Metabolic and cellular bases of sphingolipidoses

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Abstract

Lysosomes are cellular stomachs. They degrade macromolecules and release their components as nutrients into the cytosol. Digestion of sphingolipids and other membrane lipids occurs at luminal intraendosomal vesicles and IMs (intraendosomal membranes). Sphingolipid and membrane digestion needs catabolic hydrolases with the help of lipid-binding proteins [SAPs (sphingolipid activator proteins)] and anionic lipids such as BMP [bis(monoacylglycero)phosphate]. Inherited defects of hydrolases or SAPs or uptake of cationic amphiphilic drugs cause lipid accumulation, eventually leading to death, especially in inherited sphingolipid storage diseases. IMs are formed during endocytosis and their lipid composition is adjusted for degradation. Their cholesterol content, which stabilizes membranes, decreases and the level of negatively charged BMP, which stimulates sphingolipid degradation, increases. At the level of late endosomes, cholesterol is transported out of the luminal vesicles preferentially by cholesterol-binding proteins, NPC (Niemann–Pick type C)-2 and NPC-1. Their defects lead to an endolysosomal accumulation of cholesterol and sphingolipids in Niemann–Pick type C disease. BMP and ceramide stimulate NPC-2-mediated cholesterol transfer, whereas sphingomyelin inhibits it. Anionic membrane lipids also activate sphingomyelin degradation by ASM (acid sphingomyelinase), facilitating cholesterol export by NPC-2. ASM is a non-specific phospholipase C and degrades more than 23 phospholipids. SAPs are membrane-perturbing proteins which solubilize lipids, facilitating glycolipid digestion by presenting them to soluble catabolic enzymes at acidic pH. High BMP and low cholesterol levels favour lipid extraction and membrane disintegration by saposin A and B. The simultaneous inherited defect of saposins A–D causes a severe membrane and sphingolipid storage disease, also disrupting the water permeability barrier of the skin.

Introduction

SLs (sphingolipids) are amphiphilic molecules which are poorly soluble in water and aggregate in aqueous solutions. Gangliosides form stable micelles with an apparent molecular mass of more than 1000 kDa.

SLs are major membrane components of eukaryotic cells and serve as bioactive signalling components. In contrast with phosphoglycerolipids, the main building blocks of cellular membranes, GSLs (glycosphingolipids), are expressed in a cell-type-specific pattern on cellular surfaces. Gangliosides are typically expressed on neuronal membranes, GalCers (galactosylceramides) and sulfatides in myelin and kidney, globosides in visceral organs, VLC-Cers (very-long-chain ceramides) and GSL-containing VLC-Cers in keratinocytes and sperm cells.

VLC-Cers and very-long-chain GlcCers (glucosylceramides) are key components of the water permeability barrier in the epidermis of land-dwelling animals [1], and SLs containing polyunsaturated VLC-Cer moieties are essential building blocks for the maturation of sperm cells and male fertility [2,3].

Biosynthesis of sphingolipids

GSLs and SMs (sphingomyelins) are synthesized by acyl- and glycosyl-transferases bound to the membranes of the secretory pathway. Their hydrophobic membrane anchors are formed at ER (endoplasmic reticulum) membranes by six different ceramide synthases, which differ in specificity by the lengths of their acyl residues [3,4]. Glucosylation of ceramides at the cytosolic surface of Golgi membranes is followed by the formation at the luminal side of Golgi membranes of lactosylceramide, which serves as a precursor of the complex GSLs and gangliosides [5].

Although inherited biosynthetic defects have been extensively characterized in murine models [6,7], only two diseases have been identified so far in patients [8–10].

Sphingolipidoses

SL storage diseases have been known for a long time. Clinical descriptions were followed by the identification of storage compounds, analysis of their metabolism, identification of inherited catabolic enzyme and protein deficiencies, the

Key words: endocytosis, intraendolysosomal vesicle, lipid transfer protein, sphingolipid, sphingolipidosis.

Abbreviations used: ASM, acid sphingomyelinase; BMP, bis(monoacylglycero)phosphate; ESCRT, endosomal sorting complex required for transport; GlcCer, glucosylceramide; GM2AP, ganglioside GM2 activator protein; GSL, glycosphingolipid; IM, intraendosomal membrane; MLD, metachromatic leukodystrophy; NPC, Niemann–Pick type C; SAP, sphingolipid activator protein; SL, sphingolipid; SM, sphingomyelin; VLC-Cer, very-long-chain ceramide.

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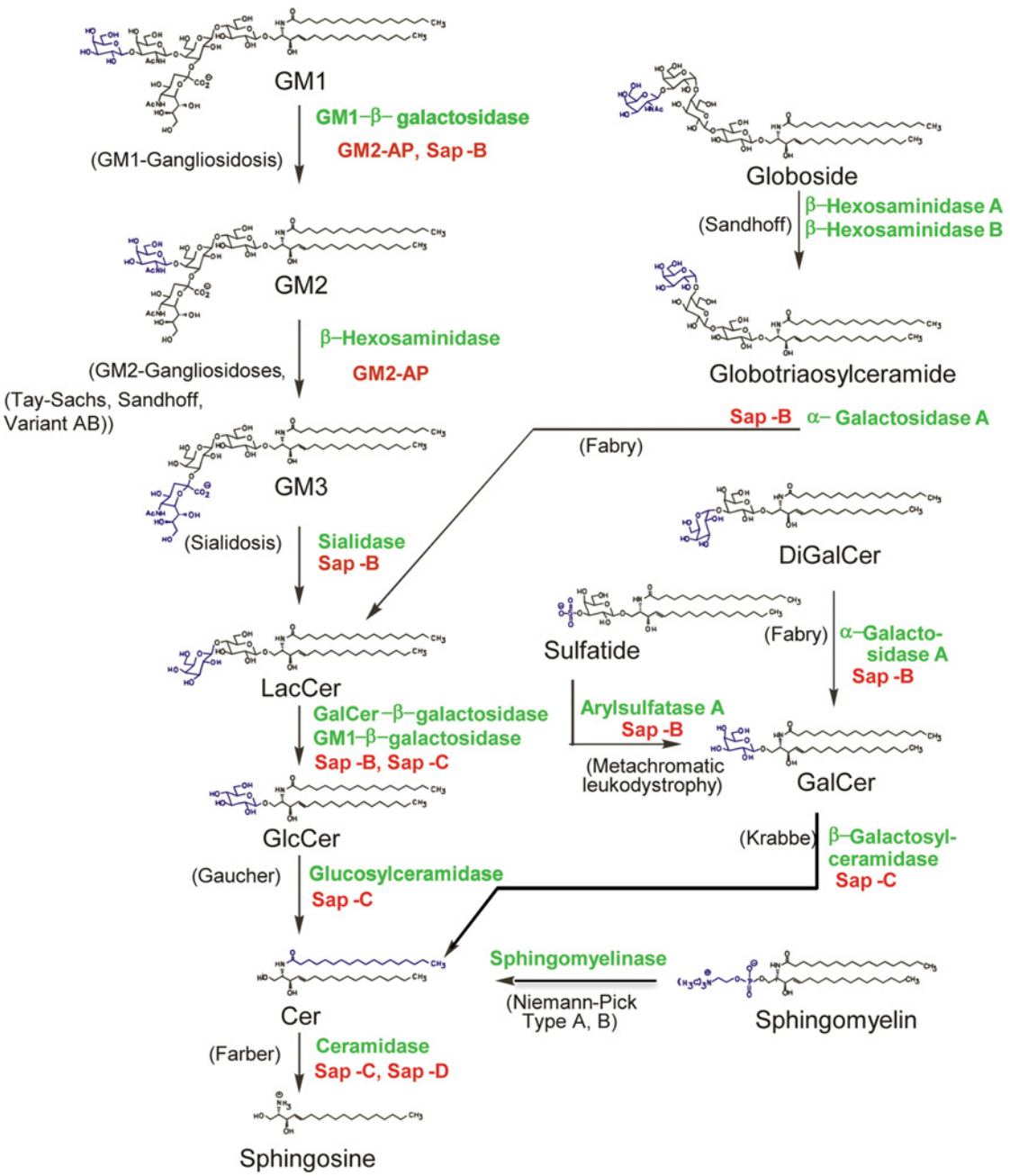
Figure 1 | **Pathway of lysosomal sphingolipid degradation**

The eponyms of known metabolic diseases and those of SAPs necessary for *in vivo* degradation are indicated. Heterogeneity in the lipid part of the SLs is not indicated. Variant AB, variant AB of GM2 gangliosidosis (deficiency of GM2AP). Data taken from [5].



analysis of the genes encoding these proteins and the identification of disease-causing mutations (Figure 1). For most of the sphingolidoses, especially the neurodegenerative storage diseases, no therapy is available. Recently, however, ERT (enzyme replacement therapy) has been successfully introduced for patients with the adult forms of Gaucher and Niemann–Pick type B diseases, which do not involve the CNS (central nervous system). Since the field of sphingolipidoses has been reviewed several times [11–14], I focus on a few basic concepts and new developments. A more detailed description can be found in earlier reviews [12,14].

Topology of endocytosis and lysosomal digestion of sphingolipids

Lysosomes are intracellular stomachs. They degrade macromolecules and release their components as nutrients

into the cytosol for use in salvage pathways and energy metabolism. Macromolecules and membrane components reach the lysosomal compartment for digestion by autophagy [15], phagocytosis [15] and endocytotic pathways [16].

GSLs and SMs of cellular surfaces are delivered to luminal intraendolysosomal vesicles or IMs (intraendosomal membranes) for digestion [17,18]. Luminal vesicles are generated during endocytosis by successive steps of vesicle budding and scission controlled by ESCRT (endosomal sorting complex required for transport) proteins [19]. They are prepared for lysosomal digestion by a lipid sorting process, which begins at the level of endosomes [16,20] by removal of membrane-stabilizing lipids. Cholesterol is removed by two sterol-binding proteins, NPC (Niemann–Pick type C)-1 and NPC-2 [21], and SM is degraded by ASM (acid sphingomyelinase). ASM is a non-specific phospholipase C that cleaves more than 23 phospholipids and is regulated by membrane lipids (V.O. Oninla, B. Breiden and K. Sandhoff, unpublished work). Anionic BMP [bis(monoacylglycero)phosphate], which stimulates SL catabolic steps substantially, is formed from phosphatidylglycerol in the luminal IMs [20,22]. Whereas the lysosomal perimeter membrane seems to be quite resistant against lysosomal degradation, IMs are digested with the help of lipid-binding proteins [SAPs (sphingolipid activator proteins)] and hydrolytic enzymes. Perimeter membranes are protected by a thick glycocalyx enriched in polylactosamine structures and by a high cholesterol level [23]. Their high lateral pressure attenuates the insertion of GM2AP (ganglioside GM2 activator protein), a lipid-binding protein essential for ganglioside catabolism [24]. Ganglioside, GSL and SM catabolism proceeds at the surface of luminal intralysosomal vesicles and membrane structures [16].

Whereas water-soluble lysosomal hydrolases can attack water-soluble macromolecules directly, the digestion of gangliosides, GSLs and membranes needs a more complex co-operation between soluble hydrolases and lipid-binding and transfer proteins co-operating on the surface of luminal intraendolysosomal vesicles [16,25]. Owing to the lipid phase problem, water-soluble glycosidases hardly attack amphiphilic GSLs as components of vesicular membranes, but need the help of membrane-perturbing and lipid-binding proteins, the SAPs. Their inherited defects cause rare, but fatal, neurodegenerative diseases [12,26]. A deficiency of prosaposin, the common precursor of four SAPs, causes membrane storage and the loss of the water permeability barrier in the skin [27].

My view on the topology of lysosomal ganglioside digestion is given in Figure 2.

Regulation of lipid trafficking and catabolic steps by membrane lipids of the late endosomes

Inherited defects of the cholesterol-binding proteins NPC-1 or NPC-2 cause the cholesterol trafficking disease Niemann–Pick type C disease, attenuating the cholesterol egress from the luminal vesicles in the late endosomes [28]. NPC-2 is a small soluble glycoprotein that can transfer cholesterol from liposomes directly to NPC-1 of the endosomal perimeter membrane or to other vesicles [28,29]. *In vitro*, the transfer of cholesterol between liposomes is stimulated by anionic BMP and other anionic phospholipids, phosphatidic acid, phosphatidylinositol, phosphatidylserine and phosphatidylcholine [30], and is strongly inhibited by SM [21]. Pre-incubation of the SM-containing vesicles with ASM releases this block (V.O. Oninla, B. Breiden and K. Sandhoff, unpublished work). This may explain the secondary cholesterol accumulation in patients with Niemann–Pick type A and B diseases, in which sphingomyelinase is deficient [31].

Currently, we assume that the luminal vesicles of late endosomes mature by a lipid-sorting process, which degrades inhibitory SM and thereby facilitates cholesterol egress by NPC-2 and NPC-1. The maturing luminal vesicles with a low cholesterol and an increasing BMP content are then degraded by lysosomal hydrolases with the help of SAPs and anionic BMP. This view is supported by further observations: cholesterol accumulation in NPC disease triggers a small accumulation of minor gangliosides, e.g. GM2 and GM3 [32]. On the other hand, increased cholesterol levels in liposomal membranes inhibit the lipid solubilization and mobilization by the SAPs tested so far, Sap A and Sap B [33,34] (Figure 2).

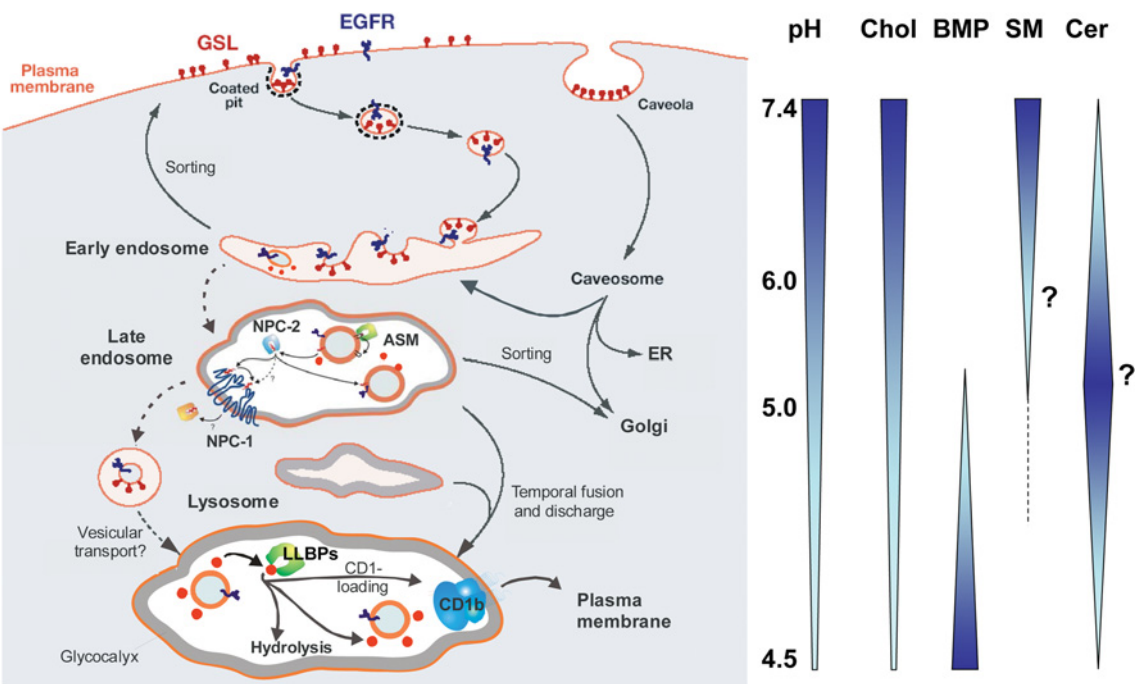
The broad specificity of glycosidases has been known for a long time, and it was no surprise to find out that GM1-β-galactosidase hydrolyses many other β-galactosides besides gangliosides GM1 and GA1 [12]. Mutations in its gene can even change its substrate specificity as discussed above and affect the catabolism of GSLs and glycosaminoglycans differently.

It was also no surprise to realize that human β-hexosaminidases have broad and partially overlapping substrate specificities. Again, some mutations in the *HEXA* gene, encoding hexaminidase A, may also change the substrate specificity of the patients' hexaminidase A: those that disrupt enzyme activity only against anionic substrates (such as GM2) and not against neutral substrates (such as globoside) cause the B1 variant of GM2 gangliosidosis [35], whereas those that affect hexaminidase A activity against all substrates including anionic and neutral substrates cause Tay–Sachs disease (variant B of GM2 gangliosidoses).

The first activator protein described, the sulfatide activator protein (now called saposin B), was discovered as an essential cofactor for the lysosomal catabolism of sulfatides [36]. Its deficiency causes a late infantile form of MLD (metachromatic leukodystrophy) with a pronounced sulfatide storage [36,37]. Saposin B forms stoichiometric soluble complexes with sulfatides which form a Michaelis–Menten complex with arylsulfatase A. Although this suggested a high lipid-binding specificity for saposin B, human saposin B is a promiscuous lipid-binding protein [38,39]. It even stimulates the hydrolysis of bacterial lipids by bacterial hydrolases [40].

Figure 2 | Topology of endocytosis, lysosomal lipid and membrane digestion

Proposed topology of endocytosis and lysosomal degradation [20]. A section of the plasma membrane is internalized by way of coated pits or caveolae. These membrane patches include GSLs (red) and receptors such as EGFR (epidermal growth factor receptor; blue). These vesicles fuse with the early endosomes which mature to late endosomes. Endosomal perimeter membranes form invaginations, controlled by ESCRT proteins [19], which bud off, forming intraendosomal vesicles. Lipid sorting occurs at this stage. The pH of the lumen is approximately 5. At this pH, ASM is active and degrades SM of the intraendosomal vesicles to ceramide, whereas the perimeter membrane is protected against the action of ASM by the glycocalyx facing the lumen. This decrease in SM, coupled with the increase in ceramide, facilitates the binding of cholesterol to NPC2 and its transport to the perimeter membrane of the late endosome where it is transferred to NPC-1 protein [28]. This protein mediates the export of cholesterol through the glycocalyx, thereby eventually reaching cholesterol-binding proteins in the cytosol. Ultimately, late endosomes fuse with lysosomes. The GSLs are harboured in intralysosomal vesicles that face the lumen of the lysosome, and are degraded by hydrolases with the assistance of SAPs. The products of this degradation are exported to the cytosol or loaded on CD1b immunoreceptors and exported to the plasma membrane for antigen presentation. Gradients of pH in the lysosol, and the content of cholesterol (Chol), BMP, SM (hypothetical) and ceramide (Cer, hypothetical) in intraendolysosomal vesicles are shown. LLBP, lysosomal lipid-binding protein. Modified from FEBS Letters, **584**(9), Kolter T, Sandhoff K, Lysosomal degradation of membrane lipids,1700–1712 [25], © 2010, with permission from Elsevier.



The other saposins are also rather non-specific lipid-binding proteins, as is the GM2AP [41].

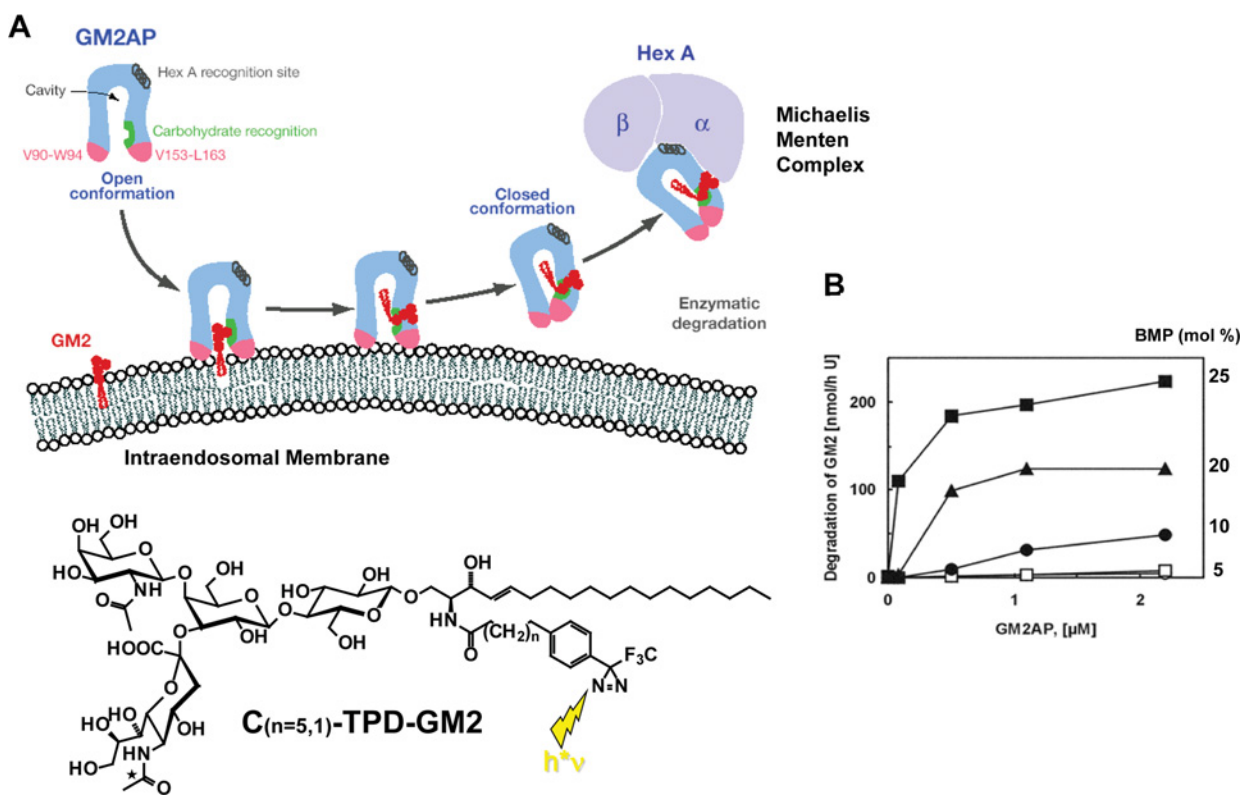
Membrane lipids are regulators of ganglioside and SL catabolism

The promiscuity of endolysosomal proteins and enzymes makes it difficult to understand the precise molecular pathology of the diseases caused by a defect of enzymes and proteins involved in SL catabolism. The complexity is increased further by the strong lipid-dependency of many endolysosomal proteins, especially since the lipid composition of many organelle membranes is mostly unknown, particularly in patients' tissues. *In vitro* experiments have shown that catabolic steps at surfaces of substrate-carrying liposomes, mimicking luminal endolysosomal vesicles, need not only low pH values, enzymes and SAPs for significant degradation rates, but also a sufficient concentration of anionic lipids such as BMP in the liposomal membranes.

Both GM2AP and saposin B can enable the hydrolysis of GM1 by β-galactosidase. Therefore a defect of neither GM2AP nor saposin B causes a GM1 accumulation, since the other unaffected protein efficiently facilitates the reaction. However, the reaction is rather slow in neutral liposomes and is stimulated 2–9-fold in the presence of saposin B by anionic phospholipids (BMP, phosphatidylinositol, dolichol phosphate, phosphatidylserine) [42]. Even at acidic lysosomal pH values, they convey negative charges to the vesicular

Figure 3 | Mechanism of GM2AP – liftase

(**A**) Model for the interaction of GM2AP with luminal lysosomal membranes in the degradation of ganglioside GM2. GM2AP interacts with the membrane by dipping the two exposed hydrophobic loops, Val^{90}–Trp^{94} and Val^{153}–Leu^{163}, into the apolar part of the membrane. Ganglioside GM2 is recognized by specific sites at the rim of the cavity. In the open protein conformation, the large hydrophobic area reaching from the apolar phase of the membrane to the activator's cavity lowers the energy barrier for lipids leaving the membrane in an upward direction. After the ceramide tail has arrived inside the activator's cavity, the inward-bending of the hydrophobic loop Val^{153}–Leu^{163} is favoured, and the conformation changes to the closed form [51]. This folding-in of the hydrophobic loop leaves a more polar patch close to the membrane. The activator is then anchored only by the Val^{90}–Trp^{94} loop. It may now rotate slightly upwards to expose all polar patches more fully to the solvent, and it may also leave the membrane and interact with the degrading enzyme. The photoaffinity label analogues $C_{(n=5,1)}$-TPD-GM2 were photoincorporated specifically into the Val^{153}–Leu^{163} region [46]. Hex A, hexaminidase A. Modified from Wendeler, M., Hoernschemeyer, J., Hoffmann, D., Kolter, T., Schwarzmann, G. and Sandhoff, K. (2004) Photoaffinity labelling of the human GM2-activator protein. Eur. J. Biochem. **271**, 614–627 with permission [52]. (**B**) BMP and GM2AP stimulate the hydrolysis of liposome-bound ganglioside GM2. The degradation of ganglioside GM2 inserted into LUVs (large unilamellar vesicles) containing 0 mol% BMP (○), 5 mol% BMP (□), 10 mol% BMP (●), 20 mol% BMP (▲) or 25 mol% BMP (■) was measured in the presence of increasing concentrations of GM2AP (0–2.2 μm). Modified with permission from Werth, N., Schuette, C.G., Wilkening, G., Lemm, T. and Sandhoff, K. (2001) Degradation of membrane-bound ganglioside GM2 by β-hexosaminidase A: stimulation by GM2 activator protein and lysosomal lipids. J. Biol. Chem. **276**, 12685–12690 [43].



surfaces, which attract and bind the protonated and positively charged hydrolases and SAPs to catalyse the hydrolytic reaction. This situation also applies to the degradation of the liposomal ganglioside GM2 by hexaminidase A in the presence of GM2AP. GM2AP is essential for the enzymatic hydrolysis of ganglioside GM2, even in the presence of higher BMP concentrations. Its absence causes the fatal neurodegenerative AB-variant of GM2-gangliosidosis *in vivo*. However, the reaction is still stimulated by anionic BMP and other anionic phospholipids, phosphatidic acid, phosphatidylinositol and phosphatidylserine more than 100-fold [43] (Figure 3). Downstream reactions are also stimulated by anionic membrane lipids. At concentrations of anionic phospholipids higher than 10 mol% in the liposomal membranes, the cleavage of GlcCer by β-glucosidase [44] and of ceramide by acid ceramidase [45] proceeds even in the absence of a SAP. Saposin D stimulates the reaction rate up to 3-fold. The rate also increases with the curvature of

the vesicular membranes of the ceramide-carrying liposomes [45].

Protracted clinical forms of sphingolipidoses and the threshold theory

An almost complete loss of catabolic activities usually results in an excessive lysosomal lipid accumulation in infantile forms of diseases, triggering pathological mechanisms.

(i) Expansion of lysosomal compartment at the expense of cytosol and other organelles.

(ii) Impaired digestion of macromolecules: inhibition of catabolic enzymes and proteins (accumulating lipids such as SM inhibit NPC-2 and lipid sorting, cholesterol inhibits saposins A and B, etc.). Accumulation of cytotoxic cationic lipids: GalSo (galactosylsphingosine) (psychosine hypothesis, Krabbe disease), glucosylsphinganine, sphingosine and sphinganine. They inhibit catabolic steps and lysosomal digestion and trigger proteolysis of ASM and other catabolic hydrolases.

(iii) Release of pro-inflammatory cytokines, activation of microglia and influx of macrophages.

Allelic mutations, however, which produce proteins with some residual catabolic activities give rise to protracted milder clinical forms often described as late infantile, juvenile or chronic diseases [46] with a wide range of clinical symptomatology. At the biochemical level, the clinical heterogeneity is paralleled by a variation of the extent and the pattern of glycolipid accumulation and by different levels of residual catabolic activities detected in cultures of patients' fibroblasts. This observation can be modelled very well on the basis of a greatly simplified kinetic model [46,47]. A nice correlation was observed between the predicted and the measured lipid substrate turnover, and also between the rate of the ganglioside GM2 turnover and the clinical course of Tay–Sachs disease and its related juvenile and adult forms. Only 10–20 % of normal GM2-cleaving activity appears to be sufficient for normal life. Similar observations were reported for MLD, Gaucher, Sandhoff and sphingomyelinase-deficient Niemann–Pick disease [48–50].

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