Sphingolipid Activator Proteins Are Required for Epidermal Permeability Barrier Formation*

(Received for publication, December 10, 1998, and in revised form, January 29, 1999)

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The epidermal permeability barrier is maintained by extracellular lipid membranes within the interstices of the stratum corneum. Ceramides, the major components of these multilayered membranes, derive in large part from hydrolysis of glucosylceramides mediated by stratum corneum β-glucocerebrosidase (β-GlcCerase). Prosaposin (pSAP) is a large precursor protein that is proteolytically cleaved to form four distinct saposin activator proteins, which stimulate enzymatic hydrolysis of sphingolipids, including glucosylceramide. Recently, pSAP has been eliminated in a mouse model using targeted deletion and homologous recombination. In addition to the extracutaneous findings noted previously, our present data indicate that pSAP deficiency in the epidermis has significant consequences including: 1) an accumulation of epidermal glucosylceramides together with below normal levels of ceramides; 2) alterations in lipids that are bound by ester linkages to proteins of the cornified cell envelope; 3) a thickened stratum lucidum with evidence of scaling; and 4) a striking abnormality in lamellar membrane maturation within the interstices of the stratum corneum. Together, these results demonstrate that the production of pSAP, and presumably mature saposin activator protein generation, is required for normal epidermal barrier formation and function. Moreover, detection of significant amounts of covalently bound ω-HCer in pSAP-deficient epidermis suggests that deactivation of ω-HCer is not a requisite step prior to covalent attachment of lipid to cornified envelope proteins.

Sphingolipid activator proteins (SAPs or saposins) are non-enzymatic glycoprotein cofactors required for lysosomal degradation of various glucosphingolipids by exohydrolases (1, 2). Proteolytic cleavage of SAP precursor (pSAP) in acidic compartments releases four homologous polypeptides (i.e. SAP-A, SAP-B, SAP-C, and SAP-D), thought to function either by direct activation of their respective enzyme or as biological detergents that lift substrates out of the membrane plane (3–5). One of the activator proteins, SAP-C, has been shown to stimulate lysosomal glucosylceramide (GlcCer) degradation by β-glucocerebrosidase (β-GlcCerase) (6, 7). A deficiency of SAP-C causes a variant form of Gaucher disease (8–10).

In terrestrial mammals, the permeability barrier that restricts excess transepidermal water loss is maintained by unique arrays of lamellar membranes localized in the interstices of the outermost stratum corneum layer of the epidermis. These lamellar arrays derive from the extruded lipid contents of lamellar bodies after fusion of these organelles with the apical plasma membrane of the outermost granular cell. The initially secreted lipids are processed by a set of co-localized lipid hydrolases into a more hydrophobic mixture, enriched in ceramides (Cer). The resultant Cer, comprising a family of compounds with complex chemical structures, together with free fatty acids and cholesterol are the dominant constituents of the stratum corneum (SC) lamellar membranes (11). Two of these species, which possess an ω-hydroxy moiety on the N-acyl fatty acid, are covalently bound to structural proteins of the cornified cell envelope forming a hydrophobic surface to terminally differentiated keratinocytes (12, 13).

The postsecretory processing of lamellar body-derived GlcCers to ceramides by β-GlcCerase is essential for maintenance of permeability barrier homeostasis (14–17). Because SAP-C stimulates lysosomal GlcCer degradation in vivo, it seems reasonable to predict that expression of pSAP would also be important for epidermal barrier formation. Decreased levels of pSAP have been detected in atopic skin, suggesting a role for SAPs in epidermal function (18). Furthermore, a 20-week pSAP-deficient human fetus, with a phenotype resembling acute neuronopathic (type 2) Gaucher disease, displayed extensive cutaneous lysosomal storage deposits and neutral glycosphingolipid accumulation in dermal fibroblasts (19). A SAP precursor-deficient mouse (pSAP−/−) has recently been created by targeted gene disruption and homologous recombination (20). Because of the deficiency of the complete precursor protein, none of the four SAPs are generated in any cell type. Initial characterization of these mice exhibited two distinct phenotypes, i.e. neonatal fatal and later onset. The pathology of the latter is complex, including severe hypomyelination and storage of multiple sphingolipids in the brain (20, 21), as well as an ichthyosiform dermatosis. Thus, in the present work, we focused on the physiologic function of pSAP in the context of cutaneous permeability barrier formation and investigated the effects of pSAP deficiency on epidermal lipid composition and structure.

* This work was supported by grants from the Deutsche Forschungsgemeinschaft (SFB 284) and the National Institutes of Health (AR39448 and AR19098). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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§ The abbreviations used are: SAP, sphingolipid activator protein; pSAP, prosaposin; β-GlcCerase, β-glucocerebrosidase; Cer, ceramide; GlcCer, glucosylceramide; N, non-hydroxy fatty acid; A, ω-hydroxy fatty acid; O, ω-hydroxy fatty acid; S, sphingosine; P, phytosphingosine; E, esterified; MALDI, matrix-assisted laser desorption ionization; SC, stratum corneum; SM, sphingomyelin; SMase, sphingomyelinase.
Animals—Generation of SAP-deficient mice through targeted gene disruption has been described previously (20). Newborn knockout mice (homozygous, n = 9; heterozygous, n = 8) as well as newborn wild-type littermates (n = 7) were studied. Genotyping was performed by polymerase chain reaction. The primers used had the following sequence: forward primer, 5′-GCC CAC AGC GGT GAG TGC-3′, matching on the pSAP genomic DNA on exon 2/intron 2; reverse primer, 5′-CAG-CAG CAG TCT GGT CAC-3′, matching on exon 3. With those primers a band of 1300 bp appeared in the pSAP +/+ mice, a band of 806 bp appeared in pSAP +/- mice, and both bands appeared in pSAP +/- mice.

Preparation of Epidermis—Whole skin was removed at autopsy from newborn mice. Epidermis was separated from dermis after floating the skin on Dispanse (Boehringer Mannheim, grade II) diluted 1:1 in Hank’s buffer at 4 °C overnight.

Light Analysis—Tissue samples were homogenized, lyophilized, and weighed. Epidermal lipids were extracted for 24 h at 37 °C in each of three solvent mixtures (chloroform/methanol/water 1:2:0.5:0.5; chloroform/methanol 1:1 (v/v); and chloroform/methanol 2:1 (v/v)). Total lipid extracts were applied to thin-layer Silica Gel 60 plates (Merek Darmstadt, Germany). All plates were washed with chloroform/methanol (9:1, v/v) before sample application. For separation of glucosylceramides, the chromatograms were developed with chloroform/methanol/water (70:30.5:3, v/v/v). Ceramides were resolved twice using chloroform/methanol (9:1, v/v) as developing solvent. For quantitative analytical TLC determination, increasing amounts of standard lipids (N-stearoyl-sphingosine (kind gift of Beiersdorf AG, Hamburg, Germany), sphingomyelin (Sigma), and glucosylceramide (purified from Gaucher spleen in our laboratory)) were applied. After development, plates were air-dried, sprayed with 8% (w/v) H3PO4, and charred at 180 °C for 10 min, and lipids were quantitated by photodensitometry (Shimadzu, Kyoto, Japan).

MALDI Mass Spectrometry of Lipids—Aliquots of lipid extracts were separated by TLC. Using MALDI mass spectrometric analysis, GlcCer A/B and GlcCer(C16-AS) accumulated 2.1-fold (p < 10^-8) over control, whereas the more polar (i.e., lower Rf value) GlcCer AB and GlcCer(C16-AS) accumulated 2.1-fold (p < 10^-4) and 2.2-fold (p < 0.02), respectively. In heterozygous mice, which demonstrate nearly 50% reduction in the expression of pSAP protein (20), only the GlcCer(EOS) fraction showed significant accumulation (1.4-fold, p < 0.03), whereas the other GlcCer fractions remained unchanged. These results suggest not only that the processing of epidermal GlcCers by acidic β-GlCerase requires the production of pSAP, and presumably mature SAP generation, but that the hydrolysis of the more hydrophobic epidermal GlcCers (e.g., GlcCer(EOS)) is more dependent upon the presence of SAP-C.

The storage of GlcCers was accompanied with a decrease in the level of epidermal ceramides. Ceramides were separated into five fractions (Fig. 1B) and later identified by MALDI mass spectrometric analysis (see below). With the exception of Cer(C16-AS), all of the other epidermal ceramides were moderately decreased in the SAP +/+ epidermis in comparison with wild-type littermates (by approximately 20%) (Fig. 1D). In contrast, the least hydrophobic ceramide, Cer(C16-AS), accumulated 1.3-fold over control (p < 0.05). Moreover, the Cer levels of heterozygous mouse epidermis were not significantly different from normal littermates (Fig. 1D). The diminished levels of epidermal Cer in conjunction with the preferential accumulation of hydrophobic GlcCer (noted above) strongly suggest a role for either pSAP itself or a mature SAP, presumably SAP-C, in the hydrolytic processing of GlcCer-to-Cer in epidermis.

We next identified each of the GlcCer and Cer fractions separated by TLC. Using MALDI mass spectrometric analysis, the most hydrophobic glycolipid, GlcCer(EOS), was found to contain ω-OH fatty acids of various chain lengths (C28–C36) and degrees of saturation with a majority being C32:0 (M + Na, 1224.7 atomic mass units) and C34:1 (M + Na, 1250.6 atomic mass units) (Fig. 2A). The ω-OH moiety of this GlcCer fraction is known to be acylated in the epidermis with linoic acid, as shown here and previously (25). GlcCer A/B (Fig. 2B) comprised a complex mixture of GlcCers (i.e., GlcCer(NS) GlcCer(NP), and GlcCer(C24,26-AS)). Non-hydroxy fatty acids...
with dominant chain length of C24:0, C25:0, and C26:0 were attached to either sphingosine or phytosphingosine. The less abundant α-hydroxy fatty acids in GlcCer A/B showed a similar pattern with C24:0-OH (M<sub>1</sub>Na, 850.4 atomic mass units), C25:0-OH (M<sub>1</sub>Na, 864.4 atomic mass units), and C26:0-OH (M<sub>1</sub>Na, 878.4 atomic mass units). The presence of significant amounts of C25 and other odd-chain fatty acids in epidermal ceramides has also previously been reported (26, 27). Because of the differences in molecular polarity, it seems likely that the upper part of the double band, GlcCer A/B, represents GlcCers with non-hydroxy fatty acids, whereas the lower part corresponds to the GlcCers containing α-hydroxylated fatty acids.

The most hydrophilic glycolipid, GlcCer(C16-AS), was identified as a homogenous GlcCer containing mainly a C16:0-OH fatty acid (M<sub>1</sub>Na, 738.5 atomic mass units, Fig. 2C). MALDI mass spectrometric analysis of ceramides revealed sphingoid and fatty acid compositions similar to those found in their corresponding glucosylceramide precursors as well. With regard to the ceramide moiety, GlcCer(EOS) appeared to correspond to Cer(EOS) having the same fatty acid pattern, again with a majority being C32:0 (M + Na, 1062.6 atomic mass units) and C34:1 (M + Na, 1088.8 atomic mass units) (Fig. 2E). Cer(NS) contained different non-hydroxy fatty acids with chain length between C24 and C26 (Fig. 2F). Cer(NP) appeared to be hydroxylated in the sphingoid backbone because it comigrated with synthetic phytoceramide on TLC chromatograms. The molecular masses indeed demonstrated an additional hydroxyl group in Cer(NP) together with various degrees of saturation of long chain fatty acids (Fig. 2G). Cer(C24,26-AS) and Cer(C16-AS) both were identified as ceramides containing α-hydroxy fatty acids attached to sphingosine. In Cer(C24,26-AS) C24:0-OH (M<sub>1</sub>Na, 688.5 atomic mass units), C25:0-OH (M<sub>1</sub>Na, 702.6 atomic mass units), and C26:0-OH (M + Na, 716.6 atomic mass units) fatty acids were found (Fig. 2H). Taken together, Cer(NS), (NP), and (C24,26-AS) comprised the lipid backbones of GlcCer A/B. The most hydrophilic ceramide, Cer(C16-AS), possessed mainly a C16:0-OH fatty acid and therefore corresponds to GlcCer(C16-AS) (Fig. 2D).

Processing of sphingomyelin to Cer by acidic sphingomyelinase (SMase) could also contribute to the pool of Cer available for barrier formation within the epidermis. A significant in vivo activation of acidic SMase by SAPs could not be concluded from lipid profiles of brain and liver from pSAP knockout mice (20). However, in the epidermis the sphingomyelin level was in-
FIG. 2. MALDI mass spectrometry of epidermal sphingolipids. Lipids were isolated from either wild-type or pSAP knockout mice by TLC and analyzed in the positive ion mode. All indicated masses represent sodium adducts of intact molecules. The likely compositions of the most prominent peaks are indicated. The fatty acids are indicated first followed by the sphingoid bases. The proposed chemical structures of individual sphingolipids are shown. The lipid backbones of GlcCer A/B are Cer(NS), Cer(NP), and Cer(C24,26-AS).
increased 1.5-fold over that in wild-type mice (p < 0.02, data not shown).

Covalently Bound Lipids in pSAP Knockout Mice—Terminally differentiated keratinocytes (corneocytes) are coated by a monolayer of ω-OH ceramides, ester-linked to proteins of the outer surface of the cornified cell envelope. This lipid-bound envelope is thought to be essential for either corneocyte cohesion and/or organization of extracellular lipid lamellae (28, 29). We characterized these lipids obtained after exhaustive prior extraction of epidermal samples followed by alkaline hydrolysis (12). To ascertain that the recovered lipids were indeed covalently attached, each sample was checked to ensure that there was no residual extractable lipids before undergoing base hydrolysis (Fig. 3A, lanes 1, 3, and 5). A mixture of long chain fatty acids, Cer(OS), and ω-OH fatty acids were released from the wild-type epidermis (Fig. 3A, lane 2). In addition to these lipids, substantial amounts of GlecCer(OS) were covalently attached to the cornified cell envelope in pSAP −/− mice (Fig. 3A, lane 4). All three ω-OH species had the same fatty acid chain length distribution with a majority being C32:1, C32:0, and C34:1 as demonstrated by mass spectrometry (Fig. 4). Furthermore, the levels of bound Cer(OS) and ω-OH fatty acids in homozygous knockout mice were significantly decreased to 80%
also be detected in pSAP

Comparably low levels of covalently bound GlcCer(OS) could previously observed in a more dramatic fashion in the Newborn pSAP knockout mice displayed an ichthyotic envelope. Mice—

Morphological Alterations in the Epidermis of pSAP Knockout Mice—Newborn pSAP knockout mice displayed an ichthyotic skin phenotype including a red and wrinkled appearance previously observed in a more dramatic fashion in the β-GlcCerase-deficient Gaucher mice (15, 17). These findings are consistent with a defect in the epidermal permeability barrier and suggest underlying defects in the critical membrane domains in the stratum corneum. Therefore, to ascertain the significance of pSAP deficiency in the epidermis, we first analyzed epidermal sections at the light microscopic level (Fig. 5). In the homozygous knockout mice, morphological alterations were limited to the stratum corneum and the stratum lucidum, which represents the interface between the upper stratum granulosum and the lower stratum corneum. The thicker stratum lucidum in the knockout mice in comparison with the wild-type mice could be the result of an accumulation of unprocessed Glc Cer. Furthermore, large parts of the stratum corneum were absent in sections of the knockout animals, possibly because of reduced mechanical stability resulting from swelling of intercellular domains.

Alterations in Epidermal Ultrastructure—To further delineate the basis for the cutaneous abnormalities induced by the deficiency of pSAP in the epidermis, micrographs from pSAP knockout, heterozygous, and wild-type littersmates were compared (Fig. 6). The pSAP-deficient epidermis (−/−) displayed a striking abnormality in lamellar membrane maturation within the interstices of the lower-to-middle stratum corneum (Fig. 6, A and B) when compared with that of normal (Fig. 6C) littersmates. The distinctive membrane unit structures seen in the normal stratum corneum (Fig. 6C) were replaced by arrays of foreshortened lamellae in loose, discontinuous aggregates in the pSAP-deficient stratum corneum (Fig. 6, A and B, arrows), resembling those seen with β-GlcCerase deficiency (14, 15). Although normal quantities of lamellar bodies were present and secreted normally in pSAP-deficient epidermis (Fig. 6E, arrowheads), their internal structure appeared to be altered from those in heterozygous or normal littersmates (Fig. 6D and inset); individual organelles often appeared enlarged and engorged with non-lamellar, electron-dense material, which in turn disrupts the internal contents normally seen in wild-type epidermis. Moreover, the secreted lamellar body-derived contents in the intercellular spaces at the stratum corneum-stratum granulosum interface in pSAP −/− animals retained a compact, spherical pattern (Fig. 6A, asterisks) rather than progressively unfurling into linear configurations, as occurred in the heterozygous and wild-type stratum corneum. These studies indicate that a profound deficiency of pSAP does not disrupt production, trafficking, or secretion of lamellar body contents into the intercellular domains. Instead, pSAP deficiency results in major abnormalities in the following: (a) the internal organization of lamellar body contents; (b) dispersal after secretion; and (c) extracellular processing of extracted lamellar body contents. Heterozygous animals, although expressing approximately 50% of the pSAP protein levels (20), did not display abnormalities in the contents or maturation of the lamellar body, suggesting that sufficient pSAP is expressed to sustain the extracellular hydrolytic activity required to generate mature lamellar membrane organization.

DISCUSSION

In this work, we isolated lipids from murine epidermis and for the first time presented a complete mass spectrometric characterization of both epidermal ceramides and corresponding glucosylceramides. Our data on the ceramides are in good agreement with previous structural characterization of murine Cer(EOS), Cer(NS), and Cer(C16-AS) (26). Because we exclusively obtained intact molecular ions (by MALDI), the fatty acid and sphingosine chain length distributions are not discernable. However, the sphingoid bases from human and pig epidermal ceramides are known to have limited chain length heterogeneities between C16 and C22 (27, 30), consistent with the substrate specificity of epidermal serine palmitoyltransferase (31). We therefore proposed likely compositions of individual sphingolipids. The structural assignment of Cer(NP), referred to here as phytoceramide, is based both on its mass spectrum and its comigration on TLC plates with synthetic phytoceramide. We found similar sphingoid and fatty acid compositions of ceramides and corresponding glucosylceramides. For example, Glc Cer(EOS) and Cer(EOS) both exhibited mainly C32:0 and C34:1 ω-OH fatty acids, strongly suggesting a precursor/product relationship between these two related epidermal lipids.

Processing of epidermal GlcCers to ceramides by the action of acidic β-GlcCerase is well known to be essential for cutaneous barrier formation and homeostasis (14–17). In pSAP −/− mice, an accumulation of all GlcCers was evident, demonstrating that optimal epidermal β-GlcCerase activity in vivo requires stimulation by SAPs (i.e. SAP-C). Furthermore, the importance of SAP-C mediation is underscored by the observation that degradation of the most hydrophobic GlcCer, GlcCer (EOS), was found to be most impaired in the absence of SAPs. SAP-C is thought to stimulate β-GlcCerase by an allosteric mechanism, resulting in trimeric complex formation between the water-soluble enzyme, the activator protein, and the membrane-associated glycolipid (1, 7). In vitro data have demonstrated the ability of SAP-C to form complexes with β-GlcCerase in the presence of phosphatidylserine (32, 33). However, in vivo binding of SAP-C to GlcCers may also contribute to the stimulation of GlcCer hydrolysis. Indeed, SAP-C has recently been shown to solubilize membrane-bound GlcCers presum-
ably facilitating their processing by water-soluble enzyme (7).

Despite the massive accumulation of GlcCers, epidermal Cer levels were only moderately reduced in newborn pSAP/−/− mice compared with wild-type littermates. One explanation for the near normal levels of Cer might be increased sphingomyelin (SM)-to-Cer hydrolysis. However, the increased SM content in the pSAP/−/− mouse epidermis reported here is also of consequence. In normal epidermis, the levels of both SM and GlcCer are high in nucleated cells but these lipids are nearly absent from the stratum corneum (34). Previous results with β-GlcCerase null allele animals also suggest that the majority of stratum corneum ceramides are derived from the hydrolysis of GlcCer precursors (15). The importance of stratum corneum SMase for barrier homeostasis has only recently been demonstrated (22). Thus, a portion of the Cer in stratum corneum normally may derive from SM, or this pathway could be up-regulated in the face of β-GlcCerase blockade. Moreover, although SAP-C has been shown to stimulate acidic SMase activity in vitro (35, 36), the role of SAPs in SM hydrolysis in vivo has not been adequately addressed. SMase activity is normal in extracts from dermal fibroblasts isolated from a patient with pSAP deficiency (8). However, the increased epidermal SM content in the absence of pSAP expression described here suggests that SAP(s) may have a role in the regulation of epidermal SMase activity. Whether increased epidermal SM contributes to the stratum corneum pathology remains to be determined.

In principle, β-GlcCerase can degrade GlcCer without SAP-C. Furthermore, an excess of β-GlcCerase activity is present in normal epidermis, as Gaucher heterozygous animals with approximately 50% of normal activity do not exhibit SC or epidermal pathology (15). Therefore, it seems likely that during embryonic development substantial amounts of ceramides are provided by slow hydrolysis of GlcCer without any loss by desquamation starting after birth. Finally, ceramides could also derive directly from de novo synthesis under pathological conditions or even physiologically. The most polar ceramide, Cer(C16-AS), was found to be elevated in the absence of pSAP, suggesting a compensatory capacity of sphingolipid biosynthetic pathways in keratinocytes.

It is well known that a monolayer of ω-OH-Cer is covalently attached to the surface of corneocytes connecting proteins of the cornified cell envelope to hydrophobic intercellular lipid

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**Fig. 6.** Ultrastructure of pSAP knockout epidermis in comparison with wild-type littermates. In the lower to mid-SC from pSAP-deficient (−/−) mouse epidermis, conventional osmium tetroxide staining reveals extruded lamellar body contents to be highly disorganized (A, open arrows), with persistence of osmiophilic material in the interstices out to at least the fourth to fifth SC cell layers (A, stars), an area routinely undeposited by osmiophilic material in normal wild-type SC tissues (not shown). Moreover, extruded lamellar body contents retain a spherical pattern in the interstices (A, asterisks), suggesting that linearization of lamellar body-derived lamellae is delayed or defective. Ruthenium staining (22) further reveals that the pSAP-deficient interstices (B, between arrows) lack the regular, compact pattern of lamellar membranes observed in normal, pSAP-replete SC (C, between open arrows). Although significant quantities of lamellar bodies are formed in pSAP-deficient epidermis (E, arrowheads), the contents have a more condensed appearance when compared with normal wild-type littermates (D, arrowheads; and inset, asterisk shows an enlarged image of a lamellar body from normal epidermis). Bars represent 0.25 (A–E) and 0.125 µm (D, inset) with final image magnifications (all are ×103) of: A, 46.6; B, 68.8; C, 62.5; D, 64.0; E, 44.8; D, inset, 120. sg, stratum granulosum.

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2 E. Proksch and J. M. Jensen, personal communication.
3 W. M. Holleran and P. M. Elias, unpublished observation.
lamellae (12). Recently, some structural proteins within the epidermis have been shown to be esterified at Glu or Gln residues with Cer(OS) (13). Although little is known about the attachment of lipids to proteins of the epidermal cornified cell envelope, it was assumed that free Cer(OS) are the direct precursors of bound Cer(OS). We have analyzed covalently bound epidermal lipids in pSAP knockout mice. Most importantly, in pSAP −/− mice a novel lipid was recovered and identified as GlcCer(OS), which had the same fatty acid chain length distribution as found in the bound Cer(OS) and in ω-OH fatty acids as well. Obviously, these lipids are metabolically related to each other. It has been an open question whether Cer(OS) is bound to amino acid side chains either via the ω-hydroxyl group or via C1 of sphingoid bases. Because GlcCer(OS) cannot be bound through C1, where the glucose moiety is located, this finding provides evidence that at least a portion of all three ω-OH species are attached to the cornified cell envelope via the terminal ω-hydroxyl group.

Because we detected small amounts of bound GlcCer(OS) in both pSAP +/− and wild-type mice, this phenomenon appears to be physiologically important. In our model, lamellar body-derived GlcCer(OS) is, in part, utilized by an enzyme that exchanges esterified linoleic acid against the side chain of a glatamate residue. Resulting bound GlcCer(OS) would subsequently be deglycosylated to bound Cer(OS) by SAP-C–assisted β-GlCerase and then hydrolyzed to bound ω-OH fatty acid and free sphingosine by SAP-D–assisted ceramidase. This metabolic relationship is supported by identical fatty acid chain length distribution in all three ω-OH species. Stimulation of lipid–bound envelope–associated processing of lipids by hydrolytic enzymes would mechanistically work by an allosteric enzyme activation rather than by solubilization of lipids, which are covalently attached and therefore cannot be lifted by an activator protein. On the other hand, it cannot be excluded that free Cer(OS) would also be transferred to proteins of the cornified cell envelope by an enzyme, which utilizes either Cer(OS) or GlcCer(OS). In pSAP −/− mice, levels of bound ω-OH fatty acid and Cer(OS) were significantly decreased to 48 and 80% of control, respectively. These data support the concept that bound GlcCer(OS) is the likely precursor of bound Cer(OS) and ω-OH fatty acid. Formation of the latter is more severely impaired than formation of Cer(OS) because, in addition to reduced substrate concentration due to SAP-C deficiency, ceramidase is missing its physiological activators, SAP-D (37) and SAP-C (4). However, it needs to be clarified in future studies whether covalently attached Cer(OS) and GlcCer(OS) are indeed substrates for ceramidase and β-GlCerase, respectively.

Finally, morphological alterations in the epidermis of pSAP −/− mice were significant and reminiscent of the alterations observed in β-GlCerase-deficient Gaucher epidermis. Electron microscopy revealed a striking abnormality in SC lamellar membrane organization. Moreover, light micrographs revealed a partial loss of stratum corneum in pSAP −/− mice, which may reflect decreased SC coherence. Thus, the accumulation of

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