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MINI REVIEW

Sphingolipid activator proteins: proteins with complex functions in lipid degradation and skin biogenesis

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Sphingolipid activator proteins (SAPs or saposins) are essential cofactors for the lysosomal degradation of membrane-anchored sphingolipids. Four of the five known proteins of this class, SAPs A–D, derive from a single precursor protein and show high homology, whereas the fifth protein, GM2AP, is larger and displays a different secondary structure. Although the main function of all five proteins is assumed to lie in the activation of lipid degradation, their specificities and modes of action seem to differ considerably. It has recently been demonstrated that the action of the proteins is highly enhanced by the presence of acidic lipids in the target membranes. These results have some interesting implications for the topology of lysosomal degradation of lipids and may provide new insights into the function of these interesting proteins, which are ubiquitously expressed in the different tissues of the body.

Recent studies indicated that the SAPs play an important role in the biogenesis of the epidermal water barrier, which has been demonstrated by the analysis of the skin phenotype displayed by SAP-knockout mice. The results obtained so far have led to some new insights into the formation of the epidermal water permeability barrier and may lead to a better understanding of this complex process.

Key words: epidermal permeability barrier/GM2-activator protein/membrane activity/saposins/sphingolipidoses

Introduction

Sphingolipid activator proteins (SAPs) are small, enzymatically inactive glycoproteins that are essential cofactors in the degradation of glycosphingolipids with short oligosaccharide headgroups. Gangliosides and glycosphingolipids that form part of the extracellular leaflet of the plasma membrane reach the lysosome after endocytosis or phagocytosis. There, they are degraded by a series of water-soluble lysosomal hydrolases that sequentially remove the respective terminal sugar residues before the ceramide backbone is hydrolyzed to sphingosine and a free fatty acid. These processes take place at the water–lipid interface because water-soluble enzymes act on membrane-bound substrates. Glycosphingolipids with long carbohydrate moieties are easily accessible to the hydrolases because the terminal sugar is situated far enough from the lipid bilayer. In contrast, the degradation of glycosphingolipids with short carbohydrate chains depends on the presence of the activator proteins, which mediate the interaction between the water-soluble enzymes and their membrane-bound substrates. To date, there are five known activator proteins. Four of them, called SAP A, B, C, and D or saposins A–D are highly homologous proteins and are produced by proteolytic processing from a single precursor protein, which is called prosaposin (pSAP) (Fürst *et al.*, 1988; O'Brien *et al.*, 1988; Nakano *et al.*, 1989; Hiraiwa *et al.*, 1993; for a review see Sandhoff *et al.*, 2001). The fifth protein, which acts on the degradation of ganglioside GM1 and GM2, is called GM2 activator protein (GM2AP). It is encoded by a different gene and does not show homology to any known protein (Hechtman, 1977; Conzelmann and Sandhoff, 1979; Li *et al.*, 1981; Klima *et al.*, 1991; Wu *et al.*, 1994; for a review see Gravel *et al.*, 2001). The mechanism by which these proteins perform their function and their unique properties have been discussed repeatedly in the past (Li and Li, 1997; Mahuran, 1998; Sandhoff *et al.*, 2001). Recently, some new experimental details on the function of the activator proteins have emerged that make a new evaluation of the data seem interesting.

Structural properties of the activator proteins

The four SAPs A–D, which are produced by proteolytic processing of a single precursor protein called prosaposin, are small proteins of about 80 amino acids. Their sequences contain six highly conserved cysteines and a conserved N-glycosylation site (Kishimoto *et al.*, 1992). The disulfide bond connectivity has been determined for SAPs B, C, and D and is identical for all three (Vaccaro *et al.*, 1995a; Tatti *et al.*, 1999). The proteins display a structure of intertwined loops, with disulfide bonds between the first and sixth cysteine in the sequence, as well as between the second and fifth and the third and forth cysteines. The bonds are necessary for the functionality of the proteins (Vaccaro *et al.*, 1995a) and are probably at least partially responsible for the high stability of these proteins against acid, heat, and proteolytic enzymes. The SAPs show homology to a growing group of proteins and protein domains called SAP-like

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proteins (SAPLIPs) (Munford *et al.*, 1995). Among this group are proteins of several species and different functions, all sharing a lipid-binding and membrane-perturbing property. For two proteins of this group, a structure determination has been successful: The structure of NK-lysin was determined by nuclear magnetic resonance measurement (Liepinsh *et al.*, 1997) and for prophytepsin, a plant aspartic protease, a crystal structure determination was achieved (Kervinen *et al.*, 1999). Both proteins show high structural similarity. They contain five α-helices that form a helical bundle that is grouped around a hydrophobic internal surface. This fold is referred to as the "saposin fold," because a similar structure is predicted for the SAPs. CD spectroscopic evidence shows that the four SAPs also contain a high amount of α-helix in their secondary structure (O'Brien and Kishimoto, 1991; Waring *et al.*, 1998). Interestingly, the first study showed a significant structural difference for SAP B in comparison to the other three activator proteins in this respect. The α-helical contents of SAPs A, C, and D were calculated to 40–53%, whereas that of SAP B was calculated to only 26%. However, a later study (Waring *et al.*, 1998) determined an α-helical content of 46% for SAP B, which is similar to that found for the other SAPs. The question whether such a difference in secondary structure does exist, and whether it affects the tertiary structure will hopefully be solved by a crystal structure analysis of the SAPs, which is still pending.

The fifth protein of the group, the GM2AP, shows some different structural features. With a molecular weight of about 20 kDa, it is larger than the SAPs. It carries one N-glycosylation and contains eight cysteines that form four disulfide bonds. The arrangement of these bonds has been determined (Schuette *et al.*, 1998) and is somewhat similar to that of the SAPs in that the first and last cysteine of the sequence are linked by a disulfide connection. The secondary structure of the GM2AP differs from that of the SAPs, though. Results from CD spectroscopy predict a high amount of β-sheet and α-helical contents between 0 and 5% (Giehl, 1997). The recently achieved crystal structure determinations of human and murine GM2AP (74% sequence identity) confirm these data (Wright *et al.*, 2000). The protein shows a unique hydrophobic β-cup structure building a spacious cavity for the ceramide moiety and possible recognition sites for the lipid-bound carbohydrate chain at the rim of this cavity. A flexible hydrophobic loop adjacent to the cavity may facilitate the interaction with the membrane and the extraction of the lipid (Wright *et al.*, 2000).

Function of the activator proteins

The determination of the *in vivo* function of the different activator proteins has not been simple and is still a matter of discussion for some of the proteins. The precursor protein, pSAP, is known to be able to endorse neurite outgrowth and to prevent the programmed cell death of neuronal cells *in vitro* (Hiraiwa *et al.*, 1997). *In vivo* it was shown to protect neurons from the effects of ischemia and other damage (Sano *et al.*, 1994). It therefore seems to have neurotrophic/neuroprotectant properties (O'Brien *et al.*, 1994; Tsuboi *et al.*, 1998; Hiraiwa *et al.*, 1999; Campana *et al.*, 1999). Smaller peptide fragments of pSAP within the amino terminal part of SAP C, called prosaptides, were reported to hold the neurotrophic sequence (Campana *et al.*, 1998; Otero *et al.*, 1999). But when the pharmacological effects of one synthetic prosaptide were investigated in the validated rabbit spinal cord ischemia model, the results showed that this prosaptide did not show neurotrophic/neuroprotectant properties, but that it exacerbated ischemia-induced behaviorial deficits (Lapchak *et al.*, 2000). These properties of pSAP will therefore have to be investigated further.

Diseases caused by a specific defect in one of the proteins are known for SAP B, SAP C, and GM2AP only. Genetic defects of SAP B lead to variant forms of metachromatic leukodystrophy with juvenile or late infantile onset (Stevens *et al.*, 1981; Kretz *et al.*, 1990; for a review see Sandhoff *et al.*, 2001). In addition to sulfatide the glycolipids lactosylceramide, globotriaosylceramide, and digalactosylceramide are excreted in increased amounts in the urine of such patients (Li *et al.*, 1985). The *in vivo* function of SAP C seems to lie mainly in the degradation of glucosylceramide, because its deficiency causes a juvenile variant of Gaucher disease (Christomanou *et al.*, 1986; Sandhoff *et al.*, 2001). Glucosylceramide is the only lipid for which a strong accumulation has been detected in the liver of those patients (Christomanou *et al.*, 1989). A deficiency in GM2AP leads to the AB-variant of GM2 gangliosidosis, which is characterized by an accumulation of ganglioside GM2 and glycolipid GA2 (Conzelmann and Sandhoff, 1978) (see also Figure 1). No isolated defects have been reported for SAP A or SAP D. A total defect of all four SAPs A–D caused by a mutation in the start codon of the SAP-precursor gene has been described, though (Harzer *et al.*, 1989; Schnabel *et al.*, 1992). Along with the lipids that are stored in SAP B and SAP C deficiency, different tissues and cultivated fibroblasts of these patients show increased levels of ceramide (Harzer *et al.*, 1989; Paton *et al.*, 1992; Bradová *et al.*, 1993).

Using fibroblasts from these patients in cell culture feeding experiments, it has been shown that SAP A stimulates the degradation of glucosylceramide by glucocerebrosidase and the hydrolysis of galactosylceramide by galactocerebrosidase (Harzer *et al.*, 1997) and that SAP D enhances the degradation of ceramide by acid ceramidase (Klein *et al.*, 1994). For several of the degradation steps that have been shown to be defective in the absence of the SAPs *in vivo*, the stimulatory functions of the SAPs on the reaction has been confirmed in *in vitro* studies. However, the use of micellar lipids as substrates in the presence of detergents might have led to artifacts. The use of liposomal lipid substrates without detergents are thought to mimic the *in vivo* situation better. Some of the latter studies have indicated additional effects of the SAPs and of the GM2AP that could not be deduced from the analysis of storage compounds in patients.

It has been demonstrated that in the presence of acidic lipids, SAP B and GM2AP are strong stimulators of the degradation of ganglioside GM1 (Wilkening *et al.*, 2000). The activation is so pronounced (up to 200-fold) that a physiological relevance has to be assumed. The reason for the failure to detect this function in patient analysis is obvious: Because two proteins perform this functions, the defect in only one of them does not lead to storage of the substrate. Moreover, GM2AP shows a significant stimulation of the β-hexosaminidases S and A catalyzed degradation of the sulfoglycolipid SM2 (GalNAcβ1-4Gal(3-sulfate)β1-4Glcβ1-1Ceramid) (Hepbildikler *et al.*, unpublished data). SAP C has been shown to significantly activate the degradation of ceramide by acid ceramidase (Linke *et al.*, 2001b) and of sphingomyelin by acid

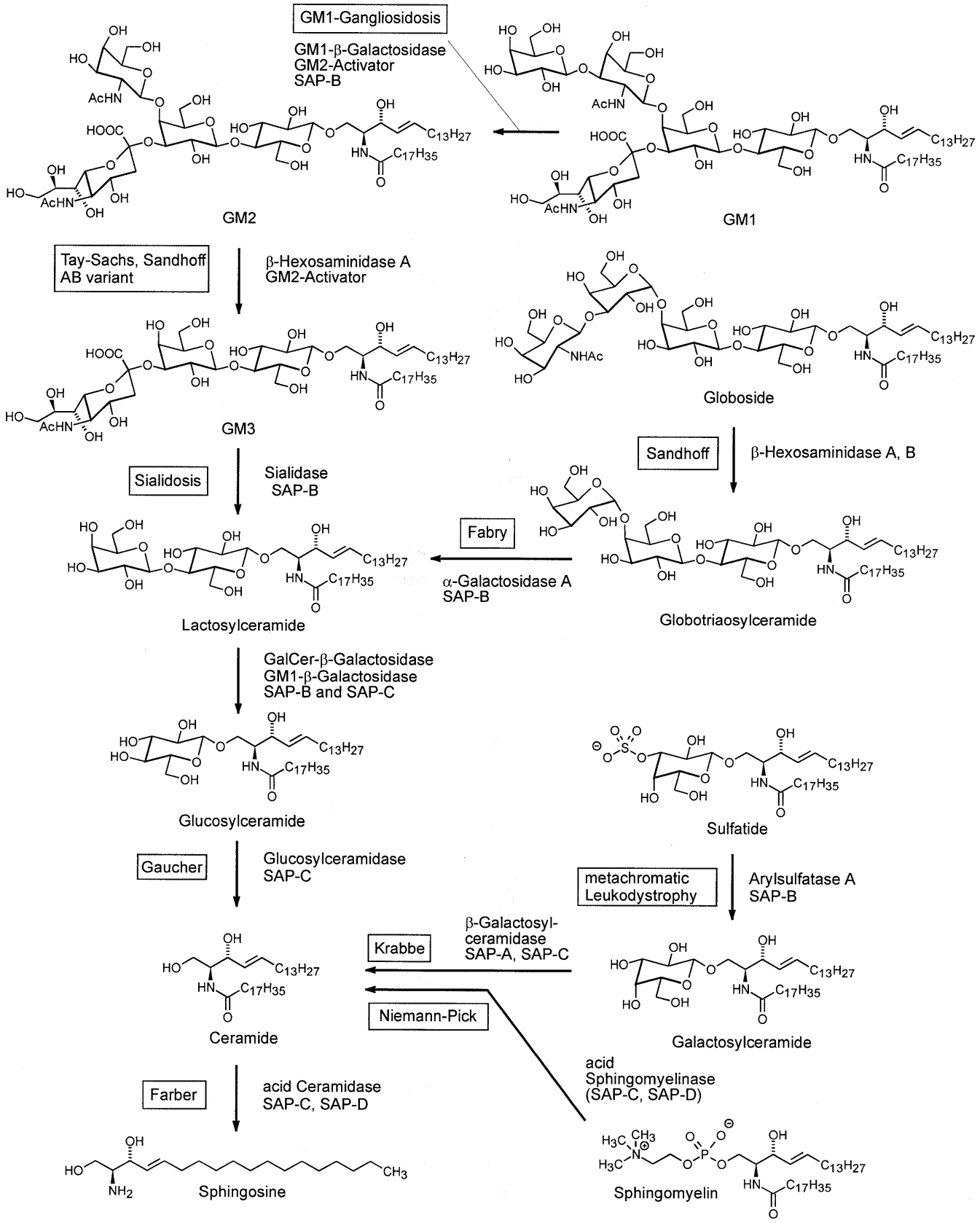

Fig. 1. Lysosomal sphingolipid degradation (Becker, 1999). The eponyms of known metabolic diseases and those of sphingolipid activator proteins necessary for *in vivo* degradation are indicated. Heterogeneity in the lipid part of the sphingolipids is not indicated. Variant AB: variant AB of GM2-gangliosidosis (deficiency of GM2-activator protein); SAP: sphingolipid activator protein; SAP-C and SAP-D in brackets are not needed *in vivo*.

sphingomyelinase (Linke *et al.*, 2001a). The physiological relevance of these activites of SAP C is not clear, because SAP D has been shown to activate the degradation of ceramide more strongly than SAP C (Linke *et al.*, 2001b). Although the activity of acid ceramidase fully depends on the presence of SAPs, acid sphingomyelinase shows activity even without SAPs. The protein contains a SAPLIP-domain in its sequence, which may activate the reaction sufficiently *in vivo* (Ponting, 1994). Further studies in this area have led to similar, but not in all cases identical results (Morimoto *et al.*, 1988, 1990). However, because these studies utilized detergents (which are not found in the natural environment) in their assay systems, their physiological importance cannot easily be assessed.

The mechanism of action of the activator proteins

Although the four SAPs are highly homologous in their sequences and seem to have similiar structures, experimental data show that their mechanisms of action presumably differ. SAP B seems to act as a physiological detergent, forming soluble 1:1 complexes with sulfatide, the storage compound in SAP B deficiency, as well as several other glycolipids (Fischer and Jatzkewitz, 1977; Li *et al.*, 1988; Vogel *et al.*, 1991; Hiraiwa *et al.*, 1992). A direct interaction of SAP B with degrading enzymes has not been demonstrated, though.

The GM2AP has also been shown to form a soluble 1:1 complex with ganglioside GM2 (Conzelmann *et al.*, 1982). Its specificity in lipid binding is higher than that of SAP B, although, apart from its main substrate GM2, the GM2AP also binds to other negatively charged lipids (Hama *et al.*, 1997) and stimulates the degradation of GA2, GM1 and SM2. Moreover, a direct interaction with the degrading enzyme β-hexosaminidase A is discussed (Kytzia and Sandhoff, 1985; Yadao *et al.*, 1997). Although both, β-hexosaminidases A and B, are capable of degrading ganglioside GM2 in the presence of suitable detergents, only β-hexosaminidase A can degrade the ganglioside in the presence of GM2AP without detergents (Conzelmann and Sandhoff, 1979; Li *et al.*, 1981), so that a specific interaction between the proteins seems probable.

The mechanism of action of SAP C mainly involves protein–protein interaction. SAP C does not form a complex with glucosylceramide, the storage compound in SAP C deficiency, but forms a 1:1 complex with the degrading enzyme glucocerebrosidase (Berent and Radin, 1981). Kinetic data support a model of allosteric activation of the enzyme (Morimoto *et al.*, 1990). In addition, SAP C has been shown to acquire hydrophobic properties at acidic pH. Under these conditions, its affinity to membranes is strongly increased (Vaccaro *et al.*, 1995b). By this mechanism, it probably facilitates the association of glucocerebrosidase with membranes, thus favoring the degradation of glucosylceramide (Vaccaro *et al.*, 1999) (see also Figure 2).

So far, little is known about the mechanisms by which SAP A and SAP D perform their respective functions, although SAP A has been shown to bind gangliosides like GM1 and GM2 (Hiraiwa *et al.*, 1992). Additionally, the activator proteins possess membrane perturbing properties. In liposomal leakage and fusion assays, it has been demonstrated that SAP C and SAP D strongly affect the bilayer integrity, whereas SAP A and SAP B only showed a minimal effect (Vaccaro *et al.*, 1995b). Film balance measurements demonstrated that GM2AP is able to reversibly insert into lipid monolayers up to a lateral pressure of 25 mN/m (Giehl *et al.*, 1999). Recent results of plasmon resonance experiments have indicated that GM2AP and SAP C are able to solubilize lipids from immobilized liposomes containing acidic lipids (Wilkening *et al.*, 1998; Werth *et al.*, 2001). However, SAP B did not show similar effects (Wilkening *et al.*, 2000; Linke *et al.*, 2001b). It is conceivable that the interaction of the SAPs with the membranes produces defects in the lateral lipid layer organisation. These lipid layer defects could favor the attack of the respective exohydrolase for lipid degradation. Recently, a similar mechanism has been demonstrated for the action of phospholipase A2 at phospholipid monolayers, which is maximal at defect sites between condensed and fluid phases of the lipid monolayer (Li *et al.*, 2000).

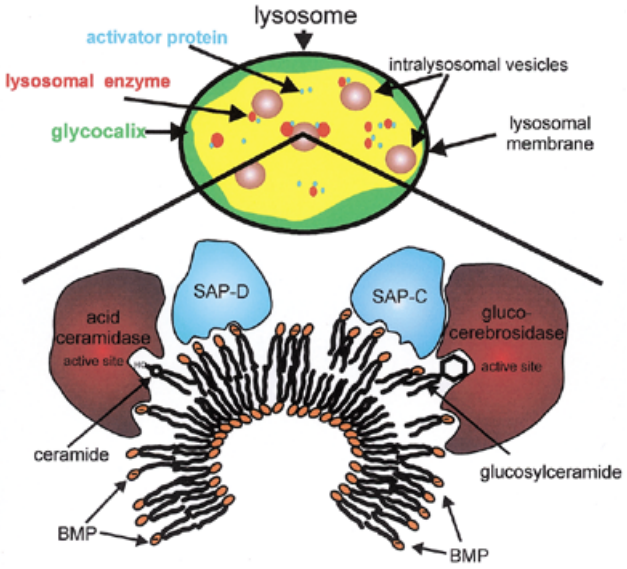

Fig. 2. Model for the degradation of membrane-bound GlcCer by glucocerebrosidase and SAP-C and Cer by acid ceramidase and SAP-D, respectively (Wilkening *et al.*, 1998). Besides the interaction of lysosomal enzyme and activator protein, the model emphasizes binding of activator protein and lysosomal enzyme to the vesicular surface containing BMP.

An interesting point is the fact that the membrane perturbing properties of the activator proteins in liposomal assays either absolutely depend on or are strongly increased by the presence of acidic lipids such as phosphatidylserine (PS), phosphatidylinositol (PI), or bis(monoacyl)glycerophosphate (BMP) in the bilayers (Morimoto *et al.*, 1990; Vaccaro *et al.*, 1995b, 1997; Wilkening *et al.*, 1998; Werth *et al.*, 2001). Strong stimulations of the reaction rates of up to 200-fold in the presence of acidic lipids have been detected for the degradation of GM1 by β-galactosidase and SAP B, and for the GM2AP-stimulated degradation of GM1 and GM2 (Wilkening *et al.*, 2000; Werth *et al.*, 2001). The reaction rates for the degradation of ceramide and glucosylceramide, stimulated by the respective activator proteins, are increased up to 4-fold and 20-fold, respectively, by the addition of BMP to the liposomes (Wilkening *et al.*, 1998; Linke *et al.*, 2001b) (see also Figure 2).

How the acidic lipids perform their stimulatory task is a question that cannot be answered in a satisfactory way at the moment. One could imagine that an interaction between the polyanionic lipid bilayer and the degrading enzymes, which are polycationic at lysosomal pH, would facilitate the reactions at the lipid–water interface. However, for the degradation of GM2 by GM2AP and β-hexosaminidase A, it has been demonstrated that the acidic ganglioside GD1A does not stimulate the reaction, whereas a strong stimulation of up to 180-fold is observed after addition of BMP, which specifically occurs in endosomes and lysosomes (Kobayashi *et al.*, 1998; Becker, 1999; Werth *et al.*, 2001). A simple concept of ionic interaction does therefore not seem to be at the basis of the observed stimulations. Moreover, it can be assumed that the mechanism by which activator proteins and acidic lipids stimulate the degradation reactions differs from one reaction to the other. In some cases a lipid that stimulates one reaction does not stimulate another, and the absolute amounts of stimulation differ widely between different activator proteins. Whether a general concept is at the basis of all the observed effects will have to be answered by more extensive studies.

In addition to the mechanistical implications, the stimulatory effect of acidic membrane components on the actions of the SAPs gives some new insight into the topology of lysosomal degradation, which will be discussed next.

The topology of lysosomal degradation

Components of the plasma membrane reach the lysosomes by vesicular flow along the endocytotic pathway. Within the lysosomes, lipids, proteins, and carbohydrates are degraded, whereas the limiting lysosomal membrane, which protects the cell from leakage of the lysosomal hydrolases into the cytosol and also consists of lipids, proteins, and carbohydrates, survives. An important question is how this differentiation between the limiting membrane and the lysosomal charge bound for degradation is achieved. To contemplate this question, it is critical to discuss the topology of the degradation. According to one hypothesis, the endocytosed plasma membrane lipids are integrated into the lysosomal membrane and degraded after successive steps of vesicle budding and fusion on the endocytic pathway (Griffiths *et al.*, 1988).

An alternative hypothesis (Fürst and Sandhoff, 1992; Sandhoff and Kolter, 1996) suggests that portions of the endosomal membrane that are enriched in components derived from the plasma membrane bud off into the endosomal lumen forming intraendosomal vesicles and other intraendosomal membrane structures. These vesicles and inner membranes are delivered to the lysosomal lumen by transient fusion of endosomes with lysosomes. Thus, components of the outer leaflet of the plasma membrane would face the "lysosol" on the outside of intralysosomal vesicles (see also Figure 2). Several experimental observations make this a probable scenario. A massive accumulation of such intralysosomal and intraendosomal vesicles has been observed in tissues and fibroblasts of a patient with complete deficiency of the SAP precursor (Harzer *et al.*, 1989; Paton *et al.*, 1992; Bradová *et al.*, 1993; Burkhardt *et al.*, 1997). In normal cells such intralysosomal membrane structures have also been observed, although they are less obvious (Futter *et al.*, 1996; Burkhardt *et al.*, 1997). In addition, biotin-labeled GM1 that was loaded into the plasma membrane of normal human fibroblasts was traced to such intralysosomal structures by electron microscopical immunocytochemistry (Möbius *et al.*, 1999).

Contemplating this topology of degradation in which endocytosed lipid material is segregated into the lumen of the lysosomes, the selective degradation of lipids is easier to understand. If the endocytosed components of the plasma membrane were integrated in the limiting lysosomal membrane, the lysosomal hydrolases would have to differentiate between lipids derived from the plasma membrane and constitutive lipids of the limiting membrane that are present in the same membrane compartment, degrading one group of lipids while leaving the others intact. It is hard to conceive how this specificity should be achieved. Moreover, the destruction of the limiting membrane could result in lysosomal hydrolases leaking into the cytosol.

Beyond that, the limiting membrane is protected by the glycocalix, which is formed by the extensive N-glycosylation of the lysosomal integrated membrane proteins (LIMPs) and lysosomal associated membrane proteins (LAMPs). It has been demonstrated recently that the lysosomal membrane proteins LAMP-1 and LAMP-2 are degraded rapidly when their glycans are removed. The stability of the protein LIMP-2, however, is largely independent on its N-glycosylation (Kundra and Kornfeld, 1999). This implies that the glycocalix protects the limiting membrane to some extent, but that its presence is not a sufficient explanation for the differentiation that is observed.

Other factors that could make the intralysosmal membrane structures more accessible for the lysosomal hydrolases than the limiting membrane include the higher curvature of the bilayers in these structures and a lipid composition that differs from that of the limiting membrane. This is implicated by the observations that were discussed in detail above, namely, the fact that acidic lipids such as PI or BMP have a strong stimulatory effect on several degradation steps that depend on activator proteins. Some of these lipids are highly enriched in endosomes and lysosomes (Stremmel and Debuch, 1976; Bleistein *et al.*, 1980), and BMP has been traced specifically to intraendosomal membrane structures by immunocytochemistry (Kobayashi *et al.*, 1998). The specific lipid composition of different membrane structures may therefore serve to differentiate between them, protecting the limiting lysosomal membrane from digestion on one hand and marking the components of intralysosomal membranes for degradation on the other.

The role of SAPs in the formation of the epidermal water barrier

The epidermis is the functional barrier between the organism and the environment. This permeability barrier protects the organism from toxins and microorganisms, but it also prevents transepidermal water loss of land-dwelling animals. To fulfill these functions, the human epidermis has a unique structure, built of several cell layers, mainly consisting of keratinocytes. In the basal layer (stratum basale), the keratinocytes contain intact cell organelles and are able to proliferate. During the migration through the following outer epidermal layers, the

stratum spinosum and the stratum granulosum (SG), the keratinocytes terminally differentiate to corneocytes, which build the outermost layer, the stratum corneum (SC) of the epidermis. The corneocytes are flattened cells without any organelles embedded in a three-dimensional network consisting of crosslinked proteins (e.g., involucrin or envoplakin), called cornified envelope (Matoltsy, 1976; Steven and Steinert, 1994; Marekov and Steinert, 1998). The corneocytes are embedded in a multilamellar extracellular matrix of long chain ceramides, free fatty acids, and cholesterol (Downing, 1992; Elias and Menon, 1991). The ceramides of the epidermis are a special family, containing different sphingoid bases and long chain fatty acids with up to 36 carbon atoms (Wertz *et al.*, 1985; Robson *et al.*, 1994; Stewart and Downing, 1999). Two types of these long chain ceramides possess an ω-hydroxy group on the N-acyl fatty acid and can be covalently attached to structure proteins of the cornified envelope (Wertz and Downing, 1986, 1987; Robson *et al.*, 1994) (see also Figure 3). These covalently bound long-chain ω-hydroxylated ceramides and the respective free fatty acids build up the corneocyte lipid envelope of the corneocytes, which are then embedded in multilamellar arrays of all epidermal ceramides, fatty acids, and cholesterol in the interstices. The entire extracellular lipid and protein complex is necessary for maintaining the permeability barrier function of the epidermis (Lampe *et al.*, 1983; Wertz and Downing, 1987; Downing, 1992).

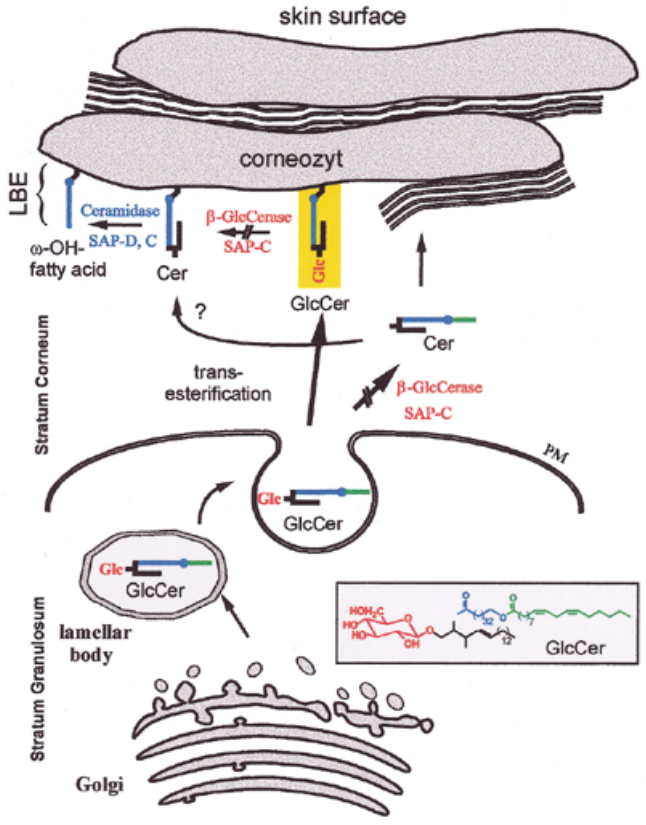

Fig. 3. Extracellular metabolism of glucosylceramide (GlcCer) and formation of the lipid-bound envelope (Schuette *et al.*, 1999). PM: plasma membrane, Cer: ceramide, consisting of sphingosine (black) and long-chain ω-hydroxyl fatty acid (blue). Linolic acid (green) is found esterified to the ω-hydroxyl moiety. Catabolic blocks in β-glucocerebrosidase- and SAP-C deficiency are indicated by the arrows with slashes through them.

The SC ceramides can be generated either by *de novo* biosynthesis or by degradation of ceramide precursors, such as glycosphingolipids, for example, glucosylceramides (GlcCer) (Holleran *et al.*, 1993), or sphingomyelins (Jensen *et al.*, 1999; Schmuth *et al.*, 2000). Recent studies show the latter way working for two ceramides that are not ω-hydroxylated. They are constituted from their respective sphingomyelins (Uchida *et al.*, 2000).

For the deglucosylation of GlcCer which is assumed to be the main pathway (Holleran *et al.*, 1994) the enzyme β-glucocerebrosidase and the respective activator protein should be essential. The importance of β-glucocerebrosidase in the epidermis has been demonstrated by the fact that a severe type of Gaucher disease results in a defective skin phenotype ("Collodian Baby") that is neonatally lethal likely due to excessive transepidermal water loss (Sidransky *et al.*, 1992; Grace *et al.*, 1999). The function of SAPs in the generation of the epidermal barrier has been analyzed in a mouse model of complete SAP deficiency (pSAP deficiency) (Fujita *et al.*, 1996). These mice show an ichthyotic skin phenotype with red and wrinkled skin (Doering *et al.*, 1999) similar to the skin of the β-glucocerebrosidase-deficient Gaucher mice (Holleran *et al.*, 1994; Liu *et al.*, 1998). Lipid analysis by thin-layer chromatography and matrix-assisted laser desorption/ionization mass spectrometry of the pSAP-knockout mouse and wild-type epidermis showed the existence of ω-hydroxylated GlcCer that corresponded in structure and chain length to the respective ceramides, indicating a precursor–product relationship between these lipid species. In the epidermis of pSAP-deficient mice the content of GlcCer, especially that of the more hydrophobic species, was elevated significantly compared to wild-type epidermis, and the amount of ceramides was decreased. Light microscopic images showed morphological alterations in the SC and the stratum lucidum (interstice between SG and SC) of pSAP deficient mice compared to wild-type mice. The stratum lucidum in the knockout mice is thicker than in wild type mice, which could be the result of an accumulation of unprocessed GlcCer. Moreover, ω-hydroxyglucosylceramides (ω-OH-GlcCer) covalently bound to the cornified envelope were discovered in the knockout mice. Their content is increased in the pSAP-deficient mice, and the contents of covalently bound ω-OH-ceramides and ω-OH-fatty acids are decreased in comparison comparison to the wild type mouse (Doering *et al.*, 1999). This demonstrates that one functioning way of generating protein-bound ω-OH-ceramides is first binding ω-OH-GlcCer to the proteins of the cornified envelope, which are then deglucosylated, forming the hydrophobic species at the protein surface that can act as a matrix for the formation of lamellar structures (Doering *et al.*, 1999; Elias and Menon, 1991). Whether an additional pathway exists, in which GlcCer is transformed into Cer before it is bound to the cornified envelope, cannot be answered at the moment. The absence of SAPs—in particular that of SAP-C, which has been shown to stimulate the degradation of GlcCer by β-glucocerebrosidase in lysosomes (Ho and O'Brien, 1971; Wilkening *et al.*, 1998)—obviously leads to a lipid composition in the SC

interstices that is not hydrophobic enough to form an functioning permeability barrier.

How the lipids, enzymes, and activator proteins reach the extracellular interstices between SG and SC is still a matter of investigation. Specialized organelles that are found in the keratinocytes in the SG, called lamellar bodies or Odland bodies, are probably at least partially responsible for this transport. Although they are important for the formation of the epidermal permeability barrier, very little is known about their generation. In general, lamellar bodies could be similar to lysosomes, and they could derive directly from the Golgi apparatus. In addition to GlcCer, which is enriched in lamellar bodies (Madison *et al.*, 1998), also a set of lysosomal enzymes have been found in these organelles. β-glucocerebrosidase activity has been shown to be increased in the isolated lamellar body fraction compared to normal epidermis (Freinkel and Traczyk, 1985; Grayson *et al.*, 1985; Madison *et al.*, 1998). Furthermore, acid lipase activity has been localized to lamellar bodies and the intercellular spaces of the SC using cytochemical and biochemical techniques (Grayson *et al.*, 1985; Menon *et al.*, 1986, 1992). These data could indicate that lamellar bodies are formed in a similar way as lysosomes. However, several studies seem to implicate that lamellar bodies derive from the Golgi. Electron microscopic images of lamellar bodies show them as elongated, oval, and oddly shaped "granules." They are more conforming with cross-sections of tubules or buds from tubules of the trans-Golgi-network than with cross-sections of vesicles (Madison *et al.*, 1998). Furthermore, GlcCer is synthezised within the Golgi apparatus by ceramide glucosyltransferase (Futermann and Pagano, 1991; Jeckel *et al.*, 1992) and could easily be packaged into the lamellar bodies and transported to the extracellular space. This is also true for the degrading enzymes.

The localization of the SAPs within the SG–SC interface has not been demonstrated yet but their presence can be deduced from the alterations that are observed in the absence of the SAPs (Doering *et al.*, 1999). Therefore it is an interesting question how they are transported to this location. They might be packaged into the Odland bodies, as it is discussed for the degrading enzymes. Alternatively, it is also possible that the SAP precursor is transported into the extracellular space directly without involvement of lamellar bodies. The protein has been shown to be secreted in high amounts by several cell types and has been detected in increased concentrations in body fluids like cerebrospinal fluid, human milk or semen (Hineno *et al.*, 1991). In this scenario, the precursor would have to be proteolytically processed in the SG–SC interface after the secretion, and therefore the respective proteases have to be present extracellularly. A third possibility for the transport of the SAPs to the extracellular space would be a direct secretion of the mature SAPs by lysosomes. This question will have to be clarified by further experiments in the future.

Apart from the stimulation of GlcCer degradation, ultrastructural analysis of the skin in SAP-precursor deficiency implies another function of the SAPs in skin formation. The comparison of micrographs from pSAP deficient mice, heterozygotes, and wild-type littermates showed that pSAP deficiency seems to disturb the dispersal of the lamellar body contents. In the intercellular spaces at the SC–SG interface the secreted contents retain a compact, spherical form rather than progressively unfurling into linear configurations, as it appears in the wild-type mice (Doering *et al.*, 1999). The interstices of the lower-to-middle SC contain patterns of foreshortened lamellae in loose, discontinuous aggregates instead of the distinctive membrane unit structures in the wild-type mice SC (Doering *et al.*, 1999). Another function of the SAPs therefore seems to be the arrangement of the extruded lipids into smooth, linear lipid arrays. How this rearrangement is achieved is not currently clear. The SAPs and the SAP precursor have been shown to bind sphingolipids and to promote lipid transport between liposomes (Vogel *et al.*, 1991; Hiraiwa *et al.*, 1992; Conzelmann *et al.*, 1982). Moreover, a highly homologous protein, the lung surfactant protein B plays a crucial role in organizing lipid structures in lung alveolae (Cochrane and Revak, 1991).

The action of SAPs in rearranging lipid structures in the epidermis may be carried out by actively transporting single lipid molecules. Alternatively, the SAPs might help destabilize the lipid "blocks" that are extruded from the lamellar bodies of the epidermis in such a way that they unfold and subsequently form the unique lipid arrays that are found in the skin. Hopefully, further experiments will help clarify these questions. This may be of considerable importance, because an understanding of this fascinating process might provide new insights into the underlying defects in skin disorders characterized by a defective permeability barrier, such as atopic dermatitis or psoriasis.

Conclusion

Although the functions of the sphingolipid activator proteins in sphingolipid degradation are widely known, their mechanisms of action and, closely related, their structures are not yet fully understood. The structural and functional properties of the SAP domains are particularly interesting with regard to the proteins of the SAPLIP family, which are involved in very different cellular processes. Apart from functions in sphingolipid degradation, lipid binding, lipid transfer, and lipid layer organization are fields in which SAPs and SAPLIPs seem to play an important role. Further studies in this direction may lead to new and possibly surprising results and could provide a more profound understanding of lipid protein interaction.

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Abbreviations

BMP, bis(monoacyl)glycerophosphate; Cer, ceramide; GlcCer, glucosylceramide; GM2AP, GM2-activator protein; LAMP, lysosomal associated membrane protein; LIMP, lysosomal integrated membrane protein; PI, phosphatidylinositol; PS, phosphatidylserine; pSAP, sphingolipid activator protein precursor; SAP, sphingolipid activator protein; SAPLIP, SAP-like protein; SC, stratum corneum; SG, stratum granulosum.

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