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Morphological Behavior of Mixed Skin Lipids

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The phase behavior of ceramides IV, the rigid palmitic acid, the fluid oleic acid and the fluid α-methyl palmitic acid in pure and mixed monolayers at the air/water interface is reported. Like natural oleic acid the synthesized α-methyl palmitic acid forms fluid-expanded films, but they are stable with time. All described mixtures were transferred to hydrophilic surface of a Si-wafer as z-type Langmuir-Blodgett monolayers. The surface morphology of these layers is characterized by atomic force microscopy (AFM). It could be shown that addition of all three fatty acids to ceramides IV changes the morphological properties of the film.

Keywords: monolayer; skin lipids; AFM; ceramides; fatty acids

INTODUCTION

The stratum corneum (SC) as outermost layer of the skin is considered as main barrier for penetration [1,2]. It is build up of corneocytes and of an intercellular layer of lipids forming a highly ordered structure of lipids [1,4]. The interest on stratum corneum lipids and corneocytes has been increased in recent years [3-6].

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For the study of phase behavior and transport properties simple models of multi-component SC lipids are used: bulk system [3,4], monolayer at the air/water interface and Langmuir-Blodgett films (LB) [4,5].

In the present work different binary mixtures between the ceramides IV and other SC lipids (palmitic acid and oleic acid) as well as the synthetic fluid α-methyl palmitic acid are studied as monolayers by Π (surface pressure) /A (molecular area) isothermes and by fluorescence microscopy. On the other hand, the LB films of the spread mixtures were characterized by AFM.

MATERIALS AND METHODS

Ceramides IV, palmitic acid, oleic acid and NBD-PC as fluorescence dye were obtained from Sigma (Germany) and used as received. The α-methyl palmitic acid was synthesized in our department of Pharmazeutical Chemistry. Monolayer investigations were carried out by a commercial film balance using the Wilhelmy system (Riegler & Kirstein, Mainz, Germany) with a fluorescence microscope (Olympus Optical Co. GmbH, Germany) for the visualization of the lateral structure. All experiments were carried out on a pure water subphase (Milli Q quality) at 20 °C. The lipids were solved in the applied concentrations in chloroform/methanol (9:1) before spreading. After spreading the lipid solution were equilibrated for 20 min to get stable reproducible films at the water surface. Then, the film was compressed with a compression speed of 0.15 cm^2/s (film balance area 400 cm^2). The films were transferred as z-type LB-films at different surface pressures to hydrophilic Si-wafers in order to characterize the lateral structure with the AFM technique. The transfer speed was about 1 mm/min. AFM measurements were performed with a Nanoscope IIIa microscope (Digital Instruments Inc., USA) under ambient conditions.

RESULTS AND DISCUSSION

Air/Water Interface

Palmitic acid and the ceramides IV form liquid condensed films on the air/water interface whereas oleic acid and α-methyl palmitic acid show a fluid-expanded film. The fluorescence micrographs of ceramides IV monolayer reveal a structure which resembles to clods that incorporate evidently the dye (Fig. 3A). The fluorescence micrograph of palmitic acid shows round shaped dark domains [7]. Oleic acid and α-methyl palmitic acid form a fluid-expanded film which is miscible with the dye. Therefore the fluorescence micrograph of the pure films are uniformly bright [7].

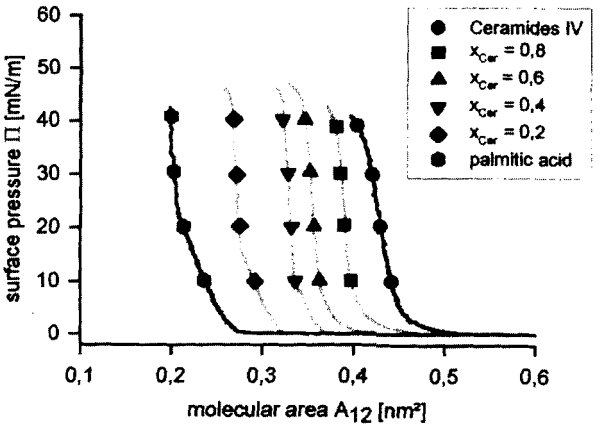

FIGURE 1 Isothermes of different mixtures of the system ceramides IV / palmitic acid; T = 298 K; subphase: water.

Addition of small amounts of palmitic acid to the ceramides IV results in a more steep and incompressible film (Figure 1). The same effect was detected in the phase behavior of mixed ceramides IV / stearic acid monolayers [6].

A quite different behavior was detected in the binary system between ceramides IV and oleic acid (Fig. 2). These films should be discussed briefly. Addition of 20 mol% of the acid to ceramides IV results in a film with a collapse pressure of about 38 mN/m (pure ceramides IV 32 mN/m).

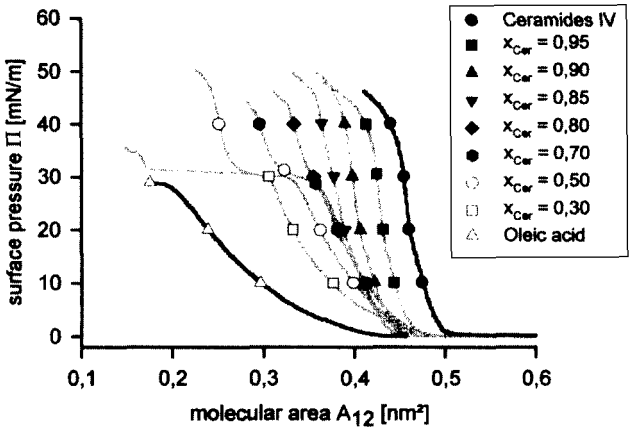

FIGURE 2 Isothermes of different mixtures of the system ceramides IV / oleic acid; T = 298 K; subphase: water.

The change of the compressibility near 28 mN/m indicates that the collapse of the oleic acid begins. This effect becomes more significant at concentration higher than 30 mol% oleic acid and results in the plateau of about 31 mN/m. The behavior of obtained isotherms of the mixture with α-methyl palmitic acid can be compared with the described system in Figure 2.

The lateral morphology of a monofilm consisting of the ceramides IV and 20 mol% palmitic acid is visualized by epifluorescence microscopy.

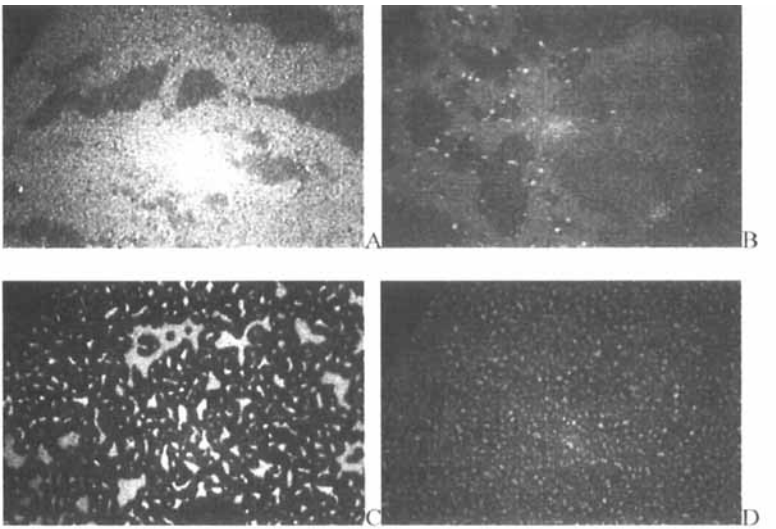

FIGURE 3 Fluorescence micrographs of **(A)** pure ceramides IV; **(B)** 80 mol% ceramides IV and 20 mol% palmitic acid; **(C)** 80 mol% ceramides IV and 20 mol% oleic acid; **(D)** 80 mol% ceramides IV and 20 mol% / α-methyl palmitic acid; surface pressure Π = 10 mN/m, T = 298 K, subphase: water.

The typical clod-structure of the ceramides IV is observed (Fig. 3A). It is obvious from Figure 3B that addition of palmitic acid results in a macroscopic phase separation. There palmitic acid is localized in dark domains which are surrounded by the ceramides IV clodes. The fluorescence dye is concentrated at the border between the two separated liquid condensed phases. Addition of the fluid expanded oleic acid (20 mo%) to the ceramides IV leads to a destruction of the typical clods (Fig. 3C). A crystalline network-like structure

and fluid areas are observed. This micrograph demonstrates a phase separation between the fluid oleic acid (bright) and the liquid condensed ceramides IV (dark).

Addition of α-methyl palmitic acid to the ceramides IV results in a homogenous distribution of small fluid areas in the ceramides IV film (Fig. 3D). This effect can be discussed as an insertion of the acid into the defect structure of the ceramides IV film or in a particular miscibility.

Langmuir-Blodgett layers

The surface of the ceramides IV layer appears very smooth, nevertheless many small defects (pin holes) can be seen (Fig. 4A). The morphology of the transferred mixed film containing 20 mol% palmitic acid is very even (Fig. 4B). The number of pin holes in the monolayer becomes strongly reduced in comparison with pure ceramides IV in Figure 4A. In the system of the ceramides IV and palmitic acid a macroscopic demixing phenomena in the scale of 100 μm can be observed.

However, by high resolution AFM small phase separated areas can be detected (Fig. 4B). This may be due to the insertion or the covering of small defects with palmitic acid molecules.

In mixtures with the oleic acid a fluidization of the films and a high tendency of phase separation at air/water interface as well as at solid surface can be detected (Fig. 4C). The small dark areas are softer and 0.7 nm deeper than the surrounding. Therefore, these areas are assumed to be formed by the phase separated oleic acid. The same effect is observed in a mixture of ceramides IV with α-methyl palmitic acid (Fig. 4D). But there can be observed the tendency of phase separation in mixtures above 40 mol% of α-methyl palmitic acid.

These results demonstrate that the different molecular structure of the fatty acids have a great influence of the phase behavior and the lateral structure of a ceramides IV film.

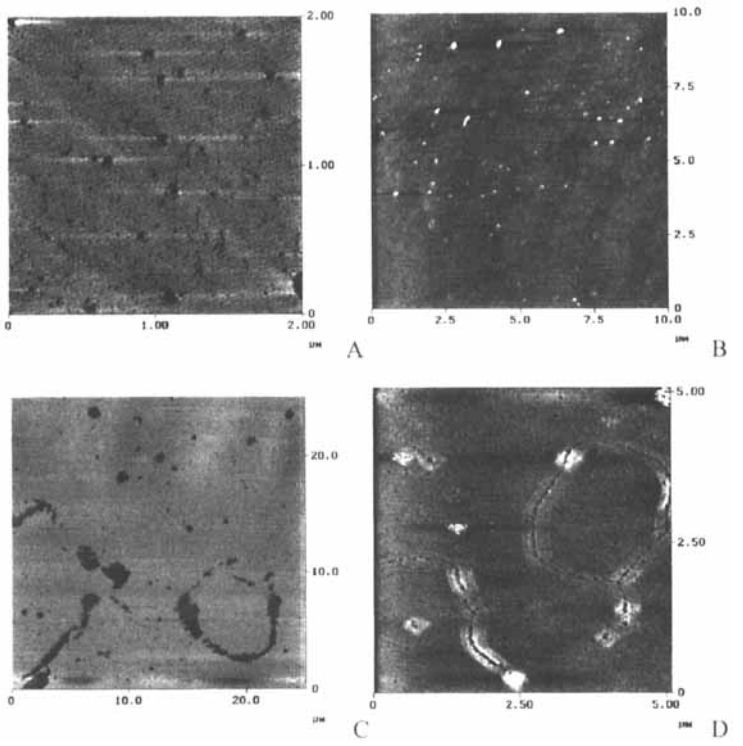

FIGURE 4 AFM images of **(A)** pure ceramides IV; **(B)** mixed monolayers of 80 mol% ceramides IV and and 20 mol% palmitic acid; **(C)** 80 mol% ceramides IV and 20 mol% oleic acid; **(D)** 50 mol% ceramides and 50 mol% α-methyl palmitic acid; pressure of transfer Π = 25 mN/m.
(See color plate VII at the back of this issue)

Acknowledgments

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