Lubricant and Bactericidal Properties of Calcium Salts of Fatty Acids: Effect of Degree of Unsaturation

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Abstract: Fatty acids containing a C18 alkyl chain such as stearic acid (C18:0 fatty acid), oleic acid (C18:1 fatty acid) and linoleic acid (C18:2 fatty acid) are common emulsifiers in skin-care products and cosmetics and are also used in skin cleansers. In this study, we prepared calcium salts (Ca salts) of the above fatty acids to determine the effect of the degree of unsaturation of the alkyl chain. Scanning electron microscopy images and X-ray diffraction patterns show that C18:0 and C18:1 fatty acid Ca salts are plate-shaped, lamellar-crystalline powder, while C18:2 fatty acid Ca salt is amorphous powder. Therefore, C18:2 fatty acid Ca salt exhibits a lower lubrication ability than do C18:0 and C18:1 fatty acid Ca salts. In addition, the bactericidal ability against Staphylococcus aureus, Staphylococcus epidermidis and Propionibacterium acnes improved with increasing degree of unsaturation. These findings suggest that Ca salts of unsaturated fatty acids have potential applications in cleansing and cosmetic products.

Key words: calcium salts of fatty acids, degree of unsaturation, crystal structure, friction property, bactericidal ability

1 INTRODUCTION

The Ca salts of surfactant molecules are useful materials for cosmetics. For example, C18:0 fatty acid Ca salt is used as a surface treatment agent in cosmetics as it exhibits a high lubrication ability. Some amino acid metal salts having lamellar crystal structures exhibit high lubrication abilities and hydrophobicity and are used as extender pigments in cosmetics. Some researchers have pointed out that the degree of unsaturation of fatty acid molecules is an important factor in their physical properties and physiological activities. Sahoo et al. showed that lubricants containing C18:2 fatty acids exhibited higher lubrication abilities on steel surfaces than did C18:0 fatty acids. In addition, unsaturated C16:1 and C18:2 fatty acids have strong bactericidal abilities against Staphylococcus aureus. However, there are few reports on the physiological activities of Ca salts of unsaturated fatty acids. Recently, we showed that C16:1 fatty acid Ca salt exhibits a high lubrication ability as well as selective bactericidal activity. In this study, we investigated the crystal structure, lubricity, and bactericidal properties of Ca salts of C18:0, C18:1, and C18:2 fatty acids to determine the effect of the degree of unsaturation. The bactericidal activities of Ca salts of fatty acids against Staphylococcus aureus, Staphylococcus epidermidis and Propionibacterium acnes were evaluated as these bacteria are associated with skin damage and acne.
(5.0 g, 50wt%) were added to the C18:0 or C18:1 fatty acid aqueous ethanol solution. The turbid solution was filtered to obtain a white precipitate. The precipitate was washed with water and acetone to remove unreacted fatty acid and calcium chloride and was subsequently dried under reduced pressure. If the powder is assumed to be a monohydrate containing two fatty acid molecules and a calcium ion, the experimental values from elemental analysis are consistent with the calculated values. The melting point and elemental composition of C18:0 fatty acid Ca salt were, respectively, 148.2–157.8°C and C, 69.17%; H, 11.74%; and Ca, 6.31%, as found by analysis. The composition of Ca(CH(CH3)18)2·3H2O was C, 69.12%; H, 11.52%; O, 12.80%; and Ca, 6.45%.

2.2.2 Preparation of C18:2 fatty acid Ca salt

The C18:2 fatty acid (0.01 mol) in the liquid state was mixed with aqueous acetone (100 mL, 50wt%). Sodium hydroxide (0.01 mol) and calcium chloride dihydrate (0.0055 mol) in aqueous acetone (5.0 g, 50wt%) were added to the C18:2 fatty acid aqueous acetone solution. The turbid solution was filtered to obtain a white precipitate. The precipitate was washed with water and acetone to remove unreacted C18:2 fatty acid and calcium chloride and was subsequently dried under reduced pressure. If the powder is assumed to be a dihydrate containing two fatty acid molecules and a calcium ion, then the experimental values from elemental analysis are consistent with the calculated values. The melting point and elemental composition were, respectively, 78–90°C and C, 68.09%; H, 10.05%; and Ca, 6.49%, as found by analysis. For Ca(CH(CH3)18)(CH = CHCH2CH3)2·2H2O, the composition was C, 68.08%; H, 10.50%; O, 15.12%; and Ca, 6.31%.

1H NMR (500 MHz, CD3OD, δ) found 0.904 (t, J = 6.80 Hz, 3H), 1.365–1.318 (m, 14H), 1.602 (q, J = 7.37 Hz, 2H), 2.059 (dt, J = 6.80, 6.80 Hz, 4H), 2.172 (t, J = 7.37 Hz, 2H), 2.770 (t, J = 6.52 Hz, 2H), and 5.371–5.289 (m, 4H).

2.3 Characterization

The calcium content of the obtained powder was evaluated using an EDXL300 X-ray fluorescence spectrometer (Rigaku, Tokyo, Japan). The melting point was measured using a melting-point apparatus (Bibby Scientific Ltd., Staffordshire, United Kingdom). 1H NMR spectra were recorded on Varian INOVA 500 spectrometers (Agilent Technologies, California, USA). All chemical shifts are stated relative to deuterated solvent signals. Chemical shifts (δ) are reported in ppm, and coupling constants (J) are reported in hertz. Scanning electron microscopy (SEM) images were obtained on a SU8000 scanning electron microscope (Hitachi High-Technologies Corp., Tokyo, Japan) using an electron beam accelerating voltage of 1 kV. For X-ray diffraction (XRD) analyses, a MiniFlex X-ray diffractometer (Rigaku, Tokyo, Japan) was used, operating at 30 kV and 15 mA to generate Cu-Kα X-rays.

2.4 Friction measurement

Friction measurements were conducted using a friction meter (Tribo Master TL201Ts, Trinity Lab Inc., Tokyo, Japan). The meter’s specifications are as follows: vertical load, 0.29 N; sliding velocity, 80 mm s⁻¹; measurement distance, 30 mm; and driving motor, AC servomotor. Powder in a 10 × 50 × 1 mm³ hole on SK-050-20 silicone rubber (SK-Electronics Co. Ltd., Tokyo, Japan) was rubbed with a polyurethane contact probe that reflected the geometric properties of a human finger to evaluate the frictional force. On the probe surface, 29 grooves with depths of 0.15 mm were carved at intervals of 0.5 mm.

2.5 Bioactive property

The bactericidal activities of the Ca salts of fatty acids were evaluated for Staphylococcus aureus, Staphylococcus epidermidis and Propionibacterium acnes. The Ca salts of fatty acids dispersed in 2wt% aqueous ethanol were added to a 50 mM phosphate buffer (10 mL, pH 6) containing the desired bacteria. The concentrations of the Ca salts of fatty acids were 100 ppm. Bacteria counts per mL in the initial state were 5.5 ± 0.3 log colony-forming units (CFU). These dispersions were spread onto SCCLP agar-gel plates, and the temporal change in the number of bacteria was monitored for 24 h. Propionibacterium acnes was cultivated under the anaerobic condition, while Staphylococcus aureus and Staphylococcus epidermidis were cultivated under the aeration condition. The anaerobic cultivation of Propionibacterium acnes was achieved in an Anaeropack (Mitsubishi gas chemical Co. Inc., Tokyo, Japan) under oxygen concentration <0.1% and carbon dioxide concentration >15%.

3 RESULTS AND DISCUSSION

3.1 Preparation and crystal structure of Ca salts of fatty acids

The C18:0, C18:1 and C18:2 fatty acid Ca salts are white powders which exhibit fluidity and hydrophobicity. If the C18:0 and C18:1 fatty acid Ca salts (0.01 g) were added to 35 g of water in a screw-cap tube with a diameter of 20.3 mm, the powder particles floated on the surface of the water and remained there for more than one month. On the other hand, in the case of the C18:2 fatty acid Ca salt, the powder particles were immersed into the water over 7 days. The appearance and odor of the powder did not change after being stored for more than one month at 25°C.
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SEM images of the C18:0 and C18:1 fatty acid Ca salts are shown in Fig. 1 (a) and (b). The C18:0 and C18:1 fatty acid Ca salts were plate-shaped powder with diameters of several micrometers and several tens of micrometers, respectively. The XRD profiles of the C18:0 and C18:1 fatty acid Ca salts are shown in Fig. 2. The peaks of the C18:0 fatty acid Ca salt at 2θ = 1.8° (d = 48 Å), 3.6° (24 Å), 5.4° (16 Å) and 7.1° (12 Å) and those of the C18:1 fatty acid Ca salt at 2θ = 1.9° (d = 45 Å), 3.8° (23 Å), 5.7° (16 Å) and 7.5° (12 Å) correspond to the (1 0 0), (2 0 0), (3 0 0) and (4 0 0) diffractions of the bilayer structures, respectively. These profiles demonstrate that the particles are in a lamellar crystalline state. Such XRD patterns of C18:0 and C18:1 fatty acid Ca salts are similar to data previously reported by Vold and Garnier.

An SEM image and XRD profile of the C18:2 fatty acid Ca salt are shown in Fig. 1 (c) and 2, respectively. The C18:2 fatty acid Ca salt was amorphous powder with diameters of several tens of micrometers. The XRD peaks of the C18:2 fatty acid Ca salt are weaker than those of the C18:0 and C18:1 fatty acid Ca salts, although peaks at similar intervals are observed at 2θ = 6.1°, 8.1° and 10.1°. These indicate that the main part of the C18:2 fatty acid Ca salt was in an amorphous state.

On the basis of the above experimental results, we can discuss the effect of the degree of unsaturation of the alkyl chain on the particle shape and crystal structure of Ca salts of fatty acids. In all the studied Ca salts of fatty acids, if the powder is assumed to be a monohydrate or dihydrate containing two fatty acid molecules and a calcium ion, then the experimental values are consistent with the calculated values. However, an increase in the degree of unsaturation changed the crystal structure: C18:0 and C18:1 fatty acid Ca salts are in crystalline state, while C18:2 fatty acid Ca salt is in amorphous state. This change was caused by the steric structure of the alkyl chain in the fatty acid. The structure of the alkyl chain at the site of unsaturation inhibited crystal formation of the Ca salts of fatty acids. This behavior has also been reported by other researchers.

3.2 Lubricant properties of Ca salts of fatty acids

The C18:0 and C18:1 fatty acid Ca salts exhibited remarkable lubricity when the powder was rubbed with the urethane rubber finger model. During the rubbing process of the Ca salts at a sliding velocity of 80 mm s⁻¹ and vertical force of 0.29 N, the dynamic friction coefficients were 0.21 ± 0.05 and 0.19 ± 0.05, respectively, which was 15 times less than that of naked silicone rubber (Fig. 3). Conversely, the dynamic friction coefficient of the C18:2 fatty acid Ca salt was 0.40 ± 0.04, which was larger than those of the others. The difference between the lubrications of these three Ca salts of fatty acids is caused by their crystal structures. In the cases of C18:0 and C18:1 fatty acid Ca salts, the lubricity is due to three factors. The first and second factors are cleavage of the lamellar layers and disintegration of the powder aggregates. These disruptions in

Fig. 1  SEM images of Ca salts of fatty acids: (a) C18:0 fatty acid Ca salt; (b) C18:1 fatty acid Ca salt; (c) C18:2 fatty acid Ca salt.

Fig. 2  Low-angle XRD patterns of Ca salts of fatty acids. Inset: Small peak of 4°–12° of C18:2 fatty acid Ca salt.
the structural system allow for absorption of the friction force between solid surfaces. The third factor is the low surface energy of the Ca salts of fatty acids. The adhesion energy between solid particles decreases with increasing hydrophobicity of the surfaces. Previously, we reported that a surfactant divalent metal salt, Ca salt of N-lauroyltaurate, forms a thin layer due to cleavage and disintegration; however, the lamellar structure still remained after deformation. Conversely, C18:2 fatty acid Ca salt is not suitable for the absorption of frictional energy by cleavage due to its amorphous structure.

3.3 Bactericidal properties of Ca salts of fatty acids

The temporal changes of the bactericidal evaluation of Ca salts of fatty acids are shown in Fig. 4. First, when saturated fatty acid C18:0 fatty acid Ca salt was added to phosphate buffers containing Staphylococcus aureus, Staphylococcus epidermidis and Propionibacterium acnes, it did not display bactericidal ability for any of the bacteria; the log CFU mL\(^{-1}\) was approximately five and did not change. Second, the monounsaturated fatty acid C18:1 fatty acid Ca salt exhibited a weak bactericidal ability for Propionibacterium acnes only. When added to the phosphate buffer containing Propionibacterium acnes, the number of bacteria decreased to 1.1 ± 2.0 log CFU mL\(^{-1}\). Finally, when diunsaturated fatty acid C18:2 fatty acid Ca salt was added to phosphate buffers containing Staphylococcus aureus, Staphylococcus epidermidis and Propionibacterium acnes, 5.4 ± 0.3 log CFU mL\(^{-1}\) of bacteria were reduced to <1 log CFU mL\(^{-1}\) after 3, 24 and 3 h, respectively. Thus, the C18:2 fatty acid Ca salt exhibited the strongest bactericidal properties of the three Ca salts of fatty acids.

Here we discuss the changes in bactericidal ability due to the degree of unsaturation. Recently, the selective bactericidal ability of C16:1 fatty acid Ca salts was found for Staphylococcus aureus and Propionibacterium acnes. In these cases, dissolved fatty acid molecules from the Ca salts became the active components for the bactericidal ability. In the present study, the bactericidal ability of Ca salts of fatty acids increased with increasing degree of unsaturation. Here we propose the significant factors influencing the bactericidal ability. The first factor is the difference in solubility: the C18:0 fatty acid Ca salt was insoluble, while the solubilities of the C18:1 and C18:2 fatty acid Ca salts were 6.0 × 10\(^{-5}\) and 3.0 × 10\(^{-4}\) mol L\(^{-1}\), respectively. Dissolved fatty acid molecules are adsorbed on the cell membrane surface, disrupting the membranes. The second factor is the steric effect. Desbois et al. reported that fatty acid molecules having cis-type double bonds are adsorbed on the cell membrane easily due to their bent structure. Therefore, unsaturated fatty acids show stronger bactericidal abilities than do saturated fatty acids.
enway et al. found that the bactericidal ability of C18:2 fatty acid is stronger than that of C18:0 fatty acid, due to its ability to penetrate into cell membranes. 

4 CONCLUSIONS

The lubricity and bactericidal properties of Ca salts of fatty acids changed with the degree of unsaturation of the alkyl chain. C18:0 and C18:1 fatty acid Ca salts are plate-shaped, lamellar, crystalline powders with particle diameters of several micrometers and several tens of micrometers, respectively. Conversely, C18:2 fatty acid Ca salt comprises amorphous powder particles. The C18:0 and C18:1 fatty acid Ca salts are suitable ingredients for powder cosmetics because they exhibited remarkable lubricity when rubbed with a urethane rubber finger model. In addition, the bactericidal properties of Ca salts of fatty acids increase with increasing degree of unsaturation. Particularly, C18:2 fatty acid Ca salt showed a superior bactericidal ability against Staphylococcus aureus, Staphylococcus epidermidis and Propionibacterium acnes. These findings suggest that these Ca salts of fatty acids have potential applications in cleansing and cosmetic products.

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