The Influence of Calcium Ions and 2-(alkoxy)1-((alkoyloxy)methyl)-ethyl-7-(4-heptyl-5,6)-dicarboxy-2-cyclohexene-1-yl) Heptanoate on a Simplified Model of the Stratum Corneum Lipids

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Addition of calcium ions to a lamellar liquid crystal of sodium oleate/oleic acid/water led to crystallization of calcium soap as detected by optical microscopy. Addition of the acid of the title (the complex acid, ca) reduced the crystallization because of the calcium ions' preferential bonding to the complex acid.

The top layer of the skin, the stratum corneum, fills an important role (1). It prevents dehydration of the body as well as excessive water uptake when immersed in water as well as serving as a defense against chemical and biological attack. This protective action is concentrated in the lipid part of stratum corneum, which constitutes approximately 10% of the nonaqueous stratum corneum. With this background it is to be expected that the composition of the lipids as well as their organization has been the focus of an intense research effort.

The composition of the lipids has been determined by thin layer chromatography (TLC) (2-5) and, although the opinion about the exact composition has varied (6), there is general agreement that fatty acids, cholesterol and ceramides are essential ingredients. The structure has convincingly been shown to be layered (7,8), with the polar groups of the lipids arranged back to back. Our group has pointed out the similarity to liquid crystals found in fatty acid/soap/water combinations (9) and built a model of the lipids on this structure (10).

Downing and collaborators have emphasized a potential role for calcium ions in transforming the spherical lipid bi-layers of basal cellular entities to the parallel multilayered structures in stratum corneum (11). This is an interesting suggestion, and we found an investigation into the role of calcium ions for the layered structure appealing against the liquid crystal/crystal transition in layered structures of this kind. This transition is essential because a crystalline arrangement of the lipids in the skin would in all probability enhance the transdermal water transport. As a matter of fact (12) crystalline hydrocarbon chain layers are effective in reducing water transport only when dislocation-free.

In this publication we analyze the influence of calcium ions on a fatty acid/soap/water liquid crystal as a partial model of stratum corneum and combine it with the fatty acid of the title to find its influence on the lipid structure.

EXPERIMENTAL

Materials. Oleic acid (99%) was Sigma Chemical Co., $Ca(OH)_2$ (99%) Fisher Scientific and $CaCl_2$ (analytical reagent) Mallinckrodt. The complex acid was provided and purified according to earlier reports (9). Calcium oleate was prepared by combining an aqueous solution of sodium oleate with an aqueous solution of $CaCl_2$. The precipitate obtained was washed twice with doubly distilled water. Sodium oleate was formed by reacting oleic acid with the stoichiometric amount of sodium ethoxide in ethanol and recrystallized twice from ethanol. Doubly distilled water was used in all sample preparations.

Preparation of samples. A liquid crystalline host was prepared by neutralization of oleic acid with sodium hydroxide to yield a 41:59 weight ratio of sodium oleate/oleic acid with a water content of 32% by weight. The effect of calcium on the sodium oleate/oleic acid mixture was studied by adding varying amounts of $Ca(OH)_2$ to the sodium hydroxide solution used in neutralization. Total calcium content ranged from 0 to 25 mol percent of the sodium oleate/oleic acid mixture. The water content remained constant at 32% for each sample.

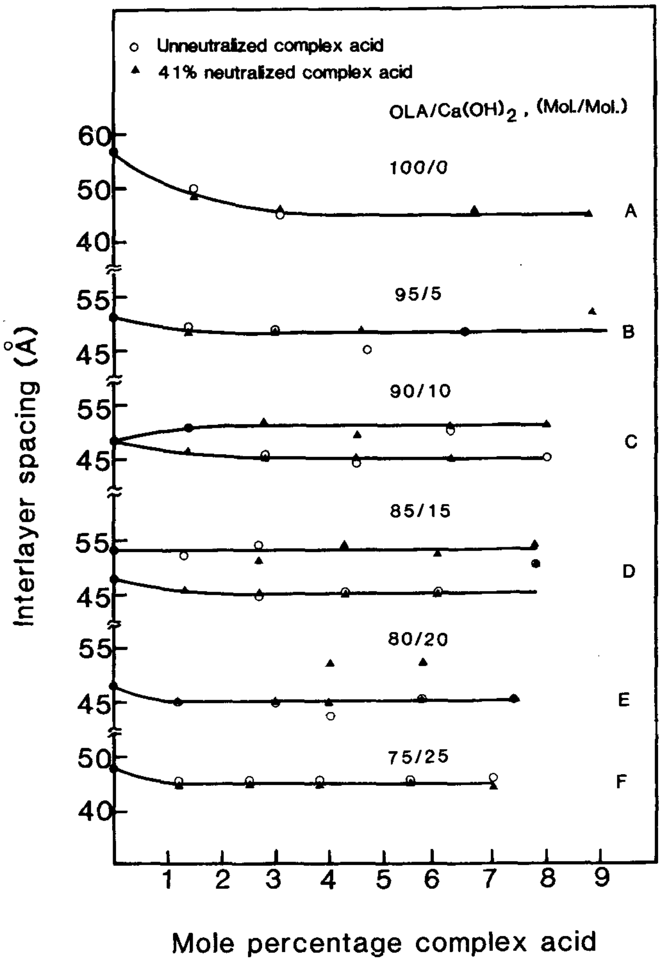

FIG. 1. The interlayer spacing versus complex acid added to a mixture containing oleic acid:calcium hydroxide in a different mole ratio. The mole percentage complex acid is counted on total lipids. The mole ratio of oleic acid to calcium hydroxide [OLA/$CA(OH)_2$] is given on the curves.

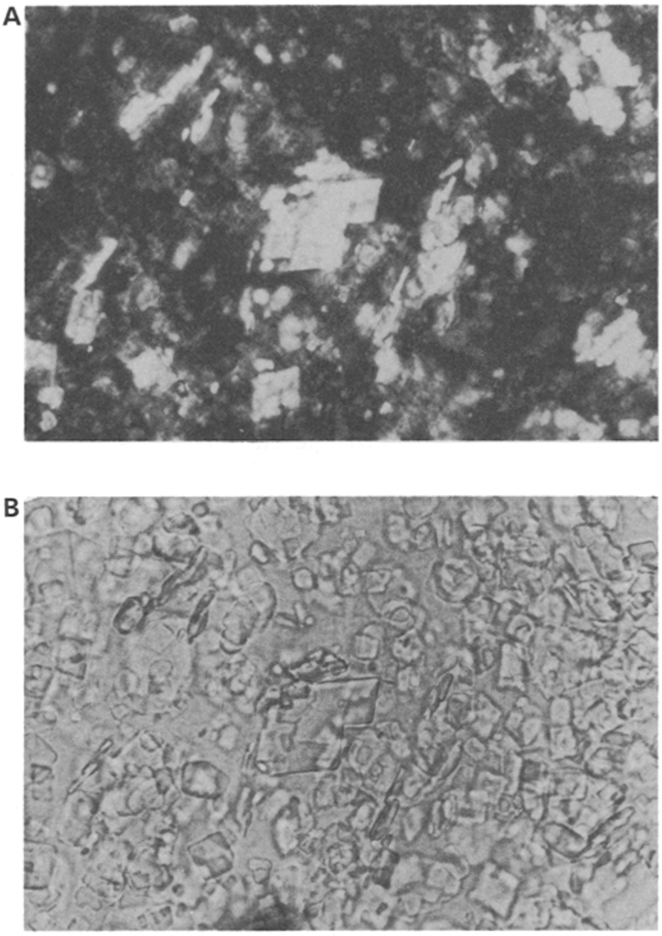

FIG. 2. Microphotograph of the liquid crystal with added calcium hydroxide. Mole percentages are oleic acid and oleate ion, 90%; calcium is 10%. A, Polarized light; B, nonpolarized light.

The complex acid was added to each of the sodium oleate/oleic acid/calcium hydroxide samples. In the first series the complex acid was added to the samples unneutralized as a free acid. In the second series it was added along with enough sodium hydroxide to neutralize it to 41% by weight.

Analysis methods. Optical microscopy was performed on an Olympus-BH polarizing microscope, attached to an automatic exposure Olympus camera (Model (C-35A) for microphotography. Samples were sheared between a slide and a cover glass to a thickness of ca. 5–10 microns and allowed to equilibrate before being observed between crossed polarizers. All microphotographs were taken at a total magnification of 400X.

Small angle x-ray diffraction measurements were obtained by use of a Kiessig low-angle camera from Richard Seifert. Nickel filtered copper radiation was used, and the reflection was determined by a Tennelec position sensitive detector system (Model PSD-1100).

RESULTS

Low angle x-ray results for the sodium oleate/oleic acid/calcium hydroxide mixtures with the unneutralized and 41% neutralized complex acid are shown in Figure 1. It should be noted that in all mixtures the oleic acid is 41% neutralized. Addition of the complex acid to the sodium oleate/oleic acid host resulted in a decrease in interlayer spacing as seen in Figure 1A. The reduction of interlayer spacing that is observed is an agreement with previous investigations being due to a disordering of the liquid crystalline structure caused by the complex acid molecule (9). Figure 1A shows a

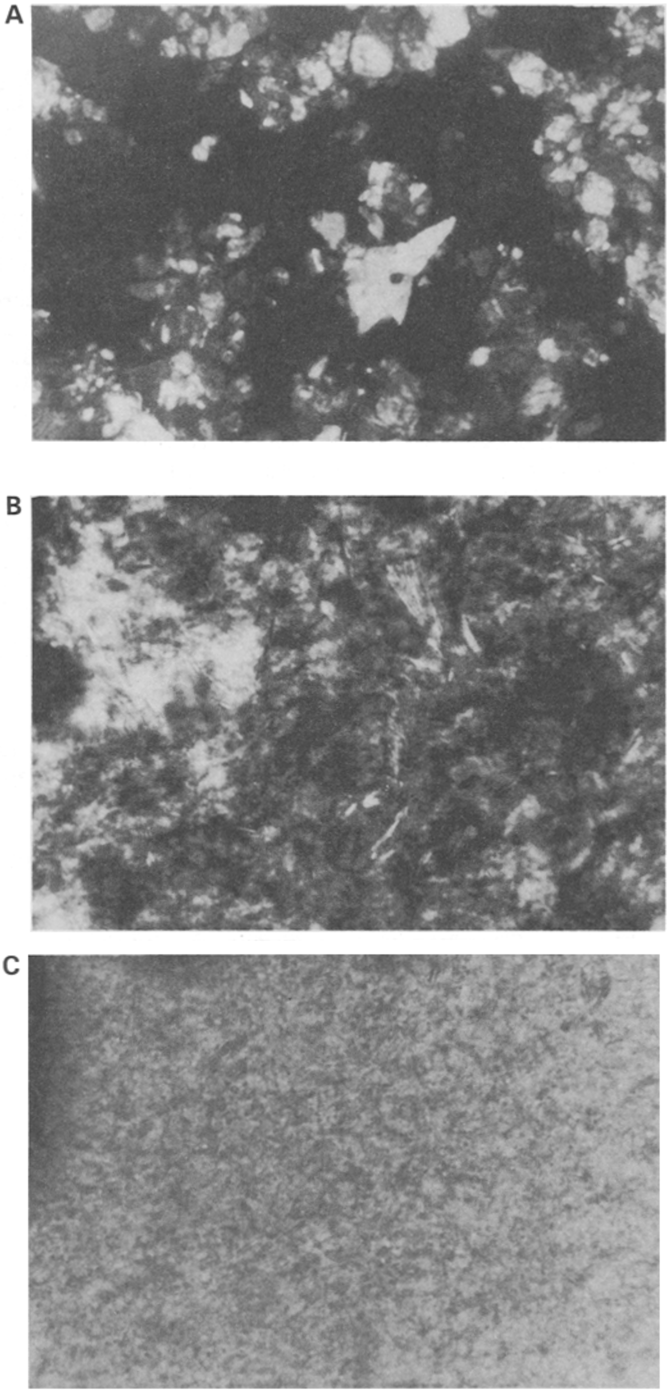

FIG. 3. Microphotograph of the liquid crystal with added calcium hydroxide and complex acid. Mole percentages are oleic acid and oleate ion, 82.5%; calcium, 9.4%; complex acid, 8.1%. A, Polarized light, nonneutralized complex acid; B, polarized light, 41% neutralized complex acid; C, nonpolarized light, 41% neutralized complex acid.

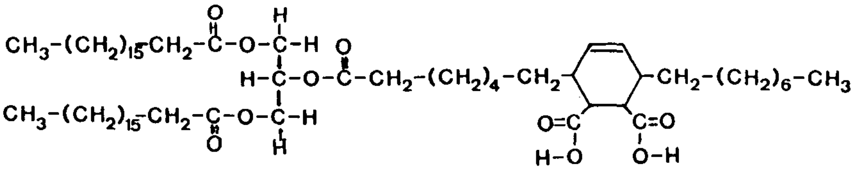

FIG. 4. The structure of the complex acid.

loss of liquid crystalline structure at about 5 mol percent of the added acid. Neutralization of 41% of the added complex acid maintains the liquid crystalline structure, Figure 1A, indicating the pH as a key factor in bilayer formation and maintenance.

An increase of the calcium hydroxide content to 10 and 15 mol percent caused the x-ray diffraction patterns of the two mixtures to split into two distinct peaks (Fig. 1C,D). The 45-Å spacing is attributed to the calcium oleate precipitating out of the mixtures; pure calcium oleate gave an interlayer spacing of 44.65 Å. The higher interlayer spacings of approximately 52-53 Å are from the liquid crystalline structure that is maintained with the 41% neutralization of the added acid.

With the addition of 20 mol percent calcium hydroxide, a higher percentage of added neutralized acid is required to obtain a liquid crystalline structure (Fig. 1E). At the highest calcium hydroxide concentration investigated, 25 mol percent, the only interlayer spacings obtained for the mixtures are from the precipitated calcium oleate, indicating that there is a limiting concentration of calcium hydroxide above which a liquid crystalline structure cannot be maintained even at high concentrations of added acid.

The microscope photographs (Figs. 2 and 3) show the crystallization of calcium oleate polarized light (Fig. 2A) and nonpolarized light (Fig. 2B). The crystals are large and easily recognized. Addition of nonneutralized complex acid (Fig. 3A) led to phase separation. The crystals were now suspended in an isotropic liquid (the blank background in the photograph, Fig. 3A). Addition of partially neutralized complex acid (Figs. 3B-3C) gave a system in which the optical pattern of the liquid crystal is still retained and the crystals present are not well defined. They cannot be recognized in nonpolarized light (Fig. 3C).

DISCUSSION

The results have a bearing on several phenomena in relation to the stratum corneum lipid structure.

At first the results show the change caused by the calcium ions. They react with free carboxylic acid forming calcium oleate crystals. These crystals are a conspicuous feature in Figures 2A and 2B. It is interesting to compare these results with those of Downing et al. (11) using electron microscopy of lipid mixtures combined with calcium ions. The interlayer spacings in our results are similar to those of Downing et al., and it appears reasonable to assume that the layered structure observed earlier (11) is in fact a crystalline calcium soap.

The results also gave information about the interaction of the complex acid and the oleic acid/oleate liquid crystalline structure. In the first instance the addition of the acid caused a reduction of interlayer spacing.

This influence on the liquid crystalline structure may be understood considering the structure of the added acid (Fig. 4). The structure of the acid is extremely bulky and would be expected to cause considerable disorder in the lamellar liquid crystalline structure. Such a disordering influence has been demonstrated earlier for an acid with similar structure (12).

Addition of the acid without prior neutralization led to complete destabilization of the liquid crystalline structure at comparatively low amounts. Figure 1A shows addition in excess of 3 mol percent counted on the oleic acid/sodium oleate to be sufficient to destabilize the structure. The liquid crystalline structure did not exist at concentrations in excess of this value. On the other hand, with addition of the partially neutralized acid the liquid crystalline structure was retained to the highest concentration of the added acid. The fact that the nonneutralized acid caused a destabilization of the liquid crystalline phase at lower concentrations than the partially neutralized one (Fig. 1A) is not connected with disordering of the liquid crystal. The reduction of interlayer spacing, which is a measure of the disordering, was identical for the neutralized and nonneutralized acid (Fig. 1A), proving this point. The destabilization is instead founded on a different and more general phenomenon which is independent of ordering. The mechanism is best illustrated by an example in which the individual components have no disordering action. The stability of lamellar liquid crystals in systems of water, soap and fatty acid is an illustrative point. It depends critically on the acid/soap ratio.

The lamellar liquid crystal in the system water, octanoic acid and sodium octanoate is stable only in the acid/(soap and acid) weight fraction range 0.42–0.64 at 40% water (13). The decisive factor for the stability is the balance between the ionic surfactant with its requirement of a comparatively large cross sectional area of its polar group and the hydrophobic amphiphile; a carboxylic acid, a long chain alcohol, etc., requiring only a small corresponding area. Hence, according to Israelachvili, Mitchell and Ninham (14), an excess of ionic surfactant with its huge cross sectional area of the polar group results in spherical or cylindrical association colloids, while an excess of the hydrophobic amphiphile gives inverse micelles. In the intermediate range a lamellar phase is formed.

With this factor in mind the destabilization of the lamellar structure by the nonneutralized acid (Fig. 1A) is a reasonable and expected phenomenon. The nonneutralized acid has a small polar group and a hydrophobic part bulky in extremum; hence its destabilizing action is pronounced according to the geometrical consideration of Israelachvili, Mitchell and Ninham (14). Its addition should move the composition efficiently toward the hydrophobic solution in such diagrams and result in a two-phase region liquid crystal plus isotropic solution. In this context the feature on Figure 3A is interesting. It is obvious that the anisotropic particles are suspended in an isotropic liquid, in accordance with the reasoning about a phase transition to the isotropic acid solution found in similar systems (13).

In addition to its influence on the original structure, the complex acid also influenced the crystallization of calcium oleate. A comparison of Figures 2 and 3 clearly demonstrates the fact that the huge crystals observed in the model without the complex acid were not found with the acid present. There are several factors to account for this influence.

The many carboxylic groups on the complex acid influence the formation of calcium oleate in two ways. In the first instance a preferential bonding of calcium to the complex acid leaves the oleate molecules unreacted; second, the multiple carboxylic groups of the complex acid will give chelation of the calcium ion, preventing a regular formation of the layered structures in the calcium oleate.

In addition, the structure of the acid virtually prevents the formation of a well defined crystalline pattern with oleic acid. Hence, even if the oleic acid should react with calcium ions, the presence of the complex acid in the structure prevents the formation of the huge crystals observed with the oleates alone.

In this context the importance of the fact that the crystallization takes place from a layered liquid crystalline structure should be emphasized. The difference of the crystallization from a solution (15–17) is significant. In a solution the rule is a selective build up of homogeneous crystals because the crystallization allows discrimination of building elements due to the rapid exchange of surface atoms or molecules with the solution. This is not the case in a lamellar liquid crystal. In such a structure the elements for crystallization are already present in layers and the exchange of elements is limited to within each layer. The translational diffusion within each layer is rapid (18–20), but the transport perpendicular to the layer is slow.

Hence, crystallization is in principle restricted to the amphiphiles present within each layer. With a layer structure in which oleic acid and a complex acid like the one in Figure 4 are statistically mixed, the necessary segregation to form homogeneous calcium oleate crystals is restricted to one layer. The consequence of this restriction is immediately evident: the formation of well defined multilayer crystals is virtually prevented.

Hence, the absence of huge, well-defined crystals after addition of the complex acid is an expected result. On the other hand, the optical patterns (Figs. 3B-3C) should not be interpreted as the absence of crystals; they and the x-ray results (Figs. 1) show only that huge, well-defined crystals are not formed.

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