Non-Equilibrium Phase-Separated State of (Palmitic Acid/Lignoceric Acid) Mixed Monolayer

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Abstract: The effect of solvent species and quantity of solvent used for spreading on the morphology of Langmuir monolayer composed of palmitic and lignoceric acids was investigated based on atomic force microscopy observations. The variation in domain size depending on the evaporation time of the spreading solution indicated that the mixed monolayer was in a non-equilibrium phase-separated state.

Key words: Langmuir method, phase separation, fatty acids, non-equilibrium

1 Introduction

Phase-separated mixed monolayers have potential applications as biosensors1 and in diverse fields such as protein engineering2 and integrated devices3. However, their development demands a good understanding of the aggregation behavior as well as the thermal and mechanical stability. Aggregation behavior, such as the miscibility of mixed monolayers, has been examined using various methods4-10. These studies revealed that the phase separation in a monolayer depends on many factors, including electrostatic interaction, molecular chirality, and the thermodynamic parameters of the subphase temperature and surface pressure. A Langmuir monolayer is prepared through the solvent evaporation process. If evaporation ends before molecular aggregation reaches equilibrium, two-dimensional molecular diffusion on the water surface is frozen after solvent evaporation, probably to become a non-equilibrium state. Whether a Langmuir monolayer is in an equilibrium state or not influences the understanding of its molecular aggregation state, such as the estimation of pair potential for phase separation. The monolayer of lignoceric acid with a longer alkyl chain is in a crystalline state, as determined by X-ray diffraction measurements and atomic force microscopic (AFM) observations20,21. Such lignoceric acid molecules, which tend to be highly aggregating, may be difficult to diffuse on the water surface after evaporation of the solvent. In this study, the effect of evaporation time, which was varied by two methods, on the phase-separated morphology of the mixed monolayer composed of palmitic and lignoceric acids was investigated based on AFM observations.

2 Experimental

2.1 Monolayer preparation from different kinds of solvent

Palmitic acid (CH₃(CH₂)₁₄COOH, C₁₆ ≥ 99%) and lignoceric acid (CH₃(CH₂)₂₂COOH, C₂₄ ≥ 99%) were obtained from Sigma-Aldrich. Toluene solution of C₁₆ and C₂₄ with an equimolar fraction was prepared at a concentration of 8.0 × 10⁻⁴ mol L⁻¹. Their chloroform solution was also prepared under the same condition. After spreading the sample solution on the water surface at a subphase temperature of 293 K, the monolayer was compressed with a microprocessor-controlled film balance system (FSD-300, USI System) to a surface pressure of 10 mN m⁻¹ and was transferred onto freshly cleaved mica by the vertical dipping method. The structure of the monolayer corresponds to a rectangular system, because the surface pressure of 10 mN m⁻¹ exceeds the plateau pressure on the surface pressure-surface area isotherm12.

2.2 Monolayer preparation with different amounts of solvent

Toluene solution of C₁₆ and C₂₄ with an equimolar concentration of 8.0 × 10⁻⁴ mol L⁻¹ was used as a spreading solution. Solution volumes of 20, 30, and 40 μL were spread by one-shot drop on the water in a petri dish of diameter 50 mm at 293 K. The solution amounts of 20, 30, and 40 μL correspond to monolayer surface areas of 0.54, 0.35, and
0.27 nm\(^2\) molecule\(^{-1}\). The monolayer was transferred onto freshly cleaved mica by the surface-lowering method.

### 2.3 Measurement of evaporation time

Water was poured into the petri dish, which was placed on a microbalance. A solvent volume of 2 \(\mu\)L was dropped on the water surface. The period for which the weight measured by a microbalance decreased to a constant value after the evaporation of the solvent was designated as the evaporation time of the solvent. In the same manner, the evaporation time of the solvent in toluene solutions of 20, 30, and 40 \(\mu\)L by one-shot drop was measured.

### 2.4 Morphological observation of monolayer

An AFM image of the monolayer was obtained with an SPA300 unit together with an SPI3700 control station (Seiko Instruments, Co.) in air. Images were recorded at scan rates of 1-4 Hz in the constant force mode. The monolayer thickness was evaluated from hole depth after piercing a hole with an area of 100 nm \(\times\) 100 nm through the monolayer with an AFM probe at an applied force of 5 nN in the repulsive force range.

### 3 Results and Discussion

Figure 1 shows the AFM images of the \((C_{16}/C_{24})\) mixed monolayer prepared from the toluene solution. The brighter and darker portions corresponding to the higher domain and the lower surrounding matrix regions of the monolayer surface, respectively, were observed at the AFM image of Fig. 1(a). The images in Fig. 1(b) and Fig. 1(c) are magnified images of the marked zone, which is the region near the interface between the domain and matrix in Fig. 1(a) and the height profile along the white line in Fig. 1(b), respectively. The height (film thickness) of the domain and matrix regions in Figs. 1(a) and 1(b) were evaluated from the height profile to be ca. 3 nm and 1.5 nm, respectively. These values were close to the calculated molecular lengths of the \(C_{24}\) (2.9 nm) and \(C_{16}\) (1.9 nm) molecule based on the extended CPK molecular model\(^{12}\). Therefore, it is apparent that the domains and the surrounding matrix are composed of the \(C_{24}\) and \(C_{16}\) molecules, respectively.

Figure 2 exhibits the AFM image of the \((C_{16}/C_{24})\) mixed monolayer prepared from the chloroform solution. The phase-separated morphology was also observed for the monolayer prepared from toluene, whereas the domain size of the monolayer was smaller than that of the monolayer prepared from toluene. The evaporation time of chloroform is short, which is probably due to the lower boiling point of 334 K (toluene: 384 K) and the larger expansion coefficient\(^{22, 23}\) of 13.2 (toluene: 6.8), as shown in Table 1. This suggests that chloroform with a short evaporation time freezes the molecular aggregation state at an earlier stage of the phase separation process, and toluene freezes at a stage where the phase separation has progressed further.

The AFM images of the mixed monolayer prepared by a one-shot drop of 20, 30, and 40 \(\mu\)L toluene solution are shown in Fig. 3. The phase-separated morphology composed of domain and matrix was observed in every image. The domain size of the monolayer prepared from a large amount of the spreading solution was, predictably, larger than that from a small amount of solution. The toluene evaporation times corresponding to 20, 30, and 40 \(\mu\)L solution were evaluated to be 55, 100, and 130 s, respectively, by measuring the change in weight during evaporation. These results indicate that the domain size increased consistently with the evaporation time of the solvent. Putting
the results of Fig. 2 and Fig. 3 together, it is reasonable to consider that the molecular aggregation state of the mixed monolayer has not yet reached equilibrium within the measured time. However, the number of fatty acid molecules corresponding to the amount of spread may affect the domain size, which will be discussed elsewhere. The molecular aggregation process of the mixed monolayer composed of highly aggregating molecules is schematically illustrated as shown in Fig. 4. In the case of the short evaporation time, the molecular aggregation is frozen at an earlier stage of the phase separation process because the molecular diffusion stops in a short time. On the other hand, when the evaporation time is longer, the molecular aggregation is frozen at an advanced stage.

### 4 Conclusion

The $\mathrm{C_{16}/C_{24}}$ mixed monolayer was composed of the domains and the surrounding matrix, and the domain size changed depending on the evaporation time. The morphological changes revealed that the mixed monolayer of fatty acids is in a non-equilibrium state. It is expected that in subsequent work, the progression of morphological growth may be tracked by obtaining morphological images at arbitrary time intervals by controlling the evaporation of the solvent.

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