

Ceramides in the skin barrier

Original Paper

Vávrová K.,[✉] Kováčik A., Opálka L.

Charles University, Faculty of Pharmacy in
Hradec Králové, Skin Barrier Research Group,
Hradec Králové, Czech Republic

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Abstract The skin barrier, which is essential for human survival on dry land, is located in the uppermost skin layer, the stratum corneum. The stratum corneum consists of corneocytes surrounded by multilamellar lipid membranes that prevent excessive water loss from the body and entrance of undesired substances from the environment. To ensure this protective function, the composition and organization of the lipid membranes is highly specialized. The major skin barrier lipids are ceramides, fatty acids and cholesterol in an approximately equimolar ratio. With hundreds of molecular species of ceramide, skin barrier lipids are a highly complex mixture that complicate the investigation of its behaviour. In this minireview, the structures of the major skin barrier lipids, formation of the stratum corneum lipid membranes and their molecular organization are described.

Keywords Skin barrier – stratum corneum – ceramide – sphingolipid

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INTRODUCTION

Ceramides, that is, *N*-acylsphingosines belong to the family of sphingolipids. Ceramides regulate several cellular processes, such as proliferation, differentiation and apoptosis (Hannun, 1996; Hannun and Obeid, 2008). In human body, ceramides are equally important outside the cell – they form multilamellar lipid membranes between corneocytes of the uppermost skin layer, the stratum corneum (Candi et al., 2005). These stratum corneum lipids prevent excessive water loss from the body and hamper the penetration of undesired substances, allergens and microbes from the environment into the body (Bouwstra and Ponc, 2006; Elias, 2008). In fact, the development of such lipid-based barrier was an essential step in the evolution of life on dry land. In this minireview, the structures of major skin barrier lipids, formation of the stratum corneum lipid membranes and their molecular organization are described.

COMPOSITION OF THE SKIN LIPID BARRIER

Human stratum corneum is composed of approximately 15–25 layers of flattened dead cells, corneocytes, which are surrounded by a lipid matrix (Elias et al., 1977). To provide a competent barrier function, the lipids in the stratum corneum are highly specialized: there are virtually no phospholipids; the major constituents are ceramides, free fatty acids and cholesterol in an approximately 1:1:1 molar ratio (**Fig. 1**). This equimolar ratio seems to be highly important for epidermal homeostasis, as alterations in this lipid composition were associated with a disturbed barrier function (Breiden and Sandhoff, 2014; Feingold and Elias, 2014; Holleran et al., 2006; Rabionet et al., 2014; van Smeden et al., 2014b). Free fatty acids in the skin barrier are 14–34 carbons long, mostly saturated and unbranched. The predominant fatty acids have 24 carbons (lignoceric acid) and 26 carbons (cerotic acid) – these two species comprise approximately 50 weight % of the stratum corneum fatty acids (Norlen et al., 1998; van Smeden et al., 2014a). Unsaturated and shorter fatty acids are present in the stratum corneum lipid membranes only in minor quantities; their concentrations increase in pathological conditions such as atopic dermatitis, probably due to flaggrin

* E-mail: katerina.vavrova@faf.cuni.cz

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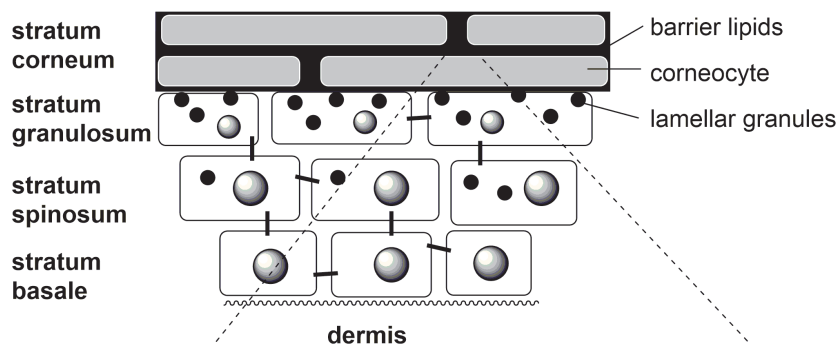
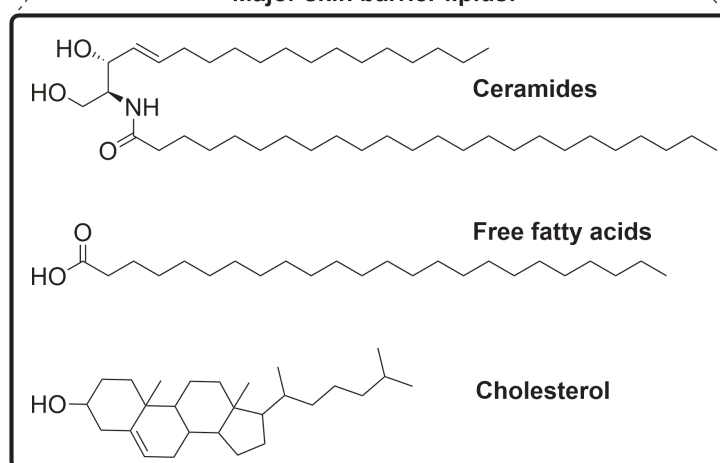
Epidermis:**Major skin barrier lipids:**

Figure 1. Schematic representation of epidermal layers, structure of the stratum corneum and the major skin barrier lipids.

deficiency (Jakasa et al., 2011; Jungersted et al., 2010; Vavrova et al., 2014).

Cholesterol is the main sterol in the skin lipid barrier. Cholesterol is also essential for the correct skin barrier function; it contributes to the correct lamellar and lateral structure and lipid fluidity (Feingold and Elias, 2014; Mojumdar et al., 2015a). Previous studies described separation of cholesterol from the lamellar phases (Mojumdar et al., 2015b; Schreiner et al., 2000); however, the physiological relevance of this separation is not known. In contrast, recent study using very high resolution electron microscopy on vitreous skin sections found no cholesterol crystals (Iwai et al., 2012).

Cholesteryl sulphate, that is, ester of sulphuric acid with cholesterol, is a minor component of the stratum corneum lipid matrix; it is present in approximately 5% of the barrier lipids (or approximately 2% of all lipids including surface lipids (Lampe et al., 1983)). The role of cholesteryl sulphate is probably not fully understood, one possible function of this molecule is that it contributes to the stratum corneum cohesion and regulates desquamation (Long et al., 1985).

CHEMISTRY OF CERAMIDES

The dominant lipids in the stratum corneum are ceramides; they constitute approximately one third of lipid molecules or 50% of the lipids by weight (van Smeden et al., 2014b). These simple sphingolipids have a relatively small polar head that does not extensively hydrate and two hydrophobic chains – the sphingoid base chain and *N*-acyl chain (fatty acid chain). The most intriguing fact in skin ceramides is their heterogeneity: 15 ceramide subclasses have been described until the present and together with the different chain lengths, this yields hundreds of distinct chemical structures (Fig. 2). For a recent review, see (Rabionet et al., 2014).

All ceramides are based on a sphingoid base, which is a long chain (usually 18 carbons) amino alcohol. The skin ceramides can contain sphingosine (S) and dihydrosphingosine (dS), which are common in eukaryotic cells, phytosphingosine (P), which is found only in some human tissues, and 6-hydroxysphingosine (H), which is specific for the epidermis (Kováčik et al., 2014). Recently, a novel tetrahydroxylated sphingoid base, which

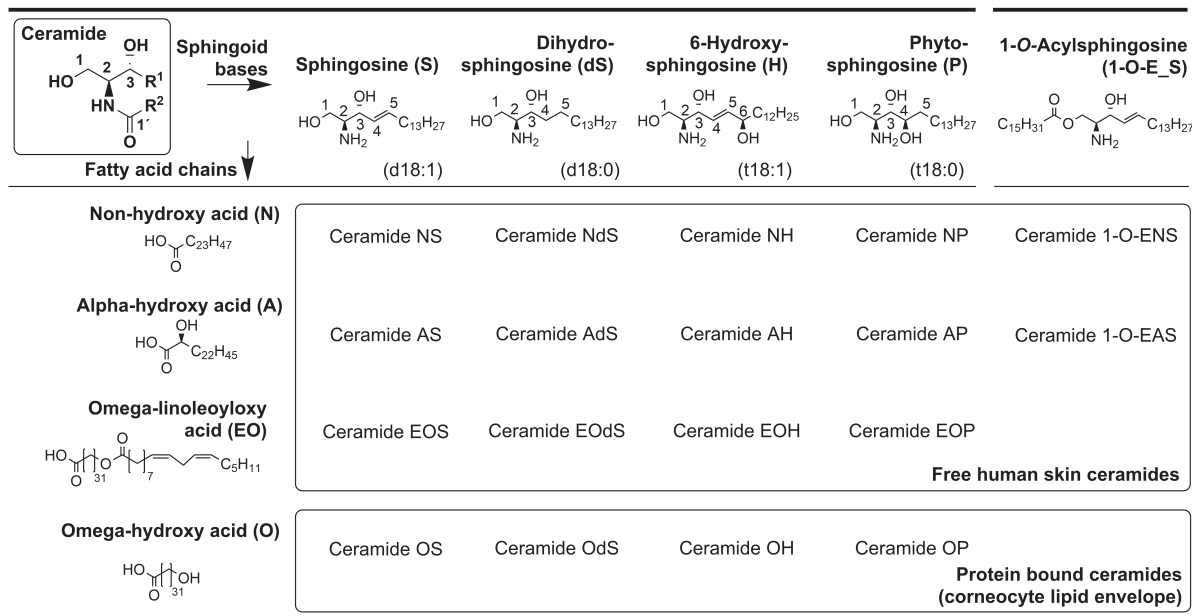


Figure 2. Chemical structures of the main components of the stratum corneum ceramides and their shorthand nomenclature.

has not been fully structurally described, has been found (t'Kindt et al., 2012). In ceramides, the primary amino group of the sphingoid base is acylated (that is, amide-linked) with a fatty acid, which is usually saturated, unbranched and very long (approximately 24 carbons) or ultralong (up to 38 carbons, mostly 30–32 carbons) (Breiden and Sandhoff, 2014; van Smeden et al., 2011). The fatty acid chain in ceramides can be without further substitution (N), α -hydroxylated (A) or, in case of ultralong chains, ω -hydroxylated (O) (Breiden and Sandhoff, 2014; Novotný et al., 2010; Rabionet et al., 2014; van Smeden et al., 2014b). The ω -hydroxyl is further ester-bound either to linoleic acid (EO) or to glutamate residues of the proteins (mostly involucrin) at the surface of a corneocyte (Candi et al., 2005). Recently, a new class of ceramides with three hydrophobic chains was discovered with the third chain ester-linked to the primary hydroxyl in position 1 of the sphingoid base (1-O-E) (Rabionet et al., 2013).

The letters in parentheses refer to the shorthand nomenclature developed by Motta (Motta et al., 1993) and expanded by Robson (Robson et al., 1994), Masukawa (Masukawa et al., 2008) and Rabionet (Rabionet et al., 2014). The ceramide structure is defined by a combination of the above-mentioned letters that specify the acyl with letters for the sphingoid part (Fig. 2). Thus, for example, *N*-lignoceroylsphingosine is ceramide NS or, more precisely, ceramide NS24, where the number 24 defines the fatty acid chain length.

FORMATION OF THE SKIN LIPID BARRIER; CORNEOCYTE LIPID ENVELOPE

The skin lipids are synthesized in the epidermal cells, keratinocytes, converted to their more polar precursors

(e.g., ceramides are converted to sphingomyelins and glucosylceramides) (Holleran et al., 2006; Jensen et al., 1999), which are stored in lamellar granules together with catabolic enzymes. At the stratum granulosum/stratum corneum interface, the lamellar granules migrate to the keratinocyte upper surface, merge with plasma membrane, and secrete their contents into the intercellular space. The enzymes are activated and convert lipid precursors to barrier lipids that eventually assemble into lamellar structures that fill the entire space between the cells (Mizutani et al., 2009; Rabionet et al., 2014; Uchida and Holleran, 2008).

Some of the glucosylceramides of the EO-subclass, or acylglucosylceramides, are oxidized at their linoleic acid tail, which is then removed and the remaining ω -hydroxyceramides are attached via their ω -hydroxyl to glutamate residues of the corneocyte proteins involucrin and loricrin (Banks-Schlegel and Green, 1981; Behne et al., 2000). These covalent lipid monolayer, the corneocyte lipid envelope (Elias et al., 2014; Wertz et al., 1989), covers the whole cell surface in the stratum corneum and is believed to act as a template for the orientation of the free barrier lipids and prevent permeable boundaries between the lipids and cells (Behne et al., 2000). Any major defects in the biosynthesis or transport of lipids of the corneocyte lipid envelope are lethal (Jennemann et al., 2012; Vasireddy et al., 2007).

MOLECULAR ARRANGEMENT OF THE SKIN LIPID BARRIER

The molecular arrangement of the stratum corneum lipids is also highly specialized: the skin barrier lipids do not form conventional bilayers but multilamellar membranes with the

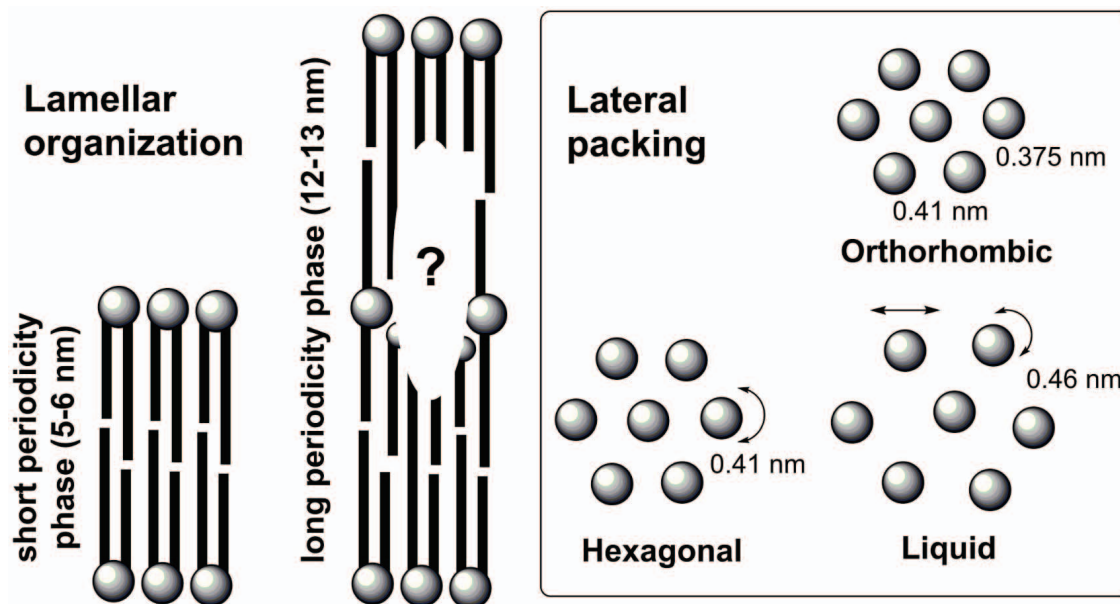


Figure 3. Lamellar and lateral organization of the stratum corneum lipid membranes.

first lipid layer covalently anchored to the corneocyte surface (Breathnach, 1975; Grayson and Elias, 1982). In contrast to phospholipids, the stratum corneum ceramides have a small polar head and very long, saturated chains with prevailing *all-trans* conformation. The prevalent lateral packing of the skin barrier lipids is orthorhombic, which is a very tight packing with highly limited motional freedom. In addition, some lipids are arranged in a hexagonal subcell with greater rotational freedom, and a minor lipid fraction is liquid (**Fig. 3**) (Bouwstra et al., 2001; Damien and Boncheva, 2010; Mendelsohn et al., 2006; Mendelsohn and Moore, 2000; Stahlberg et al., 2015). Considering the lamellar arrangement of the stratum corneum lipid multilayers, their unique feature is the formation of the so-called long periodicity lamellar phase with a repeat distance of 11.9–13.1 nm (Hou et al., 1991; Madison et al., 1987; Mojumdar et al., 2015a; Neto et al., 2011; White et al., 1988) (**Fig. 3**). This lamellar phase was first observed by electron microscopy and later confirmed by X-ray diffraction. In addition, most studies also describe the presence of a short periodicity phase (with repeat distance of 5.3–6.4 nm) (Bouwstra et al., 1995; Bouwstra et al., 1991; de Jager et al., 2004; de Sousa Neto et al., 2011; Mojumdar et al., 2014; White et al., 1988) and phase separated cholesterol (Craven, 1979; Shieh et al., 1981). Nevertheless, recent experiments on native skin barrier using very high resolution cryo-electron microscopy only detected the long periodicity phase, with the repeat distance of approximately 11 nm (Iwai et al., 2012). There is a consensus that acylceramides (that is, ceramides of the EO-subclass) are necessary for the formation of the long periodicity phase (Bouwstra et al., 1991; de Sousa Neto et al., 2011; Kessner et al., 2010; Madison et al., 1987; Mojumdar et al., 2015a; White et al., 1988). However, recent X-ray diffraction

study showed a lamellar phase with 10.6 nm periodicity in ceramide NH membranes without any acylceramide present (Kovacik et al., 2016).

Because of the multilamellar arrangement of lipids in the stratum corneum, both hairpin conformation (with both chains pointing to the same direction) and extended conformation of ceramide (or splayed-chain conformation, with the chains pointing into opposite directions) are theoretically possible (**Fig. 4**). Although the extended conformation is not common in biological membranes, it could be advantageous for the stratum corneum barrier properties (Corkery, 2002; Iwai et al., 2012). First, it would reduce the packing strain of ceramide because they have a smaller cross-section of the polar head than of the chains and there are no lipids with bulky polar heads to compensate for such unfavourable packing ratio of hairpin ceramide. Second, the extended conformation would connect the adjacent lipid lamellae and prevent permeable boundaries.

STRATUM CORNEUM LIPID MEMBRANE MODELS

The exact lipid organization in the stratum corneum has been under debate for decades (Norlen, 2013). Both lamellar phases and the lateral packing can be reproduced using the in vitro lipid membrane models (Kessner et al., 2008). Numerous previous studies using either lipid membrane models or stratum corneum described how ceramide structure influences their behaviour in membranes and permeability (Janusova et al., 2011; Novotný et al., 2009; Pullmannová et al., 2014; Rerek et al., 2001; Skolova et al., 2014; Skolova et al., 2016; Skolova et al., 2013; Stahlberg et al., 2016; Stahlberg et al., 2015) and proposed models of the arrangement (Bouwstra

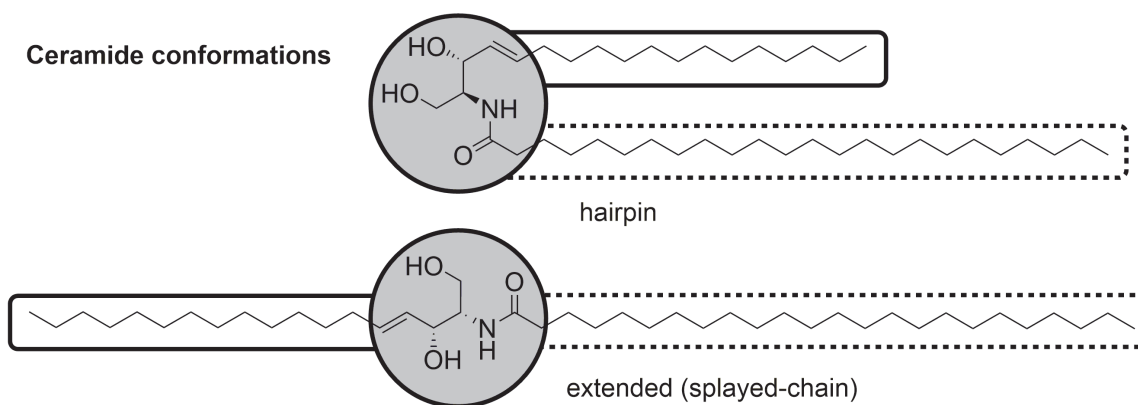


Figure 4. Two possible conformations of ceramide in the skin barrier membranes.

et al., 1991; de Jager et al., 2004; de Sousa Neto et al., 2011; Iwai et al., 2012; Mojumdar et al., 2015a; Mojumdar et al., 2015b; Mojumdar et al., 2014). However, no broad consensus has been reached, mainly because of the differences in the experimental setup, method used and, in case of models, lipid composition.

These lipid models always bear a certain level of simplification but some of them showed very good correlations with real skin properties. For example, de Jager *et al.* studied a model lipid membrane composed of ceramides EOS, NS24, NP24, AS24, NP16, AP24, free fatty acid mixture and cholesterol and found similar permeabilities of this model and isolated stratum corneum to *p*-aminobenzoic acid and its esters (de Jager et al., 2006). We compared the effects of ceramide with shortened chains (both acyl and sphingosine) in skin and simple lipid membranes composed of ceramide NS24, lignoceric acid, cholesterol and cholesteryl sulphate and found similar trends in permeabilities to the two model permeants theophylline and indomethacin (Janušová et al., 2011; Školová et al., 2016; Školová et al., 2013). Furthermore, a lipid model composed of ceramide NS24, free fatty acids and cholesterol (with or without cholesteryl sulphate) confirmed the existence of fully extended ceramide with cholesterol molecules associated with the ceramide sphingoid moiety and free fatty acids with the acyl chains (Školová et al., 2014) suggested by very high resolution cryo-electron microscopy of native skin (Iwai et al., 2012). The permeabilities of our recent complex model with ceramides EOS, EOdS and EOP, NS, NdS, NP, AS, AdS and AP, free fatty acids, cholesterol and cholesteryl sulphate (the flux values of theophylline and indomethacin were $0.24 \pm 0.02 \mu\text{g}/\text{cm}^2/\text{h}$ and $0.12 \pm 0.02 \mu\text{g}/\text{cm}^2/\text{h}$, (Opálka et al., 2016)) are in very good agreement with those of a membrane model constructed from isolated human skin ceramides (the flux values of theophylline and indomethacin were $0.40 \pm 0.05 \mu\text{g}/\text{cm}^2/\text{h}$ and $0.23 \pm 0.02 \mu\text{g}/\text{cm}^2/\text{h}$, respectively

(Pullmannová et al., 2014)) and also with permeabilities of isolated human epidermis under the same conditions (the flux values of theophylline and indomethacin were $0.15 \pm 0.05 \mu\text{g}/\text{cm}^2/\text{h}$ and $0.05 \pm 0.02 \mu\text{g}/\text{cm}^2/\text{h}$, respectively – Kováčik, unpublished data).

The main drawback of the model lipid membranes is that some ceramides are not commercially available, especially those of the EO-subclass and H-subclass. However, synthetic procedures to these lipids have recently been published (Kovacik et al., 2016; Mori and Matsuda, 1991; Muller and Schmidt, 2000; Opálka et al., 2015) and opened the possibility to further improve the stratum corneum lipid models to better mimic the real skin lipid barrier.

CONCLUSION

Ceramides are essential for our life on dry land. Major disturbances to their biosynthesis or transport could be lethal because of an extensive water loss. Even less pronounced alterations of the stratum corneum lipid composition and organization are associated with skin diseases such as atopic dermatitis, psoriasis and ichthyoses and could greatly affect the quality of life of the patients. Although topical application of skin-identical lipids or their analogues has a strong potential to repair the skin barrier in such diseases and prevent inflammation, deeper understanding of the physical chemistry of ceramides and the molecular arrangement of the skin lipid membranes is necessary for more rational design of such therapies.

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