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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,

Table 1
Temperatures and endothermic enthalpy changes of phase transitions on lipids and lipid mixtures.

Sample	~30 °C		40–50 °C		50–60 °C		60–80 °C		80–90 °C		~100 °C		Total enthalpy
	T (°C)	Δ*H* (J/g)	*T* (°C)	Δ*H* (J/g)	*T* (°C)	Δ*H* (J/g)	*T* (°C)	Δ*H* (J/g)	*T* (°C)	Δ*H* (J/g)	*T* (°C)	Δ*H* (J/g)	Change $\sum \Delta H$ (J/g)
Ceramide III[a]									*89*	21	*126*	85	106
Palmitic acd[a]							*64*	90	*82*	5			95
Cholesterol[a]			*38,43*	34	*52*(exothermic)	4			*83*	0.6			39
I(as-prepared)			*45,49*	17	*60*	0.6	*73*	13	*87*	8			39
II(70)	*29*	1			*59*	33	*69*	1	*87*	8	*102*	24	67
III(70-hyd)	*29*	4	*49*	14	*58*	2	*70*	15	*89*	13			48
IV(120)					*54*	6	*72*	10	*89*	14	*95*	4	34
V(120-hyd)			*42*	19			*70*	7	*88*	23			49
VI(75-hyd, 120)			*38*	0.4	*55*	22	*69*	2	*90*	19			43
VI(75-hyd, 120-hyd)	*30*	0.4	*43*	13	*58*	0.4	*67*	6	*85*	10			30
VIII(melt)					*55*	22	*74*	37			*98*	9	68
IX(melt-hyd)			*37,45*	14			*74*	19	*84*	6	*98*	2	41

[a] Ceramide III, palmitic acid and cholesterol were treated with the same procedure as sample VI(75-hyd, 120).

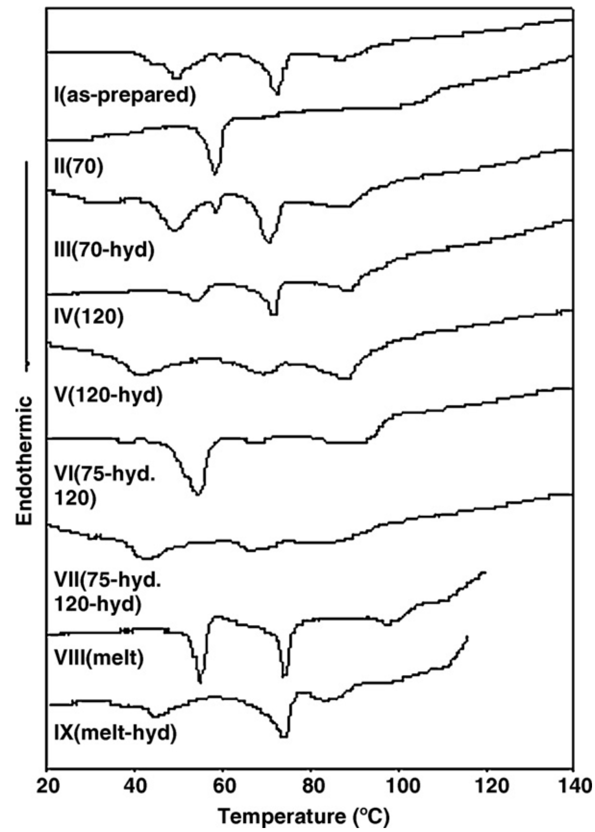

Fig. 1. Thermal behaviors of lipid mixtures (samples I–IX) prepared by different procedures.

hydrating and reheating (Fig. 2 and Table 1). It should be noted that the endothermic peaks characteristic to the melting of ceramide III and palmitic acid were not observed in lipid mixtures at any conditions, although a weak peak at the same temperature as a phase transition peak of cholesterol was observed in the lipid mixture and it depended on the preparation condition. This indicates that the component lipids in the mixture form phases consisting of

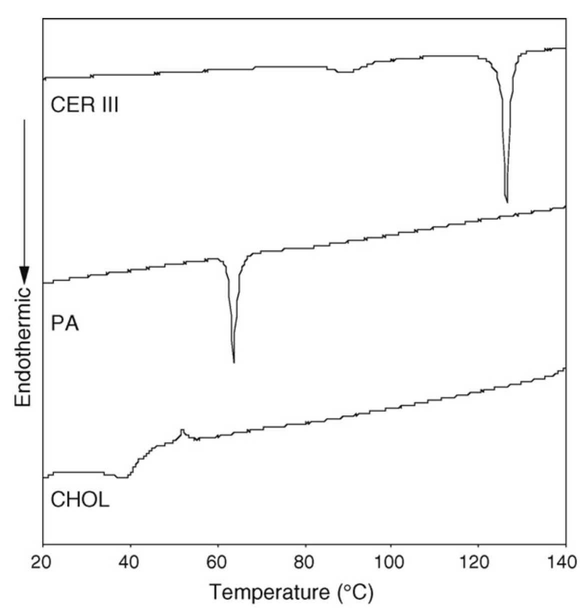

Fig. 2. Thermal behavior of ceramide III (CER III), palmitic acid (PA) and cholesterol (CHOL) prepared by the same procedure as sample VI(75-hyd, 120).

mixed components with different endothermic peaks from those of component lipids.

The thermal behavior of native skins has been reported by some groups [29–31] and temperatures of phase transition from the literatures are listed in Table 2 in comparison with the present data. Although five characteristic peaks in a DSC thermogram are observed on a human SC, peaks on porcine and pig SC are only three. This result does not astonish us, because the lipid components are different among porcine, pig and human SCs. Since the mixing ratio, 2:1:1 (in weight), of ceramide III, palmitic acid and cholesterol is controlled to be close to lipid contents in human SC, the present data can be compared with human SC rather than with porcine and pig SCs.

Although the DSC result of human SC at dry or low-hydrated (20–40%) condition displays five peaks denoted by T_1, T_x, and T_2–T_4 [31], a highest peak (T_4) around 100 °C disappears after highly hydrated. Similar tendency of the highest peak was observed even on the lipid mixture in the present work. According to the report for human SC [30], the enthalpy change of T_1 peak increases but that of T_x peak decreases with hydration. Similarly, in the present result, T_1 peak appears and T_x peak disappears after hydration. Then it is reasonable to say that the present lipid mixture, especially, treated by high temperature (more than 100 °C) reflects thermal behavior of native human SC and that T_2 and T_3 phase transitions are intrinsic for lipid mixtures, T_x and T_4 phase transitions are remarkable at the dry condition and T_1 phase transition is characteristic at the hydrated condition.

Five phase transition peaks are assigned as follows [12,31]: The T_1 peak arises from a chain packing transition from an orthorhombic to a hexagonal phase and it is enlarged at hydration condition. On the other hand, the T_x peak, which weakens or disappears after hydration, is assigned to the loss of lipid crystalline structure. The T_2 and T_3 peaks, which are common to native SC and lipid mixture, are due to the gel–liquid crystalline phase transition of lipids. The difference between native SC and lipid mixture is the intensity of T_4 peak, because this peak is attributed to the melting of lipids and proteins for native SC but only to the melting of lipids for lipid mixture, although this peak disappears after hydration. The aspects of T_1, T_x and T_4 peaks suggest that the crystalline state of lipid mixture changes after hydration.

3.2. Molecular structure in lipid mixtures

Chemical structure of lipids in lipid mixtures can be determined from the spectroscopic investigation. Fig. 3 shows FTIR absorption spectra of lipid mixtures prepared by different procedures and Table 3 lists the IR band positions with their assignments. For comparison, FTIR spectra of component lipids are provided in Fig. 4. Characteristic antisymmetric and symmetric stretching bands of CH_2 were observed at 2918 and 2850 cm^{-1} in common among ceramide III, palmitic acid and all lipid mixtures prepared, indicating the extended trans-zigzag configuration of alkyl chains [32] in orthorhombic lattice [12]. Corresponding CH_2 bending (scissors and rocking) bands also were independent of hydration at least after thermal treatment at high temperature like 120 °C. Meanwhile, an amide I (mainly C=O stretching vibration of amide derivatives [33]) mode originating in ceramide III consisted of two bands at 1647–1630 and 1621–1612 cm^{-1} but their intensities were inverted between dry and hydrated states, supporting the configuration change of head group including amide group after hydration, although pristine ceramide III displayed 1614 cm^{-1} band-rich spectra at both powder and hydrated state.

The characteristic band of palmitic acid was observed at 1703 cm^{-1} for pristine lipid and as-prepared lipid mixture but a shoulder around 1730 cm^{-1} appeared after being heated at 120 °C and hydrated, denoting the occurrence of highly hydrogen-bonded

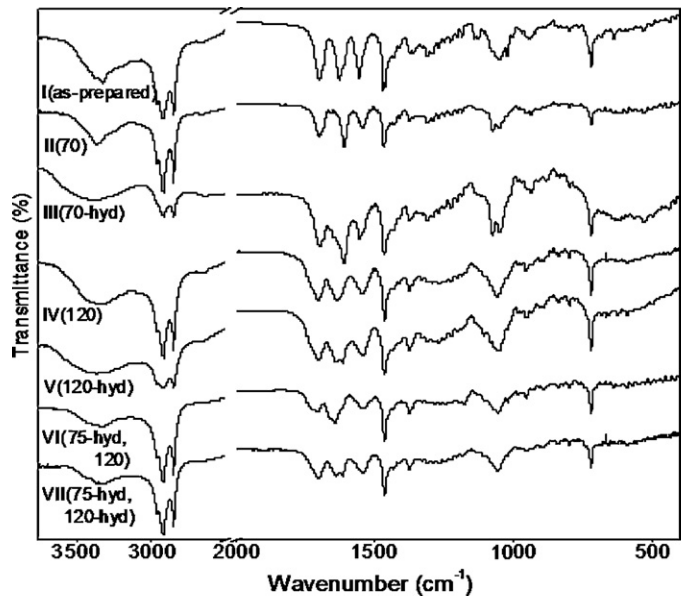

Fig. 3. FTIR spectra of lipid mixtures (samples I–VII) prepared by different procedures.

carbonyl species. Cholesterol has characteristic IR bands arising from aromatic CH, alkyl CH_2, CH_3 and COH groups, but most of these bands overlapped with corresponding bands of ceramide III and palmitic acid. Although a CH_3 symmetric deformation band at 1377 cm^{-1} alone was distinctly stronger for cholesterol than for other lipids, there was no meaningful variation on this band depending on the preparation procedure of lipid mixture. Characteristic bands of water may be intensified after hydration but the corresponding bands are superimposed on O–H and N–H bands of

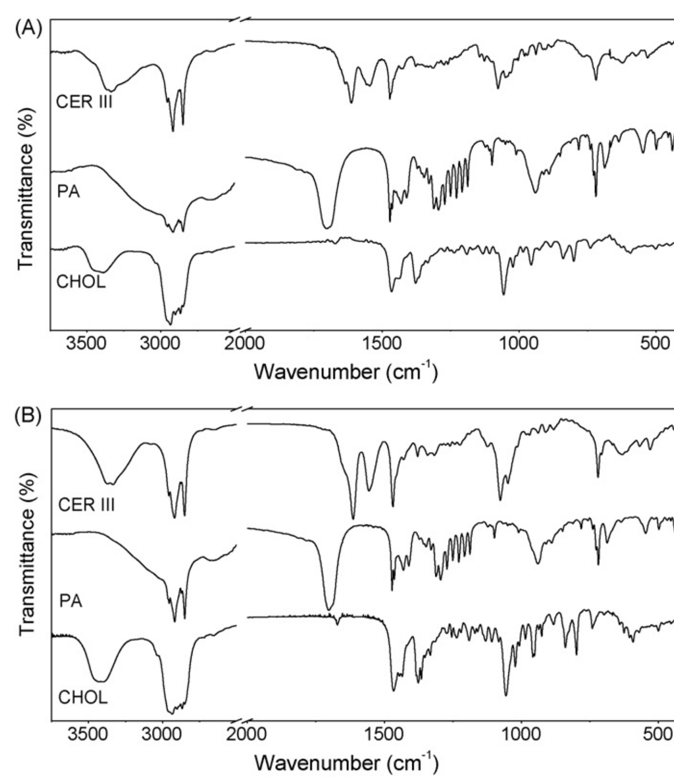

Fig. 4. FTIR spectra of ceramide III (CER III), palmitic acid (PA) and cholesterol (CHOL). (A) Pristine and (B) prepared by the same procedure as sample VI(75-hyd, 120).

Table 2
Temperatures of phase transitions of native SCs and lipid mixtures.

Sample	Condition	T_1 (°C)	T_x (°C)	T_2 (°C)	T_3 (°C)	T_4 (°C)	Reference
Percine SC	75% hydrated		60	70		98	[29]
Pig SC	Dry			68	78		[29]
	Wet			68	78	95	
Human SC	Dry	43	55	74	86	94	[30]
	Hydrated	37	55	72	83		
Human SC	20–40% hydrated	37	51–55	72	83	100	[31]
Lipid mixture	Dry (sample IV)		54	72	89	95	Present work
	Hydrated (sample V)	42		70	88		

Table 3
IR band positions of lipid mixtures and their assignments.

Assignment[a]	I(as-prepared)	II(70)	III(70-hyd)	IV(120)	V(120-hyd)	VI(75-hyd, 120)	VI(75-hyd, 120-hyd)
O–H and N–H stretching	3367, 3323	3370, 3341	3377, 3329	3363, 3325	3365, 3317	3325	3362, 3315
CH_2 antisymmetric stretching	2918	2919	2919	2918	2919	2918	2918
CH_2 symmetric stretching	2850	2850	2850	2850	2850	2850	2850
C=O (acid) stretching	1703	1703	1731sh, 1701	1726sh, 1706	1733sh, 1705	1729sh, 1706	1733sh, 1704
Amide I (80% C=O stretching)[a]	1630	1612	1634sh, 1615	1640	1643, 1618	1647, 1621sh	1644, 1619
Amide II (60% C–NH bending +40% C–N stretching)[a]	1560	1547	1559, 1543sh	1547	1544	1544	1544
CH_2 scissoring	1473	1472	1470	1469	1468	1468	1468
CH_3 symmetric deformation	1377	1377	1377	1379	1379	1379	1378
	1365	1365sh	1368sh	1367sh	1367sh	1366sh	1366sh
C–C or C–O stretching	1077sh	1076	1077				
	1051	1054	1051	1059	1054	1059	1058
CH_2 rocking	720	720	720	721	721	721	721

[a] Ref. [33].

lipids and indistinguishable. It was demonstrated from IR results that hydration caused the variation of hydrophilic region in lipid assembly.

3.3. Assembling of lipid mixtures

The assembling of lipids was compared with XRD for lipid mixtures prepared at different procedures. Fig. 5 shows the XRD patterns and Table 4 lists repeat distances of lamellar structures. Bragg peaks of as-prepared lipid mixture (sample I) indicate that three lamellar structures coexist. Those are the first group with the longest repeat distance of 4.5 nm (Q_1 = 1.39 nm^{-1}, Q_2 = 2.83 nm^{-1}, Q_3 = 4.27 nm^{-1}), the second group with 3.7 nm distance (Q_1 = 1.69 nm^{-1}, Q_3 = 5.18 nm^{-1}), and the third group with 3.5 nm distance (Q_1 = 1.79 nm^{-1}, Q_2 = 3.63 nm^{-1}) For sample II treated at medium temperature, three groups of Bragg peaks could be found but the repeat distance of major lamellar structure was shorter than that of sample I. That is, Bragg peaks of the first group with shorter lamellar periodicity of 3.9 nm were located at Q_1 = 1.59 nm^{-1}, Q_2 = 3.27 nm^{-1}, and Q_3 = 3.91 nm^{-1}, while the second and third lamellar structures had same repeat distance with sample I, indicating same ordered structures. After lipid mixture treated at medium temperature was hydrated, new Bragg peaks were additionally observed at Q = 1.28, 2.59 and 3.94 nm^{-1} (repeat distance = 4.9 nm) (sample III).

Meanwhile, it should be noticed that when as-prepared lipid mixture was treated at 120 °C, Bragg peaks with repeat distance of 4.7 nm were intensified, but those with repeat distances of 3.7 and 3.5 nm were rather diminished (sample IV). Similar XRD profile was obtained even when pre-heated and pre-hydrated lipid mixture was heated at 120 °C (sample VI). However, when samples IV and VI were hydrated (samples V and VII), repeat distance of major lamellar structure shifted to 4.8 nm and lamellar structures with repeat distance of 3.7 and 3.5 nm recovered.

XRD results at higher scattering angle are shown in Fig. 5 for lipid mixtures prepared by different procedures. In this region, the information concerning the short distance periodicity in phase such as intralayer arrangement in lamellae is obtained. Alike long distance ordering, XRD profile in this region varied with treatment at medium (70 °C) and high (120 °C) temperatures, although the variation aspect depended on the temperature. Among of characteristic Bragg peaks of sample I, Q = 14.95 and 16.72 nm^{-1} peaks weakened with thermal treatment, and alternatively a peak around 15.1–15.2 nm^{-1} remained and a peak around 16.4–16.6 nm^{-1}

Table 4
Repeat distances of lamellar structures consisting of lipid mixtures.

	Structure I′ d (nm)	Structure I d (nm)	Structure II d (nm)	Structure II′ d (nm)	Structure III d (nm)
I(as-prepared)		4.5		3.7	3.5
II(70)			3.9	3.7	3.5
III(70-hyd)	4.9		3.9	3.7	3.5
IV(120)		4.7			3.5
V(120-hyd)	4.8			3.7	3.5
VI(75-hyd, 120)		4.6			3.4
VI(75-hyd, 120-hyd)	4.8			3.7	3.5

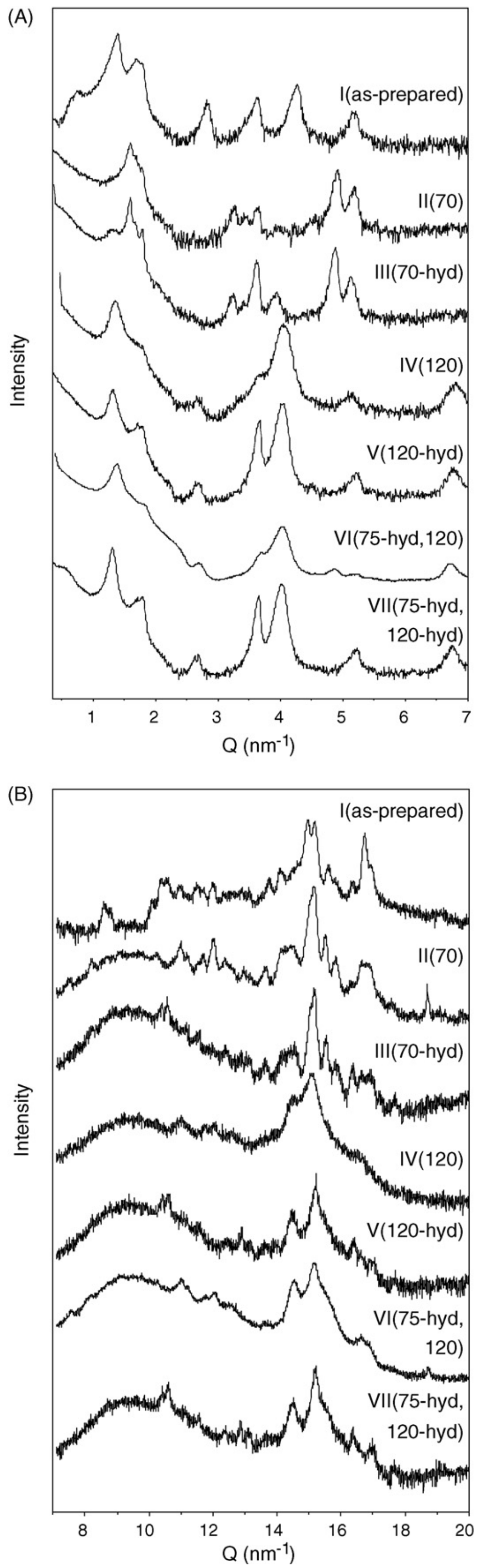

Fig. 5. XRD patterns of lipid mixtures (samples I–VII) prepared by different procedures. Q (nm^{-1}): (A) 0.4–7.0 and (B) 7.0–20.0.

became conspicuous. Both two Bragg peaks of hydrated samples (samples V and VII) take slightly higher peak positions than those of dry sample (samples IV and VI), indicating shortening of the lateral repeat distance. Another characteristic was the shift of a medium peak around 11.0 nm^{-1} to around 10.4 nm^{-1} after hydration. However this peak can be attributed to the higher order peak of long distance periodicity, that is, the eighth order peak of structure with repeat distance of 4.6 nm (dry) or 4.8 nm (hydrated), respectively.

3.4. Assembly structure of lipid mixtures

For the sake of comparison between lipid mixtures and component lipids, powder XRD of pristine lipid (ceramide III, palmitic acid or cholesterol) was also measured. Powder XRD patterns of pristine lipids are shown in Fig. 6 with patterns of lipids which were heated at 75 °C, hydrated and reheated at 120 °C as well as sample VI of lipid mixture. The position and assignment of Bragg peaks are listed in Table 5. As seen in Fig. 6 and Table 5, three crystal phases with unit distances of 4.6, 3.9 and 3.7 nm were found in powder ceramide III. Three kinds of morphologies of N-octadecanoylphytosphingosine in ceramide III group have been reported as δ, β and α phases with lamellar repeat distances of 4.49, 3.95 and 3.69 nm, respectively [28]. Ceramide III in these phases arranges with two alkyl chains straddled, and the angle between double chains is larger in the order of δ > β > α phases. Then three phases observed on ceramide III in the present work correspond to α, β and δ type structures, and a β type structure existed as majority after heating treatment. Major crystal structure of powder palmitic acid is monoclinic with a unit length of 3.6 nm and this crystal structure was invariable even after thermal treatment. Similar invariability before and after thermal treatment was maintained even for triclinic cholesterol crystal [34] with unit distance of 3.5 nm along [0 1 0] direction. It can be noted that unit distances for component lipids after thermal treatment at 120 °C are all shorter than repeat distance (4.8 or 4.6 nm) of main crystal phase in thermal (120 °C)-treated lipid mixtures. Therefore, it can be demonstrated that a main crystal in the lipid mixture is not a separate individual phase of any component lipid but it consists of a combination of component lipids. DSC result described above suggests at least the association of ceramide III and palmitic acid.

In XRD patterns at high scattering angle in Fig. 6, while the profiles of palmitic acid and cholesterol did not change even after heating at 120 °C, that of ceramide III varied partially. Moreover, when the profiles were compared among lipid mixtures and component lipids, they did not go far enough to define the local assembly structure, although resembling or common Bragg peaks were observed. This result confirms that the intralayer arrangement of lipids in lamellae of lipid mixtures is not necessarily the same as that in phases of component lipids. This is relative to the result, described above, that a main crystal in the lipid mixtures consists of association of component lipids.

Fig. 7 displays the probable models of lamellar structures in lipid mixtures prepared on the basis of results in the present work. Five structures are compatible with five phases which were observed in XRD. A main phase at the dry condition consists of “structure I”, which is constructed by mixture of three lipids. Structure I takes a ceramide III conformation with closed alkyl chains, since the observed repeat distance (4.6 nm) coincides with molecular length (2.1–2.3 nm) at this conformation. Then palmitic acid and cholesterol with shorter molecular length (1.9 and 1.6 nm, respectively) are mounted to be the lamellar orientation. After hydration (Structure I′), water penetrates into mainly hydrophilic layer and extends the repeat distance of lamellar layer. Structure II is based on β structure of ceramide III incorporating palmitic acid and cholesterol, and the repeat distance shrinks after hydration (see Structure

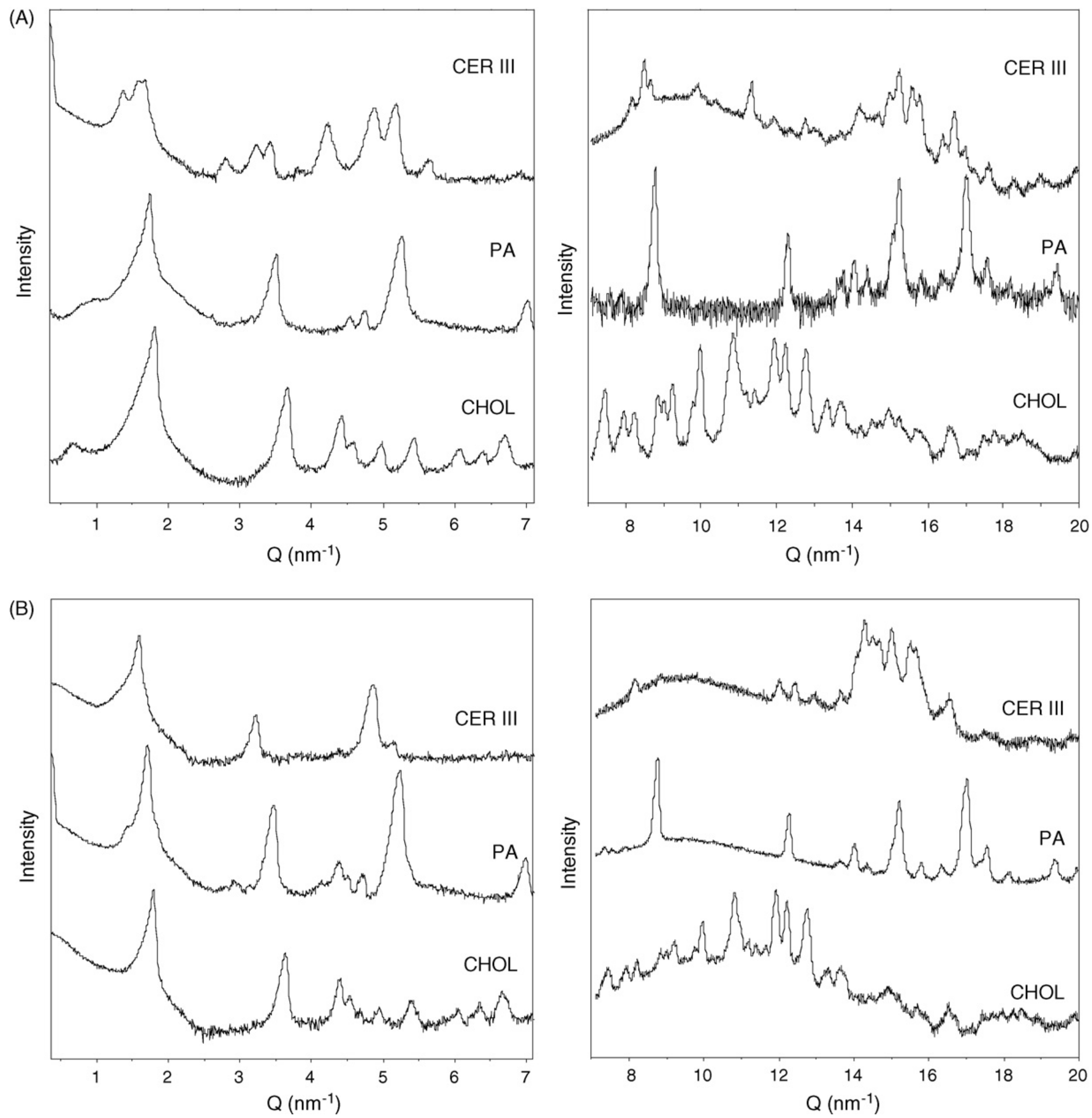

Fig. 6. XRD patterns of ceramide III (CER III), palmitic acid (PA) and cholesterol (CHOL). (A) Pristine and (B) prepared by the same procedure as sample VI(75-hyd, 120).

II′), because the hydrophilic layer of ceramide III may be laterally extended by the penetration of water and the straddled alkyl chains close slightly in the event, that is, the vertical distance shrinks. Structure III should be the isolated crystals of cholesterol, as estimated from the repeat distance of the phase.

Since Structures II, II′ and III are minor phases, the behavior accompanied by hydration can mainly get involved in the transition from Structures I–I′. Although the hydration gives rise to the configurational change of hydrophilic group in lipid mixture as estimated from the variation of an amide IR band, this comes from the penetration of water and the hydrogen bonding of water with hydrophilic group. Structure I is consistent with a short periodicity phase, as previously illustrated [13]. Moreover, although it was ascertained that both Structures I and II predominantly take orthorhombic lattice structure, hexagonal packing phase may partially coexist because of the slightly high wavenumber of a CH_2 symmetric stretching IR band and the slight frequency shift of a CH_2 scissoring band from the frequency for orthorhombic lattice, in reference to previous report [12]. The occurrence of high frequency C=O stretch mode after treatment by heat and hydration also may be related to the partial variation of crystalline phase in lipid assembly.

Table 5
Repeat distances of crystal structures consisting of lipids.

	Ceramide III				Palmitic acid		Cholesterol
	Unknown *d* (nm)	δ *d* (nm)	β *d* (nm)	α *d* (nm)	Unknown *d* (nm)	Monoclinic *d* (nm)	Triclinic *d* (nm)
Powder		4.6	3.9	3.7		3.6	3.5
Sample (70-hyd, 120)	4.9		4.0		4.4	3.7	3.5

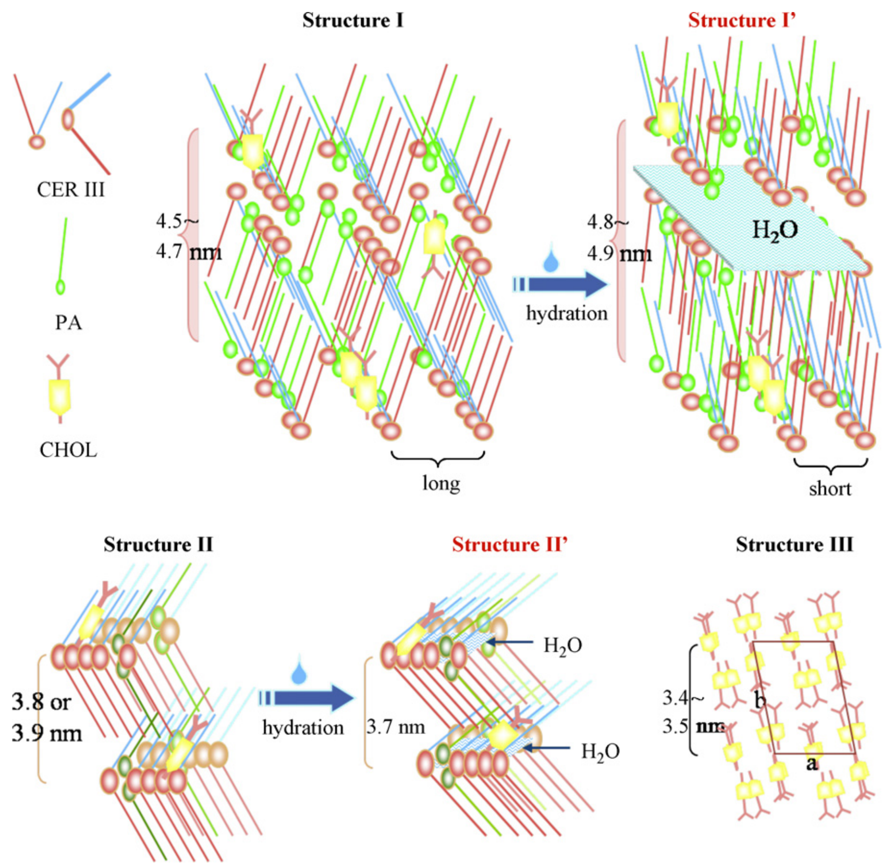

Fig. 7. Probable models of lamellar structures in lipid mixtures.

4. Conclusion

For all the expectation from cosmetic and houseware industries and dermatologists, the physicochemical investigation of native skin is still not easy and is in chaos because of the species and individual differences of skin peculiar to native materials. Especially, since SC in mammal skin plays a key role on physiology, that is, protection of living organism and prevention of its release, unified remarks on physiological characteristics of SC have been researched. The present investigation paid attention to manifest physiological properties of skin by using mimic SC consisting of mimetic lipid mixture. As a result, the association between lipid molecules was recognized and, most importantly, the suitable model SC as an alternative of the native one could be built up.

When Lipid mixtures consisting of ceramide III, palmitic acid, and cholesterol were treated at temperatures higher than 100 °C, the lipid mixtures displayed the similar thermal character to native human SC. The hydration appreciably affected the lamellar structure of the lipid mixtures: The vertical repeat distance of the lamellar structure expanded, but its lateral repeat distance slightly shortened. Moreover, the hydrophilic portion in the lamellar layer was structurally varied. It can be confirmed that hybrid phases were formed by the association between heterogeneous lipids in the lipid mixtures. Moreover, in the main phase of the lipid mixtures at the dry condition which were proposed on the basis of the obtained results, ceramide III takes a conformation with closed alkyl chains and mounts palmitic acid and cholesterol to be the lamellar orientation. After hydration, water penetrates mainly into the hydrophilic layer and extends the repeat distance of lamellar layer in the lipid mixtures. It is obvious that the clarification of structure of mimetic SC in the present work is valuable and derives correct insights on the investigation of reaction mechanism on the native skin.

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