Atomic Force Microscope Observation of Monolayers of Arachidic Acid, Octadecyldimethylamine Oxide, and Their Mixtures

Osamu Mori and Toyoko Imae*

Department of Chemistry, Faculty of Science, Nagoya University, Chikusa, Nagoya 464, Japan

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 $Monolayers of a rachidic acid, octade cyldimethylamine oxide (C_{18}DAO), and their mixtures were prepared to the state of the state$ at a surface pressure of 25 mN m⁻¹ on a mica substrate by the Langmuir-Blodgett method and were observed by use of an atomic force microscope (AFM). Arachidic acid molecules in monolayer arranged with molecular axes vertical to the mica substrate. In contrast to it, C₁₈DAO molecules in monolayer folded on the substrate. For the equimolar mixture of arachidic acid and C_{18} DAO, molecules were vertical in a part of the monolayer and folded in the other part. When the C_{18} DAO monolayer was aged at 25 mN m⁻¹, molecules were rearranged with vertical molecular axes. Molecular ordering in arachidic acid and aged C_{18} DAO monolayers was compared at a nanometer scale. Arachidic acid molecules ordered with a monoclinic unit lattice, whereas AFM could not detect a regular arrangement of C₁₈DAO molecules.

Introduction

Monolayers developed on an air-water interface form gas, liquid, and solid films, which are distinguished by measuring a surface pressure-surface area $(\pi - A)$ isotherm curve. Although many $\pi - A$ isotherms of amphiphilic monolayers were investigated, molecular arrangements in monolayers were estimated only speculatively. The scanning probe microscope (SPM) is a powerful tool to obtain the information for the molecular arrangement and orientation at an atomic order. Some workers have reported scanning tunneling microscope (STM) and atomic force microscope (AFM) images for Langmuir-Blodgett (LB) films and adsorbed monolayers of amphiphiles such as fatty acids,¹⁻⁹ dialkyldimethylammonium salts,¹⁰ lipids,¹¹ alkylcyanobiphenyl,¹² and perfluorocarbon surfactants.¹³ Arachidic acid $(CH_3(CH_2)_{18}COOH, AA)$ is a typical fatty acid with a saturated hydrocarbon chain, and its monolayer has a rather narrow occupied area² on water surface. Fatty acids partly protonated are associated into vesicles with bilayer membranes in water.^{14,15} Octadecyldimethylamine oxide (CH₃(CH₂)₁₇NO(CH₃)₂, C₁₈DAO) is a weak-base amphiphile insoluble in water at room

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Figure 1. π -A isotherm curves for monolayers of arachidic acid, C₁₈DAO, and their mixtures at 25 °C. Numerical values inserted in the figure mean the mixing molar ratio of [AA]: $[C_{18}DAO].$

temperature. Once C₁₈DAO is dissolved in water at high temperature, vesicles are formed at high temperature, and those are transformed into lamellar layers in the solution cooled down to room temperature.¹⁶⁻¹⁸ Although some characteristics of alkyldimethylamine oxides in solutions were reported, ¹⁹⁻²² the LB films have never been investigated.

In this work, π -A isotherm curves of arachidic acid, C_{18} DAO, and their mixtures are examined. Their monolayers are prepared on a mica substrate by the LB method and observed by AFM. Molecular arrangements of amphiphiles in monolayers are compared and discussed.

Experimental Section

Arachidic acid, chloroform, and standardized HCl and NaOH solutions were commercial products. $C_{18}DAO$ was a same sample as previously used.^{16-18,21}

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Figure 2. pH dependence of π -A isotherm curves for C₁₈DAO monolayers at 25 °C.

Surface pressure measurement and monolayer preparation were carried out on a LB film deposition apparatus (Nippon Laser & Electronics Lab.). Chloroform solutions of arachidic acid, $C_{18}DAO$, and their mixtures at concentrations of 10^{-3} g cm⁻³ or less were spread on water subphase at 25 °C, at which arachidic acid and $C_{18}DAO$ are insoluble in water. Surface pressure was measured under continuous compression at a rate of 10 mm min⁻¹. Monolayers were transferred onto a freshly cleaved mica substrate by the upstroke mode of the vertical dipping method at a surface pressure of 5 or 25 mN m⁻¹. Then terminal methyl groups are arranged on the monolayer surface. Monolayers were dried in vacuo for more than 3 h.

AFM was observed at room temperature with an AFM Nanoscope III (Digital Instruments, Inc.) in air and in a water-filled liquid cell. A silicon nitride tip on a cantilever with a spring constant of 0.12 or 0.38 N m⁻¹ was used. Images were recorded by the contact mode at the constant-height method. AFM was observed for two and more specimens prepared separately at the same condition, and reproducible images were obtained. Vertical distances and surface roughness of monolayer were measured from section analysis.

Results

 π -A Isotherm Curve. Surface pressures, π , of monolayers of arachidic acid, C₁₈DAO, and their mixtures on water subphase at pH 6.5 are plotted as a function of surface area A in Figure 1. The π -A isotherm curve was very sharp for arachidic acid, indicating the formation of monolayer in solid state through liquid state. The similar curve was also observed for the monolayer of arachidic acid and C₁₈DAO mixed at equimolar ratio. However, the π -A curve was expanded when the mixing ratio of C₁₈DAO was increased. The expansion was most remarkable for the monolayer of pure C₁₈DAO. It is apparent from the π -A curve that monolayers of C₁₈DAO and C₁₈DAO-rich mixtures collapsed at the liquid state without the formation of the solid film.

The occupied area, A_{iso} , per amphiphilic molecule in monolayer was evaluated from the extrapolation of the straight line at low surface areas in the π -A curve. The



Figure 3. AFM images for LB films of monolayers of arachidic acid, C_{18} DAO, and their equimolar mixture at 500 × 500 nm² area scan: (a) arachidic acid; (b) equimolar mixture; (c, d) C_{18} DAO; (a-c) 25 mN m⁻¹; (d) 5 mN m⁻¹.



Figure 4. AFM images and the section profile for a LB film of an aged C18DAO monolayer prepared at 25 mN m⁻¹.

values are listed in Table 1 with the collapse pressure π_{col} and the transition pressure π_{l-s} from liquid film to solid film. The occupied area for an equimolar mixture of arachidic acid and C₁₈DAO was slightly larger than that for arachidic acid. The collapse pressure decreased with increasing the mixing ratio of C₁₈DAO.

It is suggested that hydrophilic head groups of $C_{18}DAO$ are arranged loosely in monolayers, different from the compact arrangement of arachidic acid. The compact arrangement seems to be maintained even at the equimolar mixing of $C_{18}DAO$ molecules but is defeated with the excess addition of $C_{18}DAO$.

Figure 2 shows the pH dependence of π -A isotherm curves for C₁₈DAO monolayers. pH of water subphase in a trough was adjusted by adding HCl or NaOH solution. The pH was varied from neutral (pH 6.5) to acid (pH 4) and alkaline (pH 10). The profiles of the π -A curves were very similar, indicating the similarity in the arrangement of head groups in C₁₈DAO monolayers. The π -A curves, in particular, had no significant difference at pH 6.5 and 10, which are higher than the pK of alkyldimethylamine oxides.^{19,20} The π -A curve at pH 6.5 did not vary by the decrease of temperature down to 10 °C. The π -A curve at pH 4 was rather less expanded. Protonated C₁₈DAO molecules may be soluble in water.

AFM Images of Monolayers. LB films of monolayers were prepared at 25 mN m⁻¹ on water subphase at pH 6.5 and examined by AFM. Figure 3a gives the AFM image of arachidic acid monolayer at wide area scan (500×500 nm²). The smooth surface with a height difference less than 0.5 Å was observed. The monolayer had some holes, and the section analysis displayed an average vertical distance of 28 Å from the monolayer surface to the hole bottom. This value was in good agreement with the expected molecular length of arachidic acid (~ 27 Å) and the reported monolayer thickness for cadmium arachidate,^{2,3,5} indicating the molecular orientation vertical to the mica substrate. The occurrence of holes implys the

Table 1. Characteristics of π -A Isotherm Curves of Arachidic Acid, C₁₈DAO, and Their Mixtures

AA:C ₁₈ DAO	${mM m^{-1}}$	${m}_{1-s}^{\pi_{1-s}},$	$A_{ m iso},$ Å 2 molecule $^{-1}$		
1:0	70	32	19.6		
1:1	60	17	21.9		
1:2	59				
1:4	52				
0:1	48				

deficit of membrane, because the monolayer was prepared at the surface pressure lower than the transition pressure from liquid film to solid one which was listed in Table 1.

An AFM photograph for a LB film of equimolar-mixed monolayer of arachidic acid and $C_{18}DAO$ at 25 mN m⁻¹ is shown in Figure 3b. The monolayer surface with a height difference of 3–10 Å was rougher than that of arachidic acid. This height is larger than the difference between molecular lengths of arachidic acid and $C_{18}DAO$, suggesting the heterogeneous molecular arrangement of extended and poorly extended alkyl chains. This result is consistent with an occupied area per molecule in the equimolar-mixed monolayer larger than that in the homogeneous arachidic acid monolayer, as mentioned above. There was no hole in the equimolar-mixed monolayer, as expected for the monolayer in the solid state region.

The surface with a 2–5 Å height difference was observed for the homogeneous C₁₈DAO monolayer prepared at 25 mN m⁻¹ (Figure 3c). The poor packing of molecules on a mica substrate is supported by the π -A curve. For the C₁₈DAO monolayer at 5 mN m⁻¹ shown in Figure 3d, the roughness was less in vertical direction but more in horizontal direction. This means that molecules are lying on a mica with the wider packing area per molecule in accord with the wider occupied area on the air-water interface.



Figure 5. Top-view presentation of AFM images for a mica substrate and LB films of an arachidic acid monolayer and an aged $C_{18}DAO$ monolayer at $8 \times 8 \text{ nm}^2$ area scan: (upper) mica substrate; (middle) arachidic acid monolayer; (lower) $C_{18}DAO$ monolayer. The inserted figures are the corresponding 2D-FFT spectra in the reciprocal space of the AFM images.

The C₁₈DAO monolayer on water subphase diminished its surface area with time, during keeping a constant surface pressure of 25 mN m⁻¹. After being aged for 3 h until the decrease of surface area was not detectable, the monolayer was transferred on a mica substrate. The aged monolayer drastically varied from the nonaged one, as seen in the photograph at $5 \times 5 \,\mu\text{m}^2$ scale in Figure 4. There was the smooth surface over the wide region besides the original rough surface. The maximum height difference of ~10 Å between the smooth and rough surfaces was evaluated from the section profile in Figure 4. The AFM photograph for the smooth surface of aged C₁₈DAO monolayer was taken at 500 × 500 nm² scale, as shown in Figure 4. The monolayer with some holes seems to be comparable to the arachidic acid monolayer.

When the scanning by a strong atomic force was repeated, the monolayer was damaged by a tip, and a square hole of the scanning size was artificially created. Through such a procedure, the distance from mica substrate to monolayer surface was determined as 27 Å for the smooth surface of aged C_{18} DAO monolayer. This



C18DAO (5mN/m)



C18DAO (25mN/m)



C18DAO (25mN/m, aged)



Figure 6. Schematic representation of the molecular arrangement in LB films of arachidic acid and C_{18} DAO monolayers on mica substrate.



Figure 7. Schematic representation of the aging process for the molecular arrangement in $C_{18}DAO$ monolayer on water subphase.

height corresponds to the molecular length of C_{18} DAO.

Molecular Regularity in Monolayers. The comparison between smooth surfaces of arachidic acid and aged C₁₈DAO monolayers was carried out on AFM photographs at 8×8 nm² scale, as shown by the top-view presentation in Figure 5, in which an AFM photograph of mica surface is also included. Atoms on a freshly cleaved mica surface were periodically arranged, as previously reported.²³ The arrangement of methyl groups of arachidic acids in monolayer was also periodic, although the AFM image of C₁₈DAO monolayer did not provide any order pattern.

The difference of order pattern among mica atoms, arachidic acid molecules, and $C_{18}DAO$ molecules was demonstrated by two-dimensional fast Fourier transform (2D-FFT) spectra in the reciprocal space of the AFM images, as inserted in Figure 5. Whereas a spectrum for the mica surface displayed six spots corresponding to the

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fatty acid ^a	π , mN m ⁻¹	layer	lattice	unit cell distance, Å	$A_{ m AFM},{ m \AA}^2$	monolayer thickness, Å	ref
CdA ₂		4	rectangular or monoclinic	4.7, 5.2	24		1
CdA_2	30	2	hexagonal		20	28	2
CdA_2	35	1	hexagonal	5		28 - 33	3
CdA_2	32	3	hexagonal		18.1		4
		1	hexagonal				
BaA_2		1	disordered				5 - 7
MnA_2		1	centered rectangular	4.6, 8.7	20.0		
CdA_2	30	2-5	centered rectangular	4.8, 7.5	18.0	28	
CdA_2		1	disordered				
PbS_2		1	centered rectangular	4.5, 9.2	20.6		
CdL_2	30	5	centered rectangular	7.9 - 8.0, 4.6 - 4.9			8
CdB_2		5	centered rectangular	7.3-7.8, 4.7-4.9			
CdA_2		5	centered rectangular	7.3-7.6, 4.6-4.8			
CdS_2		5	centered rectangular	7.4 - 7.6, 4.5 - 4.7			
LA	5	1	hexagonal	4.3	21		9
SA	23	1	hexagonal	4.2			
AA	25	1	monoclinic ^b	4.9, 6.2	29.9	28	this work

Table 2.	Characteristics	of LB	Films	of Fatty	Acids	and	Their	Metal	Salts
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 a MA₂ (M = Ba, Mn, Cd), metal arachidate; PbS₂, lead stearate; CdF₂, cadmium salt of fatty acid; FA, fatty acid; L, lignoceric; B, behenic; A, arachidic; S, stearic. b The inclined angle is 77°.

hexagonal lattice, there were four spots in a 2D-FFT spectrum of the arachidic acid monolayer. The unit lattice of order pattern in the arachidic acid monolayer can be a monoclinic system with a tilt angle of 77° and unit cell lengths a = 4.9 Å and b = 6.2 Å, which were corrected for an atom-atom distance (5.3 Å²³) on a hexagonal lattice of mica. Then the packing area $A_{\rm AFM}$ per arachidic acid molecule was 29.9 Å² molecule⁻¹. The 2D-FFT spectrum of the C₁₈DAO monolayer gave no meaningful spots, as expected from the AFM image.

Discussion

Figure 6 illustrates the schematic representation of the molecular arrangement in monolayer on mica substrate. Arachidic acid molecules orient with molecular axis vertical to the mica substrate. Carboxylic acids interact through hydrogen bonding at neutral and acid pH.^{14,15} It is suggested that the strong interaction between hydrophilic head groups induces the well-organized arrangement of amphiphilic molecules on the two-dimensional area by the aid of the hydrophobic interaction between hydrocarbon chains.

In the equimolar-mixed monolayer of arachidic acid and $C_{18}DAO$, some amphiphilic molecules may be vertical to the mica substrate. The observed undulation of the monolayer surface is due to the folding of other alkyl chains in amphiphilic molecules. Therefore, the occupied area per molecule slightly increases in comparison with that of arachidic acid, as apparent from Table 1.

Since the occupied area of $C_{18}DAO$ molecule in a monolayer is large at the initial stage of the preparation, molecules must be lying in the monolayer. The lateral hydrophobic interaction allows alkyl chains to rearrange vertical to water surface through the aging time. Then the smooth monolayer surface is provided, although the regularity of molecules in a monolayer is less than that of arachidic acid. The serious difference of molecular arrangement in arachidic acid and C₁₈DAO monolayers should be caused from the hydration on an amine oxide head group. The head group is enveloped by eight hydrated water molecules.²⁴ In the initial stage of the preparation of monolayer, C₁₈DAO molecule and hydrated water molecules occupy the surface area. However, the hydrated water molecules are pulled into balk water as time passes, as illustrated in Figure 7. At the same time,

the occupied area per C_{18} DAO molecule decreases, and C_{18} DAO molecules are vertically rearranged.

It was estimated from the AFM image at nanometer scale that the packing area A_{AFM} of arachidic acid molecule in monolayer was 29.9 Å² molecule⁻¹. This value is larger than the values previously reported for metal arachidate $(18-24 \text{ Å}^2 \text{ molecule}^{-1})^{1,2,4,5,7}$ and lignoceric acid (21 Å² molecule⁻¹)⁹ with a longer alkyl chain. This suggests the nonclosed-packing of arachidic acid in a monolayer prepared in this work.

The previous AFM investigations for LB films of fatty acids are listed in Table 2. Mayer et al.¹ reported the periodic structure of rectangular (orthorhombic) or monoclinic lattice in cadmium arachidate multilayer. The LB film images of hexagonal lattice were displayed in AFM photographs by Bourdieu et al.,² Hansma et al.,³ and Radmacher et al.⁴ On the other hand, Shwartz et al.⁵⁻⁷ supported the centered rectangular lattice which included two molecules in a unit cell. The same lattice was reported for multilayers of cadmium salts of fatty acids with different alkyl chain lengths by Schaper et al.⁸ Lignoceric and stearic acids were arranged in the hexagonal lattice in monolayer, as reported by Kajiyama et al.⁹ While the repeating distance for the hexagonal lattice of fatty acids was 4.2-5 Å,^{3,9} two axes in the centered rectangular lattice were 4.5-5.1 and 7.3-9.2 Å.⁵⁻⁸ In the present work, arachidic acid molecules in monolayer were in the monoclinic orientation, where unit cell distances were longer than those by Mayer et al.¹ The inclined lattices were observed for multilayers of manganese and barium arachidates.^{6,7} Such lattices included two or three molecules in a unit cell.

The construction of periodic monolayer structure was more difficult than that of multilayer.^{3,4,7} The disordered arrangement of cadmium arachidate was reported by Schwartz et al.,⁷ while the hexagonal lattice was observed in part in the disordered arrangement.⁴ Schwartz et al.⁵⁻⁷ also reported the formation of a centered rectangular lattice in manganese arachidate and lead stearate monolayers, although they observed the disordered arrangement of barium arachidate. Hensma et al.3 found the formation of hexagonal lattice in the cadmium arachidate monolayer which was preserved for a month. The preservation process might contribute to the evaporation of solvent and/or water. The importance of the preparation process of monolayer was pointed out by Kajiyama et al.9 They succeeded in preparing the stearic acid monolayer without metal by the multistep creep method. This process

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contributes to reorientation of the monolayer. The orientation occurred with time in the C_{18} DAO monolayer in this work. Moreover, the evaporation in vacuo adopted in this work should contribute to the evaporation of water in the monolayer. Kajiyama et al.⁹ mentioned that the low surface pressure provided the better ordering of molecules when the monolayer was deposited by the continuous compression, although investigations by other workers were performed at the higher surface pressure

of 25-35 mN m⁻¹. It is concluded from the facts mentioned above that the molecular arrangement in fatty acid monolayer depends on the preparation condition.

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