Biochimica et Biophysica Acta, 1150 (1993) 98–102

BBAMEM 76021

Covalently bound lipids in keratinizing epithelia

Felicia Chang, Donald C. Swartzendruber, Philip W. Wertz and Christopher A. Squier

Dows Institute for Dental Research, University of Iowa, Iowa City, IA (USA)

(Received 14 January 1993)

Key words: Ceramide; Epidermis; Gingiva; Lipid; Palate

Covalently bound lipids have been identified and compared in keratinizing porcine epithelia including epidermis and oral epithelium from palate and gingiva. Stratum corneum was isolated by tryptic digestion, and after extensive extraction of lipids using a series of chloroform-methanol mixtures, the residual tissue was subjected to alkaline hydrolysis to release covalently bound lipids. The lipids so released were analyzed by quantitative thin-layer chromatography. Stratum corneum from each of the three anatomical sites contained ω-hydroxyceramides, ω-hydroxyacids and fatty acids. In epidermal stratum corneum the total covalently bound lipids represented 2.4% of the dry weight of the tissue, but in the oral epithelia this figure was consistently lower: 0.24% in palatal stratum corneum and 0.20% in gingival stratum corneum. Transmission electron microscopy before and after lipid extraction confirms the presence of a lipid envelope in epidermal stratum corneum and demonstrates the absence of this structure in oral stratum corneum.

Introduction

In the final stages of keratinocyte maturation, a thick band of protein is deposited on the inner surface of the plasma membrane, and the component proteins in this layer become cross-linked by isopeptide linkages through the action of a transglutaminase [1–3]. This feature has been called the cornified envelope. In the epidermis, it has recently been demonstrated that disappearance of the plasma membrane occurs simultaneously with the emergence of a lipid monolayer at the cell periphery and formation of the cornified envelope [4–7]. This lipid becomes covalently linked, apparently through ester or thioester bonds, to the outer surface of the cornified envelope and has been referred to as the lipid envelope. The covalently bound lipids in epidermal stratum corneum consist mainly of an unusual ω-hydroxyceramide with smaller amounts of ω-hydroxyacid and normal fatty acid [4]. The hydroxyacids in both the ω-hydroxyceramide and free acid fractions are unusual in that they are 30 through 34 carbons in length, so that the monolayer formed by these lipids is of the same thickness as a more conventional lipid bilayer. It has been proposed that the lipid envelope may serve as a template upon which the broad intercellular lamellae that provide the intercellular epidermal permeability barrier are formed [6,7]. In this way, covalently bound lipids may influence the organization of lipids in the intercellular spaces and thereby determine the effectiveness of the permeability barrier.

Keratinized oral epithelium, such as that covering the hard palate or the gingiva, provides an interesting comparison with epidermis. The maturation pathways in these keratinizing epithelia appear to be almost identical [8–10], and the types and amounts of lipids found in stratum corneum from epidermis and keratinized oral epithelium are grossly similar [11,12]. However, the permeability of the keratinized oral epithelium is an order of magnitude greater than that of epidermis [9], and previous studies [11,12] have indicated that this difference in permeability may be related to stratum corneum lipid content (105 vs. 47 μg lipid/mg tissue in epidermal vs. palatal stratum corneum) and possibly to subtle differences in lipid composition (0% vs. 12% phospholipids in epidermal vs. palatal stratum corneum). A large difference in the concentrations of covalently bound lipids in epidermal and palatal stratum corneum has also been noted [12], and it has been speculated that this covalently bound lipid is of significance for the organization of the intercellular lipid lamellae that are thought to determine the permeability of the stratum corneum [6,7]. The present investigation extends these previous studies to include direct comparisons of covalently bound lipids from epidermal, palatal and gingival stratum

Correspondence to: P.W. Wertz, N450 Dental Science Building, University of Iowa, Iowa City IA 52242, USA.

corneum. In addition, transmission electron micrographs of stratum corneum were recorded before and after lipid extraction to determine whether differences in the amounts of covalently bound lipids influence the organization of lipid lamellae in the intercellular spaces of the stratum corneum.

Materials and Methods

Preparation of stratum corneum [12]. Porcine tissue was obtained at a local slaughter house and epithelium was heat separated from the underlying tissue. This involved applying an aluminum cylinder heated to 65°C to the epithelial surface for 30 to 60 s after which the epithelium could be removed by gentle scraping with a stainless steel spatula. For the isolation of stratum corneum, the epithelium was suspended in 1% trypsin (Sigma, St. Louis, MO) in 50 mM phosphate buffer at pH 7.4 containing 150 mM sodium chloride (PBS) and kept at 4°C overnight. The epithelium was then rinsed with distilled water and resuspended in fresh 1% trypsin in PBS and kept at room temperature with gentle agitation for 2 h, after which the stratum corneum was rinsed extensively with distilled water to remove the digested noncornified cells and debris.

Recovery and analysis of covalently bound lipids [4]. Each piece of tissue (10–100 mg) was placed in a glass culture tube and extracted for 2-h intervals at room temperature with 10-ml portions of chloroform/methanol, 1:2, 1:1 and 2:1. This series of extractions was repeated, and the tissue was finally extracted for 24 h with 10 ml of chloroform/methanol, 2:1. The residual tissue was dried under vacuum and weighed.

The extracted and dried tissue was treated with 4 ml of 1 M sodium hydroxide in 90% methanol. After acidification to pH 4 with 6 M hydrochloric acid, the lipids released by the hydrolysis were extracted into 5 ml of chloroform. The chloroform extraction was repeated and the combined extracts from each piece of tissue were dried under nitrogen and finally under vacuum. The dried samples were redissolved in 100 μl of chloroform/methanol and analyzed by thin-layer chromatography. For the thin-layer chromatographic analyses, 20 × 20 cm glass plates coated with a 0.25-mm-thick layer of silica gel G (Adsorbosil soft layer; Alltech Associates, Deerfield, IL) were used. The adsorbent was scored into 6-mm-wide lanes and one sample or standard was applied per lane 2.5 cm from the bottom edge of the plate, which was then developed with a mobile phase consisting of chloroform/methanol/acetic acid (190:9:1, v/v). Standards included stearic acid (Sigma), ω-hydroxyacids from Carnauba wax [13] and ω-hydroxyceramide isolated from porcine epidermis [4]. The chromatograms were charred by spraying with 50% sulfuric acid and heating to 220°C as previously described [14]. The charred chromatograms were quantitated using a Bio-Rad model 620 video densitometer.

TABLE I

Covalently bound lipids in epithelial barriers

Results are presented as mean weight percent of dry tissue ± S.D. For palatal stratum corneum five samples were analyzed, whereas four samples were used for epidermis and gingiva. Results were analyzed by analysis of variance and p values in the post tests were corrected by the Bonferroni method. These analyses indicated that epidermal stratum corneum contains significantly higher ($p < 0.001$) concentrations of total bound lipid, ω-hydroxyceramide and ω-hydroxyacid than the other epithelial barriers. The concentrations of fatty acids in the different epithelia were not significantly different ($p > 0.05$).

	Epidermal stratum corneum	Palatal stratum corneum	Gingival stratum corneum
ω-Hydroxyceramide	1.6 ± 0.1	0.05 ± 0.04	0.06 ± 0.02
ω-Hydroxyacid	0.5 ± 0.1	0.04 ± 0.01	0.05 ± 0.03
Fatty acid	0.3 ± 0.1	0.15 ± 0.10	0.09 ± 0.07
Total bound lipid	2.4 ± 0.2	0.24 ± 0.10	0.20 ± 0.13

Electron microscopy [15]. Small samples of stratum corneum were fixed in either 2.5% glutaraldehyde buffered in 0.1 M sodium cacodylate or in Karnovsky's fixative and post fixed in 0.2% ruthenium tetroxide in 0.1 M cacodylate buffer. The specimens were then dehydrated in a series of acetone solutions (30, 50, 70, 95 and 100%) before embedding in Spurr's resin. Ultrathin sections were cut and collected on naked grids or on carbon-stabilized Formvar coated grids. Some sections were stained with uranyl acetate and lead citrate or with lead citrate alone prior to examination in a Zeiss EM 10 electron microscope.

Results

Table I summarizes the amounts and composition of the covalently bound lipids found in the several epithelial tissues examined. Representative structures of these lipids are summarized in Fig. 1. The ω-hydroxyacids both in the free form and in the hydroxyceramides are unusually long 30- through 34-carbon entities, whereas

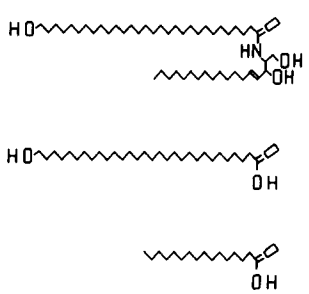

Fig. 1. Representative structures of covalently bound lipids from stratum corneum. Presented from top to bottom are ω-hydroxyceramide, ω-hydroxyacid and normal fatty acid.

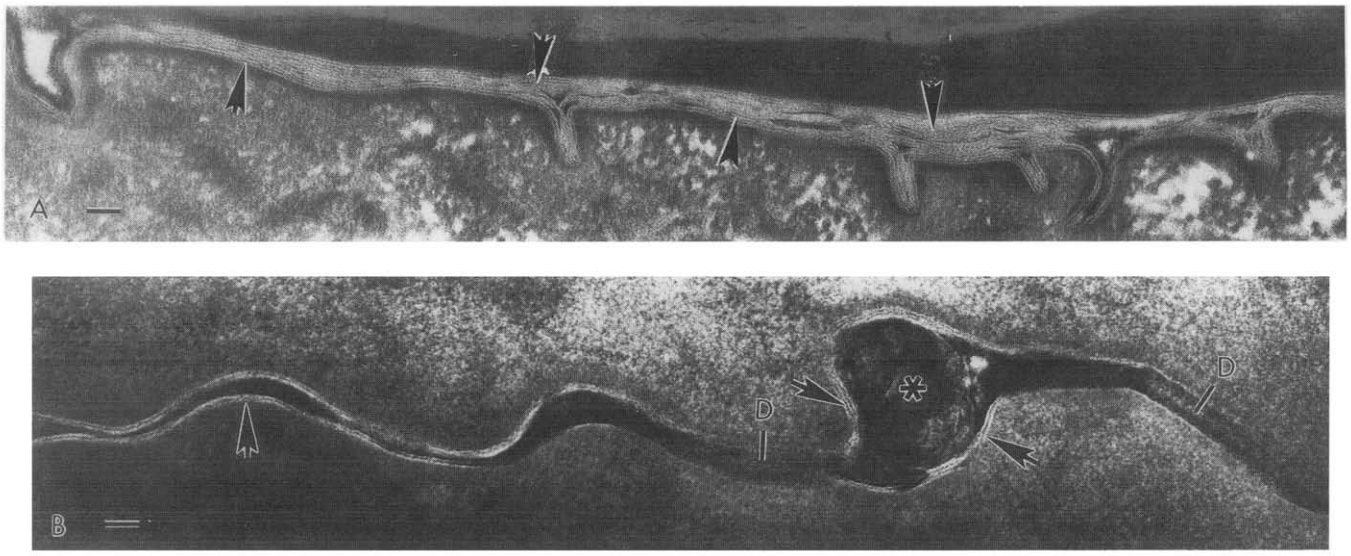

Fig. 2. Transmission electron micrographs of epidermal and palatal stratum corneum. (A) Epidermal stratum corneum. The bar represents 50 nm. Notice the extensive intercellular lamellae (arrowheads) filling the intercellular space. (B) Palatal stratum corneum. Bar = 50 nm. Dilated intercellular spaces (*) between desmosomes (D) contain broad lamellae (arrowheads) at the periphery, but the interior of these dilations contain a mixture of amorphous electron dense material and what appears to be lamellae extruded from membrane-coating granule that have not been transformed into sheets.

the simple fatty acids are mainly 16 and 18 carbons long with palmitate predominating (data not shown). Although the three keratinizing epithelia all contain the same types of covalently bound lipids, these lipids are much less abundant in the oral epithelia compared to epidermis.

Figs. 2 and 3 show intercellular regions in stratum corneum from epidermis and palatal epithelium before and after lipid extraction, respectively. After ruthenium tetroxide fixation, extensive lipid lamellae are demonstrated in the intercellular spaces of mid-to-outer epidermal stratum corneum (Fig. 2A). In this tissue, desmosomal fragments are infrequent and are confined mainly to regions near the ends of the corneocytes. Fig. 2B illustrates that an appreciable portion of the intercellular space in the outermost layers of palatal stratum corneum is occupied by desmosomes, and the number of broad lipid lamellae observed at the cell periphery between desmosomes is much less than in epidermal stratum corneum. Note also that the interior of the dilated interdesmosomal regions in this tissue appear to contain a mixture of an electron dense substance and lamellar material which resembles extruded lamellar granule contents that have apparently failed to reorganize into broad sheets as occurs in epidermis. After lipid extraction, lamellar material is removed from both epidermal and palatal stratum corneum (Fig. 3). In extracted epidermal stratum corneum, one lamella corresponding to the lipid envelope remains at the cell surface, and the intercellular

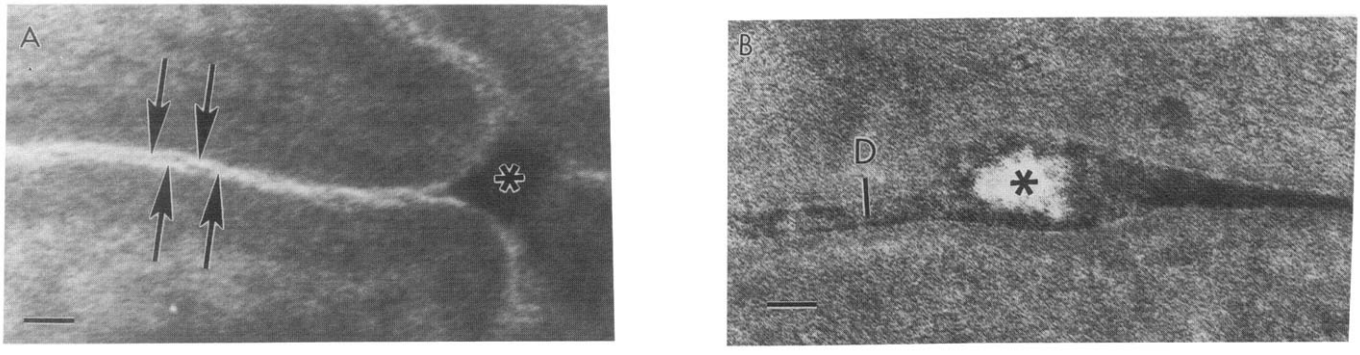

Fig. 3. Transmission electron micrographs of extracted epidermal and palatal stratum corneum. (A) Micrograph of extracted epidermal stratum corneum showing two closely apposed lipid envelopes (arrows). The bar represents 20 nm. (B) Micrograph of extracted palatal stratum corneum demonstrating the absence of lipid lamellae and lipid envelope. Desmosomes (D) and dilated interdesmosomal regions (*) containing some amorphous electron dense material remain. Bar represents 50 nm.

spaces collapse so that the lipid envelopes of adjacent corneocytes are brought into close apposition (Fig. 3A). A similar structure is not seen in extracted palatal stratum corneum (Fig. 3B). Dilated regions of both the palatal stratum corneum and epidermis remain and retain amorphous electron dense material, but the lamellar material is removed from these regions.

Discussion

It has been estimated that the amount of covalently bound lipid in epidermal stratum corneum is just sufficient to completely envelop all of the cornified cells [5]. In contrast, the present findings indicate that the amount of covalently bound lipid in stratum corneum from keratinized oral epithelium could cover only about 10% of the corneocyte surface. Even allowing for the fact that about half of the intercellular space in keratinized oral epithelium is occupied by desmosomes [16], there is not sufficient bound lipid to cover a substantial portion of the surfaces between the desmosomes. This contention is supported by transmission electron micrographs of extracted palatal stratum corneum which, in contrast to micrographs of epidermal stratum corneum, show no lucent band at the corneocyte surface. We therefore conclude that oral keratinocytes do not produce a lipid envelope comparable to that of epidermal corneocytes.

The present results do not support the proposition that covalently bound lipids of the corneocyte envelope provide a template necessary for formation of broad intercellular lipid sheets. Multiple, broad lamellar sheets are present at the corneocyte surfaces whether there is a lipid envelope, as in epidermis, or not, as in palatal epithelium, although the number of lamellae are reduced in the latter tissue. The spacing of the intercellular lamellae in epidermal stratum corneum is complex and frequently involves both 4-nm-wide and 2-nm-wide lucent bands [15,17]. For example, one common lamellar pattern in epidermal stratum corneum consists of 4-2-4-4-2-4-nm-wide lucent bands [15,17]. It has been suggested that the covalently bound lipid envelope is a participant in the formation of the 2-nm-wide lucent bands [7,17]. In support of this proposal, it was observed in the present study that 2-nm-wide lucent bands are never seen in palatal stratum corneum, where there is no lipid envelope.

The interdesmosomal regions of the intercellular space of the stratum corneum from keratinizing oral epithelium appear to be dilated. Similar dilations containing electron dense material have been described in epidermal stratum corneum after ruthenium tetroxide fixation [18], and it has been suggested that these 'lacunar dilations' arise from the breakdown of desmosomes in going from the inner to the outer stratum corneum [18]. The present results are not consistent with this suggestion since desmosomes persist into the outermost layers of the palatal stratum corneum. Although multiple broad lamellae are found along the peripheries of these dilations, the interiors of these expanded regions often contain short stacks of lamellae like those extruded from lamellar granules. In normal epidermis, such short stacks of lamellae are not observed at levels higher than the boundary between the granular layer and the stratum corneum [19], whereas in palatal stratum corneum short stacks of lamellae persist to the surface. This apparent failure of extruded lamellar granule contents to undergo transformation into broad sheets may be related to lower levels of phospholipases in the oral epithelium compared to epidermis [20]. It has previously been reported [12] that significant amounts of phospholipids are found in palatal stratum corneum, whereas phospholipids are absent from epidermal stratum corneum. Perhaps phospholipid catabolism is required in order to permit the membrane fusion necessary for conversion of short lamellar disks into broad lamellar sheets. Differences in the extent of this membrane metamorphosis may be related to the differences in permeability between epidermis and keratinized oral epithelium. A partial failure of extruded lamellar granule contents to undergo rearrangement into broad sheets has been reported to occur in the hyperproliferative epidermal disorder, congenital ichthyosiform erythroderma [21]. This situation may be more related to a high rate of proliferation and turnover than to a specific disease state.

It has been suggested that the flux of water through the skin regulates the expression of certain genes coding for lipid synthetic enzymes including β-hydroxy-β-methylglutaryl-coenzyme A reductase [22] and serine palmitoyl coenzyme A transferase [23]. In this way, barrier requirements regulate the synthesis of barrier lipids [22–25]. In this context, it might have been anticipated that the moist environment of the oral cavity would result in very limited lipid synthesis and thereby a less effective permeability barrier; however, other studies have indicated that differences in total lipid content are not sufficient to account for the differences in permeability between skin and oral mucosa [11,12]. Subtle differences in lipid composition may contribute to the regional variation in permeability [12], and the present results suggest that differences in the organization and multiplicity of lipid lamellae may be a major factor accounting for regional variation in permeability barrier function.

Acknowledgements

This work was supported in part by grant No. RO1 DEO7930 from the National Institute for Dental Research.

References

1 Brody, I. (1959) J. Ultrastruct. Res. 2, 482–511.
2 Matoltsy, A.G. (1976) J. Invest. Dermatol. 67, 20–25.
3 Simon, M. and Green, H. (1985) Cell 40, 677–633.
4 Wertz, P.W. and Downing, D.T. (1987) Biochim. Biophys. Acta 917, 108–111.
5 Swartzendruber, D.C., Wertz, P.W., Madison, K.C. and Downing, D.T. (1987) J. Invest. Dermatol. 88, 709–713.
6 Wertz, P.W., Madison, K.C. and Downing, D.T. (1989) J. Invest. Dermatol. 92, 109–111.
7 Downing, D.T. (1992) J. Lipid Res. 33, 301–313.
8 Green, H., Fuchs, E. and Watt, F. (1982) Cold Spring Harbor Quant. Biol. 1, 293–301.
9 Squier, C.A. (1991) CRC Crit. Rev. Oral Biol. 2, 13–32.
10 Wertz, P.W. and Squier, C.A. (1991) CRC Crit. Rev. Ther. Drug Carrier Systems 8, 237–269.
11 Squier, C.A., Wertz, P.W. and Cox, P. (1991) Arch. Oral Biol. 9, 647–653.
12 Wertz, P.W., Kremer, M. and Squier, C.A. (1992) J. Invest. Dermatol. 98, 375–378.
13 Downing, D.T., Krantz, F.H. and Murray, K.E. (1961) Aust. J. Chem. 14, 619–627.
14 Downing, D.T. (1968) J. Chromatogr. 38, 91–99.
15 Madison, K.C., Swartzendruber, D.C., Wertz, P.W. and Downing, D.T. (1987) J. Invest. Dermatol. 88, 714–718.
16 Schroeder, H.E. (1981) Differentiation of human oral stratified epithelia, Karger, Basel.
17 Swartzendruber, D.C., Wertz, P.W., Kitko, D.J., Madison, K.C. and Downing, D.T. (1989) J. Invest. Dermatol. 92, 251–257.
18 Hou, S.Y.E., Mitra, A.K., White, S.H., Menon, G.K., Ghadially, R. and Elias, P.M. (1991) J. Invest. Dermatol. 96, 215–223.
19 Landmann, L. (1988) Anat. Embryol. 178, 1–13.
20 Elias, P.M., Menon, G.K., Grayson, S. and Brown, B.E. (1988), J. Invest. Dermatol. 91, 3–10.
21 Ghadially, R., Williams, M.L., Hou, S.Y.E. and Elias, P.M. (1992) J. Invest. Dermatol. 99, 755–763.
22 Menon, G.K., Feingold, K.R., Moser, A.H., Brown, B.E. and Elias, P.M. (1985) J. Lipid Res. 26, 418–427.
23 Holleran, W.M., Feingold, K.R., Man, M.Q., Gao, W.N., Lee, J.M. and Elias, P.M. (1991) J. Lipid Res. 32, 1151–1158.
24 Grubauer, G., Feingold, K.R. and Elias, P.M. (1987) J. Lipid Res. 28, 746–752.
25 Grubauer, G., Elias, P.M. and Feingold, K.R. (1989) J. Lipid Res. 30, 323–333.