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Phase Behavior of Bipolar Fatty Acid Monolayers

Masud S. Huda,* Katsuhiko Fujio,† and Yuhei Uzu†

Department of Materials Science and Engineering, Graduate School of Science and Technology, Shinshu University,
Wakasato 500, Nagano 380

†Department of Chemistry, Faculty of Science, Shinshu University, Asahi, Matsumoto 390

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The phase behaviors of the monohydroxy- and dihydroxy-substituted fatty acids at an air–water interface were investigated in the presence of different substrates. All of the hydroxylated fatty acids studied, except for 2-hydroxyoctadecanoic acid (2-HOA) and 9,10-dihydroxyoctadecanoic acid (9,10-DHOA), showed an intermediate region ("plateau") for a transition from a liquid-expanded state (LE) to a liquid-condensed state (LC) in the π–A isotherms, regardless of the substrate composition. In contrast, 9,10-DHOA exhibited a pressure plateau region on only 3 M NaCl. In order to assess the effect of adding an extra polar group (i.e., a hydroxy group) for a 9,10-DHOA monolayer compared to a 9-hydroxyoctadecanoic acid (9-HOA) monolayer, the monolayer-substrate interactions were considered based on the thermodynamic parameters for the LE/LC phase transition, using a two-dimensional Clausius–Clapeyron equation. This can be explained by assuming that a greater energy was generated for breaking of hydrogen bonds between the two hydroxy groups of 9,10-DHOA and water. In this regard, the dynamic properties of the monolayer for these acids were examined using the surface pressure relaxation method as well as an equilibrium spreading pressure (ESP) measurement.

Insoluble monolayers of bipolar molecules have been extensively studied by many workers.[1—5] The primary reason for the interest in these molecules lies in the biological significance as a model of biological membranes. In order to obtain information about the "packing" of individual molecules and the intermolecular interactions of such membranes, Menger et al.[1] studied a series of synthetic fatty acids and phospholipids bearing diverse substituents on their hydrocarbon chains. Further evidence is provided by the excellent correspondence that exists between the physical states and phase transitions in both the monolayers and bilayers.[6—8] In view of these studies, well-defined bipolar monolayers as a minimum constituent unit are anticipated to be important for understanding the structures and functions of biological and reconstituted membranes.

A simple interpretation of the phase behavior of these substances, just as in the case of the 9-anthroyl fatty acid derivatives, is difficult, because one polar group is concerned with the combined effect of the hydrophilicity of the second polar group and its rather large size. Studies of simpler bipolar molecules are necessary in order to obtain a clearer view of the effect of only the hydrophilicity of the attached secondary polar group. As typical substances, fatty acid derivatives with a hydroxy substituent at varying locations would seem to be ideal for this purpose, since the hydroxy group is small.

Kellner and Cadenhead[3,4] previously reported on the surface pressure (π)–area (A) isotherms of a series of palmitic derivatives, and lately Menger et al.[1] for stearic derivatives, respectively. In these systematic studies of such systems, they suggested that most of the bipolar fatty acids revealed indicative "looping" conformations where both the hydroxy group and polar head group contact the aqueous subphase during the early stages of monolayer compression. As the compression proceeds, the hydroxy group (weaker hydrophile) is forced out of the subphase, thus bringing the chain to the vertical position. The physical transformations are usually signalled by the presence of a "plateau" region where the surface pressure is invariant to a change in the area per molecule. The π–A isotherms exhibited a strong dependence on the position of the polar hydroxy group along the chain. For example, it is interesting to note that 16-hydroxyhexadecanoic acid, as one of the representatives of α,ω-type bipolar fatty acids, does not form a stable condensed phase, while the other hydroxylated fatty acids do the reverse.

Particularly, it should be noted that spread monolayers at the air–water interface can show surface pressure relaxation phenomena, mainly because of an instability due to a desorption or collapse. Customarily, the monolayer stability limit may in some cases correspond directly to the equilibrium spreading pressure (ESP). This suggests that the mechanism of film collapse is a return to the bulk phase, or perhaps at a surface pressure higher than the ESP if the film is metastable with respect to the bulk phase. In the past it seemed that not much attention was being paid to the relaxation of such films held at a constant surface pressure. Recently, emphasis has been placed on the viscoelastic characteristics of the π–A isotherms of insoluble monolayers. In this regard, some studies have been extended to the changes in the surface properties with time to molecular organizational changes in films.[9—11] Some investigators have proposed a measurement method using new compressional modes, i.e., at constant strain rates,[12,13] or during step-wise relaxation by feed-back control.[14]

Simultaneously, substantial progress has been made concerning the structural characterization of monolayer films at an air–water interface. New and advanced developments in experimental techniques using the FTIR ATR measurement,[15] Brewster-angle microscopy[16] and so on have been extensively applied to understanding the monolayer phase behavior. As a result, molecular conformation models for the various monolayer states on the π–A isotherms are becoming relatively apparent.

Since most of the bipolar surfactants studied so far have been monohydroxy-substituted fatty acids, this investigation was undertaken to assess the effect of adding an extra polar group (i.e., a hydroxy group) at an alternative position along the hydrocarbon chain, employing 9,10-dihydroxyoctadecanoic acid and 9(10)hydroxyoctadecanoic acid (a mixture of 9- and 10-dihydroxyoctadecanoic acids). This paper reports on a study of the effects induced by temperature and substrate composition changes on the spread monolayer properties of these bipolar fatty acids; also, the thermodynamic quantities of the phase transition from the expanded to the condensed state were determined. Concomitantly, the results were compared with those obtained for monohydroxy-substituted fatty acid. Through this survey, we also examined the relaxation behavior responsible for film instability with the hydroxylated fatty acids studied.

Experimental

As monohydroxy-substituted fatty acids, 9-hydroxyoctadecanoic acid (9-HOA), 12-hydroxyoctadecanoic acid (12-HOA), 9-hydroxyhexadecanoic acid (9-HHA), and 9-hydroxytetradecanoic acid (9-HTA) were obtained from Serdary Research Lab. 16-Hydroxyhexadecanoic acid (16-HHA) was purchased from Sigma. They had a stated purity of $>99\%$, and were used without further purification. 3-Hydroxyhexadecanoic acid (3-HHA) was a product of Wako Pure Chemical Ltd. (98% purity) and was used as supplied. 9,10-Dihydroxyoctadecanoic acid (threo type, 9,10-DHOA) and 9(10)-hydroxyoctadecanoic acid (9(10)HOA): the mixture of 9-HOA and 10-HOA were ExPrices of Bromborough Ltd. They were purified by recrystallization from methanol. NaCl, HCl, H_2SO_4, urea, and sucrose were used as the substrates. Certificated-grade NaCl was dried for 24 h at 500 °C before use. All of the other substrates were of G.R. grade, and were used without further purification. The water as the substrate solution was distilled twice: first from an alkaline potassium permanganate, and then from a dilute sulfuric acid solution, respectively. The pH of the substrate solutions was always maintained at pH 6 on NaCl and pH 2 on an acidic substrate, respectively. A mixture of 1 part ethanol and 9 parts hexane was used as the spreading solvent. Certified hexane was further purified immediately before use: first by passage through a column of activated alumina, and then by distillation. The purity of ethanol was 99.5%.

The surface pressure (π) was measured by a fully computer-controlled Wilhelmy-type film balance with an accuracy of ±0.1 $\mathrm{mN\,m^{-1}}$. The compression rate was held at 3.7×10^{-2} $\mathrm{nm^2}$ $\mathrm{molec.^{-1}\,min^{-1}}$. The temperature was kept constant within ±0.1 °C during the experiment.

The equilibrium spreading pressures (ESP) was measured using a circular glass dish (area 80 $\mathrm{cm^2}$), which was situated inside of a thermostated box. A small sample was placed on the cleaned substrate surface. When the surface pressure reached a constant

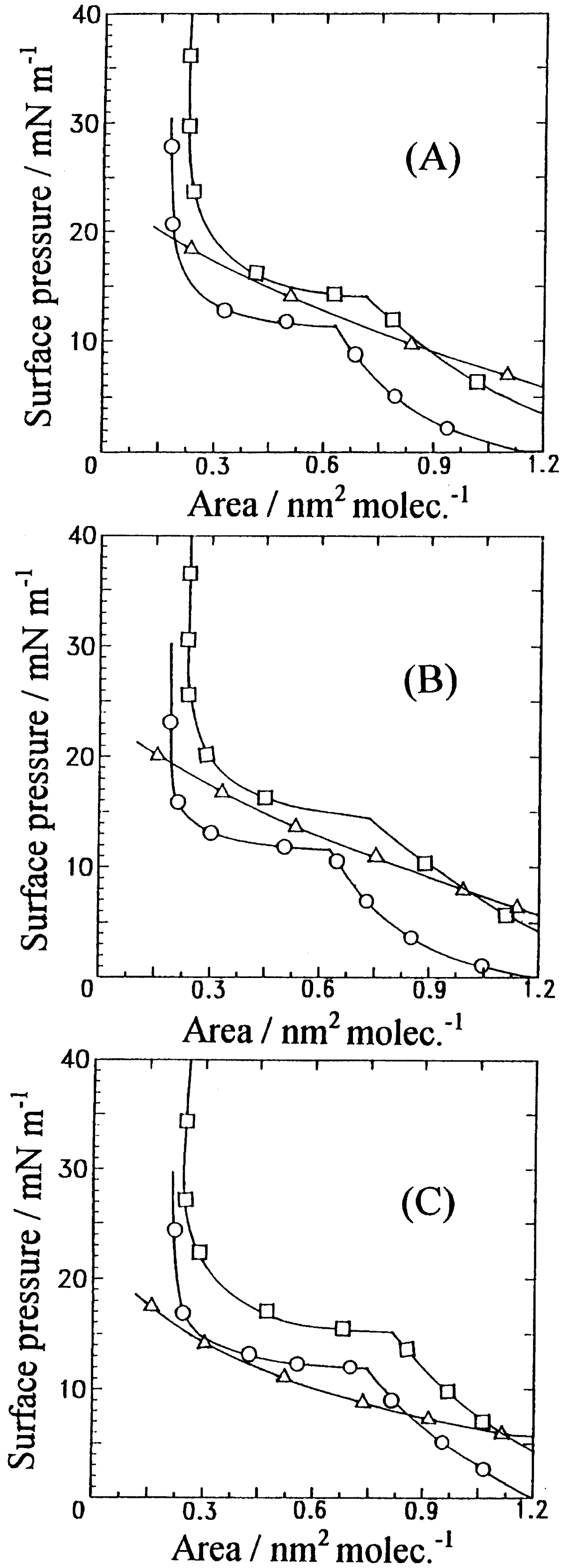

Fig. 1. π–A isotherms of 9-HOA (○), 9(10)HOA (□), and 9,10-DHOA (△) monolayers on (A) 0.01 M HCl, (B) 0.01 M H_2SO_4, (C) 0.01 M NaCl substrates at 23 °C.

Table 1. Transition Pressures and Characteristic Parameters in Hydroxylated Fatty Acid Monolayers on Different Substrates at 23 °C

Hydroxylated fatty acids	Substrates	π_t mN m^{-1}	A_C nm^2 molec.$^{-1}$	A_E nm^2 molec.$^{-1}$	A_{lim} nm^2 molec.$^{-1}$
9-HTA	3 M NaCl	21.2	0.21	0.60	0.22
3-HHA	3 M NaCl	17.4	0.21	0.31	0.21
9-HHA	3 M NaCl	16.7	0.22	0.68	0.23
16-HHA	3 M NaCl	12.1	No A_C value	1.05	No A_{lim} value
2-HOA	3 M NaCl	25.5*	0.21	0.27	0.21
9-HOA	0.01 M HCl	11.3	0.19	0.62	0.21
9-HOA	0.01 M H_2SO_4	11.6	0.19	0.63	0.20
9-HOA	0.01 M NaCl	11.7	0.24	0.74	0.25
9-HOA	3 M NaCl	12.3	0.22	0.70	0.24
12-HOA	3 M NaCl	6.5	0.27	1.11	0.29
9(10)HOA	0.01 M HCl	13.9	0.23	0.71	0.24
9(10)HOA	0.01 M H_2SO_4	14.4	0.23	0.73	0.25
9(10)HOA	0.01 M NaCl	15.0	0.25	0.81	0.25
9(10)HOA	3 M NaCl	15.8	0.25	0.79	0.25
9,10-DHOA	0.01 M HCl			No transition	
9,10-DHOA	0.01 M H_2SO_4			No transition	
9,10-DHOA	0.01 M NaCl			No transition	
9,10-DHOA	3 M NaCl	21.5	0.25	0.82	0.25

Note. π_t: surface pressure at the transition point (mN m^{-1}), π_t*: surface pressure at the inflection point (mN m^{-1}), A_C: area at liquid condensed state (nm^2 molec.$^{-1}$), A_E: area at liquid expanded state (nm^2 molec.$^{-1}$), and A_{lim}: limiting area (nm^2 molec.$^{-1}$).

value, more samples were added to the surface. The ESP was taken to be the pressure at which a further addition of samples to the pressure resulted in no change in the surface pressure within 90 min.

To measure the surface pressure relaxation, the spread films were compressed to a selected surface pressure, and then allowed to rest at a constant area. The stability limit was measured by observing the decay in the pressure as a function of time.

Results and Discussion

π–A Isotherms and Phase Transitions: The surface pressure (π)–area (A) isotherms for 9-HOA, 9(10)HOA, and 9,10-DHOA monolayers spread on 0.01 M (1 M = 1 mol dm^{-3}) HCl, H_2SO_4 and NaCl substrates at 23 °C are given in Fig. 1(A)—Fig. 1(C). These curves clearly demonstrate that both 9-HOA and 9(10)HOA can exist in the characteristic monolayer states, which are different from the states obtained for 9,10-DHOA. In this sense, the behaviors of 9-HOA and 9(10)HOA are similar to the other hydroxylated fatty acids studied, except for 2-HOA. Although these other isotherms were omitted for the sake of brevity, the characteristic parameters derived from them are included in Table 1 (see later). On the contrary, the π–A isotherms of 9,10-DHOA gave only the expanded state on acidic and low-concentrated NaCl substrates. This is attributable to the loss of monolayer molecules by dissolution through the substrate bulk. This problem can be avoided to a certain extent by using substrates of different ionic strengths.

Figure 2 shows the isotherms of 9,10-DHOA spread on a NaCl substrate at concentrations from 0.01 to 3 M. It was found that the nature of the 9,10-DHOA monolayer greatly depends on the concentration of solute ions in the aqueous substrate. On a 3 M NaCl substrate, it was observed that there was clearly a transition state of 9,10-DHOA. In general, marked condensation effects in the presence of polyvalent ions, such as Ca^{+2} and Cd^{+2} ions,[17] are expected as well as pH values in the substrate. A similar behavior may be considered with a substrate containing a relatively high concentration of NaCl, which can reduce the electrostatic repulsion between the head groups, and thereby enhance the packing efficiency, resulting in the formation of a condensed or solid film.[18]

For a comparison, the π–A isotherms of the spread monolayers of hydroxylated octadecanoic acids (9-HOA, 9(10)-HOA and 9,10-DHOA) on 3 M NaCl are shown in Fig. 3. All of the isotherms on 3 M NaCl consist of three regions, described conventionally as: (a) a liquid-expended state, (b) an intermediate transition state, and (c) a liquid-condensed state at small molecular areas, except the gaseous phase. At extremely small areas, these hydroxylated fatty acids exhibit the condensed state (steep portion of the pressure-area curve). For example, the 9-HOA monolayer occupies a limiting area (A_{lim}) of 0.24 nm^2 molec.$^{-1}$, corresponding to the cohesion between the octadecyl chain for octadecanoic acid.[19] At a surface pressure of 12.35 mN m^{-1}, a change in dπ/dA occurs, and the head groups and chain-substituents rearrange to give a highly compressible intermediate transition state; this model was speculated to correspond to a bent conformation and a vertical conformation. These typical characteristics are seen in the π–A isotherms with 9(10)HOA and 9,10-DHOA on 3 M NaCl. Especially, it is apparent that the transition pressures are higher for 9,10-DHOA than for 9(10)HOA and 9-HOA. This can be explained by the fact that 9,10-DHOA with two hydroxy groups can withstand a greater pressure than others with a single hydroxy group be-

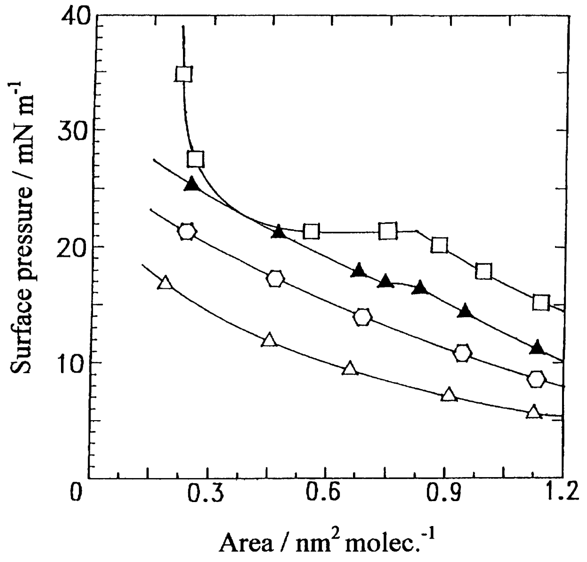

Fig. 2. π–A isotherms of 9,10-DHOA monolayer on 0.01 M NaCl (△), 1 M NaCl (○), 2 M NaCl (▲), and 3 M NaCl (□) substrates at 23 °C.

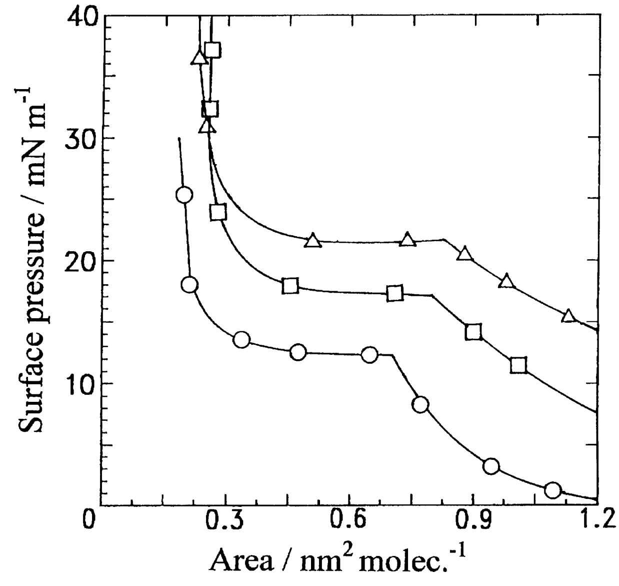

Fig. 3. π–A isotherms of 9-HOA (○), 9(10)HOA (□), and 9,10-DHOA (△) monolayers on 3 M NaCl substrates at 23 °C.

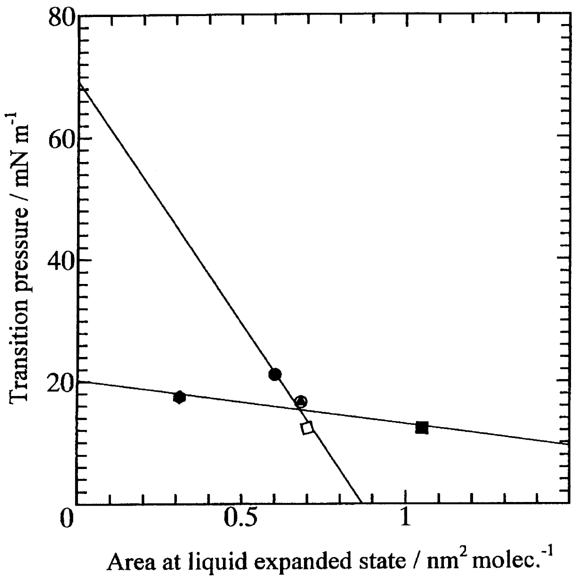

Fig. 4. Transition pressure (π_t) vs. area at liquid expanded state (A_E) of various hydroxylated fatty acid monolayers: (i) at different alkyl chain length: (●) 9-HTA, (○) 9-HHA, and (□) 9-HOA; and (ii) at the same alkyl chain length: (●) 3-HHA, (▲) 9-HHA, and (■) 16-HHA on 3 M NaCl substrates at 23 °C.

fore the hydroxy groups are forced out of the water during compression. In connection with above-mentioned conformation models, Umemura et al.[15] have recently reported an examination of the FTIR metal overlayer ATR spectra for LB films of 12-hydroxyoctadecanoic acid at various stages during compression. From the spectral intensity ratios of the symmetric CH_3 stretching band to the symmetric CH_2 stretching band, they have suggested that the average orientation of the methylene chains towards the surface normal upon compression is more pronounced in the solid condensed state than that in the plateau regions.

By employing the BM imaging method, Vollhardt et al.[16] have proposed, for 1-monostearoylrac-glycerol, that the transition state of the π–A isotherms (especially, "plateau" region) represents a first-order phase transition from a two-dimensional gaseous phase to a liquid-like condensed phase, where the formed domains increase preferentially in size rather than in number, finally reaching a tight packing with gaps in between. Such a spectroscopic and morphological study of the monolayer during compression may allow for new insights into the two-dimensional phase behavior of the monolayer, and provide reasons for reassessing the traditional designation of the π–A isotherm regions. In short, it can be shown that the exhibition of an intermediate region on an expended/condensed phase transition is closely associated with the flexibility of the hydrocarbon portion of the monolayer molecules intervening between the carboxy and hydroxy groups.

For the series of the hydroxy fatty acids studied, the surface pressure (π_t) at the transition point to the plateau region on different substrates is summarized in Table 1, together with the characteristic parameters (A_C, A_E and A_{lim}). These values were obtained using the method of Patino et al.[20] It can be seen from the A_C and A_E values that the 9-HOA and 9(10)-HOA monolayers on a NaCl substrate gave more expanded π–A curves than on acidic substrates. The same tendency was seen in the case of the octadecanoic acid spread on an alkaline substrate.[21]

As already stated, it should be emphasized that the observed π_t values for 9(10)HOA and 9,10-DHOA on 3 M NaCl substrates are relatively higher than the other hydroxy-substituted octadecanoic acid, depending on the location and number of hydroxy substituents along the chain. Apparently, 9,10-DHOA produces the largest pressure at the transition point, owing to an additional loop formation due to the ad-

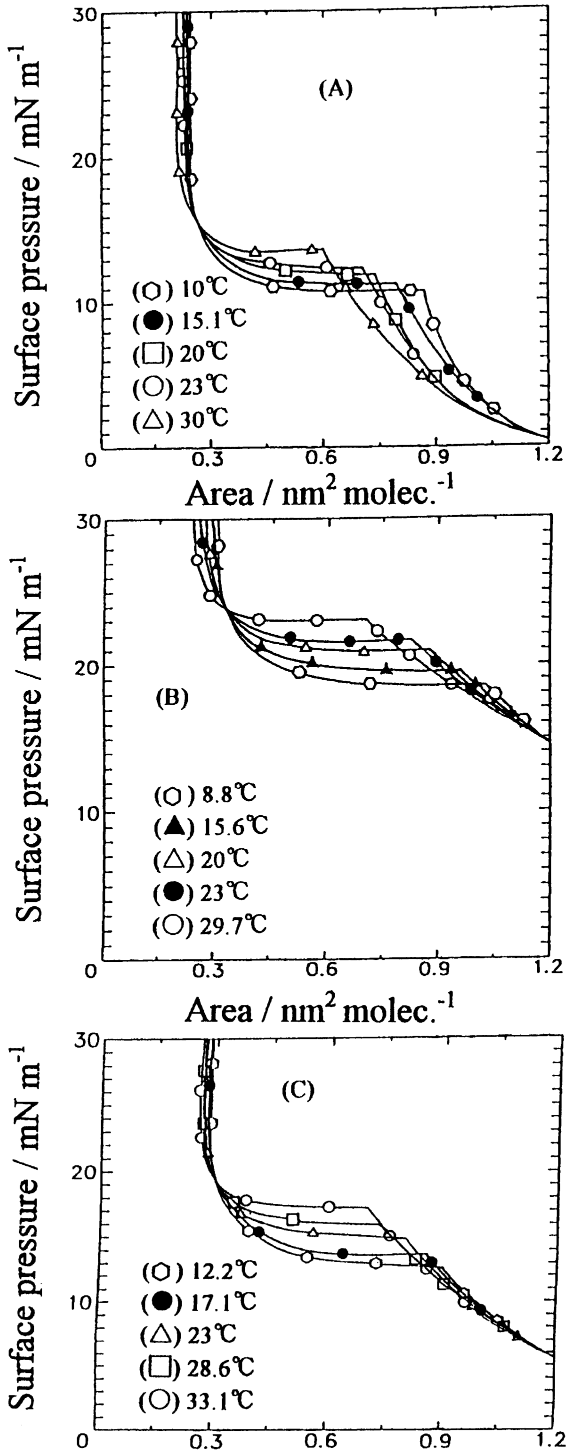

Fig. 5. π–A isotherms of (A) 9-HOA, (B) 9,10-DHOA, and (C) 9(10)HOA on 3 M NaCl substrates at different temperatures.

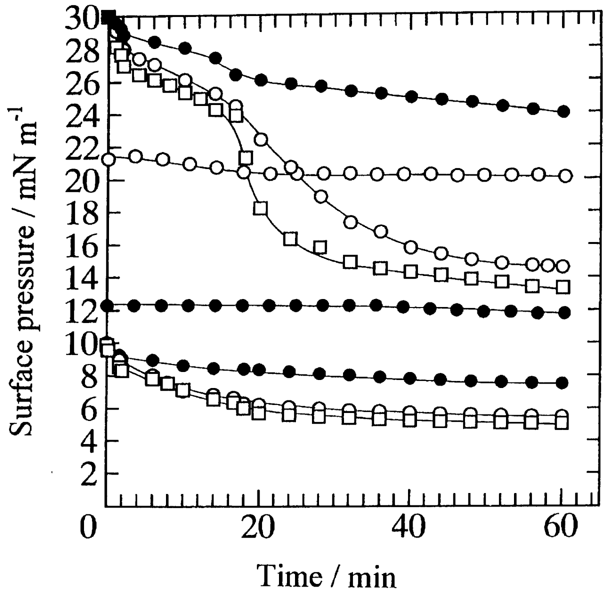

Fig. 6. Surface pressure relaxation curves after being compressed to a selected pressure on 3 M NaCl substrates at 23 °C: 9-HOA (●), 9(10)HOA (□), and 9,10-DHOA (○).

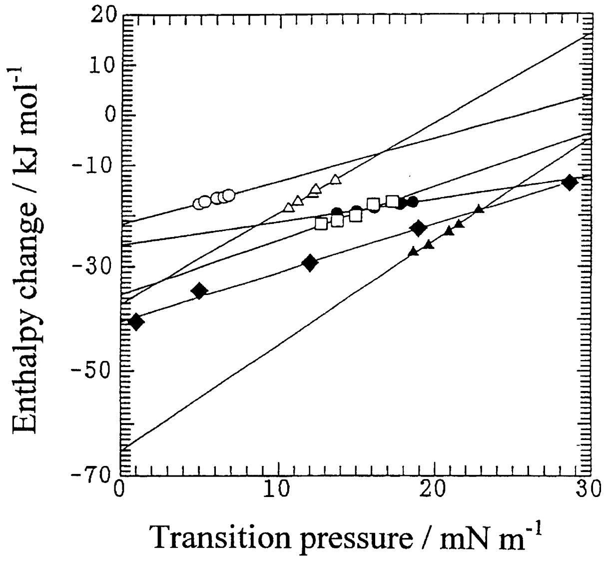

Fig. 7. Enthalpy change as a function of transition pressure. Hydroxylated fatty acids: 3-HHA (◆), 9-HHA (●), 9-HOA (△), 12-HOA (○), 9(10) HOA (□), and 9,10-DHOA (▲).

dition of an extra hydroxy group. One plausible explanation is that interactions of a polar group (–OH group) with an adjacent polar group (–COOH group) and water plays an important role in the expulsion of the hydroxy group.

Based on Table 1, the chain-length dependency of the surface pressure (π_t) at the plateau region as a function of the area per molecule (A_E), where the plateau region occurs for 9-hydroxylated fatty acids with different alkyl chains on 3 M NaCl, is shown in Fig. 4. For a comparison, the results for a series of hydroxyhexadecanoic acid homologs with a hydroxy at various positions are plotted in the figure. It can be seen that the value of π_t increases as A_E decreases, indicating the effect of the alkyl chain length. Simultaneously, the phase-transition behavior also depends on the number of methylene groups intervening between the hydroxy and carboxy groups.

Temperature Dependence of π–A Isotherms: The

π–A isotherms for six hydroxylated fatty acids (3-HHA, 9-HHA, 9-HOA, 12-HOA, 9(10)HOA, and 9,10-DHOA) were obtained over a range of temperatures from 8 to 33 °C. All of the isotherms showed a plateau typical of the π–A isotherm, which was previously ascribed to the first-order phase transition associated with a change in the monolayer conformation. As typical isotherms, the temperature dependence of the π–A isotherms for 9-HOA, 9, 10-DHOA, and 9(10)HOA on 3 M NaCl is shown in Fig. 5, respectively. It can be seen from the figures that the plateau pressure varies linearly with the temperature. For 9,10-DHOA, the plateau pressure occurs at a higher pressure region, compared to that of the monohydroxy acids. If the monolayer is unstable, the thermodynamic equilibrium of the isotherms is questionable. In this regard, we examined the surface pressure relaxation after compression of the hydroxylated fatty acid monolayer.

Figure 6 depicts the surface pressure relaxation plots for 9-HOA, 9(10)HOA, and 9,10-DHOA on a 3 M NaCl substrate at 23 °C. Previously, two criteria for stability were proposed: Cadenhead[22] suggested that films decaying in the surface pressure at a rate of no more than 1.0 mN m^{-1} min^{-1} can be considered to be stable, while Gershfeld[23] proposed a criterion of 0.1 mN m^{-1} min^{-1}. According to these two suggestions, the surface pressures of 9-HOA, 9(10)HOA, and 9, 10-DHOA decrease less than 0.3 mN m^{-1} min^{-1} within the first 60 min, even when the compression is stopped at 30 mN m^{-1}. Moreover, the surface pressure relaxation is relatively low at a low surface pressure, such as 10 mN m^{-1}, relative to the pre-collapsed state, such as 30 mN m^{-1}. Indeed, the monolayer stability depends on the monolayer structure influenced by the monolayer film-substrate interaction or film molecule interactions.

In Fig. 6, it is noteworthy that the surface pressure relaxation is nearly constant at the phase-transition pressures, such as 21.5 mN m^{-1} for 9,10-DHOA and 12.3 mN m^{-1} for 9-HOA. Little change in the surface pressure was seen, even when the compression was halted for several hours. In this respect, the compression modes have a profound effect on the shape of the π–A isotherm.[12—14] Especially, the shape of the isotherm in the postcollapse region of the curve depends on both the compression rate and the chain length of the fatty acid. From recent studies with the use of various compression modes (continuous, stepwise and constant strain rate compression) it was found that the compression rates had little effect on the transition state.[24] These facts may suggest that hydroxylated fatty acids form stable films for structural relaxation in the "plateau" regions. Despite the information obtained concerning the hydroxylated fatty acids monolayers, some questions still remain. There exists a region above the ESP which can be characterised by monolayer relaxation at a constant surface pressure. In this range the monolayer is in a state of supersaturation, and the relaxation is due to a transformation of the monolayer material to overgrown three-dimensional structures, which are quantitatively described by nucleation-growth mechanisms.[25,26] Consequently, the monolayer tends to collapse into a bulk crystalline phase at the air–water interface. Ultimately, it may be important to note that all of the monolayers are probably metastable, and that the above-mentioned criteria are simply useful rules of reflection for comparing the relative stabilities. Therefore, it might be considered that all of the hydroxylated fatty acid monolayers are metastable on a 3 M NaCl substrate. To remove this defect, much attention is being paid to stabilizing the monolayer through gentle compression, which avoids the local surface overpressure responsible for defect formation during the early stages of monolayer compression. In recent studies, Kato et al.[12,13] have shown that, from the point of film relaxation behavior above the ESP values, the shape of the π–A isotherm is governed by the balance between the relaxation times of the molecular motions in a monolayer and the "time of observation", i.e., the reciprocal of the strain rate of monolayer compression.

Table 2. The Molar Internal Energy Changes on the Phase Transition of Various Hydroxylated Fatty Acids on 3 M NaCl Substrate at 23 °C (when $\pi_t = 0$)

Hydroxylated fatty acids	Molar internal energy $-\Delta E$/kJ mol^{-1}
3-HHA	40.5±0.5
9-HHA	25.9±0.2
9-HOA	37.3±0.1
12-HOA	21.8±0.1
9(10) HOA	35.5±0.4
9,10-DHOA	65.2±0.3

The presence of stability at the plateau region in the π–A isotherms studied allows us to assume that all of data in the plateau region accompanying the LE/LC transition, apart from those of the other regions, mean to be near to the equilibrium values. We have tried to analyze the thermodynamic parameters of the transition from LE to LC on 3 M NaCl substrates. The molar enthalpy change (ΔH_t) for the transition from LE to LC was calculated by applying the two-dimensional Clausius–Clapeyron equation[27] in the form of $d\pi_t/dT = \Delta H_t/T(A_E - A_C)$, where π_t is the plateau pressure at temperature T, A_E and A_C are the molecular areas in the liquid-expanded and liquid-condensed phases, respectively. In Fig. 7, the molar enthalpy change (ΔH_t) vs. the transition pressure (π_t) is plotted for various hydroxylated fatty acids. Moreover, by using the thermodynamic equation,[27] $\Delta H_t = \Delta E + \pi_t$, the molar internal energy for the transition (ΔE) was obtained for different hydroxylated fatty acids by extrapolation to $\pi_t = 0$. The obtained molar internal energy changes are given in Table 2. All of the ΔE values were negative, indicating energy generation during the transition from LE to LC. It is of interest to note that the value of ΔE for 9,10-DHOA is about two-times greater than that for 9-HOA. As pointed out by Tachibana et al.,[5] ΔE is considered to be the sum of the energy for breaking the hydrogen bonds between the hydroxy groups on the bent chains and the water molecules, and the cohesional energy between close-packed vertical chains.

As already mentioned, the recent structural examination for the 12-HOA monolayer studied by Umemura et al.[15] does not almost give concordant results, compared to conventional molecular conformation models for hydroxylated fatty acids proposed by Kellner and Cadenhead[3,4] and others.[1,5—8] In view of Umemura's results concerning the average conformation of the methylene chains in the plateau region, it should be emphasized that the observed ΔE value is strongly dependent on the energy for breaking the hydrogen bonds between the hydroxy groups on the bent chains and the water molecules, compared to the cohesional energy. It should also be mentioned that 9,10-DHOA and 9-HOA exhibit a large difference in energy, which is involved when the film molecules change their conformations from a bent chain to a vertical chain, detaching the hydroxy groups from the interface. This implies that a higher energy was generated by breaking the strong hydrogen bonds between the two polar hydroxy substituents of 9,10-DHOA and the water molecules in the transition state, resulting from an increase in the hydrophilicity by adding an extra polar-group substituent at the 10-position along the hydrocarbon chain of 9-HOA.

Comparing 9-HOA with 12-HOA, we observed that the ΔE values of 9-HOA are higher than those of 12-HOA. Similarly, we compared 3-HHA with 9-HHA. From this observation, the greater energy is generated for forcing out the hydroxy group along with a decreasing chain length between the hydroxy and carboxy groups. Moreover, a condensed film is only obtained when the hydroxy group is substituted at the 2-position. This effect can be explained based on hydrogen bonding between the hydroxy group of 2-HOA with the carbonyl oxygen of a neighbouring 2-HOA molecule, suggesting that the strength of the combined carboxy-hydroxy polar group is greater than that of a single carboxy group. As can be seen in 9-HHA and 9-HOA, the ΔE values become larger with increasing hydroxyl alkyl chain length when the hydroxy group is at the same position along the alkyl chain, indicating an appreciable interaction between the molecules in the more coherent monolayers. For 9(10)HOA, as a mixed monolayer of 9- and 10-HOA, although a plateau region in the π–A isotherm is present, the differences in the ΔE value are small relative to 9-HOA to give a tentative interpretation.

Effect of Urea and Sucrose: Further, the study was designed to examine the action of urea and sucrose solutions on the 9-HOA monolayer. The former is described as a "structure-breaker", meaning that the addition of urea to water decreases the degree of the water structure; the latter is considered to be a "structure-maker". The π–A isotherms of 9-HOA were recorded on HCl, urea and sucrose at different temperatures, ranging 8 and 30 °C. The concentration of urea and sucrose aqueous solutions was 0.1 M. All of the isotherms exhibit the same plateau region of the LE/LC phase transition, as already described in Fig. 5. The transition pressure increases with increasing temperature, followed by a lowering of the molecular area (A_E) values. In the condensed states, the isotherms completely overlap, or shift slightly toward higher area values, following the usual expected behavior of spread monolayers. By applying the two-dimensional Clausius–Clapeyron equation,[27] the temperature dependence of the molar enthalpy change (ΔH_t) for phase transitions in the monolayer state of 9-HOA was found (Fig. 8). In addition, the molar internal energy change (ΔE) was also calculated from the figure. The values of ΔE were -29.8 ± 0.1 kJ mol^{-1} for sucrose, -28.0 ± 0.3 kJ mol^{-1} for urea, compared with -27.3 ± 0.3 kJ mol^{-1} for the HCl substrate.

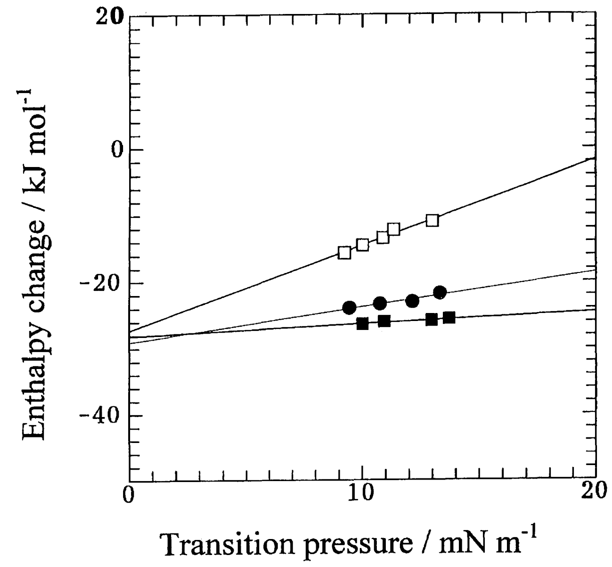

Fig. 8. Enthalpy change vs. transition pressure plot of 9-HOA monolayer spread on different substrates: 0.01 M HCl (□), 0.01 M HCl+0.1 M urea (■), and 0.01 M HCl+0.1 M sucrose (●).

The observed ΔE value of 9-HOA in the presence of the sucrose substrate is larger than those of 9-HOA on the HCl substrate, while, with urea, little difference was found for that of 9-HOA on HCl. Since sucrose contains many OH groups, it can strengthen the structure of water by means of an extended network of hydrogen bonds. It is thus suggested that the higher energy might be generated for breaking hydrogen bonds between the hydroxy groups on the bent chains and the water molecules during the LE/LC phase transition. In view of urea as a "structure-breaker", the urea in the substrate might change the phase transition, and, therefore, different effects are expected; this fact indicates a more expanded behavior of the 9-HOA monolayer, as already observed at high concentrations above 1 M urea.[28] With regards to the urea and sucrose studied, whether this phenomenon can be totally ascribed to a "structure-breaker" or to a "structure-maker" effect of the subphase induced either by urea or by sucrose, respectively, is still uncertain.

Equilibrium Spreading Pressure (ESP): Further insight concerning the stability behavior of hydroxylated fatty acids may be obtained by considering the equilibrium spreading pressure (π_e) at the air–water interface based on the respective crystalline solid forms. The ESP values on different substrates at 23 °C for the hydroxylated fatty acids used in this study are presented in Table 3. On the 3 M NaCl substrate, both a low ESP value and a high melting point were

Table 3. The Melting Point (Mp) and Equilibrium Spreading Pressure (ESP) of Various Hydroxylated Fatty Acids on Different Substrates at 23 °C

Hydroxylated fatty acids	Mp °C	Substrates	ESP (π_e) mN m^{-1}
3-HHA	82.5	3 M NaCl	17.0±0.3
9-HHA	81	3 M NaCl	15.4±0.2
16-HHA	98	3 M NaCl	1.1±0.4
9-HOA	83	0.01 M HCl	5.3±0.1
9-HOA		0.01 M H_2SO_4	5.8±0.1
9-HOA		3 M NaCl	8.9±0.3
12-HOA	81	0.01 M HCl	5.6±1.0
12-HOA		3 M NaCl	11.2±0.4
9(10)HOA	69.5	0.01 M HCl	9.3±0.3
9(10)HOA		0.01 M H_2SO_4	10.5±0.2
9(10)HOA		3 M NaCl	12.8±0.5
9,10-DHOA	93	0.01 M HCl	10.9±0.5
9,10-DHOA		0.01 M H_2SO_4	11.3±0.8
9,10-DHOA		3 M NaCl	17.1±1.0

obtained for 16-HHA, compared with other monohydroxylated fatty acids. This result had already been obtained by Kellner et al.,[3] and is in agreement with the experimental data. Since the two polar groups of 16-HHA are situated at a larger distance from each other than for 9-HHA, the 16-HHA monolayer deforms more easily than do those of 9-HHA, thus preventing the formation of an erect film conformation. If the ESP is taken as a measure of film stability, the bulk crystalline phase might be more stable for 16-HHA than for 9-HHA. In spite of the relatively high melting points, other hydroxylated fatty acids appear to have a great spreading ability due to their molecular chain–water interactions,[2] which indicate high ESP values different from that of 16-HHA. A further comparison of 9,10-DHOA with other monohydroxylated fatty acids is of interest. We have observed that the shift in the ESP value of 9,10-DHOA to a higher pressure value is very striking, except for 3-HHA. It is to be especially noted that the ESP value for 9,10-DHOA is twice as large as that of 9-HOA. This might be because the affinity between the polar group and the aqueous subphase is increased by the polarity due to the presence of the two hydroxy groups, resulting in an enhanced spreading pressure.

Based on an examination of the data in Table 3, the ESP values are relatively larger on the NaCl substrate than on acidified water substrates. Besides, the ESP values tend to decrease with increasing the alkyl chain length of the hydroxylated fatty acids, as noted in 9-HHA and 9-HOA, which is in agreement with Brooks and Alexander's findings for the fatty alcohols.[29] When the π_e and π_t data in Tables 1 and 3 are combined, it is found that π_t is always greater than π_e for all of the hydroxylated fatty acids used, except for 12-HOA on 3 M NaCl. In this connection, it has generally been suggested that, at least for hydroxylated fatty acids, π_t always occurs above π_e. The data for 12-HOA reveals a behavior which might be considered to be an exception to this statement. 12-HOA, despite the relatively high melting point, shows an ESP value which is quite high, and exhibits the transition pressure in the π–A curve below π_e. The abnormality of the LE/LC transition for 12-HOA is emphasized by a claim that this might be associated with the film stability. Thus, the film stability might be within the region where $\pi_t < \pi_e$.

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