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X-Ray diffraction study on ordered, disordered and reconstituted intercellular lipid lamellar structure in stratum corneum

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Abstract

From small angle X-ray diffraction for the stratum corneum of hairless mouse, it was obtained that in the normal stratum corneum, the 1st, 2nd and 3rd order diffraction peaks for the intercellular lipid lamellar structure appear at 13.8, 6.87 and 4.59 nm, respectively and also a broad hump for the 4th order reflection appears as observed by the previous researchers. In the damaged stratum corneum prepared by the treatment of sodium dodecyl sulfate, these small-angle diffraction peaks disappear and only the broad maxima remain around the 1st, 2nd and 3rd order diffraction peaks. These facts indicate that in the normal stratum the lamellar structure is ordered and in the damaged stratum corneum the lamellar structure is disordered. Furthermore, in the reconstituted lamellar structure obtained by immersing into the dilute suspension of the mixture of ceramide 3, cholesterol and stearic acid, the 1st, 2nd and 3rd order diffraction peaks reappear at 13.3, 6.67 and 4.44 nm, respectively. This fact indicates that the reorganization of the ordered lamellar structure takes place by adding the mixture to the damaged stratum corneum.

Keywords: Ceramide; Lamellar structure; Stratum corneum; X-Ray diffraction; Barrier recovery

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1. Introduction

Stratum corneum, constituting the outermost layer of the epidermis of skin, plays a key role of a major barrier to penetration of molecules through the skin. The stratum corneum consists of keratin-filled corneocytes that are embedded in intercellular lipid matrix arranged in a lamellar structure. The main transport of molecules takes place through the intercellular lipid lamellar and therefore, the barrier function of the skin is found in the intercellular lipid lamellar. The X-ray diffraction studies have been carried out in stratum corneum intensively. In the mouse stratum corneum, the lipid lamellar structure characterized by a repeat distance of approximately 13 nm has been observed in the small angle diffraction and furthermore, the peaks at approximately 0.38 and 0.42 nm, due to the chain packing of lipids, have been observed in the wide angle diffraction [1,2]. In this paper, using X-ray diffraction we will first show that the structure is different between the normal stratum corneum and the damaged stratum corneum and secondly that, when the damaged stratum corneum is immersed in the dilute aqueous suspension of the mixture of ceramide, cholesterol and fatty acid which are dominant components in the intercellular lipid matrix, the ordered lipid lamellar structure appearing in the normal stratum corneum is reconstituted.

2. Materials and methods

The stratum corneum samples were separated from the skin of hairless mouse by treating with trypsin. The damaged samples were prepared as follows. Irritating the mouse epidermis was caused by the closed patch method with 1 wt.% sodium dodecyl sulfate for 24 h. After removing the patch and leaving for 3 days, the damaged samples were separated from the skin. The pieces of the samples of approximately 5 mg were placed in a capillary tube with the diameter of 1 mm for the X-ray diffraction study.

To reorganize the ordered lamellar structure, the damaged sample was immersed at room temperature for 48 h in aqueous suspension of the mixture of ceramide 3, cholesterol and stearic acid, which was first resolved in chloroform to mix them, then dried and finally dispersed in water with the concentration of 0.05 wt.%. The sample was pulled out of the suspension and left in the air. The mixture is composed of ceramide 3 (Cosmoferm B.V., Delft, The Netherlands) produced by yeast cells, cholesterol (Sigma Chemical Co., St. Louis, MO, USA) and stearic acid (Sigma Chemical Co., St. Louis, MO, USA) with the molar ratio of 1:1:1. This ratio was chosen for the reason that the ratio is effective in the barrier recovery as revealed by the study of transepidermal water loss [3].

Synchrotron X-ray diffraction study was carried out at beam line 15 A of Photon Factory, Japan. All of the stratum corneum samples for the X-ray diffraction were used at room temperature without further dehydration.

3. Results

In the normal stratum corneum, we observed diffraction peaks at 13.8, 6.87 and 4.59 nm in the small angle region (see Fig. 1) and 0.42 and 0.38 nm in the wide angle region. The former is the peaks due to a lipid lamellar structure and the latter is due to hydrocarbon chain packing. These peaks coincide with the previously reported results for mouse stratum corneum [1,2]. On the other hand, in the damaged stratum corneum, only broad humps remain near the above diffraction peaks in the small angle region (see Fig. 1) and the diffraction peak intensities in the wide-angle region is reduced.

The damaged stratum corneum was immersed in the dilute suspension of the mixture in order to reorganize the ordered lamellar structure, where the fine particles of the mixture whose surface might have hydrophorbic nature were suspended in water so as not to cause aggregation and sedimentation. In Fig. 1 the intensities was normalized so as to coincide the backgrounds with each other at 0.35 nm^{-1}.

In the reconstituted lamellar structure, the diffraction peaks appear clearly at 13.3, 6.67, 4.44

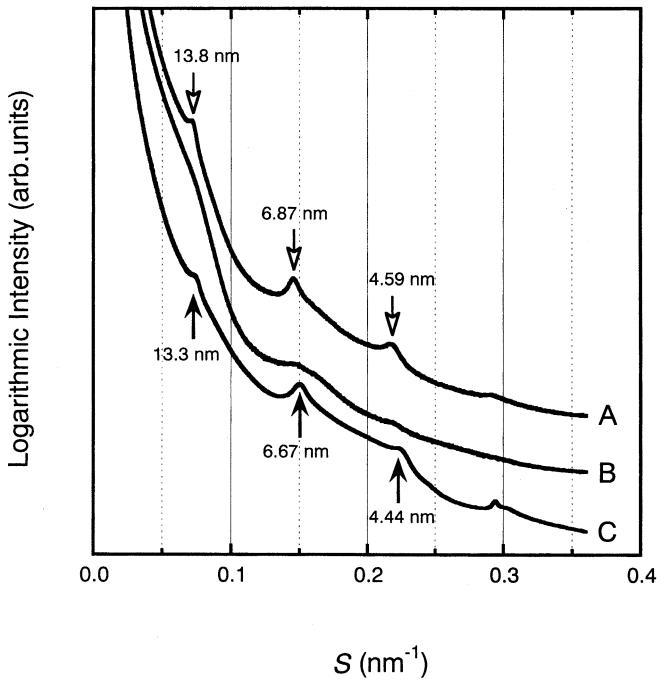

Fig. 1. Small angle X-ray diffraction of the stratum corneum of hairless mouse in the normal stratum corneum (a) in the damaged stratum corneum (b) and in the restored stratum corneum (c). So as to see easily, the above curves are shifted down successively.

and 3.33 nm (see Fig. 1) and additionally 3.39 nm that corresponds to the diffraction for cholesterol. It is surprising that the lamellar diffraction peaks in the restored stratum corneum coincide with those in the normal stratum corneum. However, it should be noted that the dilute suspension itself did not show any diffraction peaks and furthermore, even in the concentrated suspension the diffraction peaks do not appear at approximately 13 nm and its higher order peaks. In the reconstituted lamellar structure it is plausible that the slight difference of the lamellar diffraction between the normal and the restored stratum corneum is due to the difference of the components constituting lamellar lipid matrix.

4. Discussion

The studies on the in vitro reconstituted lamellar structure of the mixtures of ceramides 1 to 6 isolated from mammalian stratum corneum, cholesterol and fatty acid have been performed intensively [4–6]. In these mixtures including ceramides 1 and 2 and cholesterol with the molar ratios of cholesterol/ceramides of 1 and 2, the lamellar diffraction near 12 nm observed in vivo is reproduced. The lamellar diffraction of approximately 12 nm corresponds to that of approximately 13 nm in mouse stratum corneum. On the other hand, the mixture of bovine brain ceramide 3, cholesterol and fatty acid does not yield the diffraction near 13 nm [4]. When we measured the X-ray diffraction of the concentrated suspension of the mixture of ceramide 3, cholesterol and stearic acid, the diffraction peaks appear only in the higher angle ($S > 0.21\ \mathrm{nm}^{-1}$). As a result, the smaller angle diffraction peaks approximately 13 nm are not due to those of the diffractions for the suspension of the mixture of ceramide 3, cholesterol and stearic acid.

By immersing the damaged stratum corneum in

the solution of the mixture of ceramide 3, cholesterol and stearic acid the peak at 13.3 nm and the higher order peaks appear again (Fig. 1). The behavior indicates that the disruption and the successive restoration of the lipid lamellar structure happen in the stratum corneum. On the other hand, in the wide-angle diffraction the peak intensities at 0.38 and 0.42 nm decrease markedly in the damaged stratum corneum. However, their widths are almost unchanged. These facts indicate that, although the lamellar structure is disrupted, the nucleus of the neatly packed lipid structure partly remains even in the damaged stratum corneum. By immersing in the dilute solution of the mixture, the introduction of lipids from the mixture into the lipid lamellar structure is induced and then, the ordered lipid lamellar structure is reconstituted. This behavior can be explained as follows. In the damaged stratum corneum, the lamellar structure of lipid matrix is disordered and/or deficient and therefore, the hydrophobic part is partly exposed in water. Under such a condition, the small particles of the mixture having hydrophobic nature seem to be taken into preferentially. Until the damaged stratum corneum is reorganized sufficiently, the lipids seem to be supplied. To consider the mechanism further, it is necessary to study the effects of the time interval of the immersion in the solution.

For the components of the lipids, Bouwstra et al. [5] have pointed out that ceramide 1 plays a crucial role in the in vitro formation of the 12–13-nm lamellar structure in the ceramide/cholesterol mixtures and furthermore, without ceramide 1, only a weak diffraction peak appears approximately 12 nm. Nevertheless, in the present study by adding only ceramide 3, together with cholesterol and fatty acid to the damaged stratum corneum the diffraction peak at 13 nm reappeared. In the present restoration, it is not known whether ceramide 1 molecules remaining in the damaged stratum corneum trigger in forming the ordered lamellar structure. Otherwise, it might be the case that even in the in vitro system without ceramide 1, a weak but distinct peak appears approximately 12 nm and it might result in the 12-nm lamellar-structural formation in the restored stratum corneum. In the further investigation it is of importance to perform the X-ray diffraction study in various kinds of ceramides.

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