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Thermochimica Acta 431 (2005) 94–97
thermochimica acta
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Thermal analysis of stratum corneum of hairless mouse with attention to phase transitions near 35 °C

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Received 14 October 2004; received in revised form 20 December 2004; accepted 22 January 2005
Available online 17 May 2005

Abstract

The high-resolution differential scanning calorimetric measurement, in which the baseline was stable, was performed in the stratum corneum of a hairless mouse with the water content of 25 wt%. Near 35 °C two successive phase transitions were found at 32 and 39 °C. The latter is consistent with the transition from the orthorhombic to the hexagonal structure in a region of the intercellular lipid matrix as obtained from the former X-ray diffraction and also the former electron diffraction. To the contrary so far the phase behavior of a region having the hexagonal structure at room temperature was unknown. Based upon the present differential scanning calorimetric measurement, we suggest that this region in the intercellular lipid matrix undergoes a transition from the hexagonal to the liquid structure at 32 °C. The reason why the structural phase transition at 32 °C has not been detected easily might be due to the fact that it lies close to the transition at 39 °C and furthermore the characteristic of the X-ray or the electron diffraction in which the diffraction peak in the liquid structure is broad and frequently undetectable near 35 °C. Finally, we propose that the two domains having the orthorhombic and the low-temperature hexagonal structures at room temperature undergo transitions to the high-temperature hexagonal structure at 39 °C and to the liquid structure at 35 °C, respectively. Besides the above evidence it was firstly obtained that the total transition enthalpy due to the intercellular lipid matrix is 13 J g^{-1} in the stratum corneum of a hairless mouse.
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Keywords: DSC; Hexagonal structure; Phase transition; Orthorhombic structure; Skin; Stratum corneum

1. Introduction

The outermost layer of a skin called stratum corneum (SC) is composed of keratinized cells called corneocytes and intercellular lipids. The flattened corneocytes are embedded in the intercellular lipid matrix. The intercellular lipid matrix in SC works as not only the main barrier but also the pathway of water, drug, etc. Then, the molecular structural study on the intercellular lipid assembly is essential to solve their molecular mechanism in the function of SC.

In the present study, we pay attention to the thermodynamical event of SC near 35 °C. This is particularly interesting since the body temperature of homeothermic animals lies in the temperature range. At 25 °C in the wide angle X-ray diffraction (WAXD) of hairless mouse SC there are two sharp peaks at 0.37 and 0.42 nm and a broad diffuse peak at about 0.46 nm. At 45 °C, the orthorhombic structure is no longer observable, whereas the hexagonal structure and the liquid structure where the hydrocarbon chains are disordered take place [1,2]. In the electron diffraction (ED) of the human SC prepared at room temperature, the diffraction spots appear predominantly at 0.37 and 0.41 nm for a region with the orthorhombic structure and also at 0.41 nm for a region with the hexagonal structure, while in that prepared at 32 °C the presence of the hexagonal structure is pronounced specially in the outer parts of the SC [3]. Then, with increasing the sample preparation temperature from 30 to 40 °C the transition from the orthorhombic to hexagonal structure is induced [3]. At present, it has been known that in the region with the or-

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0040-6031/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.tca.2005.01.048

thorhombic structure at room temperature the orthorhombic to hexagonal structural transition takes place at a temperature below 45 °C [4] and on the other hand, for the region with the hexagonal structure at room temperature the phase behavior is unknown. To clarify the phase behavior of the two regions appearing at room temperature, it is useful to employ differential scanning calorimetry (DSC), which is very sensitive in the detection of phase transitions.

The phase transitions of human SC have been studied by DSC [5–7]. In the SC hydrated to 50 wt% the DSC measurement has been performed in the temperature range of 30–120 °C [6]. There are three major transitions at 65, 80 and 95 °C and in addition a small peak, not present in all the samples examined, is seen near 35 °C [6]. Subsequently, at a variety of the hydration levels, the DSC measurement has been performed in the human SC [7]. In the measurement, it has been confirmed that the transition near 35 °C is widely present and cannot be attributed of sebum production [7]. The lower three transitions at 35, 65 and 80 °C are attributed to lipid-associated structural changes [5,6] and on the other hand, the transition at 95 °C is believed to reflect protein structural changes in the corneocyte [5]. These four transition temperatures are somewhat different among a variety of the measurements for the human SCs [5–7]. Furthermore, we have to pay attention to the fact that the above transition temperatures have been observed in the human SCs and they are thought to be a little different in the SCs of the other animals since generally the details of the lipids, the proteins, etc. depend on the SCs in animals. In the present study, we perform the DSC measurement for hairless mouse SC hydrated to 25 wt% with attention to the phase behavior near 35 °C in connection with the structural change associated with hydrocarbon chain packing.

2. Experimental

Hairless mice were used. The mice were taken care according to the International Guiding Principles for Biomedical Research Involving Animals published by The Council for International Organization of Medical Science. The SC was separated from a skin of a hairless mouse by digestion of 0.1% trypsin in a phosphate buffer solution. The SC was subsequently treated with distilled water and dried under vacuum by a rotary pump. The hydration for thus obtained dried samples was performed in the following way. First the dried samples were fully hydrated in immersing into pure water. Second these were left in a closed vessel for a few hours. Third under a stream of dry nitrogen gas the samples were dehydrated until reaching the desired hydration, where the water content was estimated from weighing the sample before and after hydration. The present SC samples were prepared at the hydration of 25 wt%. This hydration was chosen based upon the following reason. From the small angle X-ray diffraction (SAXD) of hairless mouse SC it has been found that by hydration the long lamellar repeat distance is unchanged, while the short lamellar repeat distance exhibits swelling, and furthermore, the both widths of the SAXD profiles for the long and short lamellar structures become narrow simultaneously around the water content of 20–30 wt% [8]. As a result, it has been proposed that owing to the interaction between the long and the short lamellar structure the water content in the SC might be regulated so as to maintain the water content at 20–30 wt% [8]. Then, to get evidence with special attention to the physiological condition it is better to carry out the DSC measurement for the SC with the hydration of 25% (w/w).

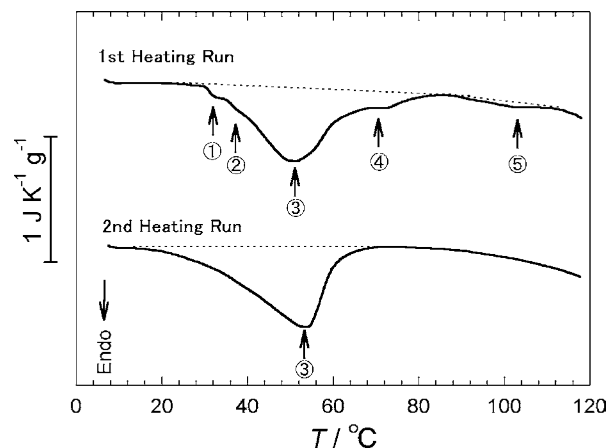

Fig. 1. Endothermic curves for the stratum corneum of a hairless mouse. The upper curve indicate the result for the first heating run and the lower for the second heating run. The dotted lines are the baseline. 'Endo' means endothermic. For the endothermic peaks denoted by circled numbers, see text.

The DSC measurement was performed in the temperature range of 5–120 °C using a DSC apparatus (Q1000, TA Instruments, DE, USA) [9]. The DSC cell was purged with dry nitrogen at 50 cm^3/min. A scanning rate was 10 °C/min. The sample mass was 10 mg. The sample was hermetically sealed into a 7.5 μl aluminum pan. This DSC apparatus has an advantage that, in addition to a first and a second thermocouples which are used to detect the temperatures at a

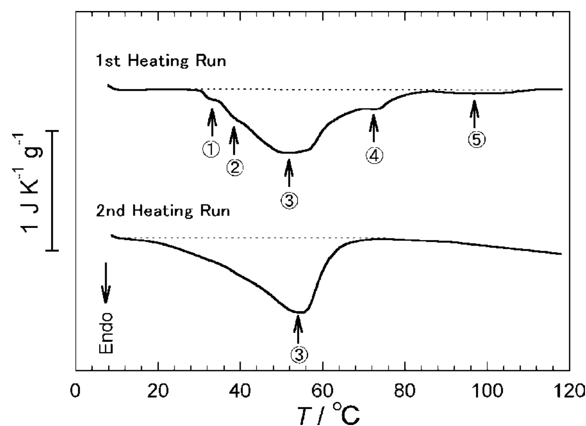

Fig. 2. Endothermic curves for the other stratum corneum of a hairless mouse, which are shown to exhibit the reproducibility. The other figure legend is the same as in Fig. 1.

sample and a reference position, respectively, as in a conventional DSC, by placing a third thermocouple between the sample and the reference positions it allows us to compensate the principal causes of poor baseline stability, such as imbalances in heat capacity, thermal resistance and heating rate between the sample and the reference positions. Therefore, we can detect subtle transitions from the endothermic curve beyond a relatively flat baseline which is shown by a dotted line in Figs. 1 and 2.

3. Results

Fig. 1 shows the endothermic curve for the hairless mouse SC. As seen in Fig. 1, in the first heating run endothermic peaks take place at ① 32 °C, ② 39 °C, ③ 51 °C, ④ 71 °C, ⑤ 103 °C and in the second heating run a broad single endothermic peak appears near 54 °C denoted by ③. In addition in the first heating run small shoulders seem to appear near 56 °C and at the other temperatures. To confirm the reproducibility of these endothermic peaks the DSC measurement was performed in the other hairless mouse SC. The result is shown in Fig. 2. The endothermic peaks take place almost in the same manner as in Fig. 1. Therefore, the reproducibility is satisfactory. It is worth to point out that in our present study we can observe two successive peaks not only at 32°C but also at 39 °C as seen in Figs. 1 and 2. This might be due to the fact that the resolution of the endothermic curve is significantly high in comparison with conventional DSC. From the analysis of Fig. 1 we obtained that in the first heating run the total transition enthalpy is 13.0 J g^{-1} for the range from 10 to 87 °C and 0.3 J g^{-1} for the range from 87 to 112 °C and in the second heating run 13.1 J g^{-1} for the range from 10 to 87 °C and not detected in the range from 87 to 112 °C. Such estimation is possible since the baseline is very stable. For the first heating run of Fig. 2, since the endothermic curve does not return to the baseline near 87 °C and then we cannot distinguish the transition enthalpies for the two temperature ranges. On the other hand, in consistent with the result of Fig. 1 in the second heating run we obtained that the total transition enthalpy is 13.4 J g^{-1} for the range from 10 to 87 °C and the endothermic peak is not detected for the range from 87 to 112 °C. This fact indicates that the reproducibility is well in a quantitative sense. As obtained from the analysis of Fig. 1 there is a good agreement in the transition enthalpies between the both heating run in the range from 10 to 87 °C, although the shape of the endothermic curve is considerably deformed by reheating.

4. Discussion

We are interested in the phase behavior of the regions composed of the hexagonal structure whose lattice constant is 0.42 nm and the orthorhombic structure whose lattice constants are 0.37 and 0.42 nm observed distinctively by the ED at room temperature [3]. Pilgram et al. have carried out the ED study on the human SC with attention to the phase transitions [3]. From the ED patterns recorded during increasing the temperature from room temperature to 90 °C, they have found that the intensity of the diffraction spot at 0.37 nm for the orthorhombic structure in the lipid packing starts to decrease at 30 °C and above 40 °C only the 0.42 nm reflection remains. This fact indicates that the orthorhombic to hexagonal transition takes place near 40 °C. This is consistent with the result obtained from the WAXD in the SC of various animals [2,4]. Then, the endothermic peak at 39 °C is thought to result from the orthorhombic to hexagonal transition.

The phase behavior of the hexagonal structure at room temperature is not clear yet. There are two possibilities to interpret the phase behavior. The one is that the hexagonal structure at room temperature merges into the hexagonal structure at 39 °C. The other is that the hexagonal structure at room temperature undergoes a phase transition to the liquid structure at a temperature below the transition temperature of 39 °C. If the former is the case, the hexagonal structure at room temperature is thermodynamically irrelevant. On the other hand, if the latter is the case, it is very likely that the phase transition at 32 °C is the hexagonal to liquid transition. This is compatible with the fact that the hexagonal structure partially undergoes a phase transition to the liquid structure at room temperature [1].

As a result, we propose that there are two domains in which the intercellular lipid assembly undergoes phase transitions distinctively. Then, the one domain undergoes the phase transition from the low-temperature hexagonal to the liquid structure at 32 °C and the other domain undergoes the phase transition from the orthorhombic to the high-temperature hexagonal structure at 39 °C. The phase behavior of the both domains is schematically shown in Fig. 3. For a further study, the detailed temperature dependence of the high-resolution WAXD is highly required. In connection with it in our preliminary experiment we observed the appearance of the liquid structure near 32 °C from the temperature dependence of the WAXD. This study is now in progress.

Based upon the DSC measurement of the human SC with the water content of about 40 wt%, Van Duzee [5] has pointed

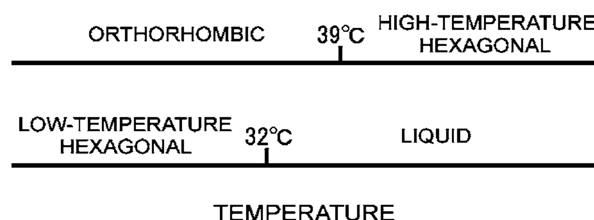

Fig. 3. Relation of the phase behaviors near 35 °C for the two domains. The upper one indicates that one domain undergoes a phase transition from the orthorhombic to the high-temperature hexagonal structure at 39 °C. The lower one indicates that the other domain undergoes a phase transition from the lower-temperature hexagonal to the liquid structure at 32 °C.

out that the transition at 85 °C (80 °C in Ref. [6]) is identified as the denaturation of α-keratin and furthermore the transition at 107 °C as the denaturation of a non-fibrous protein. From the DSC measurement of the human SC with the water content of about 30 wt% Golden et al. [6] have obtained the result that in the lipid-extracted human SC the endothermic peaks at 35, 65 and 80 °C disappear, while the endothermic peak at 95 °C remains and in reheating no endothermic peaks take place, that is, the transition at 95 °C is irreversible. Then, they have concluded that the transitions at 35, 65 and 80 °C appear to be lipid-related ones. Although the shape of the endothermic curves is different between the human and the hairless mouse SCs, it is of interest to compare them. In the hairless mouse SC the total transition enthalpy in the range between 10 and 87 °C is about 13 J g^{-1} in both the first and the second heating. This fact indicates that the transitions at 32, 39, 51 and 71 °C in the hairless mouse SC seems to be lipid-related ones. On the other hand, the transition at 103 °C in the hairless mouse SC is irreversible and then, seems to be protein-related one.

In the second heating run of the DSC almost a single endothermic peak appears. This is also similar to the behavior that takes place in the DSC for the human SC, in which the phase transition near 65 °C mainly remains in reheating [7]. We think that by such a heat treatment the sharp phase transitions become broad. Nevertheless, we can point out that the starting state lies near 10 °C and the ending state near 87 °C for the phase transitions concerned with the intercellular lipid matrix of the hairless mouse SC, since the total transition enthalpies between the two states do not change by reheating.

Lastly, the transitions at 51 and 71 °C and the other temperatures where a shoulder and a broad peak take place in endothermic curve will be discussed in the further study.

Acknowledgement

We thank Dr. Noboru Ohta of Japan Synchrotron Research Institute for the preparation of the samples of stratum corneum of hairless mouse and fruitful discussions.

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