromatic leukodystrophy[272] as well as with other sphingolipidoses.[256, 318, 319, 357, 385] Below a threhold value the substrate turnover initially falls linearly, as expected, with falling enzyme activity. Patients of the clinically heterogeneous adult form then show a significantly higher turnover of storage substances than patients of the clinically otherwise manifested juvenile form, and in turn these shows a higher turnover than the again otherwise manifested infantile form of the disease. As a result, small differences in residual enzyme activity consequently correspond to the significant differences in the respective metabolic flux.

6.13. Molecular Diagnosis: Metabolic, Enzymatic, and Genetic Procedures

The diagnosis of sphingolipidoses was initially based on clinical and pathological findings. The disease was later diagnosed on the basis of the biochemically identified storage material. The emphasis of current diagnostic procedures rests upon the determination of the enzymatic activity of the individual hydrolytic enzymes.[408, 409] Readily accessible clinical preparations of serum, leukocytes, cultured skin fibroblasts, or biopsy material serve as enzyme sources. Enzyme activities in amnion cells or chorion villi have been measured for prenatal diagnostic purposes. Metabolic procedures are irreplaceable for the diagnosis of diseases that may be attributed to the deficiency of enzymatically inactive sphingolipid activator protein. Diagnoses based on DNA analysis is

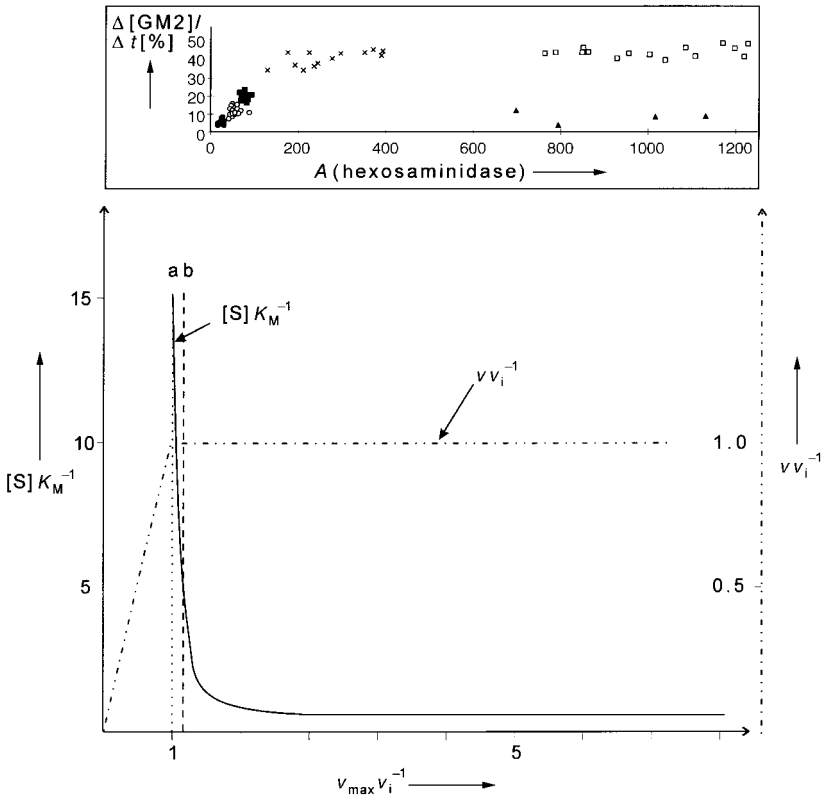


Figure 25. [223] Residual activity of a mutated enzyme and turnover of its substrate in the lysosomes.[407] The substrate concentration [*S*] is expressed as a multiple of the Michaelis constant K_M, the turnover rate v, and the maximum achievable enzyme activity (v_{max}) as a multiple of the influx rate (v_i). —— Substrate steady state concentration $[S]\,K_M^{-1}$; ••–•– Turnover rate of the substrate ($v\,v_i^{-1}$); •••• Critical threshold value of the enzyme activity (a). – – – Critical threshold value (b) of the enzyme activity with consideration of the limited solubility of the substrate. Above: residual activity *A* of hexosaminidases towards a ganglioside GM2 substrate (calculated as the amount of cleaved GM2 in vitro in $pmol\,h^{-1}mg^{-1}AU^{-1}$; AU = activator unit as defined in reference [280]) and turnover Δ[GM2]/Δt of ganglioside GM2 in fibroblast cultures of probands and patients with GM2 gangliosidoses.[272] Skin fibroblasts of normal probands and patients with different forms of GM2-gangliosidoses and their carriers were fed with a culture of radiolabeled ganglioside GM2 for three days. The cells were then harvested, homogenized in water, and the following three parameters were determined: a) total incorporation of the substrate, ganglioside GM2. b) percentage of degraded substrate, ganglioside GM2. c) activity of hexosaminidase A towards the ganglioside GM2 in the presence of the GM2-activator. ● α-chain deficiency (variant B of GM2-gangliosidosis or Tay–Sachs disease, ● infantile form; ○ α-chain deficiency, juvenile form; ■ α-chain defect, adult form; ▲ activator deficiency (variant AB of GM2-gangliosidosis). Healthy probands: × carrier of GM2-gangliosidosis; □ normal controls.

possible if the mutations within the family of the patient are known.

A particularly readily accessible enzyme source for the determination of enzymatic activity is serum, from which the activity of some, but not all hydrolases can be determined. A disadvantage is that the level of activity of lysosomal enzymes in serum is generally lower than in cellular sources, and the stability can be reduced, particularly those of mutated proteins. Blood plasma is not generally useful as an enzyme source as lysosomal hydrolases can be inhibited by anticoagulants. Leukocytes are a reliable and readily prepared enzyme source. For many questions it is advantageous to use lymphocytes, which by reason of their cellular homogeneity show a small scatter of measurement values. Cultured skin fibroblasts are another suitable enzyme source. Lysosomal hydrolases generally have low substrate specificity and their activity need not only be determined with the help of natural substrates, which are frequently difficult to handle, but also can be measured with the help of synthetic substrates (Figure 26) that have suitable fluorogenic or chromogenic properties.[408]

The demonstration of substrate storage in cultured patient cells after loading with radiolabeled substrate or catabolic substrate precursors is an additional diagnostic confirmation. For example, the degradation of ceramide[410, 411] or ceramide precursors such as sulfatide[410, 412, 413] and sphingomyelin[414] can be investigated for the diagnosis of Farber's disease. Such lipid loading studies show high degradation rates of endogenous ceramide despite existing Farber's disease. This is ascribed in part to the high residual activity of the mutated acid ceramidase or nonlysosomal localized degradation of the respective lipids.[414]

The comparison of biosynthetically labeled sphingolipids from patient cells with those of normal cells also permits the detection of sphingolipid storage. Serine radiolabeled in the 3-position allows the biosynthetic labeling of cellular sphingolipids. Sphingolipids whose degradation is disrupted contain stronger labeling after extended chase periods than those whose turnover is not impaired. A prerequisite is that a cell type is investigated in which the nondegradable lipid is also formed in significant amounts. An advantage of the method is that the enzymes and lipids occur in their natural, topologically correct subcellular environment. If lipid storage is detected with this cell system, its intensity correlates directly to the extent of the defect of the respective enzyme system. This also includes the detection of the failure of a cofactor whose deficiency is often overlooked in conventional diagnostic procedures. However, in patients whose enzyme shows a high residual activity, biosynthetic labeling is inferior to direct determination of enzyme activity.

6.14. Therapy

With the exception of the adult form of Gaucher's disease, causal therapy of sphingolipidoses is currently not possible. The objective of a number of therapeutic activities in sphingolipidoses is the re-establishment of defective degradative capacity within the lysosome. This can be achieved either directly by enzyme replacement therapy, or indirectly by gene therapy or organ transplantation. The aim of enzyme replacement therapy in sphingolipidoses is the restoration of

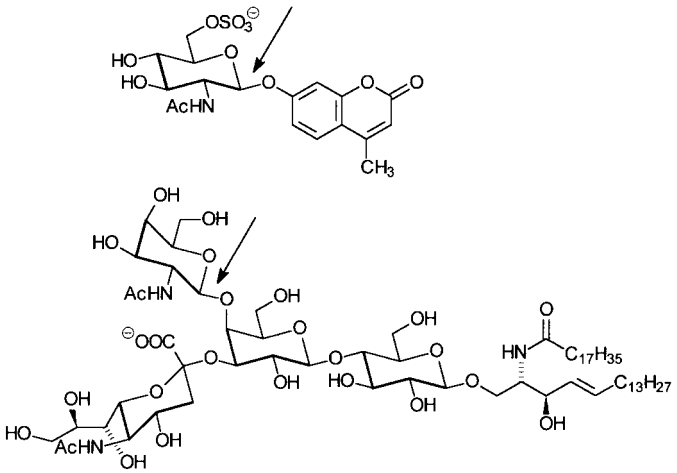


Figure 26. Structure of ganglioside GM2 and the synthetic substrate 4-methylumbelliferyl-β-D-*N*-acetylgalactosamine-6-sulfate. The arrow in each case indicates the bond that is cleaved by β-hexosaminidase A in the case of ganglioside GM2 in the presence of GM2-activator protein or surfactant.

nonexistent enzymatic activity by the exogenous addition of protein and thus alleviation of substrate storage or the prevention of further substrate accumulation. The concept of treating sphingolipidoses by the exogenous administration of the defective lysosomal enzyme is attributed to de Duve.[415] The main hurdles in its way are, on the one hand, the localization of the target cells within central nervous system in which substrate storage is to be alleviated in most sphingolipidoses and, on the other hand, for a considerable time no techniques have been available for the isolation of the required proteins in sufficient amounts and purity. The latter problem can be overcome by the availability of cDNA sequences of lysosomal proteins for recombinant DNA technology. Further problems are posed by stability and immunogenicity of the administered preparations, their half-life in humans, uptake by the respective target organs, and last but not least, the costs of such therapy. Since an adequate penetration of the blood–brain barrier[416] by systemically administered proteins cannot be presumed at the present time, enzyme replacement therapy remains for now restricted to diseases without primary impairment of the central nervous system.

The first sphingolipidosis that was treated successfully by enzyme replacement therapy is the adult form of Gaucher's disease (type I).[358] The reason is that storage of glucosylceramide is mainly limited to Kuppfer cells (hepatic macrophages) and other macrophages that express mannose receptors on their surfaces through which the exogeneously administered protein targeted at the lysosome can be incorporated.[417] There is no involvement of the nervous system because the patients have sufficiently high residual activity of the defective enzyme, glucosylceramidase, available. The initial difficulties encountered in the treatment of Gaucher's disease arose because the infused enzyme was taken up in the liver by hepatocytes, which incorporated an enzyme preparation isolated from human placenta through asialoglycoprotein receptors. The target cells of the enzyme, the Kuppfer cells, were not reached by the enzyme. If, however, an enzymatically modified enzyme preparation that exposes terminal mannose residues on its surface through partial glycosidase digestion is administered, it is incorporated by mannose receptors on the macrophages and is thus able to ameliorate a few of the symptoms of Gaucher patients. This includes regression of organ hypertrophy. The regression of skeletal deformations, which proceeds only very slowly, and the neuronopathic forms II and III remain problematical. A degeneration of the neurological state of the patient is indeed prevented in type III, but no improvement is achieved.[358, 360] Antibody formation has been reported, but neutralization of the enzyme clearly does not occur.[418]

6.14.1. Bone-Marrow Transplantation

The most significant obstacle in enzyme replacement therapy of neuropathic sphingolipidoses, namely passing the blood–brain barrier, would, in principle, be avoided by bone-marrow transplantation. Bone-marrow macrophages can cross the blood–brain barrier to a small extent and can serve as an enzyme source in the brain as microglia.[419] Although there have been individual reports of successful attempts, bone-marrow transplantation does not yet represent a successful concept for the treatment of sphingolipidoses. Bone-marrow transplantation in animal models of sphingolipidoses[420, 421] has led to an improvement in neurological symptoms and the regression of neuronal injury. The life expectancy of the twitcher mouse as an animal model of Krabbe's disease was increased by this form of treatment but recovery of the animal was not achieved.[420] A clinical improvement of "late onset" lipidosis patients (Krabbe's disease, MLD) has been reported, whereas the therapy is contra-indicated in infantile patients.[422]

6.14.2. Gene Therapy

Gene therapy applications in the treatment of sphingolipidoses are even less well advanced. Their objective is the insertion of a functional gene into the affected patient. The gene is inserted by means of suitable vectors into somatic cells that release a certain amount of the gene product into the environment. One major problem is the efficient transfer of therapeutic DNA into the central nervous system. Postmitotic cells including most neuronal cells are not transfected with retroviral vectors,[423] so that other vectors must be employed for many purposes. However, this system is also the subject matter of investigations. For example, at the cell culture level, transduction experiments have been carried out with retrovirally mediated galactosylceramidase cDNA.[424] Of the other different procedures for gene transfer,[425] replication-deficient adenoviruses deserve a special mention, which stand out in that they are suitable vectors for the introduction of recombinant genes into neuronal cells. In respect of pathogenicity and predictability of the level of expression, they appear superior to systems that are based upon the *Herpes simplex* virus. In the mouse model of a lysosomal storage disease, the Sly syndrome (mucopolysaccharidosis VII), the visceral pathology could be corrected by adenovirus-mediated gene transfer. After injection of the recombinant virus into

the lateral ventricle of mice a transient increase in β-glucuronidase activity in the brain of the experimental animals could be detected.[426, 450] Exemplary mention is also made to cell experiments in which GM2-storage in the fibroblasts of Tay–Sachs patients were eradicated after adenoviral-mediated gene transfer.[427]

6.14.3. Glycosphingolipid Biosynthesis Inhibitors

Inherited diseases may be treated fundamentally at the metabolite level. One of these methods is called "substrate deprivation" or "substrate restriction".[27, 428] One of the important factors that affect the severity and form of sphingolipidoses is the relationship of residual enzyme activity (V_{max}) to substrate influx (v_i) into the lysosome. As long as the biosynthesis of the substrate responsible for the defective degradation system continues, so does the pathological accumulation of the substrate in the lysosome. A change in the substrate influx into the lysosome (Figure 27) as well as the lysosomal degradative capacity, can affect the course of the sphingolipidosis. It should be possible to restrict influx into the lysosome by inhibition of sphingolipid biosynthesis. It should thus be possible to positively affect both the severity and form of these diseases with the help of inhibitors of biosynthesis. This method requires that a minimal residual activity of the defective enzyme is present in the lysosome. This is the case in juvenile and adult forms, whilst in the infantile form no or only a little residual activity has been found.[272] In the long term it may be assumed that nonexisting residual activity will be restored exogeneously, for example, by bone marrow transplantation, and at the same time the progression of the disease will be delayed by inhibition of substrate influx.

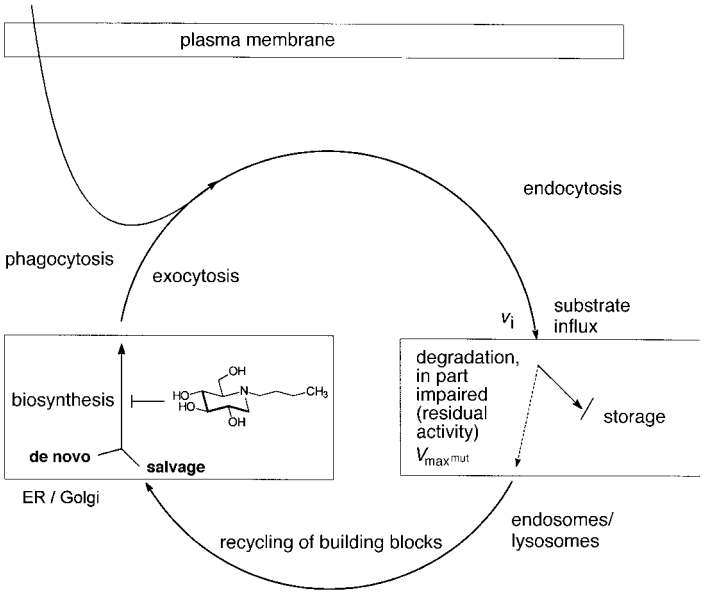


Figure 27. Substrate deprivation: the flow of substrate into the lysosome is reduced by inhibition of sphingolipid biosynthesis, whereas other therapy concepts aim to restore the degradative activity within the lysosome.

A number of low molecular weight inhibitors of GSL biosynthesis are known.[100] One active compound is of particular interest in connection with the therapy of sphingolipidoses.[429, 430] This is the already known glycosidase inhibitor, *N*-butyldeoxynojirimycin, which has been investigated in the animal model of Tay–Sachs disease.[431] Oral administration of the compound gives serum concentrations of 50 μM, which has already been demonstrated in human patients who have received this compound as part of an antiviral therapy. A 12 week treatment of Tay–Sachs mice has shown that this concentration is sufficient to achieve a 50% reduction in the storage of GM2 in the brain compared to untreated mice. Unlike untreated mice, after 16 weeks the neuronal cells of treated mice had only a few cytoplasmic membrane structures arising from lipid storage. Its low toxicity and its high bioavailability are important for the activity of the compound. It can be absorbed orally and it passes the blood–brain barrier so that sufficiently high concentrations are reached in the central nervous system to inhibit the formation of GSL to the desired extent (Figure 27).[431] The success of the compound was not totally predictable, since it not only functions as an inhibitor of biosynthesis, but also as an inhibitor of glucocerebroside degradation.

Glucocerebrosidase was purified by affinity chromatography on *N*-alkyl-deoxynojirimycin sepharose,[432] which would appear to make the suitability of the compound for the treatment of Gaucher's disease problematical. Tay–Sachs knockout mice have such a high residual enzyme activity that they do not fall ill and in this respect are suitable as a model system for checking the concept. The compound inhibits glucosyltransferase, which transfers glucose from uridine diphosphate glucose to ceramide (Section 4.3). Fundamentally, treatment with compounds of this type come into question in those patients who store sphingolipids that are derived biosynthetically from glucosylceramide. The successful use of this or similarly active compounds in human patients is at this time still hypothetical, but on the basis of the above considerations, there are realistic chances of treating sphingolipidoses successfully.

7. Future Prospects

Critical information on the pathogenesis and therapeutic methodology may be expected from animal models that were developed for the human form of sphingolipidoses.[433, 434] For example, after hints on the involvement of the immune system in the pathogenesis of Krabbe's disease, double knockout mice that showed a significantly milder disease progression were obtained from the crossing of twitcher mice (an authentic animal model for Krabbe's disease) with MHC (major histocompatibility complex) class II deficient mice.[435] Bone marrow transplantations with retrovirally modified cells has been tested in β-glucuronidase-deficient mice.[436, 437] The phenotype of the galactosialidosis mouse model could be corrected by transgenic and normal bone marrow cells.[298] These methods show that these recently available animal models are valuable aids for understanding the pathogenesis of sphingolipidoses. They are certainly of extraordinary value for the further development of novel therapeutic concepts. It may be assumed that these preclinical models will signifi-

cantly simplify the testing of concepts for the causal therapy of these diseases, and improved treatment possibility will follow as a result.

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