Biochimica et Biophysica Acta 1858 (2016) 1935–1943

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Skin barrier modification with organic solvents

Clara Barba *, Cristina Alonso, Meritxell Martí, Albert Manich, Luisa Coderch

Department of Chemicals and Surfactants Technology, Institute of Advanced Chemistry of Catalonia, Spain

ARTICLE INFO

Article history:
Received 12 November 2015
Received in revised form 29 April 2016
Accepted 9 May 2016
Available online 14 May 2016

Keywords:
Stratum corneum
Lipids
Solubilization
Barrier function
Permeability

ABSTRACT

The primary barrier to body water loss and influx of exogenous substances resides in the stratum corneum (SC). The barrier function of the SC is provided by patterned lipid lamellae localized to the extracellular spaces between corneocytes. SC lipids are intimately involved in maintaining the barrier function. It is generally accepted that solvents induce cutaneous barrier disruption. The main aim of this work is the evaluation of the different capability of two solvent systems on inducing changes in the SC barrier function. SC lipid modifications will be evaluated by lipid analysis, water sorption/desorption experiments, confocal-Raman visualization and FSTEM images. The amount of SC lipids extracted by chloroform/methanol was significantly higher than those extracted by acetone. DSC results indicate that acetone extract has lower temperature phase transitions than chloroform/methanol extract. The evaluation of the kinetics of the moisture uptake and loss demonstrated that when SC is treated with chloroform/methanol the resultant sample reach equilibrium in shorter times indicating a deterioration of the SC tissue with higher permeability. Instead, acetone treatment led to a SC sample with a decreased permeability thus with an improved SC barrier function. Confocal-Raman and FSTEM images demonstrated the absence of the lipids on SC previously treated with chloroform/methanol. However, they were still present when the SC was treated with acetone. Results obtained with all the different techniques used were consistent. The results obtained increases the knowledge of the interaction lipid-solvent, being this useful for understanding the mechanism of reparation of damaged skin.

1. Introduction

The primary barrier to body water loss and influx of exogenous substances, resides in the SC. The structure of the SC is unique, consisting of multiple layers of anucleate, proteinaceous corneocytes embedded in an expanded, lipid-enriched extracellular matrix [1]. As the lipid matrix is the only continuous pathway in the SC, the composition and organization of these lipids are of major importance for a competent skin barrier function [2,3]. The SC lipid composition differs considerably from most other biological membranes, having longer and more saturated lipids and basically no phospholipids [4]. The SC lipids are composed of an approximately equimolar ratio of cholesterol (Chol), free fatty acids (FFA) and ceramides (Cer) [5]. In addition, minor amounts of cholesterol sulfate (CholS) are present. The SC lipids are organized in regularly stacked lipid layers (lamellae).

Biological membranes typically contain a large number of different lipid species, and the permeability strongly depends on the physical state of the lipids. The organization of the lipids within the lamellae is referred to as the lateral organization. There are three possible arrangements of the lipids: a very dense, ordered orthorhombic organization, a less dense, ordered hexagonal organization, or a disordered liquid organization [6]. In most biomembranes, the lipids are in the liquid–crystalline state under physiological conditions. However, the majority of the SC intercellular lipids are primarily in the crystalline state, forming solid structures, at normal RH and ambient temperature [7,8]. Furthermore, there are several studies indicating that a small fraction of the SC lipids are suggested to be in a mobile disordered state, the liquid-crystalline, which is a fluid state [9]. The existence of fluid lipids could account for the non-negligible TEWL, which appears difficult to explain on basis only of the solid SC lipids. It could also allow for the high elasticity of the skin and for the enzymatic activity in the SC intercellular space that is unlikely to take place in a crystalline phase [10]. The lipid domain of the SC influences the diffusion properties of molecules. The high fraction of SC lipids in the solid state can assure low permeability. The presence of fluid lipids can have large consequences for the permeability by potentially forming regions with lower diffusion resistance where transport preferentially occurs [11,12].

There has been an increasing interest in the characterization of the thermal behavior of the SC lipids. The characterization of the lipid phase behavior obtained from thermal analysis is crucial for understanding the SC and SC lipids structure and their role in the selective permeability of the skin [13]. Deficiencies in any one of SC lipid species result in barrier abnormalities characterized by increased transepidermal water loss (TEWL) as well as observable alterations in the ultra structural features of the SC extracellular domains [13]. Furthermore, perturbation of the SC lipid organization increases drug

* Corresponding author.
E-mail address: cbaesl@iqac.csic.es (C. Barba).

http://dx.doi.org/10.1016/j.bbamem.2016.05.009
0005-2736/

permeability in SC lipids models. Defects in the function of the skin barrier are associated with a wide variety of skin diseases [14], in particular, patients with psoriasis [15] and atopic dermatitis [16,17] have a diminished skin barrier function.

Several studies have been carried out to determine the role of the lipids in the water permeability of the keratinized tissues such as SC and hair. Earlier studies demonstrated that changes in SC lipid composition result in differences in the SC permeability [18,19]. Furthermore, techniques such as differential scanning calorimetry (DSC) and flux of tritiated water vapour have been used to study the SC water permeability [20]. Besides, techniques such as a thermal desorption autosampler coupled to a mass spectrometer has also been used to measure the in vitro water retention of human SC as a function of a treatment applied [21].

It is best for studies of SC function to measure multiple parameters including SC water content, permeability, morphology, lipid content and composition. To determine the lipid role in the skin barrier is important to study not only the effect of a total lipid depletion, i.e. using chloroform/methanol as solvent system, but also the effect of a partial lipid extraction, i.e. using acetone as solvent. Therefore, the main aim of this work was to study the different modification on the SC barrier function due to two solvent systems. Intercellular lipids were modified by a mixture of chloroform/methanol or by acetone. After treatments, the degree of SC barrier lipids modifications was assessed by lipid composition, water sorption/desorption experiments, FSTEM images and morphological confocal Raman studies.

The different lipid structures of the extracted lipids account for the different changes in permeability when submitted to these two solvent systems. Modification of water diffusions of organic solvent treated SC can help to understand the barrier function of the SC.

2. Materials and methods

2.1. Materials

Trypsin (from porcine pancreas), cholesteryl stearate (>96%), cholesterol (>92%) and Palmitic acid (>99%) were all from Sigma-Aldrich (St. Louis, Missouri, USA). Acetone, chloroform, methanol, n-hexane, diethyl ether, and benzene (all for analysis) were purchased from Merck (Darmstadt, Germany). Formic acid (85%) was obtained from Probus (Badalona, Spain) and ceramide III from ITEQSA (Sabadell, Spain). The chemicals for the sample fixation: glutaraldehyde, paraformaldehyde and cacodylate buffer were supplied by EMS, and the glycerol by Merck.

2.2. Isolation of SC

Porcine skin (three different donors) was obtained from the unboiled back of freshly killed domestic pigs (Landrace large White race) weighing 20–30 kg. The excised skin was dermatomed to 500 ± 50 μm thickness (Dermatome GA630, Aesculap, Tuttlingen, Germany). The skin was placed in water at 70 °C for 3–4 min, and the epidermis was scraped off in sheets. Epidermal cells are subsequently removed from the overlying SC via enzymatic digestion. To isolate the SC, the epidermal sheets were incubated for 2 h at 37 °C with the epidermal side in contact with a solution of 0.5% Trypsin in PBS at pH 7.4. After 2 h, the Trypsin was removed by several washes of the SC with Milli-Q water [22]. Pieces of 10 mg of SC were prepared for the lipid extraction.

2.3. Lipid extractions

Lipid extractions were performed by treatment the SC previously isolated with 10 ml of acetone or 10 ml of the organic solvent system of chloroform/methanol (2/1 v/v) for 2 h on a rocking table and rinsed with water during 15 min. Untreated SC was left as control, submitted only to the washing protocol. To evaluate the total amount of lipids extracted, 1 ml of each of the extracts was evaporated to dryness in a P_2O_5 desiccator and weighed to a constant weight and re-dissolved in a small volume of chloroform/methanol (2/1 v/v). As Raman spectroscopy allows the study of the tissues at different depths the Raman experiments were planned to be performed with the entire pig skin (dermatomized to 500 μm). For this, skin discs ($\Phi = 25$ mm) were submitted to the lipid extraction procedures described above. Again untreated pig skin was left as control. All the samples prepared (SC and skin discs) were stored wet at 6 °C prior to continue with the planned experiments.

2.4. Lipids analyses

Lipid analyses of the different extracts were performed by thin layer chromatography (TLC) coupled to an automated flame ionization detector (FID) (Iatroscan MK-5, Iatron, Tokyo, Japan). This technique enabled the rapid separation and precise quantification of different lipid classes without sample pretreatment [23], and has been used to study the composition of different lipid extracts [24–26]. The lipids samples (10 μg/ml) and standards (2 μg/ml) were dissolved in chloroform/methanol (2/1, v/v) and were spotted (0.6–2 μl) on Silica gel S-III Chromarods using a SES (Nieder-Olm, Germany) 3202/15–01 sample spotter. Lipid content and composition were determined using an optimized TLC-FID protocol [27]:

A general lipid analysis (1 scan) was performed by eluting the rods consecutively four times using the following mobile phases: the rods were eluted twice to a distance of 2.5 cm with chloroform/methanol/water (57/12/0.6, v/v/v) to separate apolar lipids from more polar lipids such as ceramides. Successively, a third elution with hexane/diethyl ether/formic acid (50/20/0.3, v/v/v) up to 8 cm and a fourth elution with hexane/benzene (35/35, v/v) up to 10 cm were performed to separate the different apolar lipids. Each bath was prepared 30 min before development. To obtain a good vapour saturation of the development tank, a filter paper was erected along one side and wetted with solvent system. After each elution, the rods were heated for 5 min at 60 °C to dry the remaining solvent and left 10 min in a conditioned flask before the following elution.

The experimental conditions were: air flow 2000 ml/min, hydrogen flow 160–180 ml/min, and scanning speed 2–3 mm/s. Data were processed with Boreal version 2.5 software (JMBS Development, Grenoble, France). These procedures were applied to the following standard compounds: cholesteryl stearate, Chol, palmitic acid, and ceramide III to determine the corresponding calibration curves for quantification of each compound.

2.5. Differential scanning calorimetry

A differential scanning calorimeter, DSC-821 (Mettler Toledo, Columbus, OH), was used to determine the transition temperature (T_m) of the extracted lipids of the SC. Approximately 1.5 mg of sample was prepared by evaporation to dryness and stored in a desiccator under a P_2O_5 for 48 h. Samples were sealed in 40 μl aluminum punched pan. The dried sample was initially cooled from 25 °C to −100 °C and then heated from −100 °C to 100 °C, heating rate of 10 °C/min and nitrogen purging gas 50 ml/min.

2.6. Sorption experiments

Absorption and desorption curves were obtained in a thermogravimetric balance equipped with a controlled humidity chamber, the Q5000SA Sorption Analyzer (TA Instruments, New Castle, USA) [28–30]. The weight of the SC samples analyzed ranged between 5 and 10 mg. Before measuring the sorption properties, the SC samples were kept under humidity controlled conditions (22 °C and 65% RH)