1 INTRODUCTION

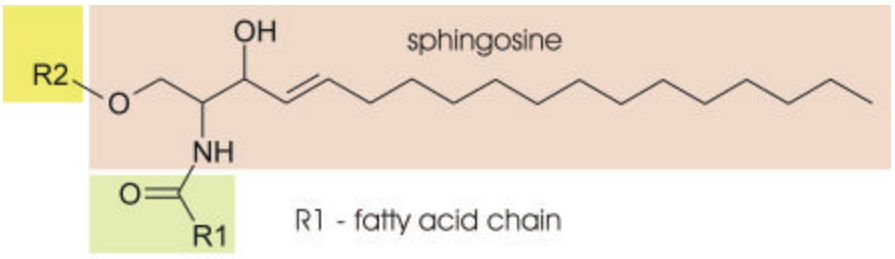
Despite the rapid progress in biochemistry, fundamental questions remain unanswered about the role of sphingolipids for health and disease. Although previously considered to be simply structural molecules, sphingolipids have recently been shown to act as signalling molecules in many key cellular functions and to play crucial roles in the regulation of pathobiological processes, such as cancer, cardiovascular and neurodegenerative disorders, inflammation, and infectious diseases. Thus sphingolipids are expected to have a significant impact in the future of health care.

1.1 Sphingolipids

Sphingolipids are derived from the aliphatic amino alcohol sphingosine (Fig. 1) and form, together with glycerophospholipids and cholesterol, important structural components of eukaryotic membranes. Ceramide is the simplest type of sphingolipid. It consists of a fatty acid chain attached through an amide linkage to sphingosine. In sphingomyelin and glycosphingolipids (GSLs), a phosphorylcholine or a carbohydrate moiety are bound to the terminal hydroxyl group of ceramide, respectively. Variations in the type, number and linkage of sugar residues within the oligosaccharide chain give rise to the wide range of naturally occurring glycosphingolipids. Glycosphingolipids can be further subdivided into cerebrosides, sulfatides, globosides, and gangliosides (Fig. 1).

Sphingolipids can be synthesized from endogenous and exogenous sphingoid bases. *De novo* biosynthesis of sphingolipids takes place in the endoplasmatic reticulum (ER) and in Golgi compartments (van Echten and Sandhoff, 1993), where the stepwise addition of activated sugar residues occurs and leads to the large number of different glycosphingolipids observed in eukaryotic cells. After the biosynthesis, GSLs reach the plasma membrane through vesicular exocytotic membrane flow (Kolter and Sandhoff, 2006). Most of the glycosphingolipids are present in the outer leaflet of the plasma membrane (Bretscher, 1973) forming species- and cell-type-specific patterns at the cell surface that change during cell growth, differentiation, viral transformation, and oncogenesis (Hakomori, 1981). Ceramide-based lipids are believed to self-aggregate in cellular membranes to form microdomains or "rafts", which are less fluid

than the bulk liquid-disordered glycerophospholipids (Cremesti et al., 2002) and play an important role in the organization of membrane structure. The integrity of rafts is, for example, critical for HIV entry, as modulation of cellular cholesterol or sphingolipid levels results in inhibition of HIV infection (Viard et al., 2004). The microdomains have also been implicated in the function of reproductive and immune systems and in molecular trafficking. In the absence of ceramide synthesis, glycosylphosphatidylinositol (GPI)-anchored proteins lose their membrane association, even though they have hydrophobic GPI-anchors (Watanabe et al., 2002)



Substituent (R2)	Sphingolipid
H-	Ceramide
$N^+(CH_3)_3CH_2CH_2(OPO_3^-)$-	Sphingomyeline
Galß-	Cerebroside
3-(OSO_3^-)-Galß1-4Glcß1-	Sulfatide
GalNAcß1-4Galα1-4Galß1-4Galß1-	Globoside
Galß1-3GalNAcß1-4Galß1-4Glcß1- ¦ Neu5Acα2 -3	Ganglioside

Figure 1. General sphingolipid structure. All sphingolipids contain a sphingoid long-chain base (e.g. sphingosine) that is linked to a fatty acid molecule (R1) through an amide bond thereby forming ceramide. The fatty acid tail R1 is between 16 and 24 carbon atoms long, but the most sphingolipids contain 16- to 18-carbon-atom-long chains. The addition of different substituents (R2) gives rise to the wide range of naturally occurring glycosphingolipids.

Furthermore, glycosphingolipids and their modified catabolites were recently found to be involved in specific functions like recognition and signaling including apoptosis (Pettus et al., 2002). Thus they could serve as possible novel targets for antiretroviral (Finnegan et al., 2004) and cancer therapy (Fehrenbacher and Jaattela, 2005).

1.2 Glycosphingolipid degradation

In most cell types, the majority of glycosphingolipids is formed within a salvage pathway (Tettamanti, 2004).

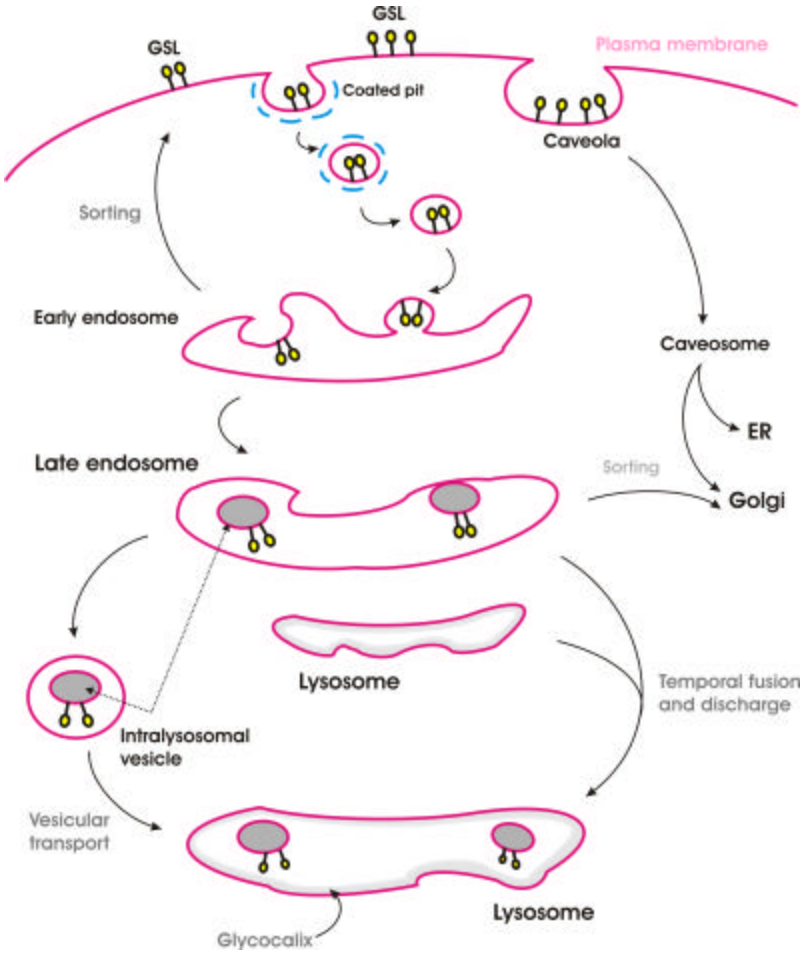


Figure 2. Endocytosis and lysosomal digestion of membranes. GSLs destined for degradation are endocytosed, sorted and traffic through the endosomal compartments to reach lysosomes. Within the lysosomes they are believed to be incorporated into intralysosomal vesicles and presented to specific acid exohydrolases for degradation. The lysosomal perimeter membrane is assumed to be protected from degradation by a thick glycocalix (Schuette et al., 2001).

GSLs destined for degradation are endocytosed, sorted and traffic through the endosomale compartments to reach the lysosome (Maxfield and McGraw, 2004) (Fig. 2). Within the lysosome, GSLs are believed to be incorporated into intralysosomal vesicles, which bud off into the lysosomal lumen and become a substrate for specific acid exohydrolases (Sandhoff and Kolter, 1996). The curvature of the vesicle membrane and its lipid composition appear to be important for GSL degradation by lysosomal hydrolases (Kolter and Sandhoff, 2006). The intralysosmal vesicles are enriched in cholesterol and anionic lipids like bis-(monoacylglycero)-phosphate (BMP) (Amidon et al., 1996) and phosphatidylinositol that attract activator proteins and hydrolytic enzymes. The degradation of glycosphingolipids occurs by stepwise action of specific water-soluble lysosomal exohydrolases, which remove the respective terminal sugar residues before the ceramide backbone is hydrolyzed to sphingosine and a free fatty acid. The sphingolipids with long sugar moieties are situated far enough from the membrane surface and are accessible to the hydrolases. In contrast, the degradation of glycosphingolipids with fewer than four carbohydrate residues requires the assistance of sphingolipid activator proteins (saposins, Sap's), which mediate the interaction between the water-soluble hydrolytic enzymes and their membrane-bound substrates.

1.3 Sphingolipid activator proteins

Sphingolipid activator proteins (saposins or Sap) are highly homologous proteins and belong to the large and divergent family of saposin-like proteins (SAPLIPs) and domains containing the "saposin fold", characterized by four or five adjacent amphipathic a-helices forming bundles stabilized by conserved disulfide bridges or by cyclization (Gonzalez et al., 2000). The saposin fold was identified in cytolytic proteins like granulysin (Anderson et al., 2003), NK-lysin from pig (Liepinsh et al., 1997), poreforming proteins from amoeba (Hecht et al., 2004), and in the bacteriocin AS-48 (Gonzalez et al., 2000). A similar helix bundle motif was also found in jellyfish lens crystallin (Piatigorsky et al., 2001), neurotrophic factors (Bornhauser et al., 2003; Kishimoto et al., 1992; O'Brien et al., 1994; O'Brien et al., 1995), and domains of several hydrolytic enzymes like human acyloxyacyl hydrolase (Staab et al., 1994), plant aspartic proteases (Egas et al., 2000; Kervinen et al., 1999; Simoes and Faro, 2004), and human sphingomyelinase (Lansmann et al., 2003).

The basic helical core structure of SAPLIPs is adapted to carry out diverse functions, but their primary feature is the interaction with lipid membranes. The four human saposins A, B, C and D are small non-enzymatic glycoproteins produced in late endosome/lysosome by sequential proteolysis of the precursor protein prosaposin (Fürst W., 1988; Hiraiwa et al., 1993a; O'Brien and Kishimoto, 1991). Within the lysosome, human saposins extract monomeric lipid antigens from lysosomal membranes and facilitate their loading onto human CD1d molecules, which present them to effector T cells (Kang and Cresswell, 2004; Winau et al., 2004; Yuan et al., 2007). Additionally, saposins were also found to be essential cofactors in the degradation of glycosphingolipids in lysosomes (O'Brien and Kishimoto, 1991), as they assist water-soluble specific exohydrolases to access their targets, membrane-bound GSLs and ceramide (Fig. 3). Inherited deficiencies of either lysosomal hydrolases or saposins lead to various mostly lethal diseases characterized by the storage of multiple GSLs in a variety of organs (O'Brien and Kishimoto, 1991).

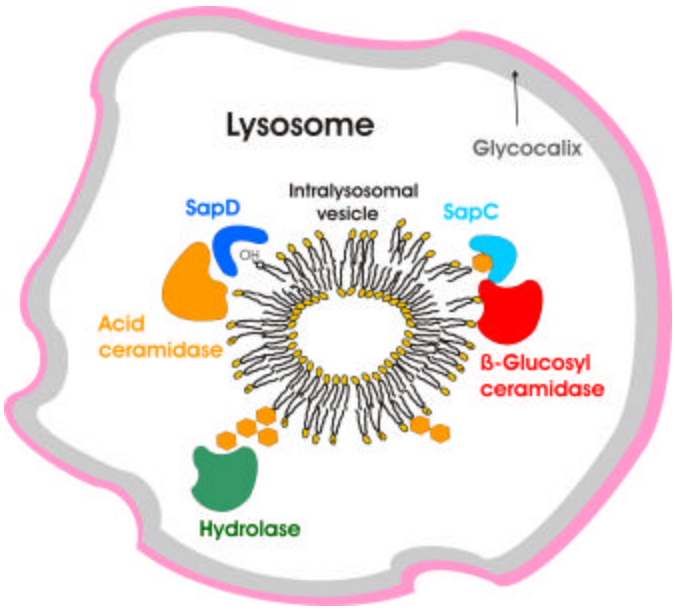


Figure 3. Model of saposin activated hydrolysis of glycosphingolipids by exohydrolases. Saposins mediate the interaction between membrane-bound GSLs with short oligosaccharide chains and water-soluble specific exohydrolases by facilitating their access to head groups of sphingolipids (Wilkening et al., 1998).

Biophysical studies have shown that the interaction of saposins with membranes is a complex process critically dependent on pH and the presence of anionic lipids (Ciaffoni et al., 2001; Ciaffoni et al., 2003). SapC is able to destabilize membranes containing phospholipids and is capable of removing lipid molecules from bilayers (Alattia et al., 2006). It is noteworthy that SapC can also induce the reverse process – the fusion of phospholipid containing vesicles at low pH (Vaccaro et al., 1994; Wang et al., 2003) – a feature not exhibited by other saposins.

The structure determination of saposins has already provided insights into their general functional mechanism (Ahn et al., 2003; Ahn et al., 2006). In all cases, ligand-free saposin adopted monomeric "compact bundle-type" structures. The solution structure of SapC bound to sodium dodecyl sulfate (SDS) micelles depicted a very different "boomerang-shape" conformation with an exposed hydrophobic surface (Hawkins et al., 2005). A similar, yet distinct, fold was found for SapB, where two V-shaped monomers form a shell around encapsulated lipids bound in the hydrophobic cavity (Ahn et al., 2003). These findings indicated that conformational flexibility is probably required for the biological functions of saposins.

1.4 Direct interactions of saposins with hydrolytic enzymes

Despite their similar structures, each saposin promotes the degradation of particular sphingolipids by a specific enzyme or set of enzymes in lysosomes, although saposins exhibits certain cross-reactivity, which is not surprising considering that a wide range of sphingolipids have to be activated by only several saposins. In particular, SapC potentatiates the activity of ß-glucosylceramidase (Christomanou et al., 1986; Harzer et al., 1997; Ho, 1971) and ß-galactosylceramidase, enzymes responsible for glucosylceramide and galactosylceramide degradation, respectively. Notably, *in vitro,* SapA was also capable to activate ß-glucosylceramidase, but it is less effective than SapC, which was also shown to promote human acid ceramidase (Linke et al., 2001), ß-galactosidase, and GM1-ß-galactosidase activities. It is not clear whether the cross-reactivity of SapC is achieved by affecting the membrane or the target enzyme, as SapC was found to interact directly with target enzymes. It protects ß-glucosylceramidase against degradation and was shown to induce conformational changes of this enzyme (Qi and Grabowski, 1998). It can also not be

excluded that SapC in turn enhances the specificity of the hydrolases to protect the cell from their adverse hydrolytic activity.

The apparent interaction of saposins with their target enzymes is likely to be weak, as a large molar excess of saposins over lipids is required to achieve maximum activity of the associated hydrolytic enzymes. Notably, a similar activation effect can be achieved when saposins are replaced by equal or larger excess of acidic detergents, suggesting that saposins rather modulate membranes structure and provide microenvironments that optimize sphingolipid hydrolysis.

1.5 Sphingolipidoses

Sphingolipidoses are inherited sphingolipid storage diseases characterized by sphingolipid accumulation in various tissues as a result of a defect of one of the exohydrolytic enzymes or activator proteins (O'Brien and Kishimoto, 1991). Together with mucopolysaccharidoses, mucolipidoses, glycoprotein- and glycogen-storage diseases, sphingolipidoses belong to rare (collective frequency is 1 in 7000-8000 live births) lysosomal storage disorders (LSDs).

A blockage of the catabolic processes ("salvage pathway") leads to cell death and contributes to the pathogenesis of these diseases, whereas most of the LSDs are associated with neurodegeneration and high mortality.

Because of the sequential degradation pathway of GSLs, defects of almost every step in their degradation have been described (Fig. 4). The most common form of sphingolipidoses, Gaucher disease, is caused by ß-glucosylceramidase or SapC deficiency leading to accumulation of glucosylceramide (Brady et al., 1965; Rafi et al., 1993). One mutation in SapC and more than 200 mutations at the β-glucosylceramidase locus have been identified in patients with Gaucher disease until now. SapC deficiency leads to an unusual juvenile form of Gaucher disease and an accumulation of glucosylceramide in the brain, liver and spleen (Schnabel et al., 1991), and a recently obtained mouse with combined deficiencies of saposins C and D developed a lethal neuronopathic phenotype (Sun et al., 2007).

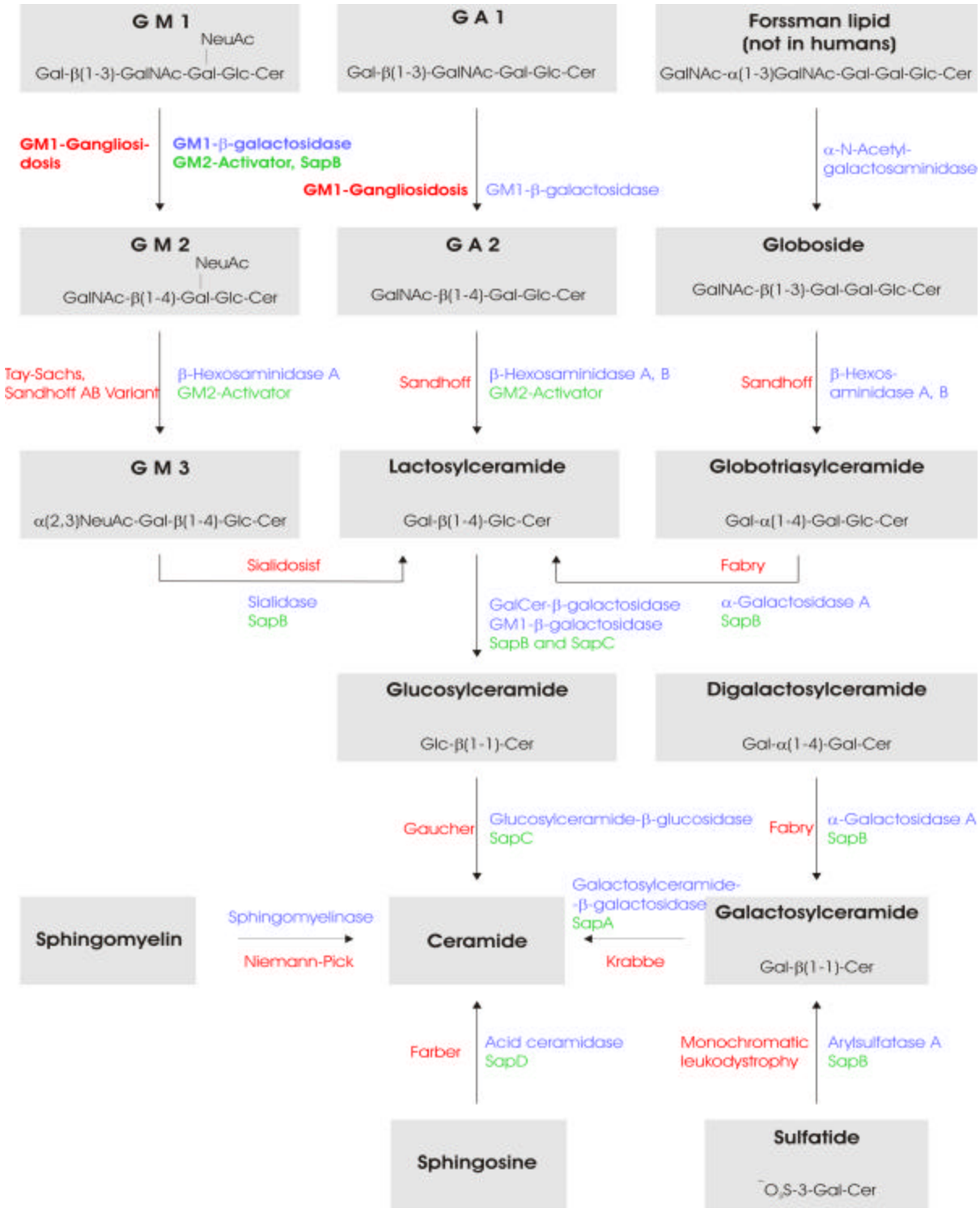


Figure 4. Degradation of selected sphingolipids in cell lysosomes. The eponyms of individual inherited diseases are given in red; activator proteins and hydrolytic enzymes required for the respective degradation step are indicated in green and blue, respectively.

Many of the sphingolipidoses are untreatable. The object of most current therapy strategies that are in use or in development is the restoration of the defective degradation capacity. Enzyme replacement therapy was successfully used in treatment of Gaucher disease and Fabry disease (Desnick, 2004). Other therapeutic approaches like gene therapy, enzyme-enhancement or substrate-reduction therapies are still under evaluation (Desnick and Schuchman, 2002; Jeyakumar et al., 2005; Platt et al., 2005).

1.6 Human acid ceramidase

Human acid ceramidase (ASAH; N-acetylsphingosine deacylase, EC 3.5.1.23) is a glycoprotein derived from the single precursor protein of 53~55 kDa, which is subsequently processed to the mature heterodimeric enzyme in the late endosome/lysosome (Bernardo et al., 1995) (Fig. 5).

The mature ASAH consists of a non-glucosylated a-subunit and a glucosylated ß-subunit covalently linked to each other by a disulfide bridge. Three of the six glucosylation sites in the ß-subunit are essential for enzyme activity (Ferlinz et al., 2001). Ceramidase is a lysosomal enzyme and targeted to endosomes and lysosomes by a mannose-6-phosphate receptor mediated pathway (Ferlinz et al., 2001). ASAH catalyzes the final step of sphingolipid degradation, the hydrolysis of ceramide into sphingosine and free fatty acid (Gatt, 1963). *In vivo,* lysosomal cleavage of ceramide requires the coordinated action of ASAH and activator proteins SapC and SapD, which render the amide bond of ceramide more accessible to the active site of the enzyme (Kishimoto et al., 1992). The pH optimum of acid ceramidase lies at 4.5, but at pH ~6 ASAH can carry out the reverse reaction and synthesizes ceramide from free fatty acids and sphingosine *in vitro* (Okino et al., 2003). Thus, acid ceramidase may play a critical role in the regulation of ceramide and sphingosine metabolism.

ASAH is indispensable for ceramide degradation. A complete knock-out of acid ceramidase genes in a mouse model led to early embryonic lethal phenotype (Li et al., 2002). An inherited deficiency of ASAH activity causes a fatal accumulation of ceramide in patients suffering from Farber disease (FD) (Farber et al., 1957). FD is

a very rare autosomal recessive disorder, and fewer than 100 cases have been reported since the first description in 1957.

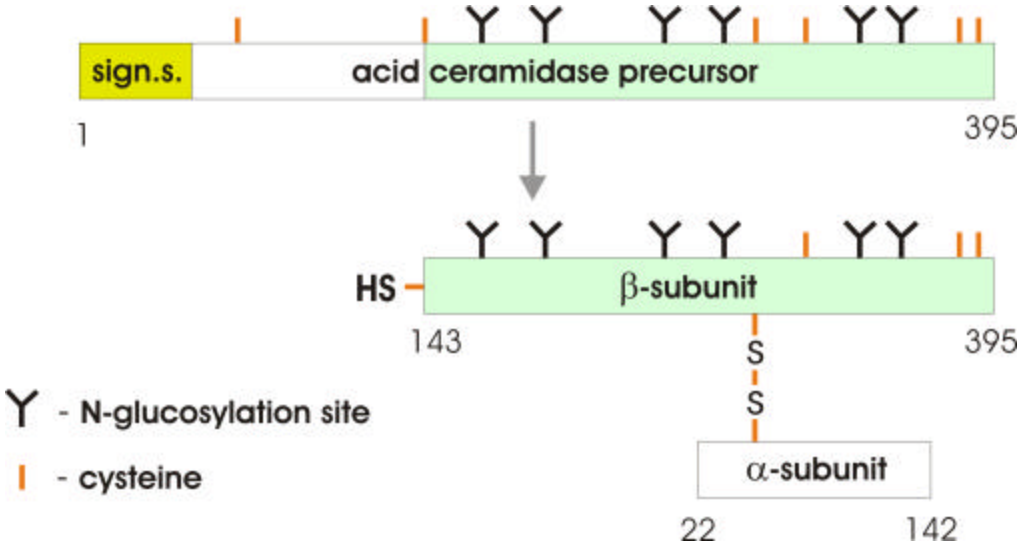


Figure 5. Processing and post-translational modification of human acid ceramidase. After removing of the signal sequence in ER, ASAH precursor is targeted to lysosomes, where it is cleaved between residues Ile142 and Cys143, thereby exposing the N-terminal cysteine, which is designed to act as a nucleophile. The mature ASAH consists of a non-glucosylated a- and a glucosylated ß-subunit covalently linked to each other by a disulfide bridge ((Schulze et al., 2007).

Patients with significant neurological involvement usually die early in infancy, whereas patients without or only mild neurological findings suffer from progressive joint deformation and contractures, subcutaneous nodules, inflammatory, periarticular granulomas, and a hoarse voice. Finally, respiratory insufficiency caused by granuloma formation in the respiratory tract and interstitial pneumonitis leads to death in the third or fourth decade of life.

Farber disease has been classified in seven subtypes (Park and Schuchman, 2006), and up to date 17 different mutations in the ASAH gene were identified in FD patients, of which 12 led to amino-acid substitution. Notably, FD of type 7 occurs in patients with intact ASAH, but with prosaposin deficiency, demonstrating the

importance of activator proteins for the activity of ASAH *in vivo* (Kishimoto et al., 1992).

1.7 Biological role of ceramide

Ceramide serves not only as a structural component of cell membranes and *stratum corneum* (Elias, 1983), but it also acts as signaling molecule to regulate fundamental cellular responses. Recent studies have shown that acid ceramidase is overexpressed in several human cancer cell lines (Kolesnick, 2002), whereas ceramide levels are significantly reduced (Selzner et al., 2001). Acid ceramidase is up-regulated in 60% of primary prostate cancer tissues, where it affects not only cell proliferation and migration, but also responses to drug therapies (Saad et al., 2007).

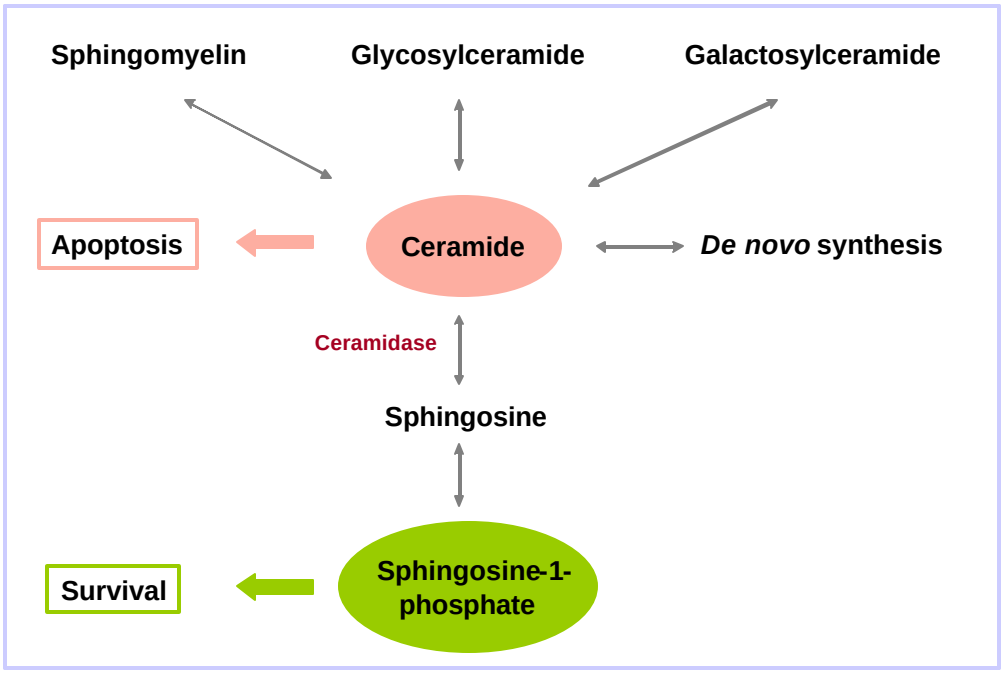


Figure 6. Apoptosis induction by ceramide. Inhibition of ceramidase activity increases the intracellular ceramide concentration and leads to apoptosis.

Knock down of the acid ceramidase using siRNA made prostate cancer cells more sensitive to pro-apoptotic antitumor drugs (Saad et al., 2007). Together with the findings that the treatment of tumor cells with cell-permeable ceramide analogs can induce apoptosis, it was assumed that the cellular balance between ceramide and

sphingosine 1-phosphate is crucial for the decision of a cell to either undergo apoptosis or to proliferate (Fig. 6), two events which are implicated in tumor development and growth. Ceramide mediates its intracellular effects through the release of cytochrome *c* from mitochondria (Yang et al., 1997), which activate the caspase-cascade, and through specific protein kinases, phosphatases and pshospholipases. Ceramide was found to activate the protein kinase CAPK (Adam-Klages et al., 1996) and phosphatase PP2A (Law and Rossie, 1995), and to inhibit phospholipase D (Jones and Murray, 1995). The modulation of the activities of these enzymes by ceramide can lead to the apoptosis.

Whereas ceramide possesses proapoptotic capacity in many cell types, sphingosine 1-phosphate acts as a counter player able to induce cell proliferation and to protect cells from undergoing apoptosis (Fig.6). Therefore, tipping the balance in favor of ceramide production, i.e. by inhibiting ceramidase, has the potential to support the pro-apoptotic action of antitumor drugs and hence represents a promising rational approach to effective cancer therapy (Huwiler and Zangemeister-Wittke, 2007). Thus, acid ceramidase was proposed to be a suitable therapeutic target, and the catalytic mechanism of ASAH is of great interest for the design of specific inhibitors. Sequence similarity searches indicated that the β-subunit of ceramidase is homologous to the conjugated bile acid hydrolase (CBAH) from *Clostridium perfringens,* suggesting that this bacterial enzyme could serve as a homology model.

1.8 Biological role of bile salts

Bile salts are produced by hepatocytes in the liver, representing the major consumption of cholesterol. In most animals, bile salts are stored in the gallbladder between the meals and are discharged into duodenum upon eating, whereas 95% of them are reabsorbed in the ileum and are reused.

Bile salts are 'biological detergents' that affect the digestion by absorption of lipids. They emulsify fats and are important for the regulation of the activity of bile-salt dependent lipases (Aubert-Jousset et al., 2004). The most abundant of the bile salts in humans are cholate and deoxycholate, and they are normally conjugated with either glycine or taurine (Fig. 7).

Once in the intestine, the bile acids may be converted to a number of metabolites by the intestinal microflora. One of the common bile salt transformations is catalyzed by bacterial bile salt hydrolases (BSH) which cleave the amide bond between the bile acid carboxyl group and amino group of either glycine or taurine. BSH activity was detected in many bacterial genera that reside in the mammalian intestine, among them C*lostridium, Enterococcus, Fusobacterium, Streptococcus* and the commercially popular "probiotic" *Bifidobacterium* and *Lactobacillu*s strains (Tanaka et al., 1999).

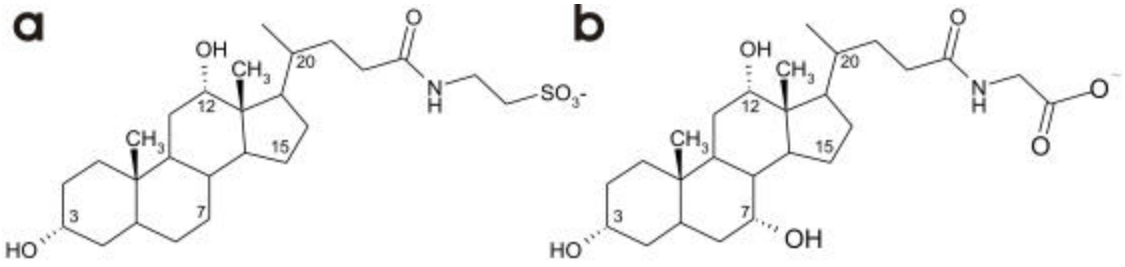


Figure 7. Chemical structures of (a) deoxycholoyltaurine and (b) choloylglycine.

Within the intestine, BSH active bacterial strains regulate the delicate balance of the cholesterol enterohepatic re-circulation by competing for bile acids with the active re-uptake in the terminal ileum (Dietschy, 1974; Kurz et al., 2003), thereby effectively lowering serum cholesterol levels (Eyssen, 1973). Deconjugated bile salts excreted more rapidly than conjugated bile acids. As a consequence, more cholesterol is needed to synthesize new bile acids, which in turn reduces the total cholesterol in the body. That is why BSH active bacterial strains have been administered orally to intervene in cholesterol circulation (De Smet et al., 1998).

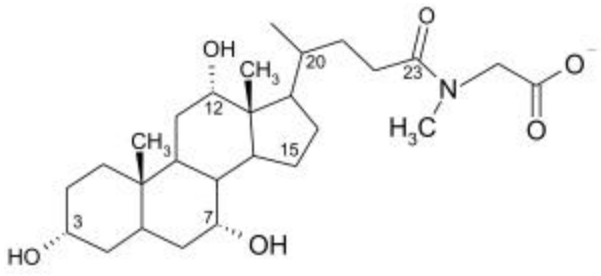


Figure 8. Chemical structure of choloylsarcosine.

The biological function of BSH enzymes remains unknown. It is believed that BSH's protect the bacteria against toxic bile salts and contribute to the ability of bacteria to infect the host. No specific BSH inhibitors are available, although the semi-synthetic bile salt choloylsarcosine (SC) (Fig. 8) could be a competitive inhibitor of bile salt hydrolases (Schmassmann et al., 1993), because it was shown to be resistant to bacterial deconjugation. SC is used in the treatment of short bowel syndrome (SBS), a disorder occurring as a consequence of a section of long segments of small intestine (Furst et al., 2005).

1.9 Conjugated bile acid hydrolase (CBAH)

Conjugated bile acid hydrolase (CBAH) is a bile salt hydrolase from *Clostridium perfringens* [E. C. 3.5.1.24] and was first shown in 1967 to have catalytic activity towards conjugated bile acids (Nair et al., 1967). The enzyme was expressed in *E.coli*, purified and characterized (Coleman and Hudson, 1995). CBAH was shown to have a native molecular weight of 147,000 Da and a subunit molecular weight of 36,100 Da, indicating its probable existence as a tetramer (Coleman and Hudson, 1995). The sequence similarity of 34% between CBAH and penicillinV acylase (PVA) from *Bacillus sphaericus* suggested that CBAH would belong to the N-terminal nucleophile (Ntn) hydrolase superfamily

1.10 Ntn-hydrolases

The N-terminal nucleophile hydrolases are characterized by a conserved aßßa-folding pattern and by autocatalytic cleavage of N-terminal residues to expose an N-terminal as nucleophile (Brannigan et al., 1995; Oinonen and Rouvinen, 2000). All the known

members of this family are synthesized as pre-proteins and catalyze the hydrolysis of amide bonds that are present in proteins or in small molecules.

In the last decade several three-dimensional structures of Ntn-hydrolases have been determined: penicillin G acylase (PGA) from *E. coli* (Duggleby et al., 1995), penicillin V acylase from (PVA) from *B. sphaericus* (Suresh et al., 1999), cephalosporin acylase (CA) from *Pseudomonas sp.* (Kim et al., 2003), proteasome subunit (Pr) from *Saccharomyces cerevisiae* (Groll et al., 1997), glutamine PRPP amidotransferase (Grpp) from *B. subtilis* (Smith et al., 1994), and human aspartylglucosaminidase (AGA) (Oinonen et al., 1995). Crystallographic and biochemical research revealed that the catalytic centers between Ntn-hydrolases are very similar and resemble the classical catalytic triad known from serine proteases.

The classical catalytic triad usually refers to the three amino acids serine, histidine and aspartate, which act together to break the peptide bond (Fig. 9a). The side chains of serine and histidine serve as a nucleophilic attacking group and general base, respectively. The role of the aspartate seems to be complex. It is believed that the aspartate increases the pK_a of the imidazole nitrogen to yield a stronger base, which in turn deprotonates the side chain of the serine residue.

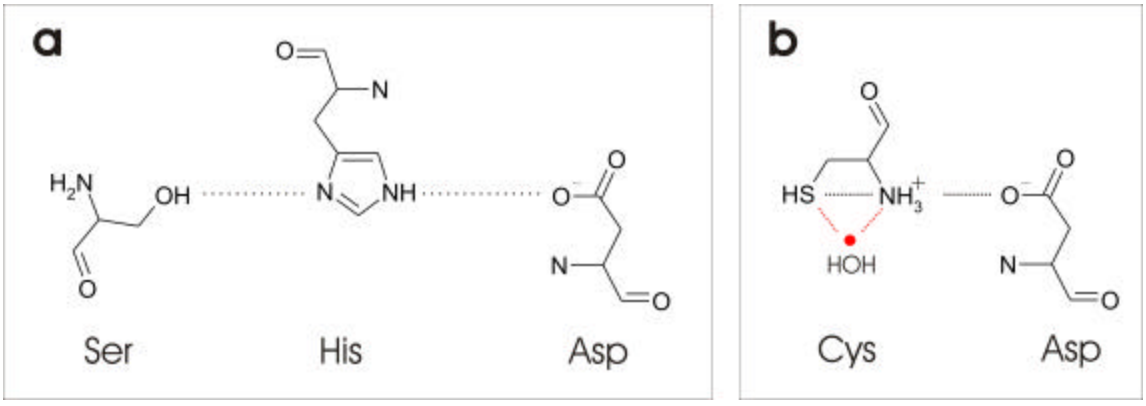


Figure 9. (a) Classical catalytic triad. The side chains of serine and histidine serve as a nucleophilic attacking group and general base, respectively, and aspartate is believed to increase the basicity of the general base. **(b) Proposed catalytic dyad in the Ntn-hydrolases.** The side chain of cysteine serves as a nucleophilic attacking group, and its α-amino group acts as a general base. The proton transfer from the hydroxyl group of serine to the α-amino group could take place

via a water molecule (shown in red). Aspartate is believed to increase the basicity of the α-amino group.

All Ntn-hydrolases have a similar mechanism of action consisting of an attack of the nucleophilic sulfur of the cysteine or oxygen of the serine or threonine hydroxyl group on the α-carbon of the scissile peptide bond. In contrast to the classical triad in serine proteases, Ntn-hydrolases employ two catalytic residues only, as the attacking nucleophilic group and the general base the free N-terminal α-amino group are from the same residue (Fig. 9b).

Despite the lack of any discernible amino-acid sequence similarity, the Ntn-hydrolases are characterized by a fold that brings the active site nucleophile in a similar position in the active site. Apart from the close catalytical similarities of the Ntn-hydrolases, a sequence homology in these proteins is not obvious. Thus, three-dimensional structure determinations in combination with biochemical data are the only reliable way to identify Ntn-hydrolases.

1.11 Biotechnological applications of Ntn-hydrolases

Penicillin acylases PGA and PVA are Ntn-hydrolases with unknown physiological role. Nevertheless, they are successfully used in the industrial production of semi-synthetic β-lactam antibiotics (Arroyo et al., 2003; Lopez-Gallego et al., 2004), where they substitute several chemical steps, thereby reducing dramatically the waste disposal costs.

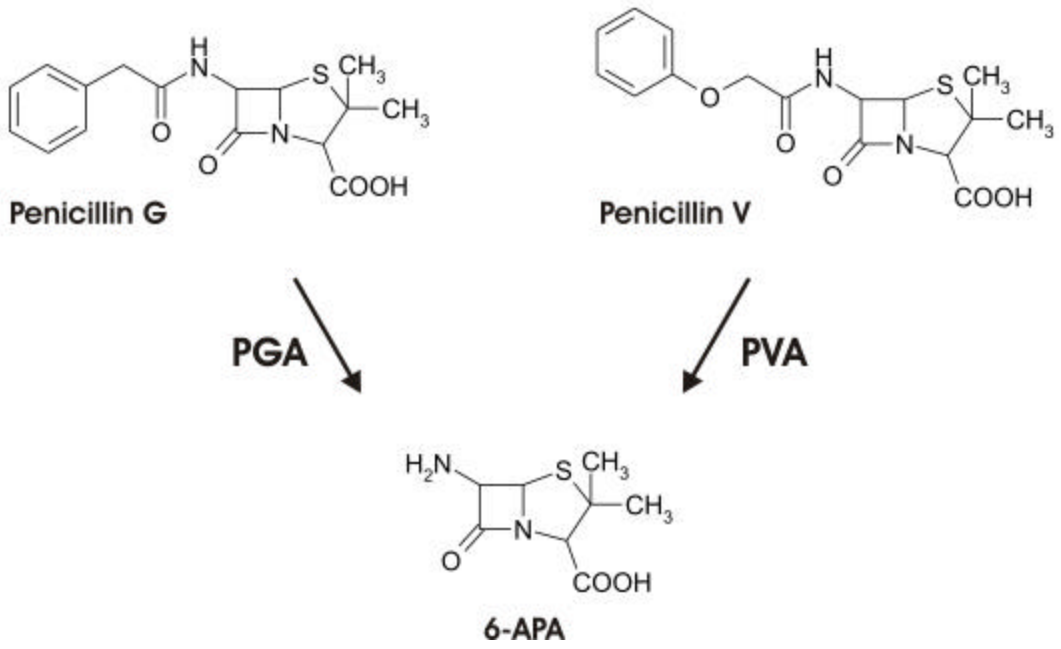


Figure 10. Processing of penicillin G and penicillin V to 6-APA (6-aminopenicillanic acid). Penicillin G acylase and penicillin V acylase catalyze the hydrolysis of penicillin G and V, respectively, to yield 6-APA which is then elaborated into a range of semi-synthetic penicillins.

As the β-lactam antibiotics account for over 65% of the world antibiotic market, PGA and PVA are the targets of protein-engineering experiments to enlarge their specificity for the biotechnological production of 6-aminopenicillanic acid (6-APA) (Fig. 10) from penicillin G and penicillin V, respectively. However, only 15% of all manufactured 6-APA worldwide is produced from penicillin V, although the use of it could be advantageous (Shewale JG, 1997). The intermediate antibiotic nuclei 6-APA is then used in the enzymatic or conventional chemical routes for the synthesis of semi-synthetic penicillins like for example ampicillin, as they can catalyze the reverse reaction at appropriate conditions (Arroyo et al., 2003).

Another natural β-lactam antibiotic, cephalosporin C (CPC) (Fig. 11), and its derivatives are the first-choice antibacterial compound in many countries.

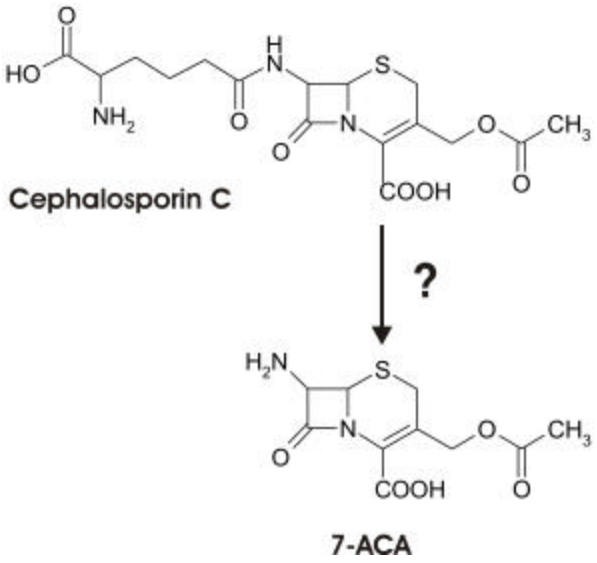


Figure 11. Desired processing of cephalosporin C (CPC) to 7-ACA (7-aminocephalosporanic acid). A one step enzymatic reaction leading directly from CPC to 7-ACA would be of great interest.

According to IMS Health (http.//www.imshealth.com), cephalosporins were ranked number 10 in the global drug sales in 2002 with estimated sales of 7.6 billion US$. In contrast to penicillins, cephalosporins are produced chemically or semi-chemically (Lit method, Bayer et al., 1999) using expensive and hazardous chemicals. The enzymatic penicillin acylase based one-step deacylation leading directly from CPC to 7-ACA, an intermediate required for the production of the β-lactam antibiotics on the basis of cephalosporin C, is of great commercial importance and currently under investigation (Gaurav et al., 2007).

Further, Ntn-hydrolases can also be employed in other useful “green” chemical reactions, such as peptide and amide synthesis (protection and deprotection of the amino groups) and in the resolution of racemic mixtures of chiral compounds (Van Langen LM, 2000). Thus, the understanding of the structural origin of the catalytic efficiency of Ntn-hydrolases provides a basis for the design of enzymes with improved conversion rates and substrate specificities. Crystallographic analysis of CBAH may provide working models for biochemically engineered changes that might alter the specificity in PVA and possibly in other Ntn-hydrolases.