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Lipids and barrier function of the skin

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The purpose of the present manuscript is to review the chemical and physical properties of epidermal lipids and to relate these properties to the formation and function of the permeability barrier of the skin. Lipids accumulate in small organelles known as lamellar granules as epidermal keratinocytes differentiate. This lipid is extruded into the intercellular spaces where it undergoes enzymatic processing to produce a lipid mixture consisting of ceramides, cholesterol and fatty acids. This intercellular lipid is uniquely organized into a multilamellar complex that fills most of the intercellular space of the stratum corneum. The barrier properties of the stratum corneum are related to the phase behavior of the intercellular lipids. It has been proposed that a structurally unusual acylglucosylceramide is thought to be involved in assembly of the lamellar granules, and a related acylceramide may have a major influence on the organization of the lamellae in the stratum corneum. ***Key words: cell membrane; ceramides; cholesterol; epidermis; keratinization.***

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LAMELLAR GRANULES AND FORMATION OF THE EPIDERMAL BARRIER

The outermost layers of the epidermis, the stratum corneum, provide a permeability barrier that prevents desiccation and thereby permits life on dry land (1). The barrier function depends upon the presence of a unique mixture of lipids in the intercellular spaces of the stratum corneum (2). As epidermal cells move from the basement membrane outward through the epidermis and toward the skin surface they undergo differentiation, one major aspect of which is the accumulation of lipids. Much of the lipid that is synthesized as the keratinocytes mature is packaged in a small organelle known as the lamellar granule (3). Toward the end of the differentiation program, the lamellar granule discharges its contents into the intercellular space. In addition to lipids, the lamellar granules delivers a battery of hydrolytic enzymes to the intercellular domains (4-6). At the time of exocytosis this lipid mixture consists mainly of phospholipids, glycolipids and cholesterol. Enzymatic processing of the lipids in the vacinity of the stratum granulosum-stratum corneum interface results in the production of a mixture of mainly ceramides, fatty acids and cholesterol. The free fatty acids are straight-chained saturated species (7). The monounsaturated fatty acids from the phosphoglycerides are transferred to cholesterol (8), and linoleic acid appears to be recycled within the viable epidermis (9). The usaturated cholesterol esters resulting from transfer of mainly oleic acid from phosphoglycerides to cholesterol in the final stages of keratinization may subsequently separate from the membrane forming lipids into a liquid phase, thus isolating the potential permeability enhancer, oleic acid.

Since the lamellar granules are lipid-rich organelles, they have a low buoyant density. This fact has been exploited to isolate subcellular fractions enriched in lamellar granules by metrizamide density gradient centrifugation (4, 6). This organelle is round to ovoid in shape, approximately 200 nm in diameter, and consists of a bounding membrane surrounding a stack of lipid lamellae and some amorphous material (3). The internal lamellae are thought to be flattened lipid vesicles (3, 10). Lipid analysis of isolated lamellar granules has revealed that this organelle is enriched in a very unusual glycolipid (6, 11). This glycolipid consists of 30- through 34-carbon ω-hydroxyacids amide-linked to a sphingosine base with linoleic acid ester-linked to the ω-hydroxyl group and glucose β-glycosidically attached to the primary hydroxyl group of the base (12, 13). This is illustrated in Figure 1. This acylglucosylceramide is the principal carrier of linoleic acid in the viable epidermis, and along with a related acylceramide, found mainly in the stratum corneum, accounts for the essential role of linoleic acid in maintaining the barrier function of the skin (14).

It has been proposed that the linoleate-containing acylglucosylceramide is involved in formation of the lamellar granules (15). There is evidence to support the contention that the lipid contents of the lamellar granules consist of stacks of flattened lipid vesicles (3, 10). The ω-hydroxyacyl portion of the acylglucosylceramide is approximately twice as long as the typical fatty acyl chain, and so this portion of the molecule is long enough to completely span a bilayer. It has been suggested that this ω-hydroxyacyl chain does span a bilayer while the linoleate tail inserts into a closely apposed section of bilayer, thus serving as a molecular rivet to link two membranes together (15). This interaction could promote both the flattening of lipid vesicles and the stacking or aggregation of flattened vesicles. In addition to the unique structure of the acylglucosylceramide and the enrichment of this glycolipid in the lamellar granules, there is abundant additional evidence to support the hypothesis that this unusual lipid is involved in lamellar granule assembly. Lamellar granules are uniquely found in keratinizing epithelia, and acylglucosylceramide has been found in the keratinizing epithelia from the regions of the hard palate and gingiva but not in the nonkeratinizing epithelia from the floor of the mouth or the buccal regions or non-epithelial tissues (16). Likewise, the epidermis from a variety of mammals and from birds and reptiles contain both lamellar granules and acylglucosylceramide; whereas, the epidermis from amphibians and fish contains neither (16). Finally, acylglucosylceramide has been shown to cause the flattening and aggregation of liposomes in vitro (17). Simpler glycolipids had no effect on vesicle morphology.

It appears that approximately one-third of the lamellar

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granule-associated acylglucosylceramide is in the internal lamellae of the granule, while two-thirds is in the bounding membrane. When the bounding membrane of the lamellar granule fuses into the cell plasma membrane prior to extrusion of the contents into the intercellular space, acylglucosylceramide is introduced to the cell periphery. All of the glucosylceramides are deglycosylated at about this point in the differentiation program, and the linoleate is removed from the pool of acylglucosylceramide at the cell surface and may be recycled. The resulting ω-hydroxyceramide, shown in Figure 1, becomes covalently attached to the outer surface of the cornified envelope, which replaces the cell plasma membrane in the final stages of keratinization (18). Half of the covalently bound hydroxyceramide molecules are ester-linked to the envelope through the ω-hydroxyl group, and the other half are ester-linked through one of the hydroxyl groups of the sphingosine base. The amount of covalently bound hydroxyceramide is just sufficient to provide a monolayer coating the outer surface of the cornified envelope (19). This covalently bound lipid layer may be significant in making individual corneocytes impermeable and may provide a template upon which the flattened vesicles extruded from the lamellar granules undergo transformation into the broad lamellae of the stratum corneum.

The acylglucosylceramide associated with the internal lamellae of the lamellar granules undergoes deglycosylation at approximately the time that it is extruded into the intercellular space and passes into the stratum corneum as acylceramide, shown in Figure 1. It has been suggested that the acylceramide plays a major role in determining the state of organization of the intercellular lamellae (20, 21). It follows that if acylglucosylceramide serves as a molecular rivet, as proposed above, to promote flattening and stacking of lipid vesicles which subsequently fuse in an edge-to-edge manner to produce the lamellae of the stratum corneum then paired bilayers should be formed. These bilayers would remain paired by virtue of the acylceramide that is derived from acylglucosylceramide acting as a molecular rivet. Furthermore the paired bilayers would be capable of interacting with other structures by extending linoleate from acylceramide molecules outward.

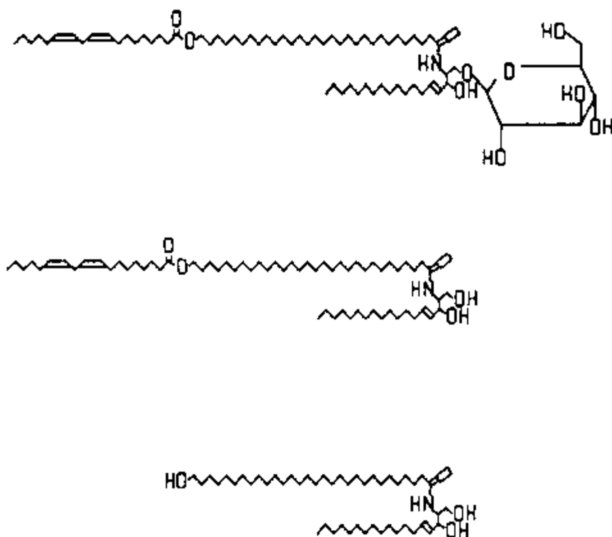

Fig. 1. ω-Hydroxyacid-containing sphingolipids from epidermis. Acylglucosylceramide (top) is associated with lamellar granules. Acylceramide (middle) is a component of the intercellular lamellae in the stratum corneum. ω-hydroxyceramide (bottom) is covalently attached to the outer surface of the cornified envelope.

STRATUM CORNEUM LIPIDS AND BARRIER FUNCTION

The major lipid classes that can be extracted from stratum corneum are ceramides, cholesterol and fatty acids, which make up approximately 50, 25 and 10 percent of the stratum corneum lipid mass, respectively (2). Small amounts of cholesterol sulfate and cholesterol esters are also present. In porcine stratum corneum there are six chromatographically separable ceramide fractions (22). The least polar of these corresponds to the above mentioned acylceramide. The second fraction in order of increasing polarity, ceramide 2, contains normal, mostly 24-, 26- and 28-carbon fatty acids amide-linked to sphingosine and dihydrosphingosine bases. The bases range from 16 through 20 carbons in length. Ceramide 3 contains the same normal fatty acids found in ceramide 3, but the base component is now a phytosphingosine. Ceramides 4 and 5 both contain α-hydroxyacids amide-linked to sphingosine and dihydrosphingosine bases. They differ in that the more mobile ceramide 4 contains mainly 24- through 28-carbon α-hydroxyacids, whereas ceramide 5 contains almost exclusively α-hydroxypalmitic acid. The most polar ceramide, found in fraction 6,consists of α-hydroxyacids amide-linked to phytosphingosines. In the human the same ceramide types are present, but there are additional variants of acylceramide, ceramide 3 and ceramide 6 in which the base component is 6-hydroxysphingosine (23, 24).

As noted above, the free fatty acids found in either porcine or human stratum corneum are straight-chained saturated species, and although the chain lengths range from 16 through 30 carbons, the most abundant species are C24:0, C26:0 and C28:0. The fatty acids may be required for formation of a lamellar phase since this is the principal ionizable lipid class in the stratum corneum.

Cholesterol is the most abundant individual lipid in the stratum corneum, accounting for approximately 25% of the stratum corneum lipid mass. This appears to be a saturating level (25).

All of the ceramides and the fatty acids found in stratum corneum are rod or cylindrical in shape, and this physical attribute makes these lipids suitable for the formation of highly ordered gel phase membrane domains. Gel phase domains will be less fluid, and thereby, less permeable than their liquid crystalline counterparts. Cholesterol is a ubiquitous membrane lipid and is capable of either fluidizing membrane domains or of making them more rigid, depending on the physical properties of the other lipids and the proportion of cholesterol relative to the other components. The role of cholesterol in the epidermal barrier is probably to provide a degree of fluidity to what could otherwise be a rigid, possibly brittle membrane system. This may be necessary for pliability of the skin.

THE DOMAIN MOSAIC MODEL AND FUTURE DIRECTIONS

There is now evidence for the existence of gel phase lipid domains within the stratum corneum from several lines of investigation, and the current picture of the physical state of the intercellular lamellae is one in which gel phase domains and liquid crystalline domains coexist. This concept was put forth as the domain mosaic model by Forslind (26). Within this model islands of gel phase domains are separated by a continuous liquid crystalline domain. Within such lamellae, polar molecules would diffuse laterally along or close to the polar head group regions, and less polar molecules would diffuse laterally within the hydrophobic interior of the bilayer. Molecules could diffuse only poorly across the gel phase domains, and diffusion across such lamellae would be more favorable at the liquid crystalline domains. For both theoretical and experimentally established reasons, the regions of greatest flux across such mixed phase lamellae would be at the phase boundaries (27). The phase boundaries have the greatest frequency of chain packing defects.

When the stratum corneum is examined by transmission electron microscopy following fixation with ruthenium tetroxide it can be seen that most of the intercellular space is occupied by lamellae (28). Smaller proportions of intercellular space contain desmosomes and amorphous material. Electron dense amorphous material is thought to represent desmosomal breakdown products, while electron lucent amorphous material is thought to represent liquid phase cholesterol esters. In the lamellar regions both the number of lamellae across the intercellular space and the pattern of lamellar organization varies. Near the ends of the corneocytes a three band arrangement with a broad-narrow-broad lucent band pattern is frequently seen. The broad bands are approximately 5 nm wide, and the narrow band is about 3 nm wide. The overall width of the entire broad-narrow-broad unit is 13 nm (28). It has been proposed that this particular lamellar arrangement is the result of an interaction between the covalently bound lipid on the outer surfaces of two adjacent corneocytes (29). The sphingosines from the covalently bound ω-hydroxyceramides evert to form a highly interdigitated zipper-like narrow lamella between the broad ω-hydroxyacyl portions of these molecules. Free lipids would fill some of the space within this structure. This interaction effectively links the adjacent cells together.

Between the broad flat surfaces of corneocytes one occasionally sees uniformly spaced, roughly 5 nm wide lamellae; however, multiples of an alternating broad-narrow-broad arrangement are dominant (30). The two most common patterns are the 6 band broad-narrow-broad-broad-narrow-broad and the 9-band broad-narrow-broad-broad-narrow-broad-broad-narrow-broad. Figure 2 shows a section of intercellular space containing 9-band and 12-band patterns. At a molecular level, it is thought that the central pair of broad bands in the six band pattern represent paired bilayers produced by edge-to-edge fusion of the flattened vesicles extruded from the lamellar granules. These paired bilayers are sometimes referred to as Landmann units. The outermost lucent bands on either side of the intercellular space are the covalently bound lipid layers, and the narrow bands intervening between the outermost broad bands and the central pair of bilayers are the interdigitated zipper layers.

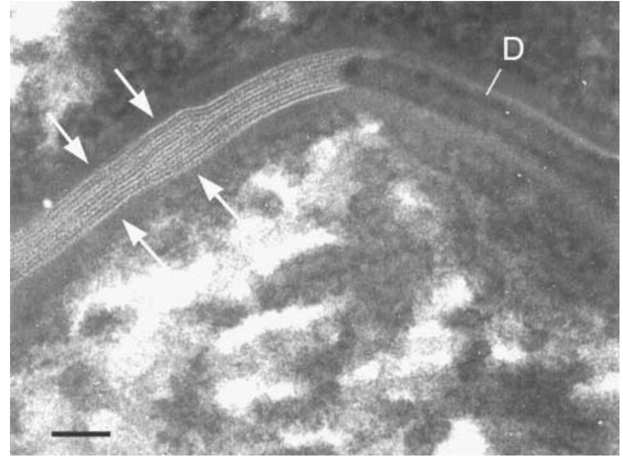

Fig. 2. Intercellular lamellae in the stratum corneum. Arrows point to the intercellular lamellae. D indicates a desmosome. Note the nine lucent band pattern to the immediate left of the desmosome, and the abrupt transition to a 12-band pattern between the arrows. The bar represents 50 nm.

Participation of acylceramide associated with the central pair of bilayers in formation of the zipper layers may be essential. In this context it has been suggested that linoleate tails from the acylceramide molecules in the central pair of bilayers extend into the narrow molecular zipper layers (31). As noted previously, the paired bilayers are thought to be linked together through a molecular rivet action of acylceramide. Again, every pair of adjacent lamellae across the intercellular space share some molecules, and the adjacent corneocytes are effectively cross-linked. The 9 band pattern contains two paired bilayers with a zipper layer between them, and the 12 band pattern contains three Landmann units with intervening zipper layers.

In fact, it has been shown that a broad-narrow-broad pattern can be produced by reconstitution of extracted stratum corneum lipids in vitro (21). One broad-narrow-broad unit has an overall width of 13 nm, and a 13 nm periodicity has been reported in the x-ray diffraction patterns of both stratum corneum and reconstituted stratum corneum lipids (20, 25). In the x-ray diffraction studies, it has been shown that if acylceramide is removed from the lipid mixture, then the 13 nm periodicity is not seen after reconstitution (20). Similarly, oral stratum corneum lipids contain a much lower proportion of acylceramide than is found in epidermal stratum corneum (32), and the broad-narrow-broad units are never seen in transmission electron micrographs of oral stratum corneum or reconstituted oral stratum corneum lipids (33, 34). These observations all support the contention that acylceramide acts as a molecular rivet in the stratum corneum and participates in formation of the zipper layers. This may be the essential role of linoleic acid in the barrier function of the skin. In fact, recent reexamination of transmission electron micrographs from essential fatty acid deficient pigs and controls revealed that in the control animals the usual broad-narrow-broad units were ubiquitous, but only uniformly spaced lamellae were evident in the deficient animals (D.C. Swartzendruber, B.B. Michniak, P.W. Wertz, unpublished observations).

The interdigitation of all of the lamellae across the intercellular spaces of the stratum corneum not only provides

for a degree of cohesion to the stratum corneum, but it almost certainly will influence lateral mobility and fluidity of the entire intercellular membrane system. Testing this hypothesis and its potential implications for barrier function presents a goal and challenge for future research.

In addition to lipid chain interdigitation, desmosomes are thought to be a major source of cohesion between stratum corneum cells, and their degradation is essential for desquamation (35, 36). Proteases have been demonstrated to degrade the desmosomal proteins (36, 37), and it appears that hydrolysis of cholesterol sulfate also accompanies cell shedding (38). Essentially nothing is known about the desmosome-lipid interface in stratum corneum. It is tempting[9] to speculate that the membrane regions in the proximity of the desmosomal plaques are enriched in cholesterol sulfate, and that the degradation of this lipid is necessary to permit proteolysis. Although direct evidence to support this speculation is presently lacking, it seems likely that the physical properties, possibly including the permeability, of the membrane regions immediately adjacent to the desmosomes are likely different from those in the bulk of the intercellular space of the stratum corneum. Elucidating the interaction between protein and lipid in the regions of the desmosomal plaques and the significance of this interaction for stratum corneum function represents another challenge for the future.

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