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Structures of Pure and Mixed Monolayers of Cholesterol and GM1 Studied by Grazing Incidence X-ray Diffraction

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Structures of Langmuir monolayers of cholesterol, ganglioside GM1, and their 1:1 (in mole) mixture at the air/water interface have been investigated at 20℃ by grazing incidence X-ray diffraction (GIXD). Film balance experiments show that cholesterol forms a condensed monolayer whereas the GM1 monolayer undergoes a first-order phase transition from an expanded fluid to a condensed phase. The mixture takes a more condensed state at 30 mN/m than that expected for the ideally mixed one, indicating the mutualphilicity between two components. The GIXD patterns exhibit for all the monolayers that the film molecules were in the hexagonal rotator phase. Molecular cell dimensions, molecular areas, and lateral and vertical coherence lengths are presented. The molecular area of cholesterol estimated from GIXD almost coincides with that obtained from the isotherm. For GM1, it is suggested that a portion of long hydrophobic chain takes part in crystallization in the monolayer because of the large and electrostatically repulsive head group, and this packing structure would make a difference between the molecular area obtained from the isotherm and that from the GIXD. The mutualphilicity is also indicated by the diffraction experiment for the mixed monolayer. However, the mixing remarkably reduces the crystallinity of the monolayer.
Key words: Cholesterol, GM1, Raft Domain, Langmuir Monolayer, Grazing Incidence X-ray Diffraction

1. INTRODUCTION

Recent advance in the membrane structural biology is the potential existence of the so-called 'lipid raft micro-domains'. The lipid rafts, originally suggested by Simons and Ikonen, are enriched in cholesterol and sphingolipids, and float on the fluid bilayer of cellular membranes [1]. It is thought that various cellular processes such as membrane transport and cell signal transduction occur at the rafts through specific membrane-protein interactions [1,2]. In the rafts as a platform of diverse cellular events, cholesterol is believed to have many key functions. Especially cholesterol would serve as a spacer and/or glue between the hydrocarbon chains of other lipids, which plays crucial roles in determining the functions and behavior of the rafts [3].

Langmuir monolayers floating on the water surface are good model systems for studying the membrane chemistry and physics [4]. Although the environment around the monolayers is not exactly the same as the real cell membranes, monolayer researches give clear information on ordering of lipid molecules, lipid-lipid and/or lipid-protein interactions, interfacial reactions, etc, at the ideally flat interface.

In the present work, Langmuir monolayers of cholesterol, GM1, and a mixture of cholesterol/GM1 (1:1) are studied by film balance and GIXD experiments. Both of cholesterol and GM1 are major raft components. GM1 contains four neutral sugar residues and a negatively charged sialic acid residue, which cause steric hindrance and electrostatic repulsion leading to formation of a weakly organized condensed phase in the monolayer as shown later. Cholesterol forms a stable, condensed monolayer on the water surface but its state is rather expanded compared with lipids having single saturated hydrocarbon chain such as *n*-alkyl fatty acids, due to the rigid steroid skeleton. Here, we focus on characterization of pure and mixed monolayers of cholesterol and GM1 at the air-water interface. The film balance and GIXD results verify the specific molecular affinity of cholesterol claimed for the cellular membranes.

2. EXPERIMENTAL

2.1 Monolayer Preparation

Cholesterol (purity ≥99%) and ganglioside GM1 (brain ovine, ammonium salt, >99%) were purchased from Sigma and Avanti Polar Lipids respectively and used as received (Fig.1). A mixture of spectro-grade chloroform (Dojin Chemicals) / methanol (Wako Chemicals) (9:1 in volume) was used as a spreading solvent. Monolayers were spread on the temperature-controlled ultrapure water surface in the PTFE Langmuir trough using a gas-tight microsyringe, and allowed to stand for about 30 min before starting compression. Surface pressure was detected by a Wilhelmy balance.

2.2 GIXD Experiments

Synchrotron GIXD experiments for the monolayers were performed using the liquid surface diffractometer on the undulator beamline BW1 in HASYLAB at DESY (Hamburg, Germany) [5]. The monochromatic X-ray

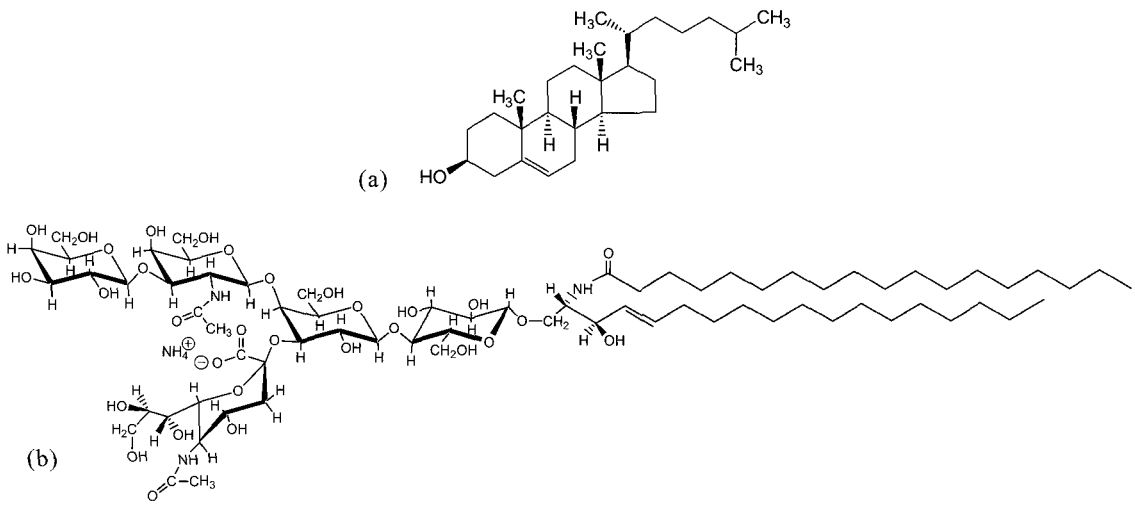

Fig. 1 Film molecules used; (a) cholesterol, (b) ganglioside GM1.

beam (λ = 0.1304 nm) was obtained by Bragg reflection form a beryllium (002) crystal. The incident angle was adjusted to $0.85\alpha_c$, where α_c (~0.13°) is the critical angle for total reflection with respect to the water surface, to obtain the high surface sensitivity under the minimized subphase scattering. The diffracted X-ray was passed trough a Soller collimator and detected by a position-sensitive detector (PSD). The GIXD patterns were recorded by scanning over a range along the horizontal component of the X-ray scattering vector, $Q_{xy} \cong (4\pi/\lambda)\sin\theta_{xy}$, where θ_{xy} is the angle between the incident and the diffracted beams projected onto the horizontal plane. The vertical component is expressed by $Q_z \cong (2\pi/\lambda)\sin\alpha_f$, where α_f is the angle between the diffracted beam and the horizontal plane. Horizontal and vertical peaks obtained were least-square fitted by Lorentzian and Gaussian functions respectively.

3. RESULTS AND DISCUSSION

3.1 π-A isotherms

Figure 2 shows surface pressure (π) - molecular area (A) isotherms for monolayers of (a) cholesterol, (b) GM1, and (c) 1:1 mixture on the ultrapure water surface at 20℃. Cholesterol reveals a very steep isotherm. This means that the molecules are in a condensed state. After spreading, the cholesterol molecules spontaneously gather each other to form condensed phase domains. This aggregation tendency could be interpreted by hydrophobic character of the molecule since it has only a single hydroxyl group as hydrophilic one in 3β position. The monolayer is rather stable, but the planner and rigid steroid skeleton makes the limiting molecular area much larger than that for lipids with a long alkyl chain.

GM1 takes a significantly expanded state on the water surface at zero surface pressure. Upon compression the surface pressure gradually increases and the monolayer undergoes a first-order phase transition from a liquid-expanded to a condensed phase starting at about 20 mN/m. The molecular area of the condensed phase, for instance ca. 53 Å^2/molec. at 55 mN/m, is larger than that expected for two close-packed alkyl chains (ca 2×19-21 Å^2/molec.) [6]. These features of the isotherm for the GM1 monolayer can be understood by the steric hindrance and the electrostatic repulsion between the head groups.

In contrast to the pure GM1 monolayer, the cholesterol/GM1 mixed monolayer shows a condensed-type isotherm. The expanded fluid phase observed for the GM1 monolayer below 20 mN/m is disappeared in the mixture. Brewster angle microscopic observation during compression showed condensed phase micro-domains ranging from ten to several tens micrometers in a fluid phase up to 0.5 mN/m and uniform surface completely covered by the condensed phase at higher surface pressure (the images are not shown). This observation suggests two components are miscible in their mixed monolayer. The average molecular area of the mixed monolayer at 30 mN/m is 42.4±1.0 Å^2/molec., approximately 18% less than that expected for the ideal

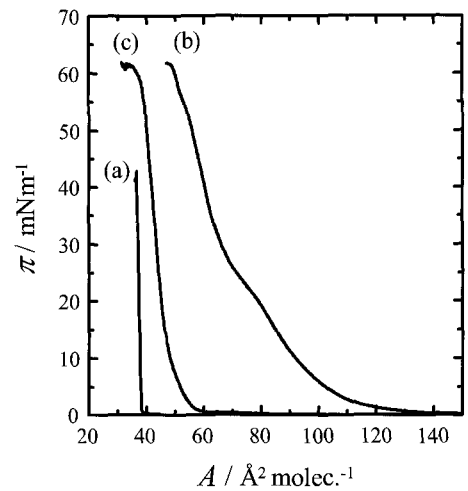

Fig. 2 π-A isotherms of (a) cholesterol, (b) GM1, and (c) cholesterol/GM1(1:1) monolayers on the ultrapure water surface at 20 ℃.

mixing state, indicating strong mutualphilicity between cholesterol and GM1 at the air/water interface.

3.2 GIXD

GIXD experiments were performed for Langmuir monolayers of cholesterol, GM1, and their 1:1 mixture on the ultrapure water. Figures 3 - 5 reveal (a) counter plots of the diffracted intensities as function of the in-plane and the out-of-plane scattering vectors (Q_{xy} and Q_z respectively), (b) in-plane intensity profiles against Q_{xy} and (c) out-of-plane intensity profiles against Q_z, for the monolayers at 30 mN/m, 20℃. The cholesterol monolayer shows a single peak at Q_{xy} = 1.101 Å^{-1} and Q_z = 0 Å^{-1} (Fig. 3), indicating the hexagonal unit cell of untilted molecules with a (= b) of 6.59 Å, the angle (γ) between the a and b axes of 120°, d-spacing (d) of 5.71 Å, and the unit cell area (A_{xy}) of 37.6 Å^2 corresponding to the molecular area in this case. These values are enough reasonable compared with those reported previously for the cholesterol monolayer at 50 Å^2 (0 mN/m) and 5℃ [7], taking into account for temperature difference and a small thermal expansion coefficient of the monolayer. The Bragg peak is apparently broader than those for monolayers of typical lipids like

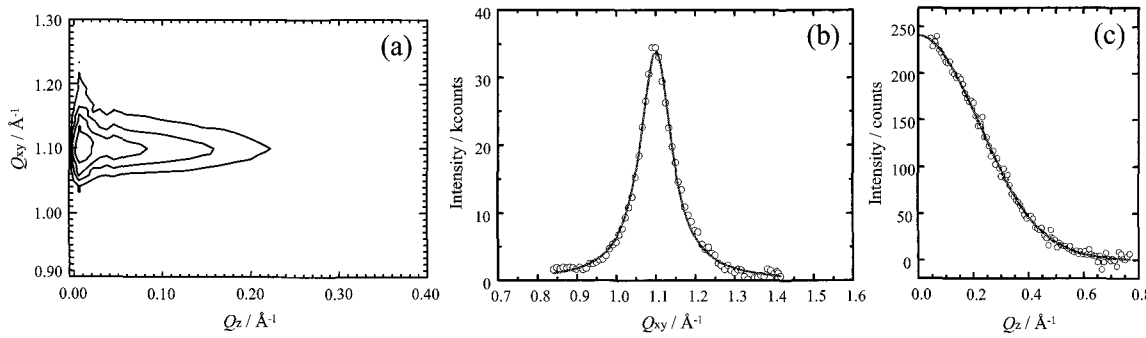

Fig. 3 GIXD for the cholesterol monolayer on the ultrapure water at 30 mN/m, 20 ℃; (a) the two-dimensional counter plot of the intensity distribution against the horizontal (Q_{xy}) and the vertical (Q_z) scattering vectors, and the baseline-subtracted (b) Bragg peak and (c) rod profiles (open circles: the observed data, solid line: the least-square fitted curves).

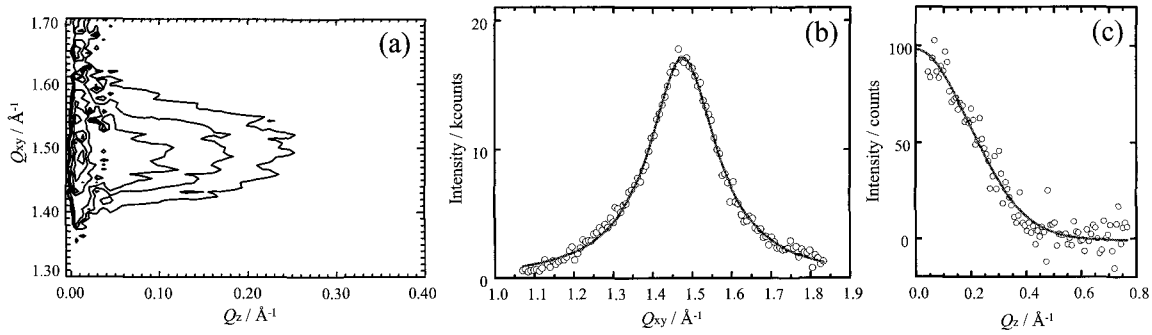

Fig. 4 GIXD for the GM1 monolayer on the ultrapure water at 30 mN/m, 20 ℃; (a) the two-dimensional counter plot of the intensity distribution against the horizontal (Q_{xy}) and the vertical (Q_z) scattering vectors, and the baseline-subtracted (b) Bragg peak and (c) rod profiles (open circles: the observed data, solid line: the least-square fitted curves).

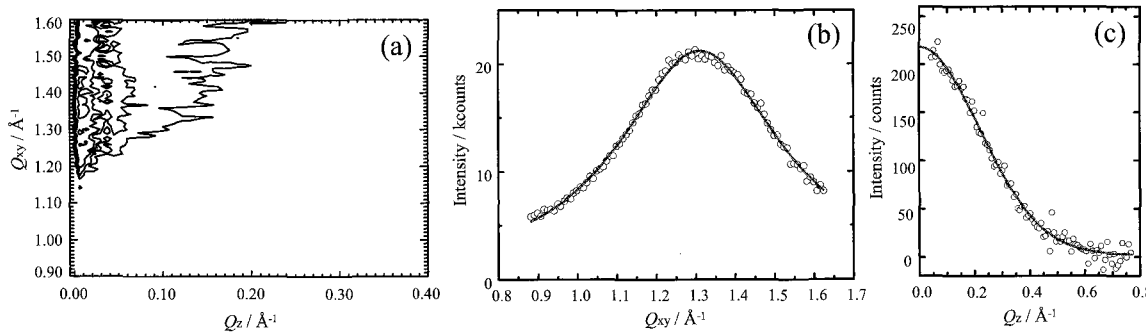

Fig. 5 GIXD for the cholesterol/GM1 (1:1) monolayer on the ultrapure water at 30 mN/m, 20 ℃; (a) the two-dimensional counter plot of the intensity distribution against the horizontal (Q_{xy}) and the vertical (Q_z) scattering vectors, and the baseline-subtracted (b) Bragg peak and (c) rod profiles (open circles: the observed data, solid line: the least-square fitted curves).

phospholipids and fatty acids with saturated long chains in the fully condensed state. The full-width at half-maximum (fwhm) of the Bragg peak (ΔQ_{xy}) corrected by instrumental resolution provides an estimate of the in-plane crystalline coherence length, $L = 0.9(2\pi/\Delta Q_{xy})$. In addition, the out-of- plane coherence length, h, corresponding to the crystalline monolayer thickness, is derived from the fwhm of the Bragg rod profile (ΔQ_z) by an equation of $h = 0.9(2\pi/\Delta Q_z)$. The obtained L and h are 59 Å and 13 Å, respectively. These values are smaller than the previously reported ones (L = 70 Å, h = 14.5 Å) [7], but the difference could be again explained by considering the temperature difference between the experiments.

The GIXD pattern for the GM1 monolayer also exhibits the feature of hexagonal molecular packing (Fig. 4). The peak maxima are Q_{xy} = 1.477 Å^{-1} and Q_z = 0 Å^{-1}, which give $a = b$ = 4.91 Å, γ = 120° and d = 4.26 Å. The molecular area ($2 \times A_{xy}$) estimated is 41.8 Å^2, which is much smaller than that obtained from the π-A isotherm (64 Å^2). The L calculated from the Bragg peak profile is 28 Å, indicating small crystalline domain. The h is 14 Å, less than that expected for fully stretched C17 or C15 chain of the GM1 molecule. These small values of A_{xy}, L, and h could be interpreted as the influence of the head group to the hydrophobic chain packing. We tentatively assume that only a part of the alkyl chain contributes to formation of the loosely packed crystalline lattice, as schematically shown in Fig. 6. This molecular arrangement would produce small molecular clusters in the monolayer, and the void space between the clusters makes the molecular area obtained from the π-A isotherm larger than that from GIXD.

For the cholesterol/GM1 (1:1) mixture, we found a considerably broad peak at Q_{xy} = 1.314 Å^{-1} and Q_z = 0 Å^{-1}, assigned to the hexagonal rotator phase with $a = b$ = 5.52 Å, γ = 120°, d = 4.78 Å, A_{xy} = 26.4 Å^2, L = 11 Å, h = 13 Å (Fig.5). The mixing of cholesterol and GM1 at the molecular level was confirmed by absence of diffraction peaks in the region where the pure cholesterol and GM1 monolayers present the peaks. The A_{xy} shrinks about 10% from the ideally mixing state, suggesting the mutualphilicity between cholesterol and GM1. However, as seen from the lateral correlation length L, the crystallinity is considerably decreased by mixing. One of the most important roles of cholesterol in the cell membranes is regulating stiffness of the membranes. In the most cases, the degree of molecular ordering in the liquid-crystalline phase is increased by incorporation of cholesterol because the rigid steroid skeleton reduces the trans-gauche isomerization of neighboring alkyl chains, whereas in the gel phase the translational and orientational ordering of the hydrocarbon chains are disturbed by cholesterol [8]. In our monolayers, consistent with the general rule, the originally low structural order of the condensed phase of GM1 is further decreased by the cholesterol incorporation. Further works are in progress to know the mixing-ratio dependent change of lattice structures in the cholesterol/GM1 monolayers using GIXD.

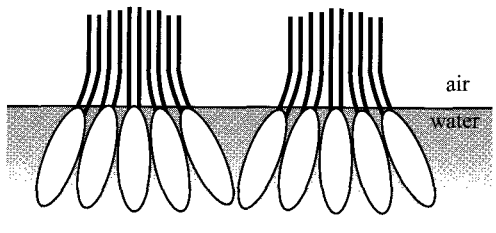

Fig. 6 A schematic drawing of molecular packing structure expected for condensed-state GM1 at the air/water interface.

4. SUMMARY

Monolayer behavior and molecular packing structures of cholesterol, GM1, and their 1:1 mixture at the air/water interface were investigated by the film balance and GIXD experiments. The cholesterol molecules are packed in the hexagonal unit cell with low crystallinity. The cell in the GM1 monolayer is also the hexagonal one but rather compact and less ordered. The mixing of cholesterol and GM1 results in formation of the more condensed monolayer. However at the same time the mixing leads to enhanced reduction of the lateral order of molecular arrangement.

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