3 Epidermal Lipids and Formation of the Barrier of the Skin

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CONTENTS

3.1 LIPIDS IN THE EPIDERMIS

3.1.1 Introduction

The evolution of life in the relatively dry terrestrial environment required the development of a waterproof integument.[1] In the terrestrial vertebrates, the stratum corneum provides the primary barrier to water loss. The barrier function of the stratum corneum depends upon a unique mixture of lipids that form lamellar structures in the intercellular spaces.[2–5] This generally consists of ceramides, cholesterol, and long chain fatty acids.

In human epidermis, the lipid end products of differentiation consist of cholesterol, 22- through 28-carbon straight chain saturated fatty acids, and nine different series of ceramides.[5,6] The building blocks from which the ceramides are composed include sphingosine, phytosphingosine, and 6-hydroxysphingosine as the base components and normal saturated fatty acids, α-hydroxyacids, and ω-hydroxyacids as the amide-linked fatty acids. In addition, the ω-hydroxyacid-containing ceramides bear ester-linked linoleate on the ω-hydroxyl group. All nine possible combinations of base–acid pairings are formed.[6] Representative structures are shown in Figure 3.1.

In addition to the free lipids found in the intercellular spaces of the stratum corneum ω-hydroxyceramides, ω-hydroxyacids, and fatty acids are covalently attached to the outer surface of the cornified envelope.[7–9] The hydroxyceramides and hydroxyacids are thought to be attached through ester-linkages involving glutamic or aspartic acid side chains,[10] while the fatty acids are thought to be attached through formation of ester linkages with serine or threonine hydroxyl groups. Evidence has been presented indicating that transglutaminase 1 may be responsible for attachment of the hydroxyceramides to the envelope.[11] Representative structures of the covalently bound lipids are presented in Figure 3.2.

The literature is replete with the use of chromatographic fraction numbers to indicate ceramide structural types. This can be very confusing because different laboratories have achieved different degrees of separation and because, even with the best resolution achieved, there is at least one fraction

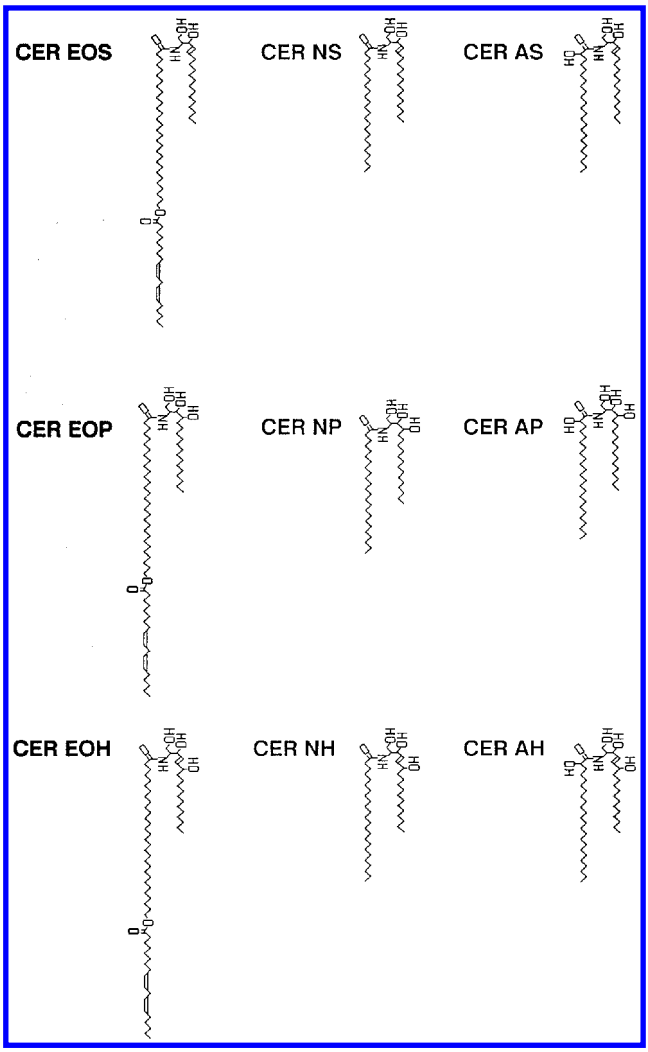

FIGURE 3.1 Representative structures of human stratum corneum ceramides.

that contains two structural types of ceramides. A solution to this problem is a nomenclature system in which the long chain base and amide-linked fatty acids are designated by single letters[12]: S for sphingosine, P for phytosphingosine, H for 6-hydroxysphingosine, N for normal fatty acid, A for α-hydroxyacid, and O for ω-hydroxyacid. The presence of an ester-linked fatty acid is indicated by a prefix E. Thus the acylceramide in which ω-hydroxyacid is amide-linked to sphingosine and linoleate is ester-linked to the ω-hydroxyl group would be designated as ceramide EOS. Similarly, the ceramide consisting of normal fatty acids amide-linked to phytosphingosine would be ceramide NP. This nomenclature is used in Figure 3.1 and Figure 3.2.

Both x-ray diffraction studies and investigations using transmission electron microscopy have indicated that the intercellular lipids are organized into 13 nm trilaminar structures.[13–15] The formation of these trilaminar units seems to require ceramide EOS,[16,17] although to a lesser extent supplementation of lipid mixtures with synthetic EOP can promote self assembly.[18] The possible role of ceramide EOH has not been studied directly; however, it is clear that the natural proportion of ceramide EOH[6] is probably insufficient to promote self assembly of 13 nm units. A transmission electron micrograph of the intercellular lipid lamellae is shown in Figure 3.3. Controversy exists regarding details of the organizational state of the intercellular lipids.[19–21]

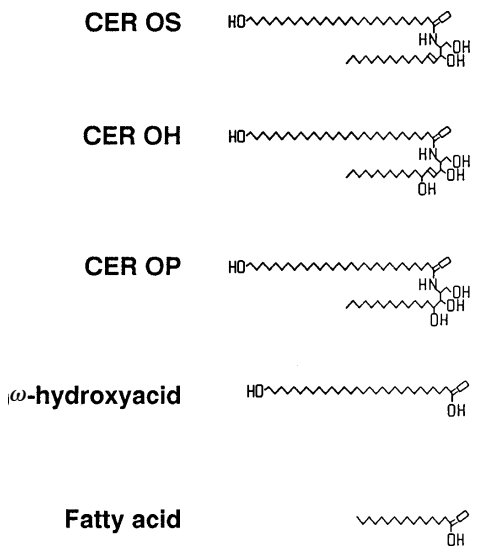

FIGURE 3.2 Representative structures of covalently bound lipids from human stratum corneum.

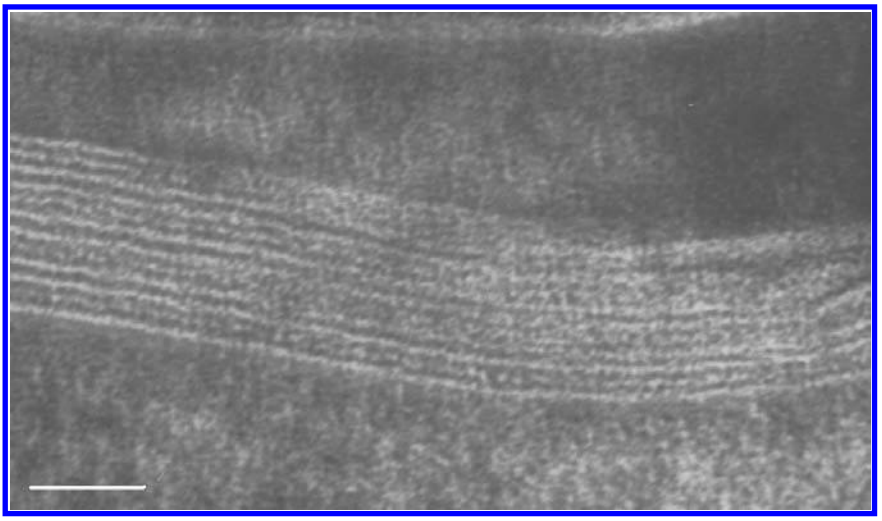

FIGURE 3.3 Intercellular lipid lamellae in the stratum corneum. Bar equals 30 nm.

Interactions between water and the polar head groups of lipid molecules are necessary for the formation of lamellar phases; however, it appears that there is no free water associated with the 13 nm trilaminar units. This is supported by the observation that this periodicity does not increase with increasing stratum corneum water content.[22] There is likely water hydrogen bonded to the polar regions of the lamellae. In contrast, the minor short periodicity swells from 5.8 to 6.6 nm as the water content of stratum corneum increases from 12 to 50%.[23] This suggests that the lipid lamellae are simple individual bilayers and free water molecules can exist between adjacent bilayers, thus causing the increase in the lamellar spacing.

3.1.2 Carbon Sources

Linoleic acid is the parent essential fatty acid of the ω-6 series, and as such, must be obtained in the diet.[24] It is primarily derived from vegetables and nontropical vegetable oils. When linoleate was

injected intradermally into porcine skin, it was initially taken up into a small, rapidly turning over pool of triglycerides.[25] It was rapidly transferred to phosphoglycerides, then to an acylglucosylceramide (glycosylated version of ceramide EOS), and finally to ceramide EOS. Basal keratinocytes have low density lipoprotein (LDL) receptors and can thereby derive cholesterol from the circulation; however, once keratinocytes move upward and out of the basal layer, the LDL receptors are internalized and degraded.[26] Except for linoleate and lipid internalized via basal cell LDL receptors, it is thought that most of the remaining carbon for epidermal lipid synthesis is derived from circulating acetate.[27] Although cultured keratinocytes have been shown to incorporate carbon from glucose into lipids, when radiolabelled glucose was injected intradermally, only the glycerol moiety of the phosphoglycerides became labeled.[27]

3.1.3 Energy Production

Adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) are required to support lipid biosynthesis. Basal keratinocytes have functional mitochondria and are thought to produce energy by β-oxidation of fatty acids.[28,29] The enzymes necessary for glycolysis are also present, but this is much less efficient than β-oxidation and the mitochondrial system. However, as cells move upward metabolism becomes increasingly more anaerobic until in the granular layer energy is produced entirely by anaerobic glycolysis with reduction of pyruvate to lactate. In fact, the mitochondria are degraded. The degradation of mitochondria and other internal membranous organelles would result in the release of calcium previously sequestered by these structures. The generation of the calcium gradient is one of the factors driving differentiation.[30]

3.1.4 Major Biosynthetic Pathways

All the major biosynthetic pathways use acetyl-CoA as the basic building block, and in each pathway the rate limiting enzyme is regulated by phosphorylation with the phosphorylated enzyme being active. In the biosynthesis of cholesterol, the rate limiting step is catalyzed by hydroxymethylglutaryl-CoA (HMG-CoA) reductase. Initially, three molecules of acetyl-CoA are condensed to produce β-HMG-CoA. HMG-CoA reductase then uses two NADPH molecules to reduce HMG-CoA to mevalonate-CoA. The remaining steps in cholesterol biosynthesis are numerous and well-documented.

The rate limiting step in fatty acid synthesis is catalyzed by acetyl-CoA carboxylase to produce malonyl-CoA at the expense of one ATP.[31] Malonate and acetate are transferred from CoA to acyl carrier protein in the cytosolic fatty acid synthetase complex, where chain extension leads to the production of palmitate. Palmitate can then be transferred back to CoA, and the chain can be extended two carbons at a time through the action of a fatty acid elongase system located in the endoplasmic reticulum. The ω-hydroxylation that produces the ω-hydroxyacids of the acylceramides is thought to be mediated by a cytochrome p450 just when the fatty acid is long enough to span the endoplasmic reticular membrane.

The rate limiting step for all sphingolipid biosynthesis is serine palomitoyl transferase, which condenses palmitoyl-CoA with serine to produce 3-ketodihydrosphingosine.[32] The keto group is rapidly reduced, and the resulting dihydrosphingosine group is N-acylated to produce a simple ceramide. The 4,5-*trans* double bond can then be introduced in the base component, and various positions can be hydroxylated to produce α-hydroxyacids, phytosphingosines, and 6-hydroxysphingosines. These hydroxylation reactions require vitamin C.[33]

3.2 LAMELLAR GRANULES

Much of the lipid that accumulates with keratinization is packaged in small organelles called lamellar granules.[4,34] These small organelles have also been called Odland bodies, keratinosomes, membrane

coating granules, lamellar bodies, and cementsomes. They are derived from the Golgi apparatus and are generally round to ovoid in shape and about 0.2 μm in diameter. They consist of a unit bounding membrane surrounding one or several internal stacks of lipidic disks. They are lipid rich, and therefore, have a low buoyant density. This property has been exploited to isolate lamellar granules from rodent and porcine epidermis.[35–37] They are particularly rich in glycolipids, especially the glucosylated analogue of ceramide EOS, and phospholipids, and contain a relatively high proportion of cholesterol. They contain little ceramide or free fatty acids. It has been suggested that glucosylceramide EOS may be involved in assembly of the internal lamellae of the lamellar granules. More recently, it has been suggested that a large portion of the lamellar granule-associated glucosylceramide EOS is actually in the bounding membrane.[5] This pool of glucosylceramide EOS would be introduced to the cell periphery when the bounding membrane of the organelle fuses with the cell plasma membrane, and could be the precursor of the covalently bound hydroxyceramide on the cornified envelope.

3.3 CATABOLISM

In addition to delivering lipids to the intercellular space between the granular layer and the stratum corneum, lamellar granules also deliver a battery of hydrolytic enzymes that convert the initially extruded phospholipid- and glycolipid-rich lipid mixture into the fatty acids and ceramides of the stratum corneum intercellular spaces.[38,39] In rodent epidermis, some of this lipid processing continues in the intercellular spaces of the stratum corneum; however, with porcine and human epidermis conversion to the mature barrier lipids is completed at the stratum granulosum–stratum corneum interface. The enzymes that mediate this transformation are mainly acid hydrolases and include a glucocerebrosidase to convert glucosylceramides to ceramides, and acid sphingomyelinase to convert sphingomyelin into ceramides and a battery of phospholipases to release fatty acids from phosphoglycerides.

3.4 COMPOSITION

The literature regarding the composition of human stratum corneum lipids has recently been reviewed.[40] In general, there is a great deal of variation among the published compositions. Some of this probably reflects differences in the analytical methods that were used; however, much of the variation reflects failures to recognize contaminants including sebaceous lipids, subcutaneous fat, and environmental hydrocarbons. When the known contaminants are factored out, it is apparent that the main stratum corneum lipids are ceramides, cholesterol, and free fatty acids in the ratio of 50:27:12 by weight. When the average molecular weights are taken into consideration, these major components are present in roughly 1:1:1 molar proportions. Mixtures of ceramides:cholesterol and fatty acids in a 1:1:1 molar ratio have been used by a number of investigators to approximate stratum corneum lipids for studies of physical properties.[41–43]

The remaining 11% of the stratum corneum lipid mass consists mainly of cholesterol sulfate and cholesterol esters.[44] The cholesterol sulfate has been implicated in regulation of the desquamation process. It has been shown that cholesterol sulfate inhibits serine proteases of the types that degrade desmosomal proteins leading ultimately to cell shedding. A sterol sulfatase must act on cholesterol sulfate to make the proteolytic degradation of the desmosomes possible. The degradation of cholesterol sulfate in association with desquamation has been demonstrated both with an organ culture model and with human skin *in vivo*. Cholesterol esters have long been cited as a hallmark of keratinization; however, these liquid phase lipids are probably not found within the intercellular lamellae. Late in the keratinization process, oleate is transferred to cholesterol to produce cholesterol oleate. This cholesterol ester is not accommodated well by membranes, and it has been suggested that it phase separates into isolated pockets within the intercellular space. The cholesterol ester deposits are

thought to be reflected in amorphous pockets within the intercellular spaces in transmission electron micrographs. The transfer of oleate to cholesterol and subsequent phase separation of cholesterol oleate may provide a mechanism for keeping oleic acid, a well-known permeability enhancer, out of the lamellar domains that provide the barrier function.

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