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Fluorescence Microscopy Investigations of the Domain Formation of Fatty Acid Monolayers Induced by Polymeric Gegenions

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The monolayer behavior of long-chain fatty acids at the air/water interface on a poly(ethyleneimine)-containing subphase was investigated. The distinct influences of the polymer in the subphase on the isothermal behavior were documented: Due to the interaction with the polymeric gegenions the usually condensed fatty acid monolayers could be altered to expanded, compressible films. Their stability was also increased, as indicated by higher collapse pressures. The monolayer morphologies were studied intensively by fluorescence microscopy: The surface textures could be widely varied via the concentration of the polymeric gegenions in the subphase, the temperature, and the chain length of the fatty acid. As a result of the improved stability derived from complexation between the fatty acid monolayer and the dissolved polymer, the monolayers could be transferred, even from relative expanded states, from the air/water interface to solid substrates without damaging the original surface morphology.

Introduction

The phase states of long-chain fatty acids in two dimensions have been of interest since the pioneering work of Langmuir and Adam on insoluble monolayers at the air/water interface.[1-3] Besides the investigations on pure water, many studies have been concerned with the influence of subphase conditions, such as temperature, pH, or metal ions, on the surface pressure/area isotherms of these fatty acid monolayers.[4-6] The alteration of monolayers or the ability to achieve specific effects on monolayers (e.g., patching) is of high interest especially in connection with their application as biomembrane models.[7]

Phase states of lipids at the air/water interface and their transitions are observable via fluorescence microscopy, if the monolayer is doped with small amounts of fluorescent probes.[8-10] This technique permits the visualization of the aggregation phenomenon in the "two-phase" region and the study of the micromorphologies of solid domains in a fluid matrix.[11-13] Moreover, it allows one to study the influence of special conditions of the monolayer (e.g., impurities and additives[14,15]) and of the subphase (e.g., pH, electrolytes[16,17]) on the form and the growth of the alkyl platelets. The interaction of proteins

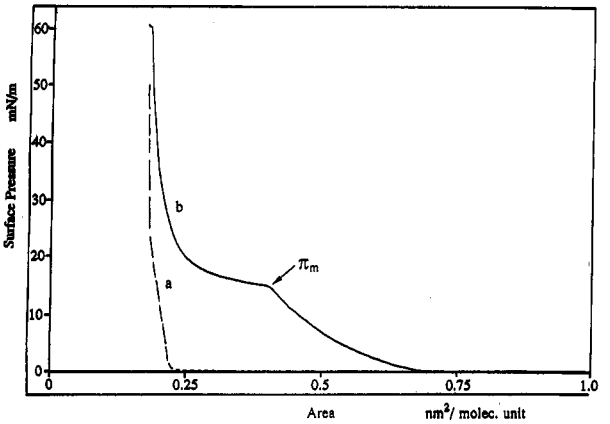

Figure 1. Surface pressure/area isotherms of stearic acid (**2**, $m = 16$) at temperature $T = 20$ °C on different aqueous subphases: (a) pure water and (b) 2.13×10^{-3} M PEI_{1800} containing water.

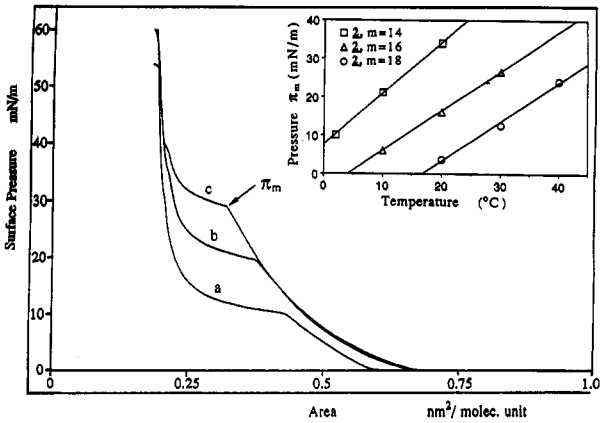

Figure 2. Surface pressure/area isotherms of palmitic acid (**2**, $m = 14$) on the 2.13×10^{-3} M PEI_{1800} containing aqueous subphase at different temperatures: (a) $T = 2$ °C, (b) $T = 10$ °C, and (c) $T = 20$ °C. The temperature dependence of main transition pressure π_m of fatty acid (**2**, $m = 14, 16, 18$) monolayers is shown in the inset.

(1) Langmuir, I. *J. Am. Chem. Soc.* **1917**, *39*, 1848.
(2) Langmuir, I. *Trans. Faraday Soc.* **1920**, *15*, 62.
(3) Adam, N. K. *Proc. R. Soc.* **1922**, *A101*, 516.
(4) Gaines, G. L., Jr. *Insoluble Monolayers at the Liquid-Gas Interfaces*; Wiley: New York, 1966.
(5) Patil, G. S.; Matthews, R. H.; Cornwell, D. G. In Monolayers; Goddard, E. D., Gould, R. F., Eds.; American Chemical Society: Washington DC, 1975.
(6) Adamson, A. W. *Physical Chemistry of Surfaces*, 4th ed.; Wiley: New York, 1982.
(7) Ahlers, M.; Müller, W.; Reichert, A.; Ringsdorf, H.; Venzmer, J. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1269.
(8) Tscharner, V.; McConnell, H. M. *Biophys. J.* **1981**, *36*, 409.
(9) Peters, R.; Beck, K. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 7183.
(10) Lösche, M.; Sackmann, E.; Möhwald, H. *Ber. Bunsenges. Phys. Chem.* **1983**, *87*, 848.
(11) Meller, P.; Peters, R.; Ringsdorf, H. *Colloid Polym. Sci.* **1989**, *267*, 97.
(12) Lösche, M.; Rabe, J.; Fischer, A.; Rucha, U.; Knoll, W.; Möhwald, H. *Thin Solid Films* **1984**, *117*, 269.
(13) McConnell, H. M.; Tamm, L. K.; Weis, R. M. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 3249.
(14) Lösche, M.; Möhwald, H. *Eur. Biophys. J.* **1984**, *11*, 35.
(15) Weis, R. M.; McConnell, H. M. *J. Phys. Chem.* **1985**, *89*, 4453.
(16) Eklund, K. K.; Vuorinen, J.; Mikkola, J.; Virtanen, J. A.; Kinnunen, P. K. J. *Biochemistry* **1988**, *27*, 3433.
(17) Helm, C. A.; Laxhuber, L.; Lösche, M.; Möhwald, H. *Colloid Polym. Sci.* **1986**, *264*, 46.

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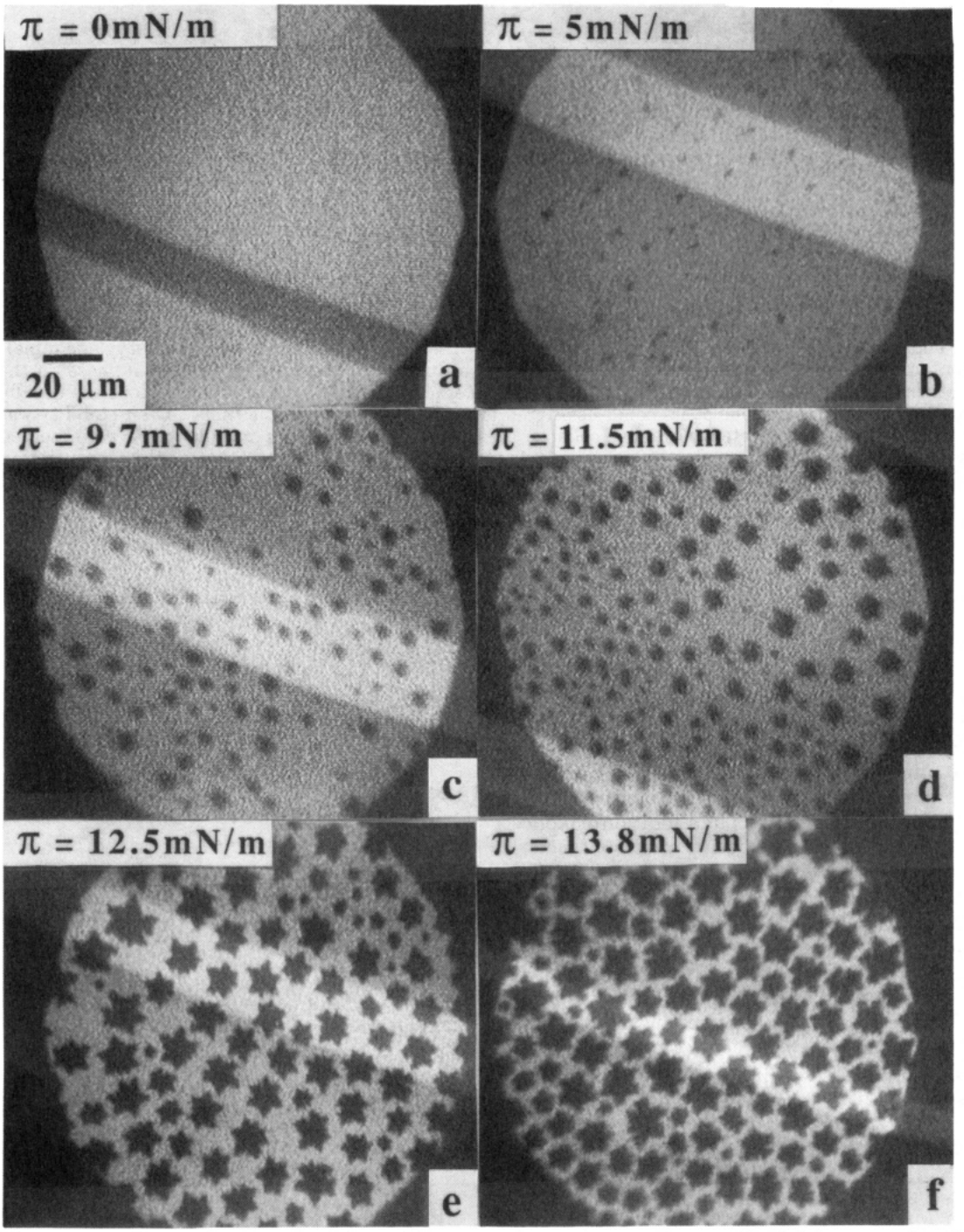

Figure 3. Fluorescence micrographs of an arachidic acid (**2**, m = 18) monolayer at T = 30 °C. Subphase contained PEI_{1800} at a concentration of 2.13 × 10^{-3} M. The surface pressure π was increased from 0 to 13.8 mN/m (upper left to lower right).

with coexisting fluid and solid membrane areas[18,19] has also been examined intensively in monolayers by this method.

The interaction of polymers with supramolecular structures such as monolayers, bilayers, or vesicles is of broad interest.[20-22] The influence of polymers, for example, polylysine on phospholipid model membranes, can provide clues as to the interaction of proteins with biomembranes.[23,24] Adsorbed polymers can stabilize lipid bilayers[25,26] as well as alter their properties.[20,27,28] Moreover, controlled polyelectrolyte interaction provides a powerful tool for the creation of amphiphilic supramolecular structures that are sensitive to environmental conditions such as temperature and pH.[29]

(18) Grainger, D. W.; Reichert, A.; Ringsdorf, H.; Salesse, C.; Davies, D. E.; Lloyd, J. B. *Biochim. Biophys. Acta* **1990**, *1022*, 146.
(19) Grainger, D. W.; Reichert, A.; Ringsdorf, H.; Salesse, C. *Biochim. Biophys. Acta* **1990**, *1023*, 365.
(20) Ringsdorf, H.; Schlarb, B.; Venzmer, J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 113.
(21) Sunamoto, J.; Iwamoto, K.; Takada, M.; Yuzuriha, T.; Katayama, K. In Polymers in Medicine; Chiellini, E., Giusti, P., Eds.; Plenum Press: New York, 1984; p 157.
(22) Sunamoto, J.; Sato, T.; Hirota, M.; Fukushima, K.; Hiratani, K.; Hara, K. *Biochim. Biophys. Acta* **1987**, *898*, 323.
(23) de Kruijff, B.; Rietveld, A.; Telders, N.; Vaandrager, B. *Biochim. Biophys. Acta* **1985**, *820*, 295.
(24) Laroche, G.; Carrier, D.; Pézolet, M. *Biochemistry* **1988**, *27*, 6220.
(25) Erdelen, C.; Laschewsky, A.; Ringsdorf, H.; Schneider, J.; Schuster, A. *Thin Solid Films* **1989**, *180*, 153.
(26) Shimomura, M.; Kunitake, T. *Thin Solid Films* **1985**, *132*, 243.
(27) Tirrell, D. A. *J. Controlled Release* **1987**, *6*, 15.
(28) Decher, G.; Kuchinka, E.; Ringsdorf, H.; Venzmer, J.; Bitter-Suermann, D.; Weisgerber, C. *Angew. Makromol. Chem.* **1989**, *166/167*, 71.

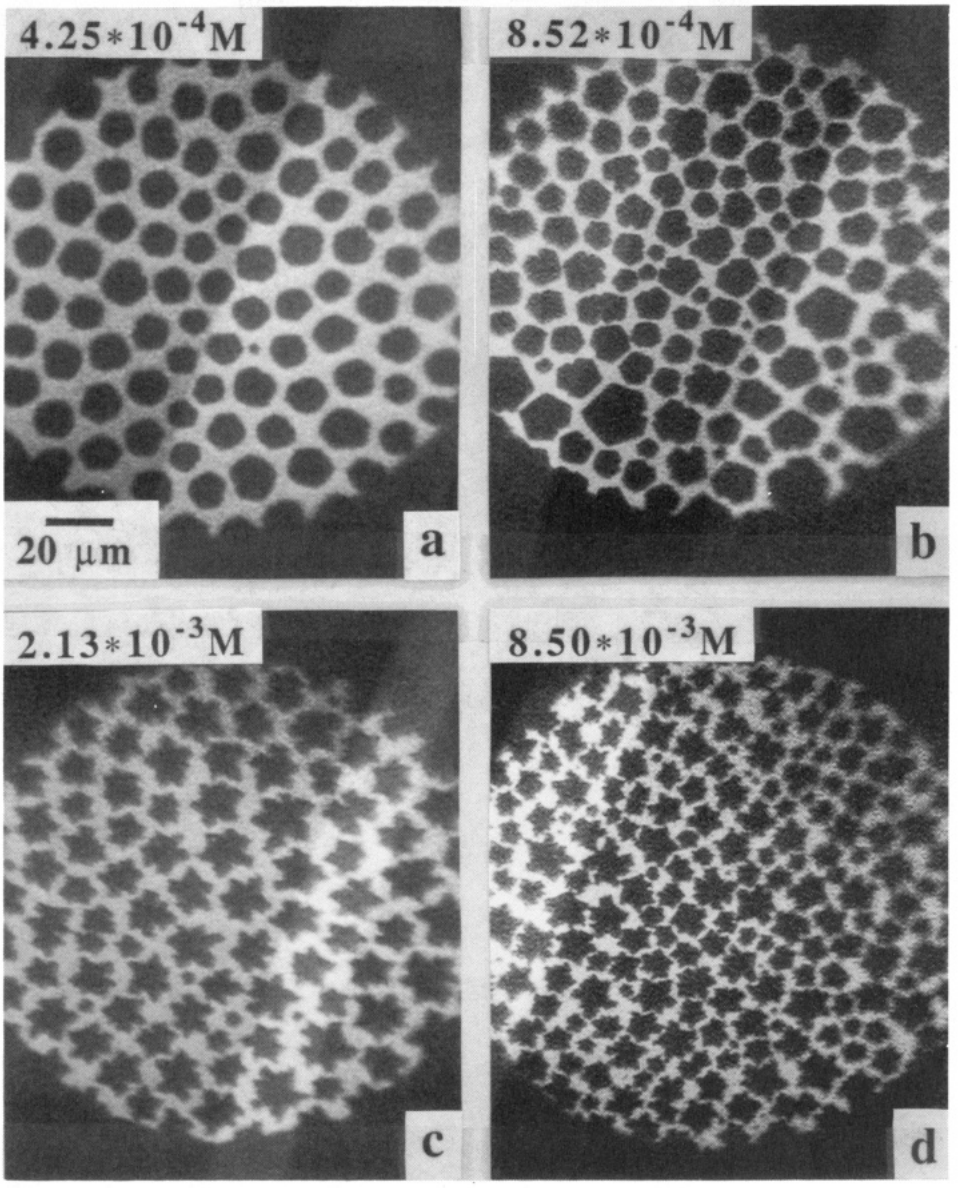

Figure 4. Fluorescence micrographs of arachidic acid (**2**, $m = 18$) monolayers for various PEI_{1800} concentrations in the subphase ($T = 30$ °C): (a) 4.25×10^{-4}, (b) 8.52×10^{-4}, (c) 2.13×10^{-3}, and (d) 8.50×10^{-3} M.

In this paper we describe monolayer experiments with long-chain fatty acids on aqueous subphases containing a water-soluble polymer. Branched poly(ethyleneimine) was used as the interacting polymeric gegenion in the subphase. Poly(ethyleneimine) can easily be modified[30] and shows strong effects on phospholipid bilayer assemblies.[31] Through the interaction with poly(ethyleneimine) **1** as

1 $n \simeq 42$

2 $m = 14, 16, 18, 20$

polymeric gegenion, the phase behavior of several long-chain fatty acids **2** at the air/water interface could be influenced distinctly. Their domain formation in the coexistence region could be studied via fluorescence microscopy[32-35] at room temperature. In addition, the temperature-dependent variation of the domain morphology was investigated and the transfer of these structured monolayers to solid supports was achieved.

Experimental Section

Materials. Fatty acids with different chain lengths (**2**; $m = 14, 16, 18, 20$) were purchased from Fluka AG and Aldrich. Their purities were checked by thin-layer chromatography. Branched poly(ethyleneimine) (99% purity) with an average molecular weight of $\bar{M}_n = 1800$ (PEI_{1800}) was purchased from Polysciences, Inc. For most experiments, a 2.13×10^{-3} M solution of PEI_{1800} was used as subphase for the monolayer experiments. Water used for all the experiments was thrice distilled and additionally passed through a Millipore filtration apparatus.

Isotherm Measurements. Measurements of surface pressure/area diagrams (π/A isotherms) were carried out with a home-designed trough[36] which was equipped with a Wilhelmy balance and controlled through an interfaced personal computer. The trough was thermostated to enable the measurements over a temperature range from 2 to 45 °C. The standard spreading

(29) Seki, K.; Tirrell, D. A. *Macromolecules* **1984**, *17*, 1692.
(30) Johnson, T. W.; Klotz, I. M. *Macromolecules* **1974**, *7*, 149.
(31) Takigawa, D. Y.; Tirrell, D. A. *Macromolecules* **1985**, *18*, 338.
(32) Moore, B. G.; Knobler, C. M.; Broseta, D.; Rondelez, F. *J. Chem. Soc., Faraday Trans.* **1986**, *82*, 1753.
(33) Moore, B. G.; Knobler, C. M.; Akamatsu, S.; Rondelez, F. *J. Phys. Chem.* **1990**, *94*, 4588.
(34) Naito, K.; Iwakiri, T.; Muira, A.; Azuma, M. *Langmuir* **1990**, *6*, 1309.
(35) Knobler, C. M. *Science* **1990**, *249*, 870.
(36) Albrecht, O. *Thin Solid Films* **1983**, *99*, 227.

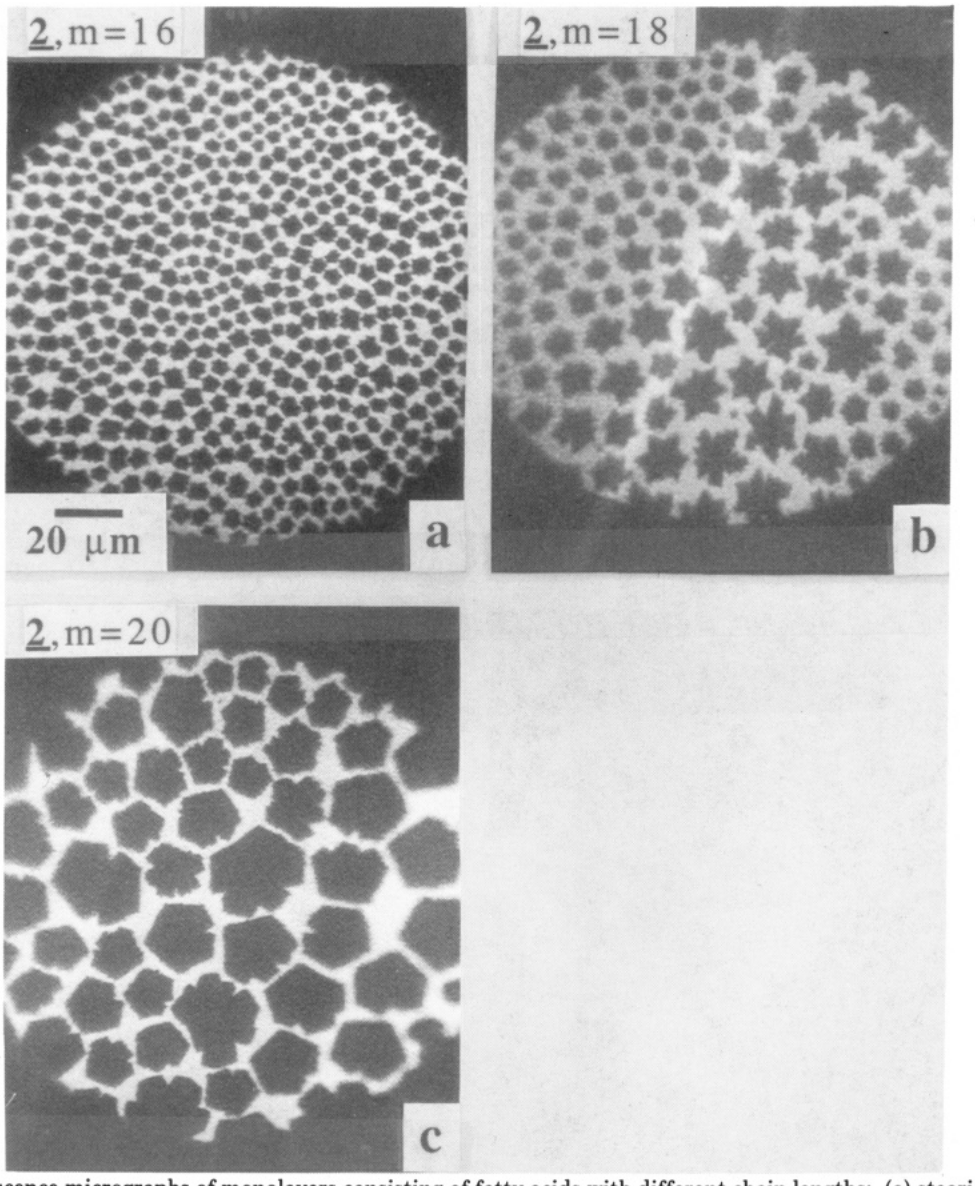

Figure 5. Fluorescence micrographs of monolayers consisting of fatty acids with different chain lengths: (a) stearic acid (**2**, m = 16, T = 16 °C), (b) arachidic acid (**2**, m = 18, T = 30 °C), and (c) behenic acid (**2**, m = 20, T = 35 °C). Subphase contained PEI_{1800} at a concentration of 2.13×10^{-3} M.

conditions were as follows: the chloroform solutions of fatty acid [$(1.2–1.9) \times 10^{-3}$ M] were spread with a microsyringe onto a surface area of 350 cm² and the films were then equilibrated for 15 min before compression. The monolayer area was reduced at a rate of 0.2 cm²/s. The PEI_{1800}-containing aqueous subphase did not show any intrinsic surface activity even at a 5 times higher concentration than that used in the experiments.

Fluorescence Microscopy of Monolayers. The fluorescence microscopy setup used in this laboratory contains a trough euipped with a Wilhelmy balance and a computer-controlled barrier system, a fluorescence microscope, and a video recording–displaying system. This setup has been described elsewhere.[37] The trough used for fluorescence microscopy measurements was also thermostated, which allowed a temperature range from 10 to 35 °C. In order to observe the monolayer by the fluorescence microscopy technique, 0.25 mol % sulforhodamine lipid was mixed with chloroform solutions of fatty acid. At this concentration, the effect of the fluorescence dye on the π/A isotherms of the fatty acids is negligible. After spreading, the films were allowed to equilibrate for 10 min before compression. The films were compressed at the rate of 0.07 cm²/s. The fluorescence micrographs shown here were taken from the video screen.

(37) Meller, P. *Rev. Sci. Instrum.* **1988**, *59*, 2225.

Results and Discussion

Influence of PEI on the π/A Isotherms of Fatty Acids. Fatty acids (**2**, $m \geq 12$) are able to form stable monolayers at the air/water interface. A "classical" π/A isotherm of a fatty acid, e.g., stearic acid (**2**, m = 16) at 20 °C, is shown in Figure 1, curve a, with pure water as the subphase. With 2.13×10^{-3} M PEI_{1800} dissolved in the aqueous subphase, the π/A isotherm of stearic acid (**2**, m = 16) is drastically changed (Figure 1, curve b). The form of the isotherm is changed from an incompressible monolayer to an expanded one with an onset of first pressure increase at relative wide surface areas. In this special case the onset is altered from 0.25 to 0.70 nm²/molecule. Moreover, in contrast to the phase transitions normally occurring for stearic acid at the air/water interface at this temperature (gas/solid, solid/solid), the monolayer undergoes an additional fluid/solid phase transition. Furthermore, the collapse pressure of the monolayer is raised compared with that on pure water, showing elevated stability of the monolayer on the PEI_{1800}-containing water subphase.

A striking feature indicated by these isotherms is the

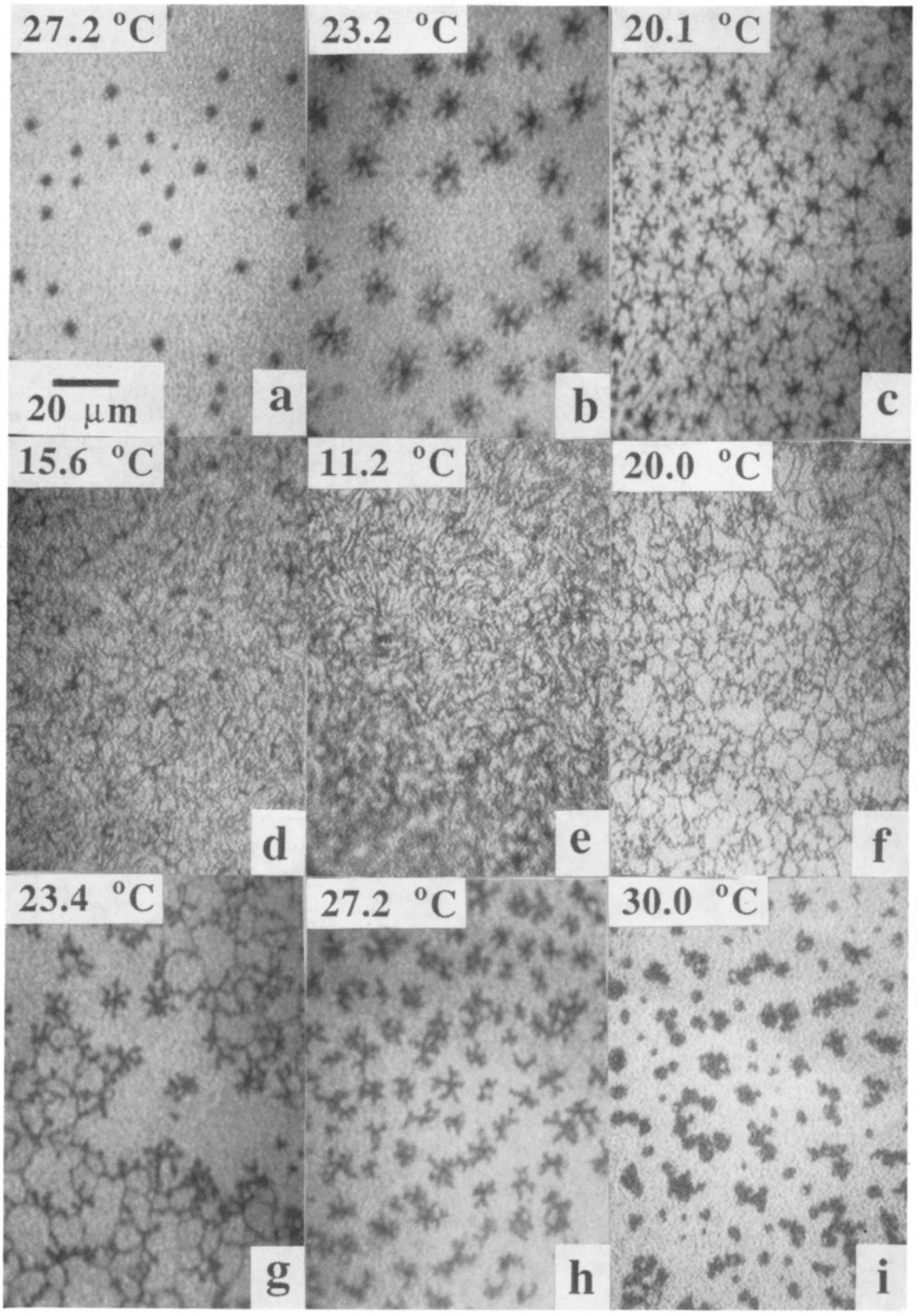

Figure 6. Fluorescence micrographs of an arachidic acid (**2**, $m = 18$) monolayer at various stages during a temperature cycle. Subphase contained PEI_{1800} at a concentration of 2.13×10^{-3} M.

increased stability of the monolayer on the one hand, combined with increased fluidity of the monolayer on the other hand. The salt formation of the polymeric amine in the aqueous subphase and the acidic monolayer leads to this unusual monolayer behavior. Variations of stability and fluidity are usually opposed:[5] metal ions (e.g., Cd^{2+}) are able to stabilize fatty acid monolayers, but the films become more condensed.

The temperature dependence of such a fatty acid π/A isotherm on a 2.13×10^{-3} M PEI_{1800} containing subphase is shown for palmitic acid (**2**, $m = 14$) in Figure 2. In this case the fluid/solid coexistence region is observable even at temperatures as low as 2 °C (A comparable π/A isotherm on a pure water subphase for this acid is only observed at 34 °C[38]). The temperature dependence of the monolayer is as expected: With increasing temperature the onset of the gas/fluid phase transition shifts to wider molecular areas, the fluid-expanded phase is enlarged, the fluid/solid coexistence region is shortened, and the collapse pressure slightly declines. In the inset of Figure 2 the temperature dependence of the main transition pressure π_m of **2** ($m = 14, 16, 18$) is displayed. A closer inspection of the isotherms within the solid-state region shows a relative high lateral compressibility at a certain molecular area, which may indicate another phase transition. This presumable phase change is accentuated with in-

(38) Fischer, A.; Sackmann, E. *J. Colloid Interface Sci.* **1986**, *112*, 1.

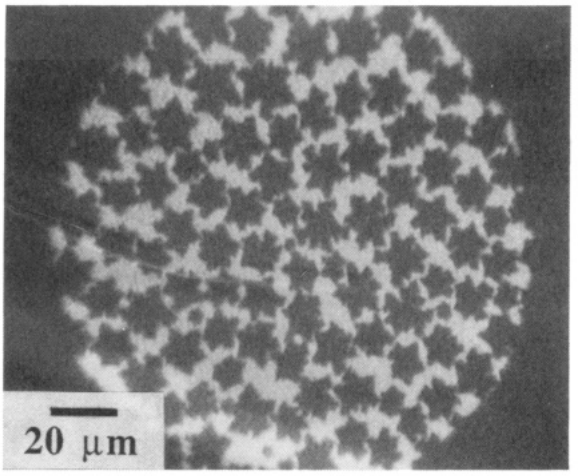

Figure 7. Fluorescence micrograph of an arachidic acid (**2**, m = 18) monolayer on a solid support (mica). The transfer was done by LB technique with one upstroke out of the coexistence region (T = 30 °C, π = 13.8 mN/m, speed of transfer 0.02 cm/s).

creasing temperature and could be analogous to the first-order solid/solid phase transition observed on pure water.[38]

Further investigations, using different average molecular weights of the poly(ethyleneimine), with altered pH of the subphase, and especially with chemically modified poly(ethyleneimine) systems, are in progress. First experiments indicate that the monolayer behavior of long-chain amines with anionic polyacids (e.g., polyacrylic acid) in the subphase shows analogous properties, e.g., defined coexistence regions.

Influence of PEI on the Surface Texture of Fatty Acid Monolayers Observed by Fluorescence Microscopy. As mentioned above, the interaction of the dissolved polymer in the aqueous subphase causes expanded states of fatty acids with different chain lengths at room temperature. Observation of the coexistence region via fluorescence microscopy reveals the presence of domains. Figure 3a–f shows the fluorescence microscopic observation of arachidic acid (**2**, m = 18) at 30 °C at different surface pressures. Standard PEI_{1800} water solution (2.13×10^{-3} M) was used as the subphase. During the fluid-analogue state a totally homogeneous monolayer is observed (Figure 3a). At the beginning of the fluid/solid phase transition the formation of tiny dark spots starts (Figure 3b). Further increase in pressure within the plateau leads to larger domains in the fluid matrix (Figure 3c–e). At the onset of the fluid/solid phase transition, the domain size stays constant (Figure 3f).

These observations on fatty acid monolayers resemble those found for phospholipids.[39] The recently published epifluorescence data for pentadecanoic acid[33,35] agrees with these results as well.

The concentration influence of PEI_{1800} in the aqueous subphase on the fatty acid (**2**, m = 18) monolayer morphology was examined at 30 °C. The lowest PEI_{1800} concentration necessary to get expanded states was found to be 4.25×10^{-4} M. With different amounts of PEI_{1800} in the subphase, the surface texture of an arachidic acid (**2**, m = 18) monolayer could be modified. The average size of the domains is not much altered when the concentration of PEI_{1800} is increased from 4.25×10^{-4} to 2.13×10^{-3} M, but the domains change their morphology from a "round" form to a "starlike" form (Figure 4a–c). Further increase in concentration of PEI_{1800} (8.5×10^{-3} M) leads to smaller "starlike" structures (Figure 4d).

Another observation worth mentioning is that the domain size of the fatty acid aggregates strongly depends on the length of the alkyl chain, as shown in Figure 5a–c. With temperature adjusted so that each fatty acid has the same transition pressure π_m (e.g., 30 °C for **2**, m = 18, and 16 °C for **2**, m = 16, result in $\pi_m \sim 10$ mN/m), the fatty acids with longer chain lengths are seen to form larger domains.

Temperature-Dependent Variation of Surface Texture of Fatty Acid Monolayers. The influence of temperature on the observed domain morphologies further enhances the possibilities of manipulating monolayers via polymeric gegenions.

Two typical domain morphologies of the fatty acids in the PEI-extended coexistence region were found: round domains (Figure 3c; **2**, m = 18) at high temperature (30 °C) and fiberlike domains (Figure 6e; **2**, m = 18) at low temperature (11 °C). At intermediate temperatures, starlike domains were observed.

Especially interesting is that these morphologies can be transformed into each other by temperature variation. This process can be followed directly via fluorescence microscopy. The change of the surface texture of an arachidic acid monolayer with temperature is shown in Figure 6a–i. The textures observed upon temperature decrease follow a distinct and repeatable pattern, and this approximate sequence of morphologies is passed through in reverse order upon temperature increase. Starting with round solid domains in the fluid matrix (Figure 6a), these compact forms disrupt with decreasing temperature and alter to snowflake forms (Figure 6b). With decreasing temperature these snowflake-like structures prolong their arms to strips and decrease in size (Figure 6c,d). The strips get longer and thinner with ongoing temperature decrease. This process continues until only tiny dark spots within a fiber matrix remain (Figure 6e). The system passes through approximately the same stages upon temperature increase until finally the almost round domains in a fluid matrix are re-formed (Figure 6f–i). The whole process takes ~4 h. To reach the thermal equilibrium, 15–30 min is needed for each step during the cooling process and 5–15 min for the heating process. In addition, the monolayer was annealed for 15 min at each step.

Similar observations have been documented for phospholipid monolayers.[39] The equilibrium shapes of phospholipid domains are apparently determined in part by a competition between electrostatic repulsion of lipid molecules and line tension of the fluid/solid boundary.[40,41]

Transfer of Fatty Acid Domains to Solid Supports. In addition to the monolayer experiments on water it could be shown that monolayers of long-chain fatty acids complexed to polymeric gegenions can be transferred to a solid support without destroying the defined surface texture.

Due to the improved monolayer stability (resulting from the interaction with PEI_{1800}), monolayers can be transferred from the air/water interface to the solid substrates by various methods, at various temperatures, and at various surface pressures without disturbing the corresponding domain shapes. Figure 7 shows one example for a fatty acid monolayer (**2**, m = 18) that has been transferred to a solid support (here MICA) via the LB technique using one upstroke out of the coexistence region, where the domains just start to touch each other (T = 30 °C, π = 13.8 mN/m, speed of transfer 0.02 cm/s). The comparison between the corresponding systems at the air/water

(39) Möhwald, H. *Thin Solid Films* **1988**, *159*, 1.

(40) Heckl, W. M.; Cadenhead, D. A.; Möhwald, H. *Langmuir* **1988**, *4*, 1352.

(41) McConnell, H. M.; Moy, V. T. *J. Phys. Chem.* **1988**, *92*, 4520.

interface (see Figure 3f) and at the air/substrate interface (see Figure 7) shows that after transfer the domains are not altered in form. In addition, the systematic temperature dependent variability of the monolayer morphology, which was observed at the air/water interface, is maintained. This apparent mobility of the supported monolayer must result from the existence of an adsorbed and mobile polymer layer immediately adjacent to the solid support. Fluorescence microscopic investigations of these transferred systems and their micromorphological variations, as well as investigations using plasmon spectroscopy and electron microscopy, are in progress.[42]

Conclusions

Adsorbed polymeric gegenions were found to change the structure of supramolecular assemblies of fatty acids in a distinct way. This may be of special interest since the adjustable monolayer structures were quite varied in terms of morphology. First studies indicated that these systems can be transferred from the air/water interface, even in very expanded states, to different solid substrates without damage to the surface texture. LB films, which possess a certain degree of mobility, while maintaining a specified inter- and intralayer structure, may serve as interesting materials for integrated optics and microelectronics devices.[43] Furthermore, supported mono- and bilayers that exhibit a degree of mobility could serve as anchors for various membrane proteins.

Registry No. Polyethyleneimine, 9002-98-6; palmitic acid, 57-10-3; stearic acid, 57-11-4; arachidic acid, 506-30-9; behenic acid, 112-85-6.

(42) Chi, L. F.; Johnston, R. R.; Kimizuka, N.; Kunitake, T.; Ringsdorf, H. *Langmuir*, submitted.

(43) Tieke, B. *Adv. Mater.* **1990**, *2*, 222.