Simple Thermodynamic Description of the Micellar-Bilayer State Transition of Assemblies Composed of \(n\)-Octyl-\(\beta\)-D-glucopyranoside and 1,2-Dioleolyl-sn-glycero-3-phosphocholine Dispersed in Aqueous Media or Supported on Solid Substrates

Ryo Ishiguro*, Keiichi Kameyama, and Tetsuro Fujisawa

Faculty of Engineering, Gifu University, 1-1 Yanagido, Gifu 501-1193, JAPAN

Abstract: In the preceding paper, we investigated a mixed assembly composed of a nonionic surfactant, \(n\)-octyl-\(\beta\)-D-glucopyranoside (OG), and an amphoteric lipid, 1,2-dioleolyl-sn-glycero-3-phosphocholine (DOPC), formed on hydrophilized solid substrates immersed in aqueous solutions containing OG and DOPC. The experimental data could be interpreted in terms of the phase equilibrium; thus, the partition equilibrium profile of OG between the bulk solution phase and the supported assembly phase was obtained, as well as that between the bulk solution and the dispersed assembly. The partition equilibrium profiles suggested that micellar-bilayer state transitions occur both in the supported assembly and in the dispersed one in a roughly synchronized manner, even though there are significant discrepancies between them. In this paper, we propose a simple thermodynamic model for the micellar-bilayer transition of the dispersed and supported assembly of OG and DOPC, assuming that the micellar and bilayer states are also pseudophases distinct from each other. Using this model, we analyzed these partition equilibrium profiles and concluded that the transition in the supported assembly should mainly be attributed to the transition in the dispersed assembly, which is partly modified by the interaction energy between the supported assembly and the substrate.

Key words: micellar-bilayer state transition, \(n\)-octyl-\(\beta\)-D-glucopyranoside, 1,2-dioleolyl-sn-glycero-3-phosphocholine, phase separation model, regular solution approximation

1 Introduction

The mixed molecular assembly comprising two kinds of amphiphilic molecules, namely a surfactant and a lipid, dispersed in aqueous medium could take either micellar or vesicular states, depending on its composition. Many researchers have utilized these properties in biological studies in various ways. Insoluble membrane proteins buried in the lipid bilayer can be extracted by suitable surfactants, accommodated by the mixed micelles\(^1,2\). On the other hand, artificial lipid bilayers or liposomes can be prepared from lipid-solubilizing micelles using a surfactant depletion method\(^3\)\(^-\)\(^5\). Furthermore, several membrane proteins can be successfully reconstituted in liposomes with the aid of surfactants, because the method involves no harsh protein-denaturing processes, such as sonication, freezing, or exposure to organic solvent\(^6\)\(^-\)\(^10\).

The micellar-vesicular state transition accompanies a remarkable structural change in the molecular assembly. Many researchers have investigated the transition process using various light scattering methods and cryo-TEM, and detected transient states such as discoidal or thread-like assemblies during the transition to propose various models for the transition\(^11\)\(^-\)\(^17\). In practice, however, another concern is preparing micellar or vesicular state assemblies with the desired composition. For this purpose, the description of the micellar-vesicular state transition in terms of chemical thermodynamics would be valuable.

Abbreviations: OG; \(n\)-octyl-\(\beta\)-D-glucopyranoside, DOPC; 1,2-dioleolyl-sn-glycero-3-phosphocholine, CMC; critical micelle concentration, CVC; critical vesicle concentration

*Correspondence to: Ryo Ishiguro, Faculty of Engineering, Gifu University, 1-1 Yanagido, Gifu 501-1193, JAPAN
E-mail: ishiguro@gifu-u.ac.jp
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Various studies on the partition equilibrium of surfactants between the bulk solution phase and the micellar or vesicular state assembly phase have been reported. Because the bulk solution phase is in equilibrium with the dispersed assembly phase, the chemical potential or activity of the surfactant in the dispersed assembly could be monitored during the micellar-vesicular state transition by quantifying the monomerically dissolved surfactant concentration in the bulk solution. These researchers pointed out that the transition occurs at specific compositions of the assemblies and that the boundary compositions of the coexisting micellar and vesicular state assemblies are different from each other.

In a previous study, we studied the formation of a molecular assembly composed of a nonionic surfactant, n-octyl-β-D-glucopyranoside (OG), and an amphoteric phospholipid, 1,2-dioleoyl-sn-3-glycerophosphocholine (DOPC), supported on hydrophilic solid substrates immersed in aqueous solutions containing OG and DOPC. We obtained the partition equilibrium profiles of OG between the bulk solution phase and the supported assembly phase, as well as that between the bulk solution and the dispersed assembly. A comparison of these two partition equilibrium profiles revealed that both the supported and dispersed assemblies exhibit micellar-bilayer transitions, and that there are significant discrepancies between them.

In this study, we assumed the phase-separation model as illustrated schematically in Fig. 1, where the micellar state and the bilayer state in the supported or dispersed assembly phases are respectively regarded as “pseudo phases” distinct from each other that are in equilibrium with the bulk solution phase comprising monomerically dissolved surfactant and lipid. Using this model, we analyzed the partition equilibrium profiles to describe the micellar-bilayer transitions in supported or dispersed assemblies in terms of chemical thermodynamics.

2 Formulation for the partition equilibrium profile of OG for a dispersed or supported assembly

Figure 2 shows the partition equilibrium profile of OG between the bulk solution phase and the dispersed assembly phase, and that between the bulk solution and the supported assembly on a hydrophilized Ge substrate, which were obtained in a previous study. In the partition equilibrium profiles, the concentration of monomerically dissolved OG in the bulk solution, \( C_{\text{1OG}} \), is plotted against the averaged mole fraction of OG in the dispersed assembly, \( X_{\text{disOG}} \), or that in the supported assembly, \( X_{\text{supOG}} \). The profile of \( C_{\text{1OG}} \) vs. \( X_{\text{disOG}} \) (open squares) was determined using equilibrium dialysis while that of \( C_{\text{1OG}} \) vs. \( X_{\text{supOG}} \) (closed circles) was determined by ATR-FTIR spectroscopy. \( X_{\text{supOG}} \) was directly estimated from the ATR spectrum on the Ge substrate, and

![Fig. 1](image-url)
Micellar-Bilayer State Transition of OG/DOPC Assembly

C_{1OG} was calculated from the ambient solution composition using the \( C_{1OG} \) vs. \( X_{\text{dis}} \) profile. In these experiments, we used aqueous solutions containing various concentrations of OG, whereas the DOPC concentration was maintained as 1.15 mM.

The \( C_{1OG} \) vs. \( X_{\text{dis}} \) profile is similar to that obtained using OG and L-\( \alpha \)-phosphatidylcholine from egg yolk, as reported by Ueno and Paternostre et al. According to them, the profile can be divided into three regions. The range of \( X_{\text{dis}} \) from 0.8–1 is the micellar state region, and that of \( X_{\text{dis}} \) from 0–0.6 is the vesicular state region. In the range of \( X_{\text{dis}} \) from 0.6–0.8, the micellar-vesicular state transition occurs, and the turbidity of the solution sharply increases when \( X_{\text{dis}} \) is lower than 0.8, indicating the formation of vesicular particles. These interpretations are consistent with the phase-separation model, where the micellar and vesicular states are pseudo phases distinct from each other. In the transition region, three "phases," namely the micellar state assembly, vesicular state assembly, and the bulk solution, coexist to restrict the \( C_{1OG} \) constant (17.7 mM) owing to the Gibbs phase rule. Furthermore, the micellar state assembly and the vesicular assembly should have their specific boundary compositions, as demonstrated in various studies, and \( X_{\text{dis}} \) is only their average value. Therefore, \( X_{\text{dis}} \) should be related to the mass ratio between the micellar and vesicular states, according to the "lever rule." The partition equilibrium profile, as shown in Fig. 2, would be useful in treating experimental systems containing water, surfactants, and lipids (Supporting Information). We interpreted the partition equilibrium profile in the micellar or vesicular state region in a manner analogous to that of Holland & Rubingh. At equilibrium, the chemical potential of the OG in the micellar or vesicular state assembly is equal to that of the monomerically dissolved OG, as follows:

\[
\mu_{1OG} = \mu_{\text{M}OG}, \\
\mu_{1OG} = \mu_{\text{V}OG},
\]

where \( \mu_{\text{M}OG}, \mu_{\text{V}OG}, \) and \( \mu_{1OG} \) are the chemical potentials of OG in the monomerically dissolved, micellar, and vesicular states, respectively. These are given as functions of the activities of the corresponding OG molecules by

\[
\mu_{\text{M}OG} = \mu_{\text{M}OG}^0 + RT \ln a_{\text{M}OG} \\
\mu_{\text{V}OG} = \mu_{\text{V}OG}^0 + RT \ln a_{\text{V}OG}
\]

Fig. 2  Partition equilibrium profiles of OG between the bulk solution phase and the supported or dispersed assembly phases. The monomerically dissolved OG concentration, \( C_{1OG} \), is plotted against the OG mole fraction in the supported assembly phase on a Ge substrate, \( X_{\text{sup}} \) (closed circles) or that in the dispersed assembly phase, \( X_{\text{dis}} \) (open squares). These were obtained from ATR-FTIR spectroscopy and equilibrium dialysis, respectively. The partition equilibrium profile for the dispersed assembly phase could be divided into three regions; the micellar state region as fitted by a solid line; the vesicular state region as fitted by a dashed line; and the transition region represented as a dotted horizontal line (\( C_{1OG} = 17.7 \) mM). These lines are described based on the phase separation model and the regular solution approximation, where phase boundaries of micellar and vesicular states are represented by open diamonds, the intersection points of the theoretical lines: \( X_{\text{dis}} = 0.803 \) and 0.616. As for the supported assembly phase, the profile for the bilayer state region is clearly observed and is fitted by a chained line, although it is modified from that for the vesicular state region of the dispersed assembly phase. \( C_{1OG} \) at the transition region for the supported assembly phase is higher than that for the dispersed assembly phase, and is more inclined, asymptotically approaching the micellar state region.
\[ \mu_{BO} = \mu_{B} + RT\ln a_{BO}. \] (2)

The \( \mu_{BO} \)'s with the superscripts \( o \) and \( a_{BO} \)'s represent the standard chemical potentials and activities of OG in the corresponding states, respectively. Here, we assume that the activity of the monomerically dissolved OG is represented by the molar concentration, \( C_{BO} \), and that the activity of OG in the micellar or vesicular state assembly is the product of the activity coefficient, \( f_{BO} \), and the mole fraction of OG in the assembly, \( X_{BO}^\text{m} \). Consequently, we obtain

\[ \mu_{B} + RT\ln C_{BO} = \mu_{B} + RT\ln (f_{BO} X_{BO}^\text{m}) , \]

\[ \mu_{B} + RT\ln C_{BO} = \mu_{B} + RT\ln (f_{BO} X_{BO}^\text{m}). \] (3)

Using the regular solution approximation\(^{25}\), the activity coefficients of OG in the micellar and vesicular state assemblies, \( f_{BO} \) and \( f_{BO} \), are given by

\[ f_{BO} = \exp \left[ \beta^m (1 - X_{BO}^\text{m})^2 \right], \]

\[ f_{BO} = \exp \left[ \beta^V (1 - X_{BO}^\text{m})^2 \right], \] (4)

where \( \beta^m \) and \( \beta^V \) are the interaction parameters between OG and DOPC in the micellar and vesicular state assemblies, respectively. By substituting Equation (4) into Equation (3) and transforming the resultant equation, we obtain

\[ C_{BO} = C_{BO}^o \exp \beta^m (1 - X_{BO}^\text{m})^2 X_{BO}^\text{m}, \]

\[ C_{BO} = C_{BO}^o \exp \beta^V (1 - X_{BO}^\text{m})^2 X_{BO}^\text{m}. \] (5)

\( C_{BO}^o \) and \( C_{BO}^o \) correspond to the CMC and CVC for the micellar and vesicular state assemblies of pure OG, respectively. We calculated the regression lines for the micellar and vesicular state regions of the partition equilibrium profile for the dispersed assembly; these are represented in Fig. 2 as solid and dashed lines, respectively. Using these regressions, we could estimate the parameters for Equation (5) that are tabulated in Table 1. \( C_{BO}^o \) was determined as 25.9 mM, which corresponds well with the CMC of pure OG\(^{23}\). \( C_{BO}^o \) was revealed to be higher than \( C_{BO}^o \), ensuring that the pure-OG vesicular state assembly remained imaginary. As for the interaction parameters, we could compare the obtained \( \beta^m \) and \( \beta^V \) with those for various sets of binary surfactant mixtures, which Holland tabulated in his review\(^{20}\). Our \( \beta \) values are comparable to those for the set composed of nonionic and ionic surfactants; the absolutes are smaller than those for anionic-cationic sets \((-10\) to \(-20\)) and larger than those for nonionic-nonionic sets \((0\) to \(-1\)).

The partition equilibrium profile for the supported assembly phase, \( C_{BO} \) vs. \( X_{BO}^\text{m} \), is roughly similar to that for the dispersed assembly phase and is also divided into the micellar state region, the bilayer state region, and the transition region, although significant modifications are observed. The transition region for the supported assembly has a higher \( C_{BO} \) than that for the dispersed assembly and has a steeper slope, gradually approaching the micellar state region; thus, the boundary of the micellar state region is more ambiguous. The profile for the bilayer state region is also characterized by fitting the theoretical curve similar to Equation (5):

\[ C_{BO} = C_{BO}^o \exp \beta^V (1 - X_{BO}^\text{m})^2 X_{BO}^\text{m}. \] (6)

The estimated parameters are presented in Table 1. \( C_{BO}^o \) is larger than \( C_{BO}^o \), suggesting that the imaginary pure-OG supported bilayer is more unstable than the pure-OG vesicle. On the other hand, \( \beta^V \) is more negative than \( \beta^V \), suggesting that the OG/DOPC interaction in the supported bilayer is more favorable than that in the vesicles.

### 3 Micellar-vesicular state transition described by thermodynamics

Using the partition equilibrium profile, we could derive the molar Gibbs free energies of the micellar and vesicular state phases as a function of their composition to interpret the micellar-vesicular state transition, as shown below. Such a diagram would enable us to conduct further systematic studies on their dependence on temperature, chemical structure of surfactant or lipid, and addition of the third component (membrane protein or minor lipid), etc., to provide a more comprehensive understanding of the micellar-vesicular state transition.

We define the averaged molar Gibbs energies of the micellar and vesicular state phases, \( G^m \) and \( G^v \) as follows:

| Table 1 Parameters for theoretical partition equilibrium profiles of OG between the bulk solution phase and various assembly phases composed of OG and DOPC, at 25°C. |
|-----------------------------------------------|
| Dispersed micellar state | Dispersed vesicular state | Supported bilayer state |
| \( C_{BO}^o \) * mM | \( \beta^m \) † | \( C_{BO}^o \) * mM | \( \beta^V \) † | \( C_{BO}^o \) * mM | \( \beta^V \) † |
| 25.9 | -4.12 | 39.2 | -2.11 | 56.5 | -5.22 |

* Concentration of the monomerically dissolved OG, \( C_{BO} \) being in equilibrium with the corresponding assembly of pure OG.
† Interaction parameter between OG and DOPC in the corresponding assembly, based on the regular solution approximation.
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\[ G^M = X_{\text{M}} \mu^M_\text{M} + (1 - X_{\text{M}}) \mu^M_{\text{DOPC}}, \]
\[ G^V = X_{\text{V}} \mu^V_\text{M} + (1 - X_{\text{V}}) \mu^V_{\text{DOPC}}, \quad (7) \]

Using Equations (3) and (4), and similar equations, \( \mu_{\text{OG}} \) and \( \mu_{\text{DOPC}} \) in the micellar state are given as
\[ \mu^M_{\text{M}} = \mu^M_{\text{DOPC}} + RTd^M(1 - X_{\text{M}})^2 + RTnX_{\text{M}}, \]
\[ \mu^M_{\text{DOPC}} = \mu^M_{\text{DOPC}} + RTd^M X_{\text{M}}^2 + RTn(1 - X_{\text{M}}). \quad (8) \]

For those in the vesicular phase, similar equations are given as
\[ \mu^V_{\text{M}} = \mu^V_{\text{DOPC}} + RTd^V(1 - X_{\text{V}})^2 + RTnX_{\text{V}}, \]
\[ \mu^V_{\text{DOPC}} = \mu^V_{\text{DOPC}} + RTd^V X_{\text{V}}^2 + RTn(1 - X_{\text{V}}). \quad (9) \]

In Equations (8) and (9), \( \mu^M_{\text{M}}, \mu^V_{\text{M}}, \mu^M_{\text{DOPC}}, \) and \( \mu^V_{\text{DOPC}} \) remain to be estimated. Therefore, we estimated them as follows. First, using Equations (3)–(5), \( \mu^M_{\text{M}} \) and \( \mu^V_{\text{M}} \) are related to each other via \( C^M_{\text{M}} \) and \( C^V_{\text{M}} \) by
\[ \mu^M_{\text{M}} - \mu^V_{\text{M}} = RTn \left( \frac{C^M_{\text{M}}}{C^V_{\text{M}}} \right). \quad (10) \]

Second, when the micellar and vesicular states coexist, \( \mu_{\text{DOPC}} \) would be common in these states, as well as \( \mu_{\text{OG}} \). In other words, \( \mu_{\text{DOPC}} \) in the micellar state phase boundary \( (X_{\text{M}} = 0.803) \) should be equal to that in the vesicular state phase boundary \( (X_{\text{V}} = 0.616) \). Therefore, we obtain
\[ \mu^M_{\text{DOPC}} + RTd^M 0.803^2 + RTn(1 - 0.803) \]
\[ = \mu^V_{\text{DOPC}} + RTd^V 0.616^2 + RTn(1 - 0.616). \quad (11) \]

Because chemical potentials essentially have their significance only in the difference between them, we define \( \mu^M_{\text{M}} \) and \( \mu^V_{\text{M}} \) to be 0 as a reference point to estimate \( \mu^M_{\text{DOPC}} \) as 1.93 kJ mol\(^{-1}\) and \( \mu^V_{\text{DOPC}} \) as 6.25 kJ mol\(^{-1}\).

Figure 3 shows \( G^M \) and \( G^V \) as functions of \( X_{\text{M}} \) to account for the micellar-vesicular state transition. In the region of \( X_{\text{M}} = 0.803–1 \), where the micellar state is observed, \( G^M \) is lower than \( G^V \). In the vesicular state region of \( X_{\text{V}} = 0–0.616 \), on the other hand, \( G^V \) is lower than \( G^M \). These properties are mainly due to the energy difference between \( \mu^M_{\text{M}} \) and \( \mu^V_{\text{M}} \), and that between \( \mu_{\text{DOPC}} \) and \( \mu^V_{\text{DOPC}} \). In other words, the pure-OG micellar state is more stable than the pure-OG vesicular state, and the pure-DOPC vesicular state is more stable than the pure-DOPC micellar state. \( \mu^V_{\text{DOPC}} \) and \( \mu^V_{\text{DOPC}} \) cannot be estimated directly because the pure-OG vesicular state and the pure-DOPC micellar state are imaginary; however, they are related to molecular geometry of OG and DOPC, such as the hydrophilic-lipophilic balance. The common tangent line of \( G^M \) and \( G^V \) indicates that the micellar state at the tangent point of \( X_{\text{M}} = 0.803 \) and the vesicular state at \( X_{\text{V}} = 0.616 \) (open diamonds) have common \( \mu_{\text{OG}} \) and \( \mu_{\text{DOPC}} \) and that the micellar and vesicular states coexist with their boundary compositions in this sandwiched region corresponding to the transition region in Fig. 2.

### 4 Influence of the solid substrate on the micellar-bilayer state transition in terms of thermodynamic quantities

As shown in Fig. 2, the partition equilibrium profile of OG for the supported assembly has characteristics similar to those of the dispersed assembly, although there are significant discrepancies. Because the supported assembly phase, the dispersed assembly phase, and the bulk solution phase are in equilibrium with one another, the chemical
potentials of each component should be common throughout the entire system, and the Gibbs–Duhem equation should hold in each phase, as written below:

\[ n^\text{OG}_\text{dis} \mu^\text{OG} + n^\text{DOPC}_\text{dis} \mu^\text{DOPC} = 0 \]

\[ n^\text{OG}_\text{sup} \mu^\text{OG} + n^\text{DOPC}_\text{sup} \mu^\text{DOPC} = 0 \]

\[ n^\text{OG}_\text{blk} \mu^\text{OG} + n^\text{DOPC}_\text{blk} \mu^\text{DOPC} = 0 \]  

Equation (12)

\( \mu \) and \( n \) are the chemical potential and the number of moles, respectively. The subscripts refer to the components, and the superscripts refer to the phases ("dis," the bulk solution phase; "dis," the dispersed assembly phase; and "sup," the supported assembly phase). Under these restrictions, the partition equilibrium profiles between the bulk solution and the assemblies should be the same, regardless of whether the assembly is dispersed in the ambient solution or supported on the substrate. Therefore, the difference between the partition equilibrium profiles shown in Fig. 2 would result from the interaction between the assembly and the substrate, which is not considered in the Gibbs–Duhem equation. Now, we discuss how the interaction energy modifies the partition equilibrium profile for the supported assembly.

In a manner analogous to the Gibbs adsorption isotherm, we define the internal energy of the supported assembly, \( E^\text{sup} \), as

\[ dE^\text{sup} = TdS^\text{sup} + pdV^\text{sup} + \mu^\text{OG} n^\text{OG}_\text{dis} + \mu^\text{DOPC} n^\text{DOPC}_\text{dis} + \varepsilon^\text{sub} dA, \]  

Equation (13)

where \( T \) is the temperature, and \( p \) is the pressure. \( S^\text{sup} \) and \( V^\text{sup} \) are the entropy and volume of the supported assembly, respectively. \( A \) is the area of the assembly/substrate interface, and \( \varepsilon^\text{sub} \) is the interaction energy between the assembly and the substrate per unit area, corresponding to the surface tension as excess energy in the Gibbs adsorption isotherm. Assuming that the supported assembly is two-dimensionally uniform along the substrate surface, we integrate the right side of Equation (13) with \( A \) to obtain

\[ E^\text{sup} = TS^\text{sup} + pV^\text{sup} + \mu^\text{OG} n^\text{OG}_\text{dis} + \mu^\text{DOPC} n^\text{DOPC}_\text{dis} + \varepsilon^\text{sub} A, \]  

Equation (14)

because all of the other extensive parameters should be proportional to \( A \). Combining Equation (13) and the total differential of Equation (14), we obtain

\[ d\mu^\text{sub} = -\frac{n^\text{OG}_\text{dis} d\mu^\text{OG} - n^\text{DOPC}_\text{dis} d\mu^\text{DOPC}}{A} \]

\[ = -\Gamma^\text{OG} d\mu^\text{OG} - \Gamma^\text{DOPC} d\mu^\text{DOPC} \]  

Equation (15)

at constant \( T \) and \( p \), where \( \Gamma^\text{OG} \) and \( \Gamma^\text{DOPC} \) correspond to the amounts of OG and DOPC in the supported assembly, which are represented as the number of moles per unit area. Equation (15) can be considered the Gibbs–Duhem equation modified by \( \varepsilon^\text{sub} \). If \( \varepsilon^\text{sub} \) were constant regardless of the composition of the supported assembly, Equation (15) would be identical to the “normal” Gibbs–Duhem equation.

Fig. 4 Relation between chemical potentials of OG and DOPC, \( \mu^\text{OG} \) and \( \mu^\text{DOPC} \) in our entire system. A solid line corresponds to \( \mu^\text{OG} \) vs. \( \mu^\text{DOPC} \) calculated from the theoretical curves of the partition equilibrium profile for the dispersed assembly phase as shown in Fig. 2, using Equations (8) and (9). At the point where \( \mu^\text{OG} = -0.942 \) kJ mol\(^{-1}\) and \( \mu^\text{DOPC} = -4.35 \) kJ mol\(^{-1}\), the curve sharply bends, reflecting the micellar-vesicular state transition (the left hand region of the point corresponds to the vesicular state region, and the right to the micellar state region). A dotted line corresponds to the imaginary curve assuming that the dispersed assembly were kept to be in the vesicular state regardless of the composition, and did not exhibit any transitions.

Therefore, the modification of the partition equilibrium profile due to the supporting substrate, as shown in Fig. 2, indicates that \( \varepsilon^\text{sub} \) exhibits some dependence on the composition of the assembly.

We tried to estimate \( \varepsilon^\text{sub} \) between the bilayer state of the supported assembly and the Ge substrate as a function of the mole fraction of OG in the supported assembly phase, \( X^\text{OG} \), using Equation (15). \( \mu^\text{OG} \), as a function of \( X^\text{OG} \) is obtained from the theoretical curve for the bilayer state of the supported assembly in Fig. 2, which is represented as a chained line, using \( \mu^\text{OG} = \mu^\text{OG}_0 + RT \ln C^\text{OG}_0 \), where \( \mu^\text{OG}_0 \) is the standard chemical potential of monomerically dissolved OG and is defined as \( -RT \ln C^\text{OG}_0 \) by Equation (3) and the definition \( \mu^\text{OG}_0 = 0 \) as written above. In addition, according to the regular solution approximation for the dispersed assembly, \( \mu^\text{OG} \) and \( \mu^\text{DOPC} \) are determined as functions of \( X^\text{OG} \) and are expressed by Equations (8) and (9), respectively, which restricts the relation between \( \mu^\text{OG} \) and \( \mu^\text{DOPC} \), as shown in Fig. 4. Consequently, we obtained \( \mu^\text{OG} \) and \( \mu^\text{DOPC} \) as functions of \( X^\text{OG} \) as shown in Fig. 5(a). At the same time, we estimated \( \mu^\text{OG} \) and \( \mu^\text{DOPC} \) as functions of \( X^\text{OG} \) as shown in Fig. 5(b), using the total weight of the supported assembly per unit area, \( w^\text{sup} \), determined in a previous
Fig. 5  (a) Chemical potentials of OG and DOPC, $\mu_{\text{OG}}$ and $\mu_{\text{DOPC}}$, as functions of the OG mole fraction in the supported assembly, $X_{\text{OG}}^{\text{sup}}$. $\mu_{\text{OG}}$ (solid line) was calculated from the theoretical curve for the bilayer state of the supported assembly (chained line in Fig. 2), and $\mu_{\text{DOPC}}$ (dashed line) was calculated from $\mu_{\text{OG}}$ using Fig. 4. (b) Amounts of OG and DOPC in the supported assembly per unit area, $\Gamma_{\text{OG}}$ (solid line) and $\Gamma_{\text{DOPC}}$ (dashed line) as functions of $X_{\text{OG}}^{\text{sup}}$. These were calculated from the total weight of the supported assembly per unit area, $w_{\text{sup}}$, determined in a previous study\(^\text{23}\), with complementing the missing data in the range of $X_{\text{OG}}^{\text{sup}} = 0$–0.45 by the quadratic regression curve (Supporting Information II). (c) The interaction energy between the bilayer state of the supported assembly and a Ge substrate, $\varepsilon_{\text{sub}}$ (solid line) as a function of $X_{\text{OG}}^{\text{sup}}$. This was estimated by numerical integration of Equation (15) using data in (a) and (b). Dotted lines in (a) and (c) represent the imaginary $\mu_{\text{DOPC}}$ and $\varepsilon_{\text{sub}}$, assuming that dispersed assembly were kept to be in the vesicular state regardless of the composition, as represented by a dotted line in Fig. 4.

As shown in Fig. 5(c), the larger $X_{\text{OG}}^{\text{sup}}$ was, the higher the affinity of the supported bilayer toward the Ge substrate was, as reflected by the negative slope of $\varepsilon_{\text{sub}}$ ($X_{\text{OG}}^{\text{sup}}$) in the bilayer state region. The variation in $\varepsilon_{\text{sub}}$ in the bilayer region is on the order of $10^{-9}$ kJ cm$^{-2}$, which corresponds to a difference of only a few kJ mol$^{-1}$ between OG and DOPC regarding their contributions to $\varepsilon_{\text{sub}}$. $\varepsilon_{\text{sub}}$ is considered to involve the bonding energy between the substrate and the OG or DOPC. Many researchers have demonstrated that supported lipid bilayers are formed on the substrate surface via a water layer with a thickness of several nanometers\(^\text{27-31}\). Therefore, the bonding OG or DOPC to the substrate surface was ascribed to a hydrogen bonding network, each of which was considered to be several tens of kJ/mol. Because the bonding energy also depends on the
chemical properties of the substrate, the partition equilibrium profile in the bilayer region should vary according to the type of substrate, even though it might have a minor effect. In addition, $\varepsilon_{\text{sub}}$ would involve the energy required to deform the assembly structure to be fit for the flat surface. Sun & Ueno\textsuperscript{a} and Ueno et al.\textsuperscript{c} reported that the small unilamellar vesicles of phospholipids containing some amount of nonionic or amphoteric surfactants tended to grow by fusing with each other, suggesting that these surfactants promote the flexibility of the lipid bilayer structure. This effect would also contribute to the negative slope of $\varepsilon_{\text{sub}} (X_{\text{dis}})$ in the bilayer state region.

Because the ambient solution system is much larger than the supported assembly phase, the chemical potentials of the entire system are determined from the partition equilibrium between the dispersed assembly phase and the bulk solution phase. When the dispersed assembly is in the vesicular state, the corresponding region of the $\mu_{\text{DOPC}}$ vs. $\mu_{\text{OG}}$ profile in Fig. 4 is "roughly" consistent with the partition equilibrium profile for the bilayer state of the supported assembly, resulting in a relatively small change in $\varepsilon_{\text{sub}}$ with the $X_{\text{dis}}$. However, once the composition of the dispersed assembly passes through the micellar-vesicular state transition point, the $\mu_{\text{DOPC}}$ vs. $\mu_{\text{OG}}$ profile for the micellar state region is no longer consistent with the partition equilibrium profile for the bilayer state of the supported assembly, resulting in a large change in $\varepsilon_{\text{sub}}$ with the $X_{\text{dis}}$. We also estimated the imaginary $\mu_{\text{DOPC}}$ vs. $\mu_{\text{OG}}$ profile, and the imaginary $\mu_{\text{DOPC}}$ and $\varepsilon_{\text{sub}}$ as functions of the $X_{\text{dis}}$, assuming that the dispersed assembly was always in a vesicular state regardless of the composition, as shown by the dotted lines in Figs. 4, 5(a), and 5(c). In this case, a marked change in $\varepsilon_{\text{sub}}$ does not occur until at least $X_{\text{dis}} = 0.8$, suggesting that the transition from the bilayer state to the micellar state in the supported assembly would be observed only at much higher $X_{\text{dis}}$. Therefore, the micellar-bilayer state transition in the supported assembly is mainly attributed to the transition in the dispersed assembly.

5 Limitation of the Phase-separation Model

Our approach, based on the phase-separation model, involves several oversimplifications. According to the Gibbs phase rule, for example, when micellar and vesicular state assemblies coexist, $C_{\text{dis}}$ should be constant. In practice, Fig. 2 shows that $C_{\text{dis}}$ in the micellar-vesicular-state transition region changes slightly with $X_{\text{dis}}$; for example, when $X_{\text{dis}} = 0.59, C_{\text{dis}} = 16.4 \text{ mM}$. Furthermore, $C_{\text{dis}}$ slightly increases as $X_{\text{dis}}$ increases, and is 18.8 mM when $X_{\text{dis}} = 0.81$. This phenomenon demonstrates the limitations of the proposed model.

We assumed that the mixed micellar or vesicular state assembly was a "pseudo phase" and that the number concentration of the assembly particle did not influence the phase equilibrium. However, when we regarded the micellar and vesicular particles as chemical species, and gave them free energies derived from the configuration entropy, the chemical potentials of OG and DOPC would depend on the number concentration of the micellar or vesicular particles. In fact, the partition equilibrium profile shifts to a higher $C_{\text{dis}}$ as the preparation concentration of DOPC increases, as noted in Supporting Information III. Therefore, for a stringent experiment and analysis, we should prepare a partition equilibrium profile using the desired concentration ranges of surfactants and lipids. In a previous study\textsuperscript{c}, we prepared the partition equilibrium profile for the dispersed assembly and that for the supported one using a series of OG/DOPC solutions with $C_{\text{DOPC}} = 1.15 \text{ mM}$. Furthermore, as shown in Fig. 3, the troughs of the molar Gibbs free energy landscape of the micellar and vesicular assemblies are shallow, and even at the midpoint of phase separation, namely $X_{\text{dis}} = 0.72$, the gain of free energy due to phase separation is only $\sim 0.1 \text{ kJ/mol}$. Therefore, any micellar and vesicular particles with $X_{\text{dis}} = 0.6-0.8$ could exist probabilistically, even though the probabilities would depend on their association numbers. The distribution of the compositions of the micellar and vesicular assemblies should also be considered.

Therefore, to confirm the validity of our micellar-vesicular phase-separation model, we also constructed a thermodynamic description of the assembly composed of OG and DOPC without explicitly assuming the micellar-vesicular transition.

As for the equilibrium between the dispersed assembly phase and the bulk solution phase, the chemical potential of the OG is described by

$$\mu_{\text{OG}} = \mu_{\text{OG}}^{\text{bulk}} + RT\ln C_{\text{OG}} = \mu_{\text{OG}}^{\text{lipid}} + RT\ln \left( f_{\text{DOPC}}^{\text{lipid}} X_{\text{dis}}^{\text{lipid}} \right),$$

and that of DOPC is described by

$$\mu_{\text{DOPC}} = \mu_{\text{DOPC}}^{\text{bulk}} + RT\ln \left( f_{\text{DOPC}}^{\text{lipid}} (1 - X_{\text{dis}}^{\text{lipid}}) \right).$$

In these equations, $\mu_{\text{OG}}^{\text{lipid}}$ and $\mu_{\text{DOPC}}^{\text{lipid}}$ correspond to the chemical potential of OG in the pure-OG micelles and DOPC in the pure-DOPC vesicles, respectively. In this treatment, the micellar state was not explicitly distinguished from the vesicular state. For this reason, the complicated behavior of the partition equilibrium profile in Fig. 2 can be ascribed to the activity coefficients in the dispersed assembly, $f_{\text{DOPC}}^{\text{lipid}}$ and $f_{\text{DOPC}}^{\text{micelle}}$, as functions of $X_{\text{dis}}$. From the $C_{\text{dis}}$ vs. $X_{\text{dis}}$ profile in Fig. 2, we estimated $\mu_{\text{OG}}$ and $f_{\text{DOPC}}^{\text{lipid}}$ as functions of $X_{\text{dis}}$ using Equation (16), as shown in Fig. 6. We also estimated $\mu_{\text{DOPC}}$ by numerically integrating

$$d\mu_{\text{DOPC}} = \frac{-X_{\text{dis}}^{\text{lipid}}}{1 - X_{\text{dis}}^{\text{lipid}}} d\mu_{\text{OG}},$$

which is derived from the Gibbs–Duhem equation in the dispersed assembly phase, and $f_{\text{DOPC}}^{\text{lipid}}$, using Equation (17).

Note that, in the numerical calculations, we defined $\mu_{\text{OG}}^{\text{lipid}}$.
Micellar-Bilayer State Transition of OG/DOPC Assembly

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[Figure 6](#) Thermodynamic analysis of the dispersed assembly without assuming the micellar-vesicular phase separation. (a) Chemical potentials of OG and DOPC, $\mu_{\text{OG}}$ and $\mu_{\text{DOPC}}$, as functions of the mole fraction of OG in the dispersed assembly, $X_{\text{OG}}^{\text{dis}}$, were calculated from $C_{\text{OG}}$ vs. $X_{\text{OG}}^{\text{dis}}$ profile (open squares) in Fig. 2 using Equation (16), and $\mu_{\text{DOPC}}$ (closed triangles) was calculated from $\mu_{\text{OG}}$ by integrating Equation (18). (b) Activity coefficients of the OG and DOPC in the dispersed assembly, $f_{\text{OG}}$ (open squares) and $f_{\text{DOPC}}$ (closed triangles), as functions of $X_{\text{OG}}^{\text{dis}}$. These were calculated using Equations (16) and (17).

and $\mu_{\text{DOPC}}$ as 0, which corresponds to the definition employed in Figs. 3 and 4.

Using $f_{\text{OG}}$ and $f_{\text{DOPC}}$, we calculated the Gibbs free energy change of mixing OG and DOPC in the dispersed assembly, $\Delta G^{\text{mix}}$, using the equation below:

$$\Delta G^{\text{mix}} = \Delta G^{\text{ideal}} + \Delta G^{\text{nonideal}}$$

$$= RT \left[ X_{\text{OG}}^{\text{dis}} \ln X_{\text{OG}}^{\text{dis}} + (1 - X_{\text{OG}}^{\text{dis}}) \ln (1 - X_{\text{OG}}^{\text{dis}}) \right]$$

$$+ RT \left[ X_{\text{DOPC}}^{\text{dis}} \ln f_{\text{DOPC}}^{\text{dis}} + (1 - X_{\text{DOPC}}^{\text{dis}}) \ln f_{\text{DOPC}}^{\text{dis}} \right].$$

In this equation, $\Delta G^{\text{ideal}}$ and $\Delta G^{\text{nonideal}}$ represent the contributions of ideal mixing and non-ideality due to intermolecular interactions, respectively. Figure 7 shows $\Delta G^{\text{mix}}$, $\Delta G^{\text{ideal}}$, and $\Delta G^{\text{nonideal}}$ as functions of $X_{\text{OG}}^{\text{dis}}$. Although $\Delta G^{\text{nonideal}}$ exhibits two distinct minima, the upward convex region between them completely cancels out the downward convex shape of $\Delta G^{\text{ideal}}$. Consequently, $\Delta G^{\text{mix}}$ changes linearly in the range of $X_{\text{OG}}^{\text{dis}} = 0.6–0.8$. We conclude that Fig. 7 is essentially identical to Fig. 3, and that the linear region of $\Delta G^{\text{mix}}$ corresponds to the common tangent line in Fig. 3, that is, the $\Delta G^{\text{mix}}$ curve is actually separated into two different types of curve at $X_{\text{OG}}^{\text{dis}} = 0.6–0.8$, where the phase transition occurs.

Using the correlation between $\mu_{\text{OG}}$ and $\mu_{\text{DOPC}}$ as shown in Fig. 6(a), we investigated the influence of the solid substrate on the assembly in a manner similar to that described in Section 4. Figure 8(a) shows $\mu_{\text{OG}}$ and $\mu_{\text{DOPC}}$ as functions of the mole fraction of OG in the supported assembly, $X_{\text{OG}}^{\text{sup}}$, and Fig. 8(b) shows $\varepsilon^{\text{mix}}$ as a function of $X_{\text{OG}}^{\text{sup}}$. It should be noted that $\varepsilon^{\text{mix}}$ in Fig. 8(b) was evaluated by defining $\epsilon^{\text{sub}} = 0$ at $X_{\text{OG}}^{\text{sup}} = 1$, whereas $\epsilon^{\text{sub}}$ in Fig. 5(c) was defined as $\epsilon^{\text{sub}} = 0$ at $X_{\text{OG}}^{\text{sup}} = 0$. For comparison, we also plotted $\varepsilon^{\text{mix}}$ in Fig. 5(c) in Fig. 8(b), which was displaced vertically to be superimposed. Figure 8(b) indicates that $\varepsilon^{\text{mix}}$ of the supported bilayer calculated based on the micellar-vesicular phase separation model and that without any explicit assumption agree well in terms of the slope in the

Fig. 7 Gibbs free energy change of mixing OG and DOPC in the dispersed assembly as a function of the mole fraction of OG, $X_{\text{OG}}^{\text{dis}}$, which was derived using Equation (19) without assuming the micellar-vesicular phase separation. Open squares, Gibbs free energy change of mixing OG and DOPC, $\Delta G^{\text{mix}}$; solid line, the contribution of the ideal mixing, $\Delta G^{\text{ideal}}$; open circles, that of the non-ideality due to the intermolecular interactions, $\Delta G^{\text{nonideal}}$. $\Delta G^{\text{mix}}$ in the range of $X_{\text{OG}}^{\text{dis}} = 0.6–0.8$ exhibits linear, represented by a dotted line.

Fig. 8(a) shows $\mu_{\text{OG}}$ and $\mu_{\text{DOPC}}$ as functions of the mole fraction of OG in the supported assembly, $X_{\text{OG}}^{\text{sup}}$, and Fig. 8(b) shows $\varepsilon^{\text{mix}}$ as a function of $X_{\text{OG}}^{\text{sup}}$. It should be noted that $\varepsilon^{\text{mix}}$ in Fig. 8(b) was evaluated by defining $\epsilon^{\text{sub}} = 0$ at $X_{\text{OG}}^{\text{sup}} = 1$, whereas $\epsilon^{\text{sub}}$ in Fig. 5(c) was defined as $\epsilon^{\text{sub}} = 0$ at $X_{\text{OG}}^{\text{sup}} = 0$. For comparison, we also plotted $\varepsilon^{\text{mix}}$ in Fig. 5(c) in Fig. 8(b), which was displaced vertically to be superimposed. Figure 8(b) indicates that $\varepsilon^{\text{mix}}$ of the supported bilayer calculated based on the micellar-vesicular phase separation model and that without any explicit assumption agree well in terms of the slope in the
and pure lipid, which are closely related to their molecular geometries. Furthermore, our approach has versatility and extensibility, because the Gibbs free energy diagram could integrate additional experimental data, for example, the effects of the types of surfactant and lipid, the additional lipophiles including the third lipid and membrane protein, and the environment, including temperature, pressure, solvent composition, and solid substrates, as discussed in this paper. Therefore, it is feasible to modify our model to involve the law of mass action using statistical mechanics.

6 Conclusion
In a previous paper\textsuperscript{23}, we investigated the phase equilibrium between the supported OG/DOPC assembly phase on hydrophilized substrates and its ambient aqueous solution system consisting of the dispersed assembly phase in the micellar or vesicular state and the bulk solution phase comprising the monomerically dissolved OG. The obtained partition equilibrium profiles of OG between the bulk solution phase and the dispersed or supported assembly phases suggest that a micellar-bilayer transition occurs both in the dispersed and supported assemblies, even though there are significant modifications.

In order to interpret the difference in the partition equilibrium profile depending on whether the assembly is dispersed or supported on substrates, we constructed a simple thermodynamic model to describe the micellar-bilayer transition, assuming a phase-separation model in which the micellar state and the bilayer state are pseudo phases distinct from each other. The micellar state region and the bilayer state region in the partition equilibrium profile were interpreted using the regular solution theory, which is often used in studies on mixed surfactant systems\textsuperscript{24, 26}, to obtain the Gibbs free energies of the micellar and bilayer states as a function of the composition.

Based on this model, we successfully interpreted the partition equilibrium profile for the supported bilayer, considering the restriction of the chemical potentials of OG and DOPC due to the ambient solution system, and additional energy, $\varepsilon_{\text{sub}}$, the interaction energy between the assembly and the substrate. As shown in Fig. 5(c), if the supported assembly retained the bilayer state over the entire range of $X_{\text{OG}}$, $\varepsilon_{\text{sub}}$ would increase sharply at the transition point of the dispersed assembly. This suggests that the supported assembly cannot adopt the bilayer state under the thermodynamic restraint of the ambient micellar solution system and transforms to the adsorbed micellar state.
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Conflicts of Interest Statement

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Supporting Information

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