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Characterization of mimetic lipid mixtures of stratum corneum

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ABSTRACT

Lipid mixtures consisting of ceramide III, palmitic acid, and cholesterol were prepared at different thermal and humidity conditions. The lipid mixture, treated at temperature higher than 100 °C, displayed the similar thermal character to native human Stratum Corneum (SC), although hydration changed structural characters of the lipid mixtures as well as human SC: Hydration gave rise to the variation of lamellar distances in lipid mixtures such as lengthening of vertical repeat distance and slight-shortening of the lateral repeat distance. It also generated the configurational transition of amide groups. Since these variations depending on the heating and hydrating processes do not occur on pristine lipids, it can be confirmed that the lipid mixture forms hybrid phases by the association between heterogeneous lipids.

1. Introduction

Stratum Corneum (SC) in mammal skin takes on an important role for protecting living organisms and preventing their release. It is constructed with the brick-and-mortar structure, that is, corneocytes in SC are embedded in lipid lamellar layers [1]. The intercellular lipid matrix of SC affords the barrier function of human skin, and it is mainly arranged with two repeating distances of 6.4 nm (in short periodicity phase) and 13.4 nm (in long periodicity phase) [2,3]. Besides, the lamellar structures with spacing of 4–4.5 nm also exist in human SC [2]. The lipid lamellae in SC are directed nearly along the surface of the corneocytes with orthorhombic and hexagonal lateral packings [1,4]. The distribution of such packing phases varies with the depth of human SC and the temperature. Moreover, orthorhombic phase shows lower permeability than hexagonal phase.

Lipids in human SC are mainly classified into ceramides, free fatty acids and cholesterol. Different skins have different compositions of lipids in SC: Ceramides I and IV containing ω-hydroxy acid are often absent in young dry skin [5]; cholesterol has higher content in lamellar ichthyosis skin than in normal skin [1]. It is also obvious that the component and composition of lipids in SC affect seriously the health of skin to make it non-dry and non-diseased. The investigation on model SC has provided reliable evidence to verify that the arrangement of lipid is susceptible to the composition [6–12]. Ceramide I contributes to the formation of long periodicity phase, although its fraction is only 8.3% of the total ceramides in human SC [13,14], while cholesterol is an additional important component on the formation of long periodicity phase [9]. It is also reported that head groups of ceramides play a role on the stabilization of orthorhombic lamellar arrangement [15], and free fatty acids contribute to the formation of orthorhombic phase [16].

In this context, there are some differences between lipid mixtures prepared with synthetic and natural ceramides. For instance, there are additional phases with repeat distances of 3.7 and 4.3 nm in the lipid mixture from synthetic ceramides, which are separate domains of crystalline ceramides [10]. Most of the researches focus on phase behavior of lipid mixtures with multiple components by using Fourier transform infrared (FTIR) absorption spectroscopy [12,17], Raman microspectroscopy [18], NMR [19] and small and wide angle X-ray scattering (SAXS, WAXS) [20,21]. Nevertheless, excessive components are not accessible to clarify the interaction between lipid molecules and the mechanism of molecular assembling. Therefore, some model SCs with simple compositions have been investigated. Multilayer lipid films of only four components

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(ceramide VI, palmitic acid, cholesterol and cholesterol sulfate) are prepared, and it is found from the neutron diffraction technique that humidity or hydration of lipid mixture raises an important effect on the structure of lipid membrane, that is, two phases are formed at high humidity, while only one phase is formed at low humidity [22]. Meanwhile, higher content (more than 4%) of cholesterol sulfate in the lipid mixture is not appropriate, since the fraction of cholesterol sulfate is less than 2% in human SC [23].

A ternary system including native lipid (bovine brain ceramide III), palmitic acid and cholesterol has attracted large interest to investigate phase behavior by FTIR absorption spectrometry [24,25]. It is found that in this ternary system, the composition involves deeply in the interaction between different lipid molecules [26]. Especially, a ratio of ceramide III/palmitic acid above 2 allows the interaction between two components. The phase behavior of ceramide III is complicate and different phases are obtained by different procedures [27]. In the nature of things, the phase variation is considered to result from the change in arrangement of lipids in the model SC. Hitherto, five phases have been found with different repeat distances of 3.73, 3.95, 4.59, 4.65 and 5.15 nm [28].

In the present work, the study aimed at investigating the effect of preparation procedure on the thermal behavior and assembling of lipids in the mixture prepared from ceramide III, palmitic acid and cholesterol. In this ternary system, three lipids were mixed by weight ratio, in concord with their existential quantities in human SC, which are in equimolar ratio. The investigation was mainly concerned with effects of thermal treatment and hydration (humidity) on the preparation procedure of lipid mixture. Actually, it has been reported that the thermal behavior of native SC is affected by process, that is, reheating of porcine SC causes alterative thermal behavior [29]. To explore the effect of preparation procedure on thermal behavior and assembling of lipid mixture contributes to recognize the mechanism of interaction between lipid molecules, to elucidate the functions of SC at the biomolecular level and to build up the suitable model SC as an alternative of the native one.

2. Materials and methods

2.1. Materials

Ceramide III (95.9%) was supplied from Evonik Degussa (Essen, Germany). Palmitic acid (95.0%) and cholesterol (Guarantee reagent) were purchased from Nacalai Tesque (Kyoto, Japan).

2.2. Preparation of lipid mixtures

2.2.1. Method I

Ceramide III, palmitic acid and cholesterol (2:1:1 weight ratio) were dissolved in a mixed solvent of chloroform and methanol (5:1 volume ratio) to prepare a lipid solution at a concentration of 1 wt%. A lipid solution (1.0 cm^3) was spread on a 76 mm × 26 mm slide glass and dried under air atmosphere to obtain a lipid mixture (sample I(as-prepared)). Sequentially, the lipid mixture was heated at 70 and 120 °C for 1 h under nitrogen atmosphere in a glass tube oven (GTO-200, SIBADA) and then cooled down to room temperature to obtain samples II(70) and IV(120), respectively. Samples II and IV were hydrated for 3 days at the weight ratio 1:3 of sample:water to obtain samples III(70-hyd) and V(120-hyd), respectively.

2.2.2. Method II

Sample I was heated at 75 °C for 30 min under nitrogen atmosphere in a glass tube oven and, after being cooled down immediately, the lipid mixture was hydrated by immersing in water for 15–20 min. Finally, it was heated at 120 °C for 1 h under nitrogen atmosphere and cooled down immediately to obtain sample VI(75-hyd, 120). Sample VI was hydrated for 3 days at the ratio 1:3 of sample VI:water to obtain sample VI(75-hyd, 120-hyd).

2.2.3. Method III

Ceramide III, palmitic acid and cholesterol were mixed at a weight ratio of 2:1:1. Then the mixed powder was heated up to 100 °C for 1 h in vacuum to melt into liquid in a glass tube oven. With cooling down to room temperature, wax-like solid (sample VIII(melt)) was obtained. The solid was hydrated for 20 h at the ratio 1:3 of solid:water to obtain hydrated solid (sample IX(melt-hyd)).

2.3. Characterization of lipid mixtures

Thermal behavior of lipids was recorded on a differential scanning calorimeter (DSC) (Seiko Instrument Inc., Tokyo, Japan) equipped with an SII SSC/5200H analyzer. The lipid sample (0.6–10 mg) was put into a silver DSC capsule. The capsule was sealed and placed in the DSC cell along with a vacant one as a reference. Then the sample was heated from 20 to 140 °C at 2 °C/min. The assembly of lipids was characterized by using an X-ray diffractometer (D8 Advance, Bruker) with CuKα radiation (λ = 0.154 nm) operating at 20 mA and 40 kV. Lipid samples were placed on an X-ray diffraction (XRD) glass or a plastic plate in a sample holder. FTIR absorption spectra of lipids using KBr pellet were recorded on a FTIR spectrometer (Bio-Rad Digilab FTS-60A) in transmittance mode in the range of 4000–400 cm^{-1} with 64 scans at 1 cm^{-1} resolution. The data were ascertained for their reproducibility by repetition of measurement.

3. Results and discussion

3.1. Thermal behavior of lipid mixtures

The DSC curves of lipid mixtures (samples I–IX) are shown in Fig. 1. The temperature and enthalpy change of phase transition on lipid mixtures are listed in Table 1. Obviously, lipid mixtures prepared at different conditions displayed different thermal behavior. In the DSC of lipid mixture (sample I) just prepared from the lipid solution, there were two strong and three weak endothermic peaks. When this as-prepared lipid mixture was heated at medium temperatures (70 °C), a main peak occurred at 59 °C (see a curve of sample II). A similar DSC curve was observed even at 65–85 °C heating (data are not shown). When the lipid mixture pre-heated at 70 °C was hydrated, a peak at 59 °C lessened and endothermic peaks at 49, 70, and 89 °C appeared instead (see sample III). A similar DSC curve was obtained even for lipid mixtures (samples IV and V) after being heated at 120 °C and hydrated, although a peak at 59 °C completely disappeared and two peaks at high temperatures (72 and 89 °C) appeared already for the lipid mixture without hydration (see a curve for sample IV).

If the lipid mixture pre-heated at 75 °C and pre-hydrated was heated at 120 °C and hydrated, the DSC curves (see samples VI and VII) came near to curves (samples IV and V) of lipid mixture directly heated at 120 °C and hydrated. Separately, mixed powder was completely melted by heating at 100 °C and cooled down to room temperature. Then the DSC curve presented characteristic peaks at 55 and 74 °C. Similar curve was obtained even after melting at 120 °C under nitrogen atmosphere (data are not shown). After hydration of solid, the DSC peak at 55 °C disappeared but peaks at 45 and 84 °C appeared. This behavior was similar to the variation of a mixed lipid prepared from a lipid solution.

In DSC curves of component lipids, main peaks existed at 126, 64, and 38 °C for ceramide III, palmitic acid, and cholesterol, respectively, and these peaks do not change even after treated by heating,