Solid-Supported Assembly Composed of \(n\)-Octyl-\(\beta\)-D-glucopyranoside and 1,2-Dioleoyl-sn-glycero-3-phosphocholine in Equilibrium with Its Ambient Aqueous Solution System Including Dispersed Assembly

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Abstract: In the last few decades, the preparation of solid-supported lipid bilayers by immersing a solid substrate in an aqueous solution where the lipid is dissolved with the aid of a surfactant, followed by dilution of the solution, has been reported. In this study, we attempted to interpret the evolution of supported surfactant/lipid assemblies towards the supported lipid bilayer in terms of a phase equilibrium between the supported assembly phase and its ambient solution system consisting of the dispersed surfactant/lipid assembly phase and the bulk solution phase comprising monomeric surfactant and lipid. We characterized the supported assembly formed on hydrophilized Ge or mica substrates in equilibrium with aqueous solutions containing various concentrations of the nonionic surfactant, \(n\)-octyl-\(\beta\)-D-glucopyranoside (OG) and the amphoteric phospholipid, 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), using interaction-force-profile measurements by atomic force microscopy (AFM), and attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR). We also investigated the ambient solution system using equilibrium dialysis to obtain the partition equilibrium profile of OG between the bulk solution and dispersed assembly phases in the micellar or vesicular states. These studies indicate that the properties of the supported assembly depend on the composition of the dispersed assembly and concentration of monomERICally dissolved OG. Further, a type of micellar-bilayer state transition occurs in the supported assembly, roughly synchronized with that in the dispersed assembly.

Key words: solid-supported assembly, \(n\)-octyl-\(\beta\)-D-glucopyranoside, 1,2-dioleoyl-sn-glycero-3-phosphocholine, phase equilibrium model, micellar-bilayer state transition

1 Introduction
Solid-supported lipid bilayers have received attention as a model system with various potential uses, such as a platform for studying biomembranes, sensor tips using membrane proteins, etc.1-4. These are physically stabilized by supporting substrates and may be characterized by several surface-sensitive techniques. The lipid bilayers are formed on the solid support via a water layer or hydrated polymer layer with a thickness of several nanometers, which ensures the lateral fluidity of lipids5-10. Tethered lipid bilayers, which contain lipids with functional groups anchored to the solid substrate via a hydrophilic spacer, have also been11-16. These tethered bilayers possess higher physical stability and electrical sealing ability, which enables impedance or other electrical measurements.

Solid-supported lipid bilayers have been prepared using various procedures. The most familiar is using aqueous lipid vesicle dispersions. Kalb et al.17 prepared a solid-supported bilayer by putting the first monolayer using the Langmuir-Blodgett method, followed by vesicle fusion. Keller and Kasemo18, Reviakine and Brisson19, Benes et al.20, and Tero et al.21 observed the direct adsorption of vesicles to the hydrophilized solid substrate followed by their transformation to a bilayer. Since the processes of vesicle adsorption and fusion are irreversible and kinetical-

Abbreviations: OG; \(n\)-octyl-\(\beta\)-D-glucopyranoside, DOPC; 1,2-dioleoyl-sn-glycero-3-phosphocholine, ATR-FTIR; attenuated total reflection Fourier transform infrared spectroscopy, AFM; atomic force microscopy, CMC; critical micelle concentration

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ly controlled, the composition of the supported bilayer can be controlled only by the composition of the vesicles. Further, these authors reported the halting of vesicle adsorption and fusion, depending on the chemical properties of the substrates or lipids and vesicle size.

On the other hand, Tiberg et al. proposed a method using surfactant-solubilized lipid solution. They immersed a hydrophilized substrate in an aqueous surfactant/lipid solution and then diluted the solution with pure water to prepare a supported lipid bilayer on the substrate. This process was interpreted in analogy with the micellar-vesicular state transition within the molecular assembly dispersed in aqueous solution, as follows. First, surfactant/lipid mixed micelles and monomerically dissolved surfactant molecules coexisted in the ambient solution, and the mixed micelles were adsorbed to the substrate, forming a supported assembly in equilibrium. When the solution was diluted, a part of the surfactant molecules was transferred from the supported assembly into the bulk solution as monomers. Consequently, the lipid content in the supported assembly increased, and finally, the assembly transformed into a supported lipid bilayer. The process of supported bilayer formation was observed by ellipsometry, interaction-force-profile measurement, and neutron reflection, which confirmed that the composition of the supported bilayer evolved during the continual dilution process. Furthermore, they succeeded in introducing another lipid as the third component into the pre-formed supported bilayer using the correspondingly prepared mixed micelle. Other researchers have also reported that surfactants enable the introduction and exchange of lipid molecules in supported bilayers. These observations suggest that the formation of solid-supported bilayers mediated by a surfactant is a reversible process and can be interpreted using equilibrium thermodynamics.

In this study, we investigated the formation of a solid-supported lipid bilayer in terms of the phase equilibrium between the supported assembly phase and its ambient solution system comprising the dispersed surfactant/lipid assembly phase (in the micellar or vesicular state) and bulk solution phase containing monomerically dissolved surfactant and lipid, as illustrated in Fig. 1. Using a nonionic surfactant, \( n \)-octyl-\( \beta \)-D-glucopyranoside (OG) and an amphoteric phospholipid, 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), we characterized the supported assembly composed of OG and DOPC on a Ge substrate immersed in an ambient solution system containing various concentrations of OG and DOPC via attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR). We also measured the mechanical properties of the supported assembly on a mica substrate using atomic force microscopy (AFM). Simultaneously, we elucidated the ambient solution system using equilibrium dialysis to obtain the partition equilibrium profile of OG between the bulk solution and the dis-

![Fig. 1](image)

**Fig. 1** Phase equilibrium model for our experimental system. The "supported assembly phase" composed of OG and DOPC is formed on a substrate immersed in an ambient solution system. The ambient solution system is constituted from the "dispersed assembly phase" composed OG and DOPC, and the "bulk solution phase" comprising monomeric OG and DOPC. Both the supported assembly and the dispersed assembly can take either "micellar state" or "bilayer (vesicular) state". Assuming that the bulk solution phase, the dispersed assembly phase, and the supported assembly phase are in equilibrium with one another, chemical potentials of OG and DOPC are common in the entire system.
supported assembly, which enabled us to estimate the composition of the dispersed assembly and concentration of monomerically dissolved OG. Based on these data, we demonstrate the control of the properties of the supported assembly by the ambient solution system.

2 Materials and Methods

2.1 Materials

OG and DOPC were purchased from Dojindo Molecular Technologies, Inc., and Sigma-Aldrich Co., respectively and used without further purification.

2.2 Sample preparation

To prepare aqueous solutions containing various concentrations of OG and DOPC, we first prepared two types of stock solutions: one containing 25.4 mM DOPC and 171 mM OG, and the other containing only 171 mM OG. We mixed these stock solutions with pure water in an appropriate ratio to obtain the sample solution with the desired OG and DOPC concentrations just before the experiment. It should be noted that no vesicular particles formed in these stock solutions; therefore, uniformity within these stock solutions was confirmed to avoid uncertainties in the OG and DOPC concentrations in the sample solution.

2.3 Infrared spectroscopy

Infrared spectra were measured using a Fourier transform infrared spectrometer (Perkin-Elmer System 2000). Each spectrum was obtained by accumulating 100 scans at a resolution of 4 cm⁻¹. During the measurement, the inner compartment of the spectrometer was purged with N₂ gas dried over synthetic zeolites (A-4, Fujifilm Wako Pure Chemical Co.).

Transmission spectra were obtained using a TGS detector and a liquid cell with a pair of CaF₂ windows and a piece of 0.05 mm Teflon spacer. Attenuated total reflection (ATR) spectra were obtained using an MCT detector and a trapezoidal Ge prism with an incidence angle of 45° and 10 internal reflections (Spectra Tech Inc.) accommodated by a homemade thermostatic liquid chamber. Just prior to each experiment, the prism was polished with diamond paste of 0–1 μm (Tokyo Diamond), cleaned with acetone and pure water, and hydrophilized with O₂ plasma using a desk-top quick coater, SC-704 (Sanyu Electron Co.), up to a water contact angle of approximately 3°.

2.4 Atomic force microscopy

For AFM, we used SPA-400 (Seiko Instruments Inc.) and a liquid chamber. We also used a triangular cantilever of Si₃N₄ (OMCL-TR400PSAHW, Olympus Co.), with a spring constant of 0.09 N m⁻¹, total length of 100 μm, and pyramidal probe of 0.40 μm high. Just before use, the cantilever was washed overnight in 0.1 N HNO₃ aqueous solution. Freshly cleaved mica was used as the substrate.

3 Results

3.1 Quantitative analysis of infrared ATR spectra for supported assembly

3.1.1 Infrared ATR spectra measurement

Figure 2(a) shows the infrared ATR spectra measured on a Ge prism covered with aqueous solutions containing 27.4 mM OG and various concentrations of DOPC at 25.0 ± 0.1°C. These measurements were carried out 1 h after placing the solutions on the prism surface. The absorption peaks ascribed to OG or DOPC increased with the DOPC concentration while those ascribed to water molecules decreased, indicating that OG and DOPC form a supported assembly on the Ge substrate, excluding water molecules.

To quantify the amounts of OG and DOPC in the supported assemblies, we analyzed the ATR spectra. However, absorbance of these spectra is weak, even compared with the “false” spectrum which is attributed to the sealing materials (silicone rubber) used to attach the liquid chamber on the Ge prism or the organic material coating the optical system within the spectrometer instrument, and emerges
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Fig. 2  (a) Infrared ATR spectra of aqueous OG/DOPC solutions on a Ge substrate. Each spectrum was obtained at 25.0 ± 0.1°C, 1 h after placing the solution containing 27.4 mM OG and various concentrations of DOPC: 0, 0.24, 0.55, 0.93, 1.27, and 2.74 mM. With increasing the DOPC concentration, absorption peaks ascribed to C-H stretching (3000–2800 cm⁻¹), C=O stretching (1800–1700 cm⁻¹), C-H bending (1500–1400 cm⁻¹), and fingerprint region (1300–900 cm⁻¹) increase as pointed by upward arrows, and those ascribed to water molecules (3700–2900 and 1750–1600 cm⁻¹) decrease as pointed by downward arrows. (b) Energy spectrum of pure water used as reference in measuring spectra in (a). For quantitative analysis of the supported assembly spectra, we only used the meshed region (1280–1276, 1244–1186, 1174–1155 cm⁻¹), where interfering absorption bands other than OG and DOPC do not exist.

owing to the minor error on subtracting the reference spectrum shown in Fig. 2(b). Therefore, in the present study, we only processed the meshed region in Fig. 2 (1280–1276, 1244–1186, 1174–1155 cm⁻¹), where such interfering absorption bands are absent.

3.1.2 Theory of ATR spectroscopy

The ATR spectra obtained in this study were quantitatively analyzed according to the theory of Harrick and du Pre²⁴⁻³¹. The absorbance of a sample layer on an ATR prism depends on the refractive indices of the prism (phase 1, n₁), sample layer (phase 2, n₂), and ambient medium (phase 3, n₃), angle of incidence (θ), number of internal reflections (N), and amplitude of the electric field of the evanescent wave in the neighborhood of the prism surface. The electric field amplitude decayed exponentially with distance from the prism surface. The decay length or penetration depth of the evanescent wave (d_p) is given by

\[ d_p = \frac{\lambda}{2\pi n_{ij}\sqrt{\sin^2 \theta - n_{ij}^2}}, \]

where \( n_{ij} = n_i/n_j \) and \( \lambda \) represent the refractive index ratio and wavelength, respectively.

When the thickness of the sample layer is sufficiently smaller than \( d_p \), the electric field amplitudes of the evanescent wave in the "small-thickness" sample layer, \( E_i^{\text{rel}} \) (relative to those of the incident light) can be expressed as:

\[ E_i^{\text{rel}} = \frac{2\cos \theta \sqrt{\sin^2 \theta - n_{ij}^2}}{\sqrt{1 - n_{ij}^2 \sin^2 \theta}}, \]

where \( \theta \) is the angle of incidence, and \( n_{ij} \) is the refractive index ratio of the sample layer/prism interface. The absorbance, \( A \), is expressed as:

\[ A = \sum_i C_i k_i A_i^{\text{rel}}, \]

where \( C_i \) and \( k_i \) are the amount per unit area and the absorption coefficient, respectively, of the component \( i \) in the sample layer.

When the thickness of the sample layer is much larger than \( d_p \), the relative electric field amplitudes at the "large-thickness" sample layer/prism interface, \( E_i \) are

\[ E_i = \frac{2\cos \theta \sqrt{\sin^2 \theta - n_{ij}^2}}{\sqrt{1 - n_{ij}^2 \sin^2 \theta}}, \]

and

\[ E_i^{\text{rel}} = \frac{2\cos \theta \sqrt{\sin^2 \theta - n_{ij}^2}}{\sqrt{1 - n_{ij}^2 \sin^2 \theta}}. \]

In this case, the electric field amplitude decayed with \( d_p \) in the sample layer. Therefore, \( A \) is given by

\[ A = \sum_i N_i k_i C_i \left( E_i^{\text{rel}} + E_i^{\text{rel}} + E_i^{\text{rel}} \right)^2, \]

where \( N_i \) is the concentration of component \( i \) in the sample layer.

3.1.3 Absorption coefficients of OG and DOPC

As mentioned above, quantitative analysis of the ATR spectrum requires the absorption coefficient \( k_i \). To determine \( k_{\text{OG}} \) and \( k_{\text{DOPC}} \), we measured the transmission infrared spectra of aqueous solutions of OG and DOPC. Based on Lambert–Beer’s law, we calculated \( k_{\text{OG}} \) and \( k_{\text{DOPC}} \) as functions of the wavenumber (ν) by adopting the least squares fitting of these spectral data, permitting a linear baseline with an arbitrary slope for each spectrum. The absorption
3.1.4 Other necessary parameters

We describe the other parameters required for the ATR spectrum analysis. The refractive indices of Ge and H$_2$O as functions of ν were adopted from the literature$^{32,33}$ and represented as quadratic functions:

\[
n_{\text{Ge}} = 6.234 \times 10^{-3} \nu^2 - 5.942 \times 10^{-3} \nu + 4.003,
\]

\[
n_{\text{H}_2\text{O}} = -4.180 \times 10^{-7} \nu^2 + 1.216 \times 10^{-3} \nu + 4.230 \times 10^{-1}.
\]  

We used 1.4 as the refractive index of the supported assembly composed of OG and DOPC, as it has been frequently adopted as that of lipid bilayers$^{40}$. The incident angle θ was determined to be 45° from the geometric shape of the ATR prism. The number of internal reflections, N, which was also determined mainly from the geometric shape of the prism as 10, required modifications because the edge of the prism surface was buried in the liquid chamber and hence, inaccessible to the sample layer. Therefore, we measured the ATR spectra of the standard sample (10–50 mg/mL bovine serum albumin dissolved in D$_2$O) to estimate N as 7.14, according to our previous study$^{39}$. Using these parameters and Equation (1), $d_0$ was calculated to be (4.9–5.4) x 10$^{-7}$ nm in the range of 1280–1155 cm$^{-1}$. It should be noted that these $d_0$ values are much larger than the expected thickness of lipid bilayers of several nanometers.

3.1.5 Quantifying OG and DOPC in supported assembly

In Fig. 2(a), the absorbance of the ATR spectrum involves contributions from OG and DOPC in the supported assembly, as well as those in its ambient solution. Using the above equations and parameters, we resolved the ATR spectrum into individual contributions as:

\[
A^\text{obs} = A^\text{sup} + A^\text{DOPC} + A^\text{amb},
\]  

where $A^\text{amb}$ is the absorbance observed in the ATR spectrum, while $A^\text{amb}$ is attributed to OG and DOPC in the ambient solution. Because the OG and DOPC concentrations in the ambient solution are already known, $A^\text{amb}$ can be calculated using Equation (5). $A^\text{DOPC}$ and $A^\text{OG}$ originate from OG and DOPC in the supported assembly on the substrate, respectively. Using Equation (3), these are represented as linear functions of the amounts of OG and DOPC per unit area, $\Gamma^\text{OG}$ and $\Gamma^\text{DOPC}$, respectively. Therefore, to determine $\Gamma^\text{OG}$ and $\Gamma^\text{DOPC}$ we adopted the least squares fitting of the theoretical spectra described by Equation (7) on the experimental data, which permits a linear baseline with an arbitrary slope for each spectrum. Figure 3 shows examples of the resolution of the ATR spectrum.

Figure 4 shows the amounts of OG and DOPC in the supported assembly on a Ge substrate. Figures 4(a) and 4(b) represent $\Gamma^\text{OG}$ and $\Gamma^\text{DOPC}$, respectively, as functions of the DOPC concentration in the ambient solution, $C^\text{DOPC}$, while the OG concentration ($C^\text{OG}$) was maintained at 27.4 mM. As $C^\text{DOPC}$ increases up to 0.6 mM, $\Gamma^\text{OG}$ increases, and then slightly decreases with further increase in $C^\text{DOPC}$, whereas $\Gamma^\text{DOPC}$ increases monotonically. On the other hand, Figs. 4(c) and 4(d) show that as $C^\text{OG}$ decreases with a constant $C^\text{DOPC}(1.15$ mM), $\Gamma^\text{OG}$ slightly decreases and $\Gamma^\text{DOPC}$ increases monotonically. These changes in $\Gamma^\text{OG}$ and $\Gamma^\text{DOPC}$ with $C^\text{OG}$ and $C^\text{DOPC}$ exhibit practical reversibility, except when $C^\text{DOPC}$ is very low, indicating that the supported assembly is in equilibrium with the ambient solution system. The reversibility observed at low $C^\text{DOPC}$ may be attributed to the very low rate of OG-rich supported assembly formation.
These data were determined using the ATR spectra measured 1 h after the ambient solutions were placed on the prism surface. It should be noted that the spectrum reached a steady state approximately 20–40 min after sample placement, as described in Supporting Information III. In addition, we estimated the total weight of the supported assembly per unit area, $w_{\text{sup}}$, from the data in Figs. 4 and 5 as a function of the mole fraction of OG in the supported assembly, $X_{\text{OG}}$. Figure 5 shows that $w_{\text{sup}}$ is several $10^{-7}$ g cm$^{-2}$ throughout the entire $X_{\text{OG}}$ range, which roughly corresponds to a surface density of $3.6 \times 10^{-7}$ g cm$^{-2}$ for the pure DOPC bilayer.

As demonstrated above, quantitative ATR-FTIR spectroscopy enables us to determine the amounts of several different kinds of adsorbents separately, using the difference between the wavenumber dependence of their absorption coefficients. In this regard, ATR-FTIR surpasses other methods of quantitative surface adsorption analysis, such as quartz crystal microbalance and surface plasmon resonance.

### 3.2 Interaction force/distance profile of supported assembly

We investigated the mechanical properties of the sup-
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The supported assembly formed in aqueous solutions containing 27.4 mM OG and various concentrations of DOPC. As the DOPC concentration increased, the force profile makes a transition from the “micellar state” type (open symbols) to the “bilayer state” type (closed symbols), and that a type of micellar-bilayer state transition occurs in the supported assembly on the substrate, as well as in the dispersed assembly in the ambient solutions.

3.3 Partition equilibrium profile of OG between bulk solution and dispersed assembly

Under our experimental conditions, the dispersed assembly in the micellar or vesicular state composed of OG and DOPC is formed in the ambient solution, and assuming equilibrium within the entire system, the chemical potential of OG in the dispersed assembly is equal to that in the bulk solution, as illustrated in Fig. 1. For the vesicular state assembly, various sizes of large unilamellar vesicles are expected to be formed as thermodynamically stable species via the surfactant-depletion process during equilibrium dialysis[41-44]. We investigated the partition equilibrium of OG between the bulk solution and dispersed assembly phases using data from the equilibrium dialysis experiment. Here, we define "partition equilibrium profile" in this paper, where the concentration of monomerically dissolved OG in the bulk solution, \( C_{\text{OG}} \), is plotted against the average mole fraction of OG in all of the dispersed assemblies, \( X_{\text{OG}} \), as shown in Fig. 7. In preparing the data points represented by open square symbols in Fig. 7, \( C_{\text{OG}} \) was estimated as the OG concentration in the outer solution in the equilibrium dialysis experiment, determined by refractive-index measurement, using

\[
\Delta n_{\text{sol}} = \frac{dn}{dC} C_{\text{OG}} \Delta C_{\text{OG}}
\]

where \( \Delta n_{\text{sol}} \) is the refractive index increment of the outer solution. In reality, a small amount of pure OG micelles is formed in the outer solution, even at concentrations lower than the CMC. Therefore, we corrected \( C_{\text{OG}} \) by removing the contribution of pure OG micelle formed in the outer solution, based on the static light scattering experiment, as described in Supporting Information IV. Notably, DOPC was not detected in the outer solution by the phosphorus assay. \( X_{\text{dis}} \) was estimated as follows. In the inner solution, the dispersed assembly composed of OG and DOPC and monomerically dissolved OG coexist. Therefore, we obtain

\[
0.76 \text{ mM}, \text{ steric force was observed at a distance of approximately } 2 \text{ nm, and increased monotonically as the probe approached. When the DOPC concentration exceeded } 1.15 \text{ mM, the steric force was observed at a distance of approximately } 6 \text{ nm and increased until the probe abruptly penetrated the supported assembly layer at } 2.5 \text{ nm. The force required for this "breakthrough" increased with DOPC concentration. Such breakthroughs in force/distance profiles have been generally observed in supported lipid bilayers[23, 39]. Based on these observations, we concluded that as the DOPC concentration increases, the force profile makes a transition from the "micellar state" type (open symbols) to the "bilayer state" type (closed symbols), and that a type of micellar-bilayer state transition occurs in the supported assembly on the substrate, as well as in the dispersed assembly in the ambient solutions.}
\[ \Delta n_{in} = \left( \frac{dn}{dc} \right)_{OG} C_{OG} + \left( \frac{dn}{dc} \right)_{DOPC} C_{DOPC} \] 

where \( \Delta n_{in} \) is the refractive index increment of the inner solution, and \( C_{OG} \) and \( C_{DOPC} \) are the concentrations of OG and DOPC included in the dispersed assembly, respectively. Among them, \( C_{OG} \) was already estimated above, and \( C_{DOPC} \) was determined separately by a phosphorus assay. Therefore, we could determine \( C_{OG} \) to finally estimate \( X_{OG}^{sup} = \frac{C_{OG}}{C_{OG} + C_{DOPC}} \).

Ueno and Paternostre et al. reported the partition equilibrium profiles using OG and L-\( \alpha \)-phosphatidylcholine from egg yolk, which resembled that shown in Fig. 7. According to them, the profile could be divided into three regions. The regions \( X_{OG}^{sup} = 0.8-1 \) and \( X_{OG}^{sup} = 0-0.6 \) represent the micellar state region and vesicular state region, respectively. The micellar-vesicular state transition occurred in the range of \( X_{OG}^{sup} = 0.6-0.8 \). In fact, the turbidity of the solution increased sharply when \( X_{OG}^{sup} \) was lower than 0.8, indicating the formation of large unilamellar vesicle particles.

### 3.4 Equilibrium between the supported assembly and ambient solution system

Using the phase-equilibrium model illustrated in Fig. 1, the amounts of OG and DOPC in the supported assembly dependent on the ambient solution composition, as shown in Fig. 4, could be simply interpreted. The "supported assembly phase" is considered to be in equilibrium with the "ambient solution system" which can be divided into the "dispersed assembly phase" and the "bulk solution phase" which comprises monomerically dissolved OG and DOPC. In practice, the amount of DOPC dissolved in the bulk solution phase is too small to be detected. Because these thermodynamic phases are in equilibrium with one another, the chemical potentials of OG and DOPC are common through-
out the entire system. Using the partition equilibrium profile shown in Fig. 7, we determined the concentration of monomerically dissolved OG in the bulk solution phase \(C_{\text{OG}1}\) and the mole fraction of OG in the dispersed assembly phase \(X_{\text{OG}m}\) from the total OG and DOPC concentrations, each of which is a measure of the "thermodynamic activity" of OG in each phase. Therefore, we re-plotted the amounts of OG and DOPC in the supported assembly on a Ge substrate in Fig. 4 against \(X_{\text{dis} \text{OG}}\) in Fig. 8. In Fig. 8, as \(X_{\text{OG}m}\) increases, \(\Gamma_{\text{OG}}\) slightly increases except when \(X_{\text{OG}m} = 0.9–1\) and \(\Gamma_{\text{DOPC}}\) decreases, regardless of the net composition in the ambient solution (constant \(C_{\text{OG}}\) or constant \(C_{\text{DOPC}}\)). These observations suggest that the composition of the supported assembly is determined by that of the dispersed assembly.

We also prepared the partition equilibrium profile of OG between the bulk solution phase and supported assembly phase on a Ge substrate, as shown in Fig. 7, where \(C_{\text{OG}1}\) is plotted against the mole fraction of OG in the supported assembly phase, \(X_{\text{OG}m}\) (closed circles), which is comparable to that for the dispersed assembly (open squares). The partition equilibrium profile for the supported assembly phase is roughly similar to that for the dispersed assembly phase and is also divided into the micellar state region, bilayer state region, and transition region, although significant modifications were observed. The transition region for the supported assembly has higher \(C_{\text{OG}1}\) than that for the dispersed assembly. This coincides roughly with that for the supported assembly on a mica substrate represented as a meshed region, which is estimated from the interaction force measurement in Fig. 6, corresponding to the condition 0.76–1.15 mM DOPC and 27.4 mM OG. In addition, the transition region has a steeper slope that gradually approaches the micellar state region, so that the boundary of the micellar state region is ambiguous. The profile for the bilayer state region in the supported assembly has a larger mole fraction of OG than that for the vesicular state region in the dispersed assembly phase at the same \(C_{\text{OG}1}\). These properties suggest that the supporting substrate causes the bilayer state to include more OG.

4 Conclusion

In this study, we characterized a supported assembly composed of OG and DOPC on hydrophilized substrates in equilibrium with its ambient aqueous solution system containing the dispersed OG/DOPC assembly as well as the monomerically dissolved OG. The properties of the supported assembly are controlled by the composition of the dispersed assembly and the concentration of the monomerically dissolved OG, indicating that this phenomenon could be interpreted as a phase equilibrium.

We also obtained the partition equilibrium profiles of OG between the bulk solution phase and supported or dispersed assembly phases, which suggests that a type of micellar-bilayer state transition occurs in both the supported assembly and the dispersed assembly. The partition equilibrium profile of OG for the supported assembly has characteristics resembling that of the dispersed assembly, al-
though there are significant discrepancies, probably due to the interaction with the substrate. For a clearer understanding, the energetics of the micellar-bilayer transition based on such experimental data should be constructed.

Supported lipid bilayers are expected to be useful in a wide range of fields, and our fundamental study would lead to the development of their preparation and handling.

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Conflicts of Interest Statement

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Supporting Information

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