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Human stratum corneum lipid organization as observed by atomic force microscopy on Langmuir–Blodgett films

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Abstract

The barrier function of skin ultimately depends on the physical state and structural organisation of the stratum corneum extracellular lipid matrix. Ceramides, cholesterol and a broad distribution of saturated long-chain free fatty acids dominate the stratum corneum lipid composition. Additionally, smaller amounts of cholesterol sulfate and cholesteryl oleate may be present. A key feature determining skin barrier capacity is thought to be whether or not different lipid domains coexist laterally in the stratum corneum extracellular lipid matrix. In this study, the overall tendency for lipid domain formation in different mixtures of extracted human stratum corneum ceramides, cholesterol, free fatty acids, cholesterol sulfate and cholesteryl oleate were studied using atomic force microscopy (AFM) on Langmuir–Blodgett (LB) films on mica. It is shown that the saturated long-chain free fatty acid distribution of human stratum corneum prevents hydrocarbon chain segregation. Further, LB-films of human stratum corneum ceramides express a pattern of connected elongated domains with a granular domain interface. The dominating effect of both cholesterol and cholesterol sulfate is that of increased ceramide domain dispersion. This effect is counteracted by the presence of free fatty acids, which preferentially mix with ceramides and not with cholesterol. Cholesteryl oleate does not mix with other skin lipid components, supporting the hypothesis of an extra-endogenous origin. In the system composed of endogenous human ceramides and cholesterol plus 15 wt% stratum corneum distributed free fatty acids, i.e., the system mimicking most closely the lipid composition of the stratum corneum extracellular space, LB-films on mica express lateral domain formation.

Keywords: Skin barrier; Ceramides; Cholesterol; Free fatty acids; Cholesterol sulfate; Cholesteryl esters

1. Introduction

The main function of the skin is to serve as a physical barrier at the interface between the body and the environment. This barrier capacity is largely a function of the physical state and structural organisation of the extracellular lipid matrix of stratum corneum (Blank, 1952; Michaels et al., 1975; Forslind, 1994; Norlén, 2001). Several models for the structural organisation of the lipid matrix have been proposed, e.g., the domain mosaic model (Forslind, 1994), the laminglass model of Pascher (cf. Norlén, 2003), the sandwich model (Bouwstra et al., 2000), the single gel-phase model (Norlén, 2001) and the assymetric bilayer model (McIntosh, 2003). In the stratum corneum extracellular space the relative amount of ceramides is 40–50 wt%, cholesterol 20–33 wt%, free fatty acids 7–13 wt% (predominantly long-chain saturated), cholesterol sulfate 0–7 wt% and cholesteryl esters 0–20 wt% (Wertz et al., 1987; Norlén et al., 1998; Wertz and Norlén, 2003).

One of the main challenges in skin barrier research is to determine the possible existence of lipid domain formation versus lipid intermixing in the stratum corneum extracellular

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lipid matrix (Forslind, 1994; Norlén, 2001; Bouwstra et al., 2002; Norlén, 2003). This is because (a) permeabilities of biological membranes in the liquid crystalline state may be 2–3 orders of magnitude higher than for corresponding membranes in the crystalline gel-state (Carruthers and Melchior, 1983), (b) membrane permeabilities may be further enhanced at the interface between different domains (Papahadjopoulos et al., 1973; Cruzeiro-Hansson and Mouritsen, 1988; Clerc and Thompson, 1995; Xiang and Anderson, 1998), and (c) liquid crystalline lipid domains may be induced to form oil-continuous or bicontinuous lipid structures with resulting dramatically increased skin permeability (Engström et al., 1995).

Broad distributions of saturated, long hydrocarbon chains and high cholesterol content is characteristic of stratum corneum lipid composition. In lipid *in vitro* systems these features generally favour the dissipation of domain line boundaries, bringing e.g., phosphatidylcholine monolayers from two-phase coexistence to an apparent one-phase system (Slotte, 1995). Non-random microscopic distribution of molecules (e.g., cholesterol) may, however, occur even if the lipid system macroscopically behaves as a single phase. One example is the ripple phosphatidylcholine–cholesterol bilayer that macroscopically can be described as a single phase although cholesterol is not evenly distributed in the structure (Copeland and McConnell, 1980; Mortensen et al., 1988).

One way to gain new insights into the lateral organisation of the lipid matrix of the stratum corneum extracellular space may be by atomic force microscopy (AFM) on Langmuir–Blodgett (LB) lipid layers deposited on solid substrates (cf. Ten Grotenhuis et al., 1996; Schaffer et al., 1999; Ekelund et al., 2000; Sparr et al., 2001). AFM on deposited monolayer films benefits from high lateral and vertical resolution (a few Ångströms) and simplicity of sample preparation (no decoration or conducting coating is needed). Another strength of that technique is that differences in material properties (e.g., friction, viscoelasticity etc.) between different lipid domains can be identified in parallel with topographic surface measurements.

Langmuir lipid monolayers on water are considered to be excellent model systems for biomembranes as a biological membrane can be considered as two weakly coupled monolayers (Kaganer et al., 1999, p780). However, due to different adhesive interactions of the monolayer transferred to a solid substrate with respect to the monolayer floating on water, the mixing behaviour of floating and transferred monolayers is different, leading to a possible substrate-mediated condensation of monolayers upon transfer (Spratte and Riegler, 1994). This effect is however minimized when the film molecules are transferred from condensed phases at high surface pressures (Rana et al., 1994). Furthermore, the recorded condensation into close-packed islands during transfer of fatty acids in the liquid expanded state is less pronounced at low pH (Sikes and Schwartz, 1997). Accordingly, in order to minimize the effect of possible lipid condensation during deposition, stratum corneum lipid monolayers were prepared at low subphase pH (3.0) (which also ensured a complete deionisation of free fatty acids) and transferred after compression to the liquid condensed state (22 mN/m).

The bulk pH of the stratum corneum and upper viable epidermis has been measured to 4.0–4.5 (Denda et al., 2000) and to 5.0–7.0 (Öhman and Vahlquist, 1994), respectively, and the local pH of the stratum corneum extracellular lipid matrix (in mice) to ~6 (Hanson et al., 2002). The local proton concentration immediately adjacent to the lipid headgroups of the layered extracellular lipid matrix remains, however, undetermined. A subphase pH of 3.0 may therefore not necessarily be "unphysiological". Nonetheless, comparison between neutral (7.0) and low (3.0) subphase pH were performed for all non-free fatty acid containing lipid systems included in the study. For free fatty acid containing lipid systems comparison between subphase pH 3, 5, 6 and 8 were performed. Further, the structural organisation of transferred lipid trilayers was compared to that of transferred lipid monolayers. Also, monolayers and trilayers transferred onto mica substrates were compared to corresponding systems transferred onto glass substrates. The glass surface has the advantage of being non-structured while the mica surface has the advantage of being flat at the molecular level. The lipid trilayer system consists of a lipid bilayer supported on a lipid monolayer surface, thus simulating more closely the situation in vivo.

In a pioneering work by Ten Grotenhuis et al. (1996) monolayers (transferred to silicon wafers) of extracted pig stratum corneum ceramides, alone and in mixtures with cholesterol and lignoceric- and palmitic acid, respectively, were investigated by contact mode AFM. In the present study we extended these studies to monolayers (transferred to mica) of extracted *human* stratum corneum ceramides, cholesterol, saturated long-chain FFA, cholesterol sulfate and cholesteryl oleate. The objective was to investigate human stratum corneum lateral lipid organisation in Langmuir–Blodgett films using contact mode and vibrating tip mode AFM, which allows for uniquely high lateral and vertical resolution with respect to lipid domain formation.

2. Material and methods

The study was approved by the authors' Institutional Review Board and was conducted according to Declaration of Helsinki principles.

2.1. Skin samples

All participants were judged devoid of skin disease by a dermatological examination. Subjects with susceptibility for dry skin, atopic dermatitis, asthma, contact dermatitis or allergy at present, in childhood or in the biological family were excluded.

Full thickness human breast skin from 5 healthy females (21–45 y, median = 26 y) was obtained from reconstructive surgery. The skin material was subsequently kept in a