Biochimica et Biophysica Acta 1808 (2011) 1529–1537

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Is an orthorhombic lateral packing and a proper lamellar organization important for the skin barrier function?

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ARTICLE INFO

Article history:
Received 29 July 2010
Received in revised form 18 October 2010
Accepted 22 October 2010
Available online 28 October 2010

Keywords:
Stratum corneum
Lipid
Hexagonal
Orthorhombic
Lamellar phase
Arrhenius

ABSTRACT

The lipid organization in the stratum corneum (SC), plays an important role in the barrier function of the skin. SC lipids form two lamellar phases with a predominantly orthorhombic packing. In previous publications a lipid model was presented, referred to as the stratum corneum substitute (SCS), that closely mimics the SC lipid organization and barrier function. Therefore, the SCS serves as a unique tool to relate lipid organization with barrier function. In the present study we examined the effect of the orthorhombic to hexagonal phase transition on the barrier function of human SC and SCS. In addition, the SCS was modified by changing the free fatty acid composition, resulting in a hexagonal packing and perturbed lamellar organization. By measuring the permeability to benzoic acid as function of temperature, Arrhenius plots were constructed from which activation energies were calculated. The results suggest that the change from orthorhombic to hexagonal packing in human SC and SCS, does not have an effect on the permeability. However, the modified SCS revealed an increased permeability to benzoic acid, which we related to its perturbed lamellar organization. Thus, a proper lamellar organization is more crucial for a competent barrier function than the presence of an orthorhombic lateral packing.

1. Introduction

The uppermost layer of the human skin, the stratum corneum (SC), consists of flattened protein-rich dead cells (corneocytes) surrounded by intercellular lipids. The intercellular lipid domains in the SC form the only continuous pathway through the SC and are suggested to act as the main barrier for diffusion of substances through the SC [1]. The main lipid classes in the SC are ceramides (CER), cholesterol (CHOL) and free fatty acids (FFA) [2–6]. The lipids are arranged in two coexisting lamellar phases; a long periodicity phase (LPP) with a repeat distance of around 13 nm and a short periodicity phase (SPP) with a repeat distance of around 6 nm [7,8]. Furthermore, at the skin temperature of around 30-32 °C in human SC the orthorhombic lateral packing is dominantly present, although a subpopulation of lipids also forms a hexagonal lateral packing. When increasing the temperature of SC, a transition is noticed from an orthorhombic to a hexagonal lateral packing between 30 and 40 °C. Both the lateral and lamellar lipid organization are considered to play an important role in the barrier function of the skin [9–11]. A detailed analysis of the lipid composition revealed that the FFA have a wide chain length distribution, in which the chain lengths of 22 and 24 carbon atoms are most abundantly present [12]. In addition, there are eleven subclasses of CER identified in human SC [2,5,6].

As the lipids play a crucial role in the barrier function, a large number of studies have been performed to understand the complex lipid phase behaviour underlying the skin barrier function. These studies, performed using isolated as well as synthetic CER mixtures, have markedly contributed to our present knowledge on the SC lipid organization and the role the lipid subclasses play in the lipid phase behaviour [10,13–20]. However, in these studies no information was obtained about the relation between lipid organization and skin barrier function. In order to study this, we developed a SC lipid model consisting of a porous substrate covered by a lipid film prepared from synthetic CER, CHOL and FFA. This lipid membrane mimics the lipid organization and lipid orientation in SC closely and is referred to as the stratum corneum substitute (SCS) [21–23]. As the lipid composition can easily be modified, this lipid membrane allows us to study the relationship between lipid composition, molecular organization and barrier function in just one model. In a previous study using the SCS, it was observed that the LPP plays an important role in the skin barrier function [21]. However, only little information is available on the role the orthorhombic lateral packing plays in forming a proper skin barrier function. One of the key parameters to monitor the skin barrier function is the trans epidermal water loss (TEWL). In a very recent study the TEWL has been related to the degree of orthorhombic lateral packing present in SC in vivo in humans [24].

In the present study we examine whether the formation of the very dense orthorhombic packing and the formation of the characteristic lamellar phases observed in SC are crucial for the lipid barrier function in SC. As model compound we use benzoic acid (BA), a

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doi:10.1016/j.bbamem.2010.10.015

medium lipophilic low MW molecule. To examine the lipid organization in the SCS models, Fourier transform infrared spectrometry (FTIR) and small-angle x-ray diffraction (SAXD) are used. To determine the importance of the orthorhombic lateral packing for the SC lipid barrier, diffusion studies are performed during a step-wise increase in temperature from 15 to 45 °C, sampling the temperature of the orthorhombic-hexagonal phase transition. To determine whether a simultaneous change in lateral packing and in the lamellar phases has a profound effect on the SC lipid barrier, a SCS with short free fatty acids is prepared, referred to as the short FFA SCS. This composition was selected as short chain FFAs are encountered in SC of human skin equivalents [25].

2. Materials and Methods

2.1. Materials

In these studies we used 5 CER subclasses, see Fig. 1. The CER subclasses consist of either a sphingosine (S) or phytosphingosine (P) base, whereas the acyl chain is a nonhydroxy (N), α-hydroxy (A) or ω-hydroxy chain [26]. The acyl chain length is either 16 carbons (C16), 24 carbons (C24) or 30 carbons (C30). The corresponding nonhydroxy and α-hydroxy CER are denoted as CER NS (C24), CER NS (C16), CER NP (C24) and CER AP (C24). In an additional CER subclass a linoleic acid is ester linked to the ω-hydroxy group (indicated by EO) with a sphingosine base. This CER is denoted as CER EOS (C30). These ceramides were generously provided by Cosmoferm B.V. (Delft, The Netherlands). Myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0), behenic acid (C22:0), tricosanoic acid (C23:0), lignoceric acid (C24:0), cerotic acid (C26:0) and cholesterol were purchased from Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany). The deuterated FFA with chain length of C16:0 and C22:0 were obtained from Larodan (Malmö, Sweden) and C14:0, C18:0 and C20:0 were purchased from Cambridge Isotope laboratories (Andover MA, USA). Benzoic acid, trypsin (type III, from bovine pancreas), and trypsin inhibitor (type II-S from soybean) were obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands). Dialysis membrane disks (cutoff value of 5000 Da) were obtained from Diachema (Munich, Germany). Nuclepore polycarbonate filter disks (pore size 50 nm) were purchased from Whatman (Kent, UK). All organic solvents are of analytical grade and manufactured by Labscan Ltd. (Dublin, Ireland). All other chemicals are of analytical grade and the water is of Millipore quality.

2.2. Isolation of SC from human skin

SC was isolated from abdominal or mammary skin, which was obtained from the hospital within 24 h after cosmetic surgery. After removal of the subcutaneous fat tissue, the skin was dermatomed to a thickness of approximately 250 μm using a Padgett Electro Dermatome Model B (Kansas City KS, USA). The SC was separated from the epidermis by trypsin digestion [0.1% in phosphate-buffered saline (PBS), pH 7.4], after overnight incubation at 4 °C and subsequently at 37 °C for 1 h. The SC was then placed in a 0.1% solution of trypsin inhibitor and washed twice with Millipore water. Until use, the SC was stored in a silica-containing box under gaseous argon in the dark to prevent oxidation of the intercellular SC lipids. Before FTIR measurements, the SC was rehydrated for 24 h at 100% relative humidity.

2.3. Preparation of the lipid mixtures

For the preparation of the SCS, synthetic CER, CHOL and FFA were used in equimolar ratio. For the SCS, the following synthCER composition was selected (see also Fig. 1): CER EOS (C30), CER NS (C24), CER NP (C24), CER AS (C24), CER NP (C16) and CER AP (C24) in a 15:51:16:4:9:5 molar ratio, similar as observed in pig SC [14]. For the

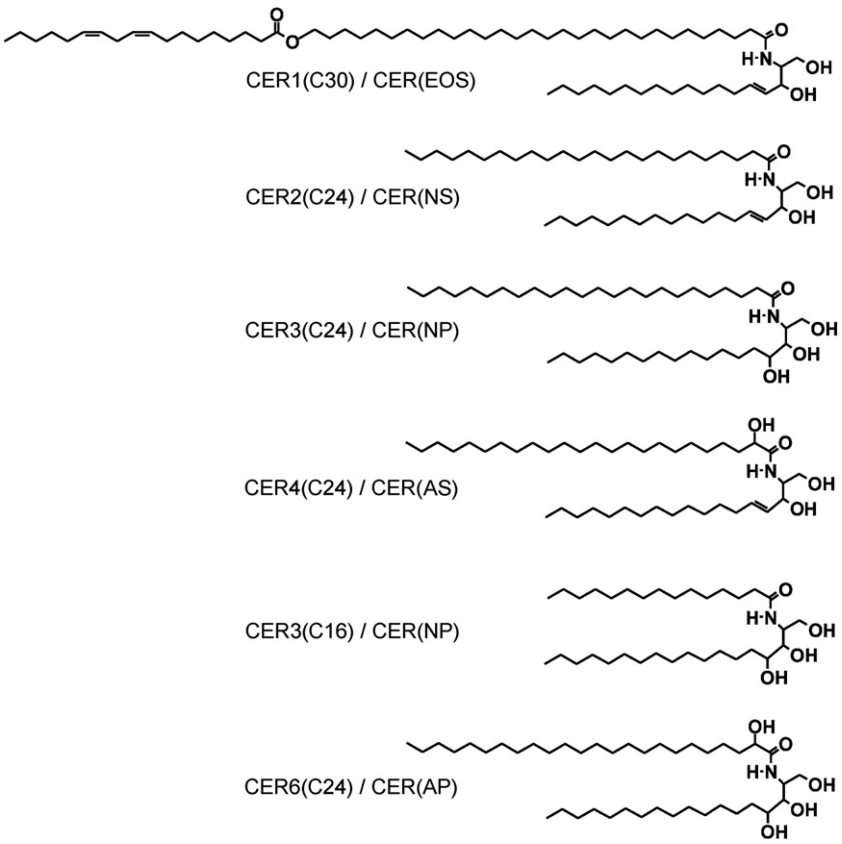

Fig. 1. Molecular structure of the synthetic CER used in the SCS. The nomenclature is according to Motta et al [26].