Surface phase separation and collapse of the stearate anion–alkaline earth cation complex

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Abstract  The surface properties of fatty acid and fatty acid–alcohol mixtures were examined at 22–24°C. At pH 12, sodium stearate forms a rigid surface film that generates an equilibrium spreading pressure of 16.5 dynes/cm. At pH 12, stearate–alkaline earth cation films collapse at the air–water interface and do not generate significant equilibrium spreading pressures. The rate of film collapse depends on the counterion decreasing in the sequence Ba$^{2+}$ > Sr$^{2+}$ > Ca$^{2+}$. Stearate–stearic acid mixtures form solid (condensed) films that are relatively stable and behave initially as homogeneous surfaces in their selectivities for counterions. Stearate–oleyl alcohol mixtures form fluid (expanded) films that are unstable. Lateral phase separations occur rapidly in fluid films and the stearate–alkaline earth cation phase collapses. The rate of film collapse in the fluid mixtures also depends on the counterion decreasing in the sequence Ba$^{2+}$ > Ca$^{2+}$. These surface properties suggest how a lipid anion may function as an ionophore in the translocation of alkaline earth cations.

Supplementary key words  stearyl alcohol · oleyl alcohol · calcium · strontium · barium · equilibrium spreading pressure · field strength · counterion selectivity · ionophore

MATERIALS AND METHODS

Stearic acid, stearyl alcohol, and oleyl alcohol (Applied Science Laboratories, State College, PA) were spread on aqueous subphases as n-hexane solutions. n-Hexane was purified as previously described (1). The aqueous subphase contained 0.025 N sodium hydroxide and either 0.3 mM calcium chloride (Mallinckrodt, AR), barium chloride (Mallinckrodt, AR), or strontium chloride (Baker and Adamson, Reagent). The measured pH of all subphase solutions was 12.

$\Pi$ was measured by the Wilhelmy plate technique utilizing a Cahn R. G. recording balance as previously described (1) (Cahn Instruments, Paramount, CA). $\Pi$–$\Pi$ isotherms were generated at different compression rates utilizing a Teflon Langmuir trough (50 × 10 × 1, cm) and a movable Teflon barrier driven by a high-torque variable speed motor (1). All measurements were made at 22–24°C.

RESULTS AND DISCUSSION

$\Pi_E$ and the $\Pi$–$\Pi$ isotherm

$\Pi_E$ was determined by spreading in a covered Petri dish a 10-fold excess of stearic acid on subphase
Open symbols designate films compressed at 17 Å²/molecule per min. Closed symbols designate films compressed at 3 Å²/molecule per min. Arrows point to II values generated immediately when stearic acid is spread at 20 Å²/molecule on subphases containing the cation specified in the figure.

Fig. 1. II–A isotherms at 22–24°C for stearic acid spread on subphases containing sodium hydroxide (0.025 N) and an alkaline earth cation (0.3 mM). The cation is specified in the figure. Open symbols designate films compressed at 17 Å²/molecule per min. Closed symbols designate films compressed at 3 Å²/molecule per min. Arrows point to II values generated immediately when stearic acid is spread at 20 Å²/molecule on subphases containing the cation specified in the figure.

solutions containing 0.025 N sodium hydroxide and various alkaline earth cations. Stearic acid films are completely ionized on 0.025 N sodium hydroxide (5). Surface films of stearate spread on sodium hydroxide alone and surface films of stearate spread on sodium hydroxide containing either 0.5 mM calcium chloride or 0.3 mM barium chloride all showed particles that resembled a white scum on standing for 16 hr. A small jet of air moved the scum freely when the subphase contained either Ca²⁺ or Ba²⁺. Stearate films spread on Ca²⁺ or Ba²⁺ gave very low II values of around 0.5 dynes/cm. II in this range is not significant, particularly since the Wilhelmy plate is sensitive to changes in contact angle. Thus, surface viscosity and II data both indicated that stearate films collapsed when the subphase contained Ca²⁺ or Ba²⁺.

A small jet of air showed that the scum was trapped in a rigid surface film when the stearate was spread on a subphase that contained only sodium hydroxide. These sodium stearate films generated a significant II of 16.5 ± 1.5 dynes/cm. Thus surface viscosity and II data showed that sodium stearate formed a stable monolayer with a II value of 16.5 dynes/cm.

Film collapse was examined in a second series of experiments utilizing the Langmuir trough. The stearate films (pH 12) were compressed rapidly until they generated approximately 20 dynes/cm and then compression was stopped. Films spread on a Ba²⁺ subphase collapsed rapidly, with II decreasing 15 dynes/cm in one min. Films spread on a Ca²⁺ subphase collapsed slowly, with II decreasing 2 dynes/}

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layer collapses to a bulk phase as the water of hydration is removed. Since the rate constant for the removal of water from the inner coordination sphere increases in the sequence $\text{Ca}^{2+} < \text{Sr}^{2+} < \text{Ba}^{2+}$ (11–13), water of hydration is most rapidly removed from the $\text{Ba}^{2+}$ ion and the rate of film collapse should occur in the $\text{Ba}^{2+} > \text{Sr}^{2+} > \text{Ca}^{2+}$ sequence that was found in these experiments (Fig. 1).

The rate of film collapse resulted in $\Pi$–$A$ data that could be interpreted to indicate that surface films condensed in an apparent $\text{Ba}^{2+} > \text{Sr}^{2+} > \text{Ca}^{2+}$ sequence when films are compressed at a slow rate (Fig. 1). The effect of the collapse rate can be minimized by initially spreading sufficient stearic acid to give $20 \, \text{Å}^2$-molecule and measuring the instantaneous $\Pi$ of the film (see arrows in Fig. 1). Since the most condensed film generates the lowest $\Pi$ in a constant area study, it is apparent in this experiment that binding (condensing) occurs in the anticipated strong field sequence $\text{Ca}^{2+} > \text{Sr}^{2+} > \text{Ba}^{2+}$ for the completely ionized stearate film.

Counterion binding in mixed lipid films

In previous studies with stearic acid, we showed that counterion binding changed from the weak field sequence $\text{Ba}^{2+} > \text{Sr}^{2+} > \text{Ca}^{2+}$ to the strong field sequence $\text{Ca}^{2+} > \text{Sr}^{2+} > \text{Ba}^{2+}$ when the pH of the subphase was increased from 6 to 9 (4, 5). Mixed lipid films containing stearic acid and the stearate anion are formed in this pH region (3, 5). However, it is difficult to interpret the data obtained with stearate–stearic acid films. The relative amounts of acid and anion cannot be measured directly in the film. Skimmed films have been examined (4, 14) but lateral phase separations may occur during the skimmin process. Finally, stearic acid–stearate films contain acid–soap complexes (3, 5, 15) that may affect counterion binding. These problems can be overcome with mixed lipid films containing only the stearate anion (subphase at pH 12) and stearyl alcohol.

Counterion binding to mixed lipid stearate–stearyl alcohol films is examined in Fig. 2. The 1:4 stearate–stearyl alcohol mixture selected $\text{Ba}^{2+}$ in preference to $\text{Ca}^{2+}$, indicating the anticipated weak field surface. The 1:1 stearate–stearyl alcohol mixture selected $\text{Ca}^{2+}$ in preference to $\text{Ba}^{2+}$, indicating the anticipated strong field surface. These data show that stearate–stearyl alcohol mixtures form homogeneous films and they confirm our interpretation of binding selectivity in stearate–stearic acid films (8, 5).

Lateral phase separation in mixed lipid films

Counterion selectivity (Fig. 2) shows that stearate–stearic acid and stearate–stearyl alcohol mixtures initially form homogeneous films. If these films separated rapidly into discrete stearate and stearic acid or stearyl alcohol phases, a change in relative composition would not alter counterion selectivity. Furthermore, the mixed films are relatively stable, yet a discrete stearate phase spread on $\text{Ba}^{2+}$ will rapidly collapse (see Fig. 1).

Lateral phase separations occur in synthetic lipid bilayers (7–9) and in biological membranes (16). These membranes are fluid since they contain lipids composed largely of unsaturated fatty acids. Stearic acid and stearyl alcohol films, and mixed lipid stearate–stearyl alcohol films are solid (condensed) at room temperature. Oleyl alcohol and mixed lipid stearate–oleyl alcohol films are fluid (expanded) at room temperature (see Fig. 3 for comparisons). If lateral phase separations occur in fluid films, they should be found in stearate–oleyl alcohol mixtures.

$\Pi$–$A$ isotherms (Fig. 3) show that 1:1 stearate–stearyl alcohol mixtures initially formed stable films on both $\text{Ca}^{2+}$ and $\text{Ba}^{2+}$ subphases. The 1:1 stearate–oleyl alcohol film behaved in a very different manner. When this lipid mixture was spread on a $\text{Ba}^{2+}$ subphase, the film collapsed as $\Pi$ approached 20 dynes/cm and the $\Pi$–$A$ isotherm exhibited a plateau (Fig. 3). This plateau was followed by an increase in $\Pi$ generating a region of the $\Pi$–$A$ isotherm that corresponded exactly to pure oleyl alcohol (see X in Fig. 3). This region of the $\Pi$–$A$ isotherm is readily explained by the collapse and removal of barium stearate from the surface film.

The collapse rate for a calcium stearate film was much slower than the collapse rate for a barium stearate film (Fig. 1). Indeed, a plateau was not generated in the $\Pi$–$A$ isotherm of a 1:1 stearate–oleyl alcohol film spread on a $\text{Ca}^{2+}$ subphase (Fig. 3).
Since calcium stearate collapses slowly, the formation of a calcium stearate surface micelle can be demonstrated only by a constant II--variable A technique. This technique employs a moving barrier maintained at 17 dynes/cm with a piston oil composed of castor oil (17).

Stearate--stearyl alcohol films spread on a Ca\(^{2+}\) subphase did not decrease in surface area and stearate--stearyl alcohol films spread on a Ba\(^{2+}\) subphase decreased only 4% in 5 min when the films were maintained at 17 dynes/cm. The surface area of the stearate--oleyl alcohol mixture decreased 25% in the first minute when the subphase contained Ba\(^{2+}\). The surface area also decreased, but only 6% of the first minute, when the subphase contained Ca\(^{2+}\). These data show that lateral phase separation occurs at a finite rate in fluid stearate--oleyl alcohol films and this phase separation is followed by collapse when the films are spread on either Ba\(^{2+}\) or Ca\(^{2+}\).

**Model system for cation transfer between aqueous and nonaqueous phases**

Starks (18) and Dehmlow (19) have recently reviewed the use of organic soluble cations such as quaternary ammonium or phosphonium cations for the transport of anions from an aqueous phase to an organic phase. We suggest that the fatty acid anion may operate in an analogous manner in the transfer of alkaline earth cations from an aqueous phase to a nonaqueous phase. The fatty acid anion--alkali metal cation complex forms a stable surface film. When alkaline earth cations are added to the system, the fatty acid anion--alkaline earth cation complex collapses and is, therefore, removed from the surface. This property of the fatty acid anion makes it particularly attractive as an ionophore for alkaline earth cation transport.

Anionic phospholipids such as cardiolipin and phosphatidic acid can act as ionophores for the translocation of divalent cations in a Pressman cell (20). However, the flip-flop between adjacent monolayers is a relatively slow process for compounds such as the choline phosphoglycerides (21) and, as a consequence, it has not appeared reasonable for membrane phospholipids to act as ionophores (22). We suggest that translocation may involve a process analogous to the collapse and removal of the fatty acid anion--alkaline earth complex from a surface film. This hypothesis is supported by the observation that filters impregnated with phosphatidyl serine change from hydrophilic to hydrophobic surfaces in the presence of Ca\(^{2+}\) (7). Furthermore, the Ca\(^{2+}\) channel in an excitable membrane is more permeable to weakly bound Ba\(^{2+}\) than to the more strongly bound Ca\(^{2+}\) (23). This observation is consistent with our rate data for the collapse (translocation) of an anion--alkaline earth complex.

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**REFERENCES**


