Solution of fatty acids from monolayers spread at the air-water interface: identification of phase transformations and the estimation of surface charge

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ABSTRACT The contraction or decrease in area of fatty acid monolayers maintained at a constant surface pressure of 16 dynes/cm was studied as a function of fatty acid chain length, unsaturation, temperature, and the hydrogen ion concentration in the subphase. The data were consistent with the hypothesis that fatty acid solution from the monolayer into the subphase was the mechanism for film loss. Autoxidative reactions did not contribute significantly to film loss since contraction occurred with saturated fatty acid monolayers and with unsaturated fatty acid monolayers in an anaerobic environment. The decrease in area per unit time or the solution rate was inversely proportional to chain length and directly proportional to the degree of unsaturation. Arrhenius plots showed activation energies of 1.5–2.5 kcal mole^{-1} for tetradecanoic, octadecenoic, and octadecadienoic acids, and 25 kcal mole^{-1} for hexadecanoic acid. The solution rate from the monolayer increased in a sigmoidal fashion with an increase in subphase pH, and the apparent surface pK$_a$ was estimated as the point where the solution rate was half-maximum. Apparent surface pK$_a$ values were: hexadecanoic acid, 9.7; octadecenoic acid, 8.3; tetradecanoic acid, 7.9; and octadecadienoic acid, 8.0.

SUPPLEMENTARY KEY WORDS surface film · surface pressure · surface pH · apparent surface pK$_a$ · temperature · solubility · activation energy · autoxidation · tetradecanoic · hexadecanoic · octadecanoic · octadecenoic · octadecadienoic

MONOLAYERS OF fatty acids and monolayers of lipids obtained from membranes have provided useful models for the study of membrane-related processes. In particular, surface pressure and surface area measurements have been important in monolayer penetration studies (1–3), and surface potential and surface viscosity measurements have provided information on ionic conformations and interactions in lipid head groups (4–7). However, loss of lipid from monolayers has received little attention although this may affect the interpretation of surface measurements (8).

Porter, Henick, and Clifford (9) recently described a procedure by which the loss of fatty acids from monolayers may be studied while the monolayer is maintained at a constant surface pressure. Their procedure employs a free floating barrier which separates a fatty acid monolayer from a piston oil. The piston oil, being present in excess, exerts a constant pressure on the fatty acid monolayer, and the disappearance of fatty acid from the interface is measured as the decrease in monolayer area. Porter et al. (9) investigated octadecadienoic acid films and concluded that autoxidation, followed by the solution of short-chain cleavage products in the subphase, was the mechanism of film loss. In the present paper we report studies on the effect of chain length, unsaturation, anaerobic environment, temperature, and subphase pH on this phenomenon. The major mechanism of film contraction for both saturated and unsaturated fatty acids was solution from the monolayer into the subphase, and the solution rate depended on the structure of the monolayer and its surface charge. Autoxidation did not contribute greatly to the contraction of unsaturated fatty acid monolayers.

METHODS

Apparatus

The trough, 10 × 50 × 1 cm, was milled from a solid Teflon block. A floating barrier, 3 × 10 × 0.1 cm, was cut from polystyrene to a 0.5 mm clearance on each side of the trough. The trough was partially immersed in a covered temperature-controlled water bath for aerobic experiments. For anaerobic experiments at room temperature, the trough and barrier were enclosed in a polyethylene glove bag (I^{2}R, Cheltenham, Pa.) which contained fatty acids (Applied Science Laboratories Inc., State College, Pa.), subphase solutions, castor oil (E. R. Squibb & Sons, New York), and pipets. The glove bag was flushed 5 times with nitrogen before the monolayer was applied, and a positive nitrogen flow was maintained throughout the experiment.

Subphase Solutions

Double distilled water from a glass still was used to prepare all solutions. The subphase contained 0.1 M NaCl, 10 mM tris–acetate buffer or 18.3 mM Tris buffer, and 0.1 mM EDTA which was added to remove any polyvalent cations which may have been introduced with the other reagents. The pH was varied between 3 and 12 by additions of HCl and NaOH. Tris and tris–acetate provided adequate buffering in the pH 5–9 range. The NaCl concentration was kept at 0.1 M to maintain a reasonably constant ionic strength at the different hydrogen ion concentrations. When anaerobic conditions were required, solutions were boiled for 30 min and were then sparged with nitrogen during cooling. Solutions were added to a trough which was enclosed in the glove bag and maintained under nitrogen. In some experiments 0.2% hydroquinone was added to remove any trace of oxygen in the subphase.

Measurement of Film Loss at Constant Pressure

The trough was filled with subphase, and the fatty acid and castor oil were applied to the interface on opposite sides of the floating barrier. Castor oil was applied first with a thin steel applicator. Care was taken to insure that a few small lenses of castor oil were always present. This forced the freely floating barrier to the end of the trough, although castor oil sometimes leaked around the barrier when it was first applied, and a small space remained between the barrier and the end of the trough. This space was cleaned by suction. Sufficient fatty acid in hexane solution was added to the interface to move the floating barrier and to produce a monolayer with an initial area of 200–400 cm^2 and with a surface pressure of 16 dynes/cm on each side of the barrier. In some experiments, particularly those with octadecadienoic acid under aerobic and anaerobic conditions, fatty acid alone was added directly from the vial using a thin glass applicator to avoid possible oxidation in the hexane solution. The absence of monolayer leakage around the moving barrier was demonstrated by occasionally sprinkling talc on either side of the barrier and observing that no streaming of talc particles occurred around the barrier. Furthermore, the surface areas of insoluble and nonvolatile compounds such as octadecanol, cholesterol, and tripalmitin were the same at 16 dynes/cm when surface pressure was maintained either with a piston oil or moving barrier in the standard Wilhelmy plate technique. The castor oil maintained a constant surface pressure of 16 dynes/cm, the equilibrium spreading pressure of castor oil, as measured occasionally by a Cahn RG recording balance. The initial film area, A_0, and the film area at time intervals, A_t, were measured, and ΔA, or $A_0 - A_t$, was calculated.

RESULTS

The decrease in film area as a function of time for several fatty acid monolayers is shown in Fig. 1. The rate of decrease in area was inversely proportional to chain length (14:0 > 16:0 > 18:0) and directly proportional to unsaturation (18:2 > 18:1 > 18:0). Highly fluid monolayers of lipids such as cholesterol and egg lecithin (General Biochemicals, Chagrin Falls, Ohio) showed no film loss over extended periods of time (30–60 min).

Film losses were similar when octadecadienoic acid was spread on a trough exposed to the air and when octadecadienoic acid was spread on a trough enclosed in a nitrogen atmosphere (Fig. 2). In several experiments the octadecadienoic acid ampule was opened under nitrogen in the glove bag just before use. In other experiments octadecadienoic acid from an ampule that had been opened for several weeks and stored under nitrogen was used. Similar results were obtained.

The effect of the hydrogen ion concentration in the subphase on film loss was studied by spreading fatty acid monolayers on acid and alkaline subphases (Fig. 3).

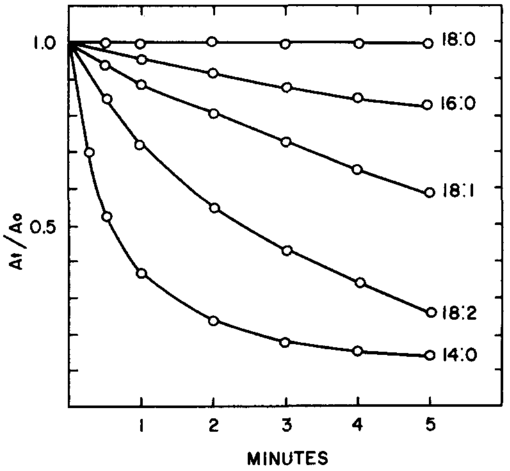

FIG. 1. Effect of chain length and unsaturation on disappearance of fatty acids from a monolayer. The subphase contained 0.1 M NaCl, 0.1 mM EDTA, and 0.01 M tris-acetate buffered at pH 8.4. The temperature was 27°C.

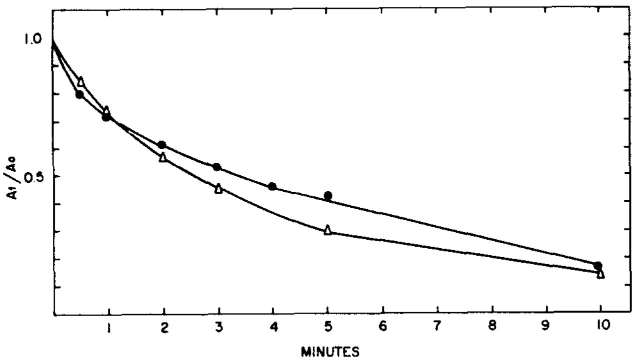

FIG. 2. Effect of oxygen on the disappearance of octadecadienoic acid from a monolayer. ●, nitrogen atmosphere; △, air. The subphase is described in Fig. 1 except that the subphase for the anaerobic experiment also contained 0.2% hydroquinone. The temperature was 31°C.

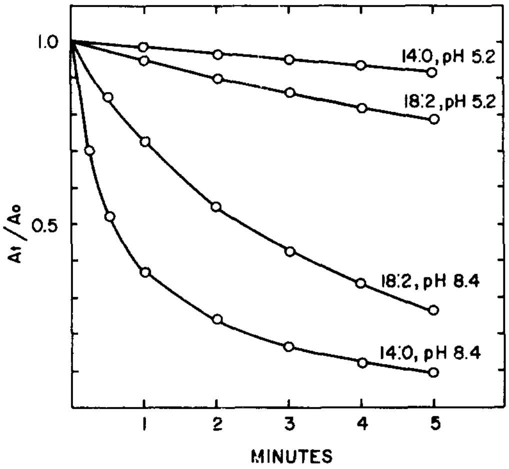

FIG. 3. Effect of subphase pH on disappearance of fatty acids from a monolayer. The subphase and temperature are described in Fig. 1.

The fraction of the initial film remaining at time t, represented by A_t/A_0, was much less when the film was spread on alkaline subphases than when the film was spread on acid subphases. When the film loss was large, the rate of film loss decreased with time, and A_t/A_0 approached a constant value (see 14:0 at pH 8.4 in Fig. 3). The initial rate of film loss was, therefore, approximated from the relative change in monolayer area from 0 to 1 min in an experiment, $(\Delta A/A_0)$ per min. These rates were low for all fatty acids when they were spread on acid subphases and increased markedly in a sigmoid-shaped curve when the pH of the subphase was increased (Fig. 4).

The rate of the film loss from monolayers was also studied as a function of temperature (Fig. 5). Rates increased linearly for tetradecanoic, octadecadienoic, and octadecenoic acid as the temperature was raised from 5° to 35°C. Hexadecanoic acid had a definite break in the curve between 20° and 25°C, at which point the curve rose very steeply. Calculations of activation energy by the Arrhenius equation gave 1.5, 1.8, and 2.5 kcal mole^{-1}, respectively, for tetradecanoic, octadecadienoic, and octadecenoic acid between 5° and 35°C, and 25 kcal mole^{-1} for hexadecanoic acid between 20° and 35°C.

DISCUSSION

Several possible mechanisms have been proposed to explain contraction of monolayers. These include evaporation from the monolayer (10), collapse into a bulk phase (11, 12), solution into the subphase (13–17), and autoxidation of unsaturated compounds followed by solution of the short-chain reaction products (9).

Evaporation

Evaporation of fatty alcohols from monolayers has been described by Brooks and Alexander (10). It is unlikely that evaporation contributed significantly to the loss of fatty acids from monolayers in the present study since the rate of film loss was nearly zero at low pH and increased with increasing pH. Ionized carboxylate groups which are formed with increasing pH should interact strongly with the polar subphase. This increasing pH and increasing interaction, which should retard evaporation, or have no effect on evaporation, actually enhanced film loss.

Collapse into Bulk Phase

Studies involving film stability and the equilibrium spreading pressure (π_E) are discussed by Heikkila, Kwong, and Cornwell (12). When a film is maintained at surface pressures greater than π_E, the film is unstable and collapses into the bulk phase with a decreased area at the air-water interface. The π_E for octadecanoic acid at 27°C is 7.5 dynes/cm (12), and collapse probably could explain the slow contraction of this monolayer when it was maintained at 16 dynes/cm on acid and alkaline subphases. We found that the π_E values for octadecenoic and octadecadienoic acid at 27°C were 29 and 27 dynes/cm, respectively. Since these values were greater than the 16 dynes/cm generated by the piston oil, collapse into the bulk phase did not explain film loss with these unsaturated compounds. A similar argument could be extended to tetradecanoic and hexadecanoic acids which have π_E values at 27°C of 13–17 dynes/cm and 10–14 dynes/cm, respectively (12, 18). If collapse were responsible for the contraction of these monolayers, contraction would be relatively slow since the π_E values are very similar to the π_E of the castor oil. Since saturated and unsaturated films with higher Π_e values contract more rapidly than the octadecanoic acid monolayer, a different mechanism besides collapse is necessary to explain film contraction.

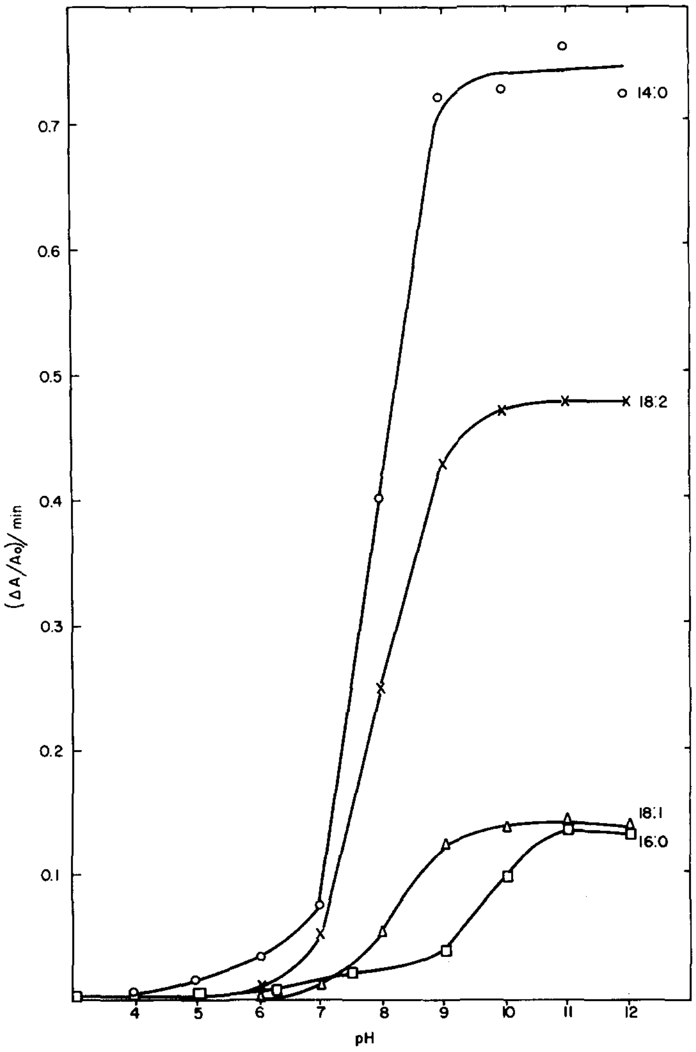

FIG. 4. The effect of subphase pH on the initial rate of fatty acid disappearance from a monolayer. The subphase contained 0.1 M NaCl, 0.1 mM EDTA, and 0.018 M tris-acetate buffer. The temperature was 25°C.

Solution of Monolayers into Subphase

Two results of the present study strongly support the hypothesis that monolayer contraction occurred through fatty acid solution into the subphase. First, film losses were directly proportional to the solubilities of saturated fatty acids in water (19). Second, the rate of the film loss was inversely proportional to the hydrogen ion concentration in the subphase, and the sigmoid function of rate vs. pH indicated that the contraction rate was directly proportional to carboxyl group ionization and the increased solubility of carboxylate anions. The solubility of octadecanoic acid and hexadecanoic acid films was suggested by Sebba and Briscoe (13) to explain variations in their surface pressure measurements. Ter Minassian-Saraga (14) found that the rates of dodecanoic and tetradecanoic acid solution from films decreased with time, an observation that was confirmed in our study. She attributed this decrease to the fact that the contraction rate was dependent upon both the solution rate and the diffusion of the soluble species within the subphase. She postulated that the rate of film contraction became slower with time due to the buildup of fatty acid molecules in the portion of subphase just beneath the interface and the formation of a pseudo-saturated solution in this area. To test her hypothesis, she agitated the subphase of a dodecanoic acid film and found that the rate of solubility was constant with time. Ter Minassian-Saraga (14), and later Motomura, Shibata, Nakamuro, and Matuura (15) used the solution of fatty acid molecules from monolayers held at constant surface pressures to determine diffusion constants, while Gershfeld (16) showed that the solution of monooctadecyl phosphate monolayers held at constant surface pressure was a diffusion limited process. In addition, Gershfeld (17) used

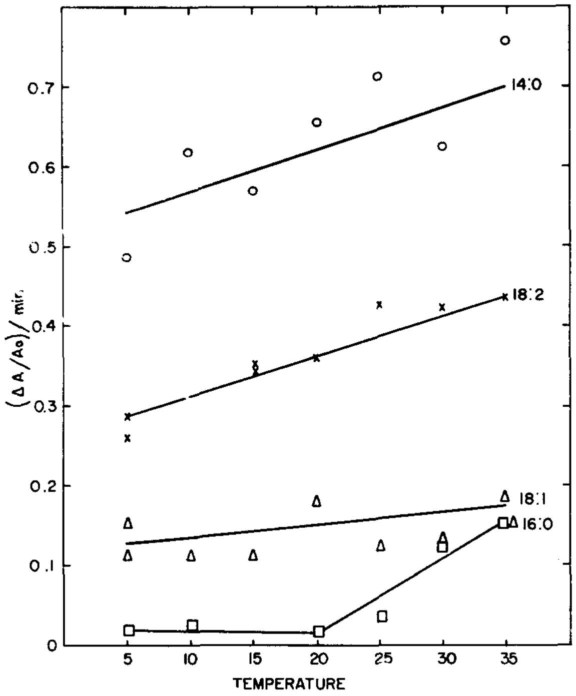

FIG. 5. Effect of temperature on the initial rate of fatty acid disappearance from a monolayer. The subphase buffered at pH 8.0 is described in Fig. 4.

solution data to estimate London–van der Waals dispersion forces in monolayers.

Autoxidation

Porter et al. (9) suggested that autoxidation followed by solution of short-chain reaction products explained the contraction of octadecadienoic acid monolayers. However, it is apparent from the present results that simple solution is primarily responsible for the contraction of octadecadienoic acid films, since contraction was not significantly inhibited when octadecadienoic acid was spread as a monolayer in an anaerobic environment. Furthermore, film losses occurred with saturated fatty acids which are not susceptible to autoxidation and with octadecenoic acid which is less susceptible to oxidation than octadecadienoic acid. The hydrogen ion concentration in the subphase had the same general effect on all fatty acids studied. The greater contraction rate of films on alkaline subphases was, therefore, caused not by autoxidation but rather by solubility.

Effects of Temperature and Subphase pH

Measurements of film contraction rates may be used to investigate pH and temperature effects on monolayers. The rate of film loss was a sigmoid function of bulk phase pH which resembled a titration curve (Fig. 4). If the solution rate was related to the number of carboxylate ions in the monolayer, as is likely, the pH at which the fatty acid was half-ionized could be estimated from rate data by determining the pH at which the rate was half-maximum. By this method, the apparent pK_a of hexadecanoic acid was 9.7, of octadecenoic acid, 8.3, of tetradecanoic acid, 7.9, and of octadecadienoic acid, 8.0. This point was several pH units to the alkaline side of the point where soluble fatty acids are half-ionized in aqueous solutions although Schmidt-Nielsen (20) and Mattson and Volpenhein (21) showed that apparent pK_a of micellar solutions of fatty acids was approximately 9. Similar pK_a data for saturated fatty acids were estimated by Peters (22) who measured the interfacial tension of fatty acids absorbed at a benzene-water interface (pK_a hexadecanoic acid, 8.5, pK_a octadecanoic acid, 8.5). Christodoulou and Rosano (23) estimated pK_a values of 9.0 for octadecanoic and docosanoic acids from surface potential and surface area data, and these same investigators discussed the contribution of acid soaps, described by Goddard and Ackilli (24), to the observed pK_a shift. Davies and Rideal have suggested that this effect may be explained by the difference between surface and bulk phase pH values (25). An alternative explanation would be that the ionization property of the head group is affected and that the surface and bulk pH values are actually the same.

Phase transitions in fatty acid monolayers have been studied by a number of investigators, and phase diagrams for saturated fatty acids have been prepared (12, 26). Of the fatty acids studied here, only hexadecanoic acid appeared to undergo a phase change as measured by solution rates in the temperature range used. This would be expected since tetradecanoic, octadecenoic, and octadecadienoic acids form liquid monolayers between 5° and 35°C (8). Thus, the increase in solution rate between 15° and 25°C for hexadecanoic acid may be related to the CS $\rightarrow$ L_2 phase transition (26). The high activation energy of solution obtained for hexadecanoic acid also indicated that solution is occurring from a different phase than solution from tetradecanoic, octadecenoic, and octadecadienoic acid films.

Charged groups on a surface sometimes exhibit anomalous reactivities. For example, Papahadjopoulos and Weiss (27) found that liquid crystalline vesicles of phosphatidyl ethanolamine did not react with 2,4,6-trinitrobenzenesulfonic acid. They suggested that reactivity was affected more by salt linkages than surface pH since the surface pH changes calculated by the Hartley–Roe equation were not large. The early studies by Peters (22), recent experiments by Christodoulou and Rosano (23), and the data reported in our study show that surface pH changes may be large and that these changes could account for altered reactivity.

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