Theoretical Possibility of Cuplike Vesicles for Aggregates of Lipid and Bile Salt Mixture

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Received: August 5, 2002

The aggregate forms of bile salt and lipid mixture were studied by a statistical mechanical theory. The free energies of disclike mixed micelles, cuplike vesicles, and of rodlike micelles were defined and the minimized free energies were compared to study the stable forms of these aggregates. In addition to the adsorption affinity free energy of bile salt to the vesicle membrane, the following two energy terms were taken into account: one is the bending energies of the cuplike vesicle and rodlike micelle, and the other is the enhancement of the affinity free energies of bile salt from aqueous solvent to the curved membranes. By postulating the spontaneous curvature, which is assumed to be proportional to the surface coverage of bile salt, the cuplike vesicle and the rodlike micelle were shown to be mechanically frustrated. Despite the bending energy cost, the cuplike vesicle was shown to be the stable aggregate form in a certain concentration region. In this concentration region the molecular weight of the aggregate is expected to increase monotonically, while the radius of gyration is expected to possess a maximum value. This is consistent with the light scattering measurement made by Egelhaaf and Schurtenberger (*J. Phys. Chem.* **1994**, *98,* 8560).

Introduction

The aggregation problem of bile salt and lipid is related to biochemical and biomedical problems, such as the digestion of lipid, pathogenesis of cholesterol gallstones, and so on. Bile salts are highly soluble in water and solubulize lipid and their aggregated forms have been assumed to be disclike vesicles, spherical vesicles, and/or rodlike micelles. So far, many experimental work has been done on geometrical forms of aggregates in mixed solutions of lipid and bile salt;[1−16] however, there appeared several theoretical analyses on this problem.[3,4,9,14] Theoretical treatments have been based on the assumption of the adsorption isotherm of bile salt to the rim of discs and the membrane of discs or vesicles.[3,4,9,14]

In this paper, the author proposes that a cuplike vesicle of partial sphere could be a stable aggregate beside discs or spherical vesicles for the lipid and bile salt mixture. The possibility of cuplike vesicles has not seriously been considered partly because of the preconception that the energy cost of bending of the matrix membrane for cuplike vesicles would be expected to be high compared to that of the disclike mixed micelle. By optical microscopes, Hotani and co-workers observed cuplike vesicles of a mixed system of lipid and protein (talin).[17] The shape of the cup changed as a function of the concentration of talin. The author and co-workers successfully explained these observed data of cuplike vesicles by a statistical mechanical calculation.[18] This report is an extension of our theory to the mixture of lipid and talin.

The dilution procedure has been applied for the study of the shape change of the aggregates of the lipid bile salt system. Intuitively, we expect that the change of the radius of the rim of cuplike vesicle might easily adjust the change of adsorbed bile salts to the rim during the dilution procedure. Our analysis will show the theoretical possibility of the stable cuplike vesicles. Thus, the existing light scattering data on the radius of gyration and others had better be reanalyzed.

Instead of postulating the chemical potential balance of monomer bile salts in aqueous solution and those in aggregates as in previous reports, the chemical potential balance will be derived by minimizing the total free energy of the system. Also, the bending energy of the vesicles will be taken into account to evaluate the energy cost of curved cuplike vesicles. In the next section, we will first reproduce the results of old theories on the disclike mixed micelles by using our theory. Next, the formula for the cuplike vesicles will be formulated. Last, the rodlike micelle will also be analyzed. In the last section, we will compare our theory with the experimental data observed so far.

Theoretical Formulation

The previous theories on the disclike micelles or mixed micelles postulated the adsorption isotherm of bile salt to the disc surface. In this paper, we will define the free energy of the total system and will derive the adsorption isotherm by minimizing the free energy. Thus, we will be able to evaluate whether the assumed system is stable or not by comparing the minimized free energies of different systems.

1. Disclike Vesicles. Here we will treat the disclike vesicles and will confirm the validity of our theory by reproducing the previous theoretical studies. To analyze the system of lipid and bile salt in aqueous solution, we first define the free energy F of the total system. Let X and N_L be the total numbers of bile salts and lipid, respectively, and the total volume of the system is denoted as V. Let N be the total number of adsorbed bile salts and N_b be the number of bile salts adsorbed at the disc surface. Therefore, the remaining $X - N$ bile salts are dissolved as monomers in the aqueous solution and $N - N_b$ bile salts are adsorbed at the rim of the disc. The schematic picture of the system is shown in Figure 1.

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10.1021/jp026682p CCC: $22.00
Published on Web 11/23/2002

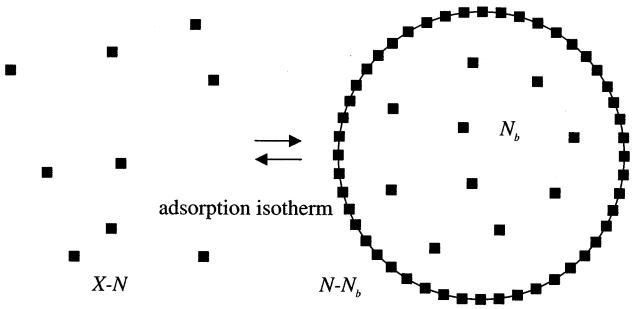

Figure 1. Schematic drawing of disclike micelle.

The free energy F is written down as follows:

$$F = (X - N)kT \log c_{X-N} + (N - N_b)kT \log c_r + NbkT \log c_{bm} - (N - N_b)\epsilon_r - N_b(\epsilon_b + kT \log 2) \quad (1)$$

where c_{X-N}, c_r, and c_{bm} are the concentrations of monomers in the solution, of the rims of the discs, and of the inside discs, respectively, and are given as follows:

$$c_{X-N} = \frac{X - N}{V} \quad (2)$$

$$c_r = \frac{1}{b^3} \quad (3)$$

$$c_{bm} = \frac{N_b}{b(N_L a + N_b b^2)} \quad (4)$$

In eqs 2−4, a bile salt molecule is regarded as a cube with side b and the surface area per lipid in the disc membrane is denoted as a. The asymmetric shape of the bile salt molecule will be taken into account to the affinity free energy to the curved surface of cuplike vesicles and rodlike micelles later in this section. In eq 1, the factors ϵ_r and ϵ_b are the affinity free energies per bile salt from water to the rim and to the membrane of the disc, respectively. In eq 3, we assumed that the coverage of the rim by bile salts is full as previous authors also postulated, which is due to the strong tendency to avoid the exposure of hydrophobic tail of lipid to water. Thus, the energy ϵ_r is expected to be larger than ϵ_b. The last term, kT log 2 on the right-hand side (RHS) of eq 1 comes from the entropy gain due to the random distribution of bile salts between both the monolayer membranes of the disclike mixed micelle. In the next subsection, we will assume asymmetric distribution of bile salts for curved membrane surfaces. We should be careful about the fact that the assumption of the disclike shape is valid only when the radius of the rim is much larger than the molecular length. Thus, our theory in itself possesses the limit of the applicability.

Let R_0 be the radius of the disc and n be the number of discs in the system. Then, the following relations hold:

$$2\pi n R_0 = b(N - N_b) \quad (5)$$

$$\pi n R_0^{\,2} = N_L a + N_b b^2 \quad (6)$$

From eqs 5 and 6, the radius R_0 is determined as

$$R_0 = \frac{N_L a + N_b b^2}{2b(N - N_b)} \quad (7)$$

The numbers N and N_b are determined so as to minimize the free energy F. First, differentiation of eq 1 with respect to N results in the following:

$$\frac{\partial F}{\partial N} = -kT \log c_{X-N} - kT + kT \log c_r - \epsilon_r = 0 \quad (8)$$

Equation 8 is rewritten as follows:

$$\frac{\partial F}{\partial N} = \mu_{rim} - \mu_{bulk} = 0 \quad (9)$$

where

$$\mu_{bulk} = kT \log c_{X-N} \quad (10)$$

$$\mu_{rim} = -\epsilon_r + kT \log(c_r/e) \quad (11)$$

are the chemical potentials of monomer bile salt in aqueous solution and that of bile salt at the rim of disclike vesicle. From eqs 9−11, we obtained the threshold concentration, c_{th} of bile salt which is expressed as

$$c_{th} = \frac{1}{eb^3} \exp\left(-\frac{\epsilon_r}{kT}\right) \quad (12)$$

The concentration c_{th} is the threshold equilibrium concentration that corresponds to IMC (intermicellar concentration) defined by Mazer and others.[9] Similarly, the number N_b is determined from the following:

$$\frac{\partial F}{\partial N_b} = kT \log c_{bm} - kT \log c_r + kT + \epsilon_r - \epsilon_b - xkT - kT \log 2 = 0 \quad (13)$$

where the surface coverage x of bile salt in the matrix lipid membrane is defined as

$$x = \frac{N_b b^2}{N_L a + N_b b^2} \quad (14)$$

and x is determined from eqs 8, 12, 13, and 14 as

$$x \exp(-x) = 2c_{th} b^3 \exp\left(\frac{\epsilon_b}{kT}\right) = \frac{2}{e} \exp\left(-\frac{\epsilon_r - \epsilon_b}{kT}\right) \quad (15)$$

Finally, the minimized free energy F_{min} becomes

$$F_{min} = F_{N=0} + XkT \log\left(\frac{X - N}{X}\right) + NkT - N_b x_L kT \quad (16)$$

where x_L (=1 − x) is the surface coverage of lipid in the matrix membrane. In eq 16, $F_{N=0}$ is the free energy of monomer solution of bile salts without disclike micelles. Equation 16 can be rewritten as

$$f = \frac{F_{min} - F_{N=0}}{XkT} = \log\left(\frac{c_{th}}{c}\right) + 1 - \frac{c_{th}}{c} - \frac{ac_L x}{b^2 c} \quad (17)$$

where c (=X/V) is the concentration of bile salt in the system and c_b (=N_b/V) is the concentration expressed in the usual way. The first three terms of the RHS of eq 17 are negative, a decreasing function of c and has a inflection point at $c = 2c_{th}$.[18] Thus, the formation of disclike micelles lowers the total free energy of the system, which has not been proven in the previous theoretical studies.

When the dilution proceeds so as to keep the ratio of the concentration of lipid to that of bile salt constant, the radius of

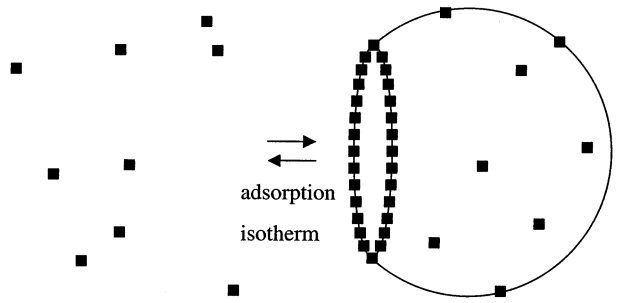

Figure 2. Schematic drawing of cuplike vesicle.

the disc is expected to become larger and larger indefinitely. As is known, however, the observed radius remains finite.

2. Cuplike Vesicle Formation. In this subsection, we will evaluate the free energy of the cuplike vesicles by taking account of the bending energy of the vesicle. Now we assume the cuplike vesicle as a partial sphere, which is a sphere cut by a plane. Let R and r be the radius of curvature of the partial sphere and the radius of the rim of the cup, respectively. The schematic pictures of cuplike vesicles are shown in Figure 2. As the molecular shape of bile salt is asymmetrical, we postulate that the affinity free energy ϵ_b is enhanced when the membrane surface is curved. The asymmetrical adsorption of bile salt causes the reduction of entropy and we will express this fact approximately by deleting the term kT log 2 per adsorbed bile salt to the curved membrane compared with that in the planer disc in eq 1. Thus, the free energy F of the cuplike vesicle system is expressed as

$$F = (X - N)kT \log c_{X-N} + (N - N_b)kT \log c_r + N_b kT \log c_b - \epsilon_r(N - N_b) - \left(\epsilon_b + \frac{\epsilon_1}{R}\right)N_b + E_b \quad (18)$$

where ϵ_1 is the energy parameter that expresses the enhancement of the affinity free energy of bile salt due to the curvature $1/R$. The identical notations are used for other parameters to the disc system. Also, the term E_b in eq 18 is the bending energy expressed as

$$E_b = \frac{n\kappa}{2}\int\left(\frac{2}{R} - \frac{2}{\rho_0}\right)^2 dA = 4\pi n\kappa p^2\left(\frac{1}{R} - \frac{1}{\rho_0}\right)^2 \quad (19)$$

where n, κ, and ρ_0 are the number of cuplike vesicles, the bending modulus of the membrane, and the radius of the spontaneous curvature, respectively. The integral is taken over by the membrane surface of the cup. By introducing the parameter, p, the surface area per cuplike vesicle is denoted as

$$\int dA = 2\pi p^2 \quad (20)$$

In other notation, the above area is rewritten as

$$\int dA = 2\pi R(R \pm \sqrt{R^2 - r^2}) \quad (21)$$

where plus sign for cups with longer depths than hemisphere and minus sign for cups with shallower depths[18] (also see Figure 2). From eqs 20 and 21 we obtain

$$R^2 = \frac{p^4}{2p^2 - r^2} \quad (22)$$

By definition, the values of r and p are related by the following two equations:

$$2\pi nr = (N - N_b)b \quad (23)$$

$$2\pi np^2 = N_L a + N_b b^2 \quad (24)$$

Now we define the parameter α as follows:

$$\alpha = \frac{(N - N_b)b}{2(N_L a + N_b b^2)} \quad (25)$$

The parameters p and R are expressed as

$$p = \sqrt{\frac{r}{2\alpha}} \quad (26)$$

$$R = \frac{1}{2\alpha\sqrt{\frac{1}{\alpha r} - 1}} \quad (27)$$

Because the spontaneous curvature $1/\rho_0$ is expected to increase as x increases, we assume that

$$\frac{1}{\rho_0} = \frac{x}{\rho} \quad (28)$$

where $1/\rho$ is the spontaneous curvature of the bile salt molecule. Finally, the bending energy E_b is rewritten as

$$E_b = 4\kappa b(N - N_b)\left[\frac{1}{r} - \alpha + \frac{x^2}{4\rho^2\alpha} - \frac{x}{\rho\alpha}\sqrt{\frac{1}{\alpha r} - 1}\right] \quad (29)$$

Eventually, there remain three independent variables, N, N_b, and r. Apparently the total free energy becomes a function of them and also of α,

$$F = F(N, N_b, r, \alpha) \quad (30)$$

As the factor α is a function of N and N_b as expressed in eq 25, these three independent variables of N, N_b, and r are determined so as to minimize the free energy F.

Now, we minimize of the free energy. First, we minimize F by N as follows:

$$\frac{\partial F}{\partial N} = -kT\log c_{X-N} - kT + kT\log c_r - \epsilon_r - N_b\epsilon_1\frac{\partial}{\partial N}\left(\frac{1}{R}\right) + \frac{\partial E_b}{\partial N} = 0 \quad (31)$$

where

$$\frac{\partial E_b}{\partial N} = \frac{E_b}{N - N_b} + \frac{\partial E_b}{\partial \alpha}\frac{\partial \alpha}{\partial N} \quad (32)$$

$$\frac{\partial \alpha}{\partial N} = \frac{\alpha}{N - N_b} \quad (33)$$

$$\frac{\partial E_b}{\partial \alpha} = 4\kappa b(N - N_b)\left[-1 - \frac{x^2}{4\rho^2\alpha^2} + \frac{x}{2\rho r\alpha^2}\frac{1}{\sqrt{\frac{1}{\alpha r} - 1}}\right] \quad (34)$$

and

$$\frac{\partial}{\partial\alpha}\left(\frac{1}{R}\right) = \frac{\frac{1}{\alpha r} - 2}{\sqrt{\frac{1}{\alpha r} - 1}} \tag{35}$$

The final expression of eq 31 will be shown later because it is related to the derivative of F with respect to r.

Next, we minimize F with respect to N_b as follows:

$$\frac{\partial F}{\partial N_b} = kT \log c_{bm} + kTx_L - kT \log c_r + \epsilon_r - \epsilon_b - \frac{\epsilon_1}{R} - N_b\epsilon_1\frac{\partial}{\partial N_b}\left(\frac{1}{R}\right) + \frac{\partial E_b}{\partial N_b} = 0 \tag{36}$$

with the formulas

$$\frac{\partial E_b}{\partial N_b} = -\frac{E_b}{N - N_b} + \frac{\partial E_b}{\partial \alpha}\frac{\partial \alpha}{\partial N_b} \tag{37}$$

$$\frac{\partial \alpha}{\partial N_b} = -\alpha\left(\frac{1}{N - N_b} + \frac{x}{N_b}\right) \tag{38}$$

Again, to show the final result of eq 36 will be postponed until after the next calculation.

The minimization of F with respect to $1/r$ becomes

$$\frac{\partial F}{\partial\left(\frac{1}{r}\right)} = -\epsilon_1 N_b \frac{\partial}{\partial\left(\frac{1}{r}\right)}\left(\frac{1}{R}\right) + \frac{\partial E_b}{\partial\left(\frac{1}{r}\right)} \tag{39}$$

By use of eqs 27 and 29, eq 39 becomes

$$\frac{\partial F}{\partial\left(\frac{1}{r}\right)} = -\frac{\epsilon_1 x}{\sqrt{\frac{1}{\alpha r} - 1}} + 8\kappa b^2\alpha\left(1 - \frac{xb}{2\alpha\rho\sqrt{\frac{1}{\alpha r} - 1}}\right) = 0 \tag{40}$$

The above equation can be rewritten as

$$\frac{1}{r} = \alpha + \frac{C^2}{\alpha} \tag{41}$$

where

$$C = \frac{x}{2\rho}\left(1 + \frac{\rho\epsilon_1}{4\kappa b^2}\right) \tag{42}$$

From eqs 27, 41, and 42, the curvature $1/R$ of the membrane becomes

$$\frac{1}{R} = \frac{x}{\rho}\left(1 + \frac{\rho\epsilon_1}{4\kappa b^2}\right) \tag{43}$$

From the definition of eqs 19 and 28, we can say that the obtained curvature in eq 43 is larger than the spontaneous curvature x/ρ, which is caused by the enhanced affinity free energy of bile salt to the curved membrane rather than to the planer disc. Due to the enhancement of $1/R$ in eq 43 rather than the spontaneous curvature, x/ρ, the cuplike vesicle is mechanically frustrated. Equation 41 shows that the value of r takes a maximum value at $r = R$. The reduction of α by the dilution procedure causes the following: first dishlike vesicles enhance their size, become hemisphere at $r = R$, then the radius of rim r reduces, finally they become almost spherical vesicles. The details of this fact will be discussed in the next section.

By using eqs 32−35 and 41, the last two terms of the RHS in eq 31 cancel each other. Thus, the eq 31 becomes identical to eq 8, which means that the threshold concentration for the system of cuplike vesicles is same as that of a disclike vesicle. Also, by the use of eq 41, eq 36 becomes

$$\frac{\partial F}{\partial N_b} = kT(\log c_{bm} + 1 - \log c_r - x) + \epsilon_r - \epsilon_b + \frac{\epsilon_1 x}{\rho}\left[1 + \frac{\epsilon_1\rho}{4\kappa b^3}\left(2b - \frac{1}{\alpha}\right)\right] = 0 \tag{44}$$

The last term of the RHS in eq 44 could take negative values when dilution proceeds (the value of α becomes small). Then, the value of c_{bm} is larger than that of disclike vesicles. In this case the cuplike vesicles are more stable than disclike micelles. By further dilution, the cuplike vesicle approaches to a spherical shape.

By using eqs 31, 34, 40, and 44, the minimized free energy F_{min} eventually becomes

$$F_{min} = F_{N=0} + kT\left[X \log\left(\frac{X - N}{N}\right) + N - N_b x_L\right] + \frac{N_L a}{8\kappa b^4}(x\epsilon_1)^2 \tag{45}$$

In the expression similar to eq 17, the free energy difference per bile salt molecule g is defined and becomes as follows:

$$g = \frac{F_{min} - F_{N=0}}{XkT} = \log\left(\frac{c_{th}}{c}\right) + 1 - \frac{c_{th}}{c} - \frac{ac_L x}{b^2 c}\left(1 - \frac{x{\epsilon_1}^2}{8\kappa b^2 kT}\right) \tag{46}$$

Now, we will evaluate whether cuplike vesicles are more stable than disclike vesicles or not by comparing the values of g (eq 46) with f (eq 17) in the next section.

3. Rodlike Micelles. Egelhaaf and Schurtenberger[15] made static and dynamic light scattering experiments and concluded that the rodlike micelles could be a more probable form in the concentrated region of bile salt. Now, we will try to extend our model to this rodlike micelle system. One of the theoretical problems is the extension of eq 19 to this system. Strictly speaking, the bending energy written in the form of eq 19 makes sense only when the radius of curvature is much larger than the size of constituent molecules. Also, the bending modulus κ is expected to change largely when x changes. However, we will use eq 19 when the rodlike micelle possesses radius of curvature in the order of molecular size. In the theoretical study, however, the theoretical consistency of the format is important to compare the free energies of the different system. As the expected length of the rod is very long,[15] we simply assume a long single rodlike micelle in the system. By assuming the radius of curvature as R and the length of the rod as L, eq 19 can be rewritten as

$$E_b = \frac{n\kappa}{2}\int\left(\frac{2}{R} - \frac{2x}{\rho}\right)^2 dA = 2\pi\kappa RL\left(\frac{1}{R} - \frac{x}{\rho}\right)^2 = 2\kappa(N_L a + Nb^2)\left(\frac{1}{R} - \frac{x}{\rho}\right)^2 \tag{47}$$

And the free energy F becomes

$$F = (X - N)kT\log c_{X-N} + NkT\log c_b - \left(\epsilon_b + \frac{\epsilon_1}{R}\right)N + E_b \tag{48}$$

where

$$c_b = \frac{N}{b(N_L a + Nb^2)} \tag{49}$$

The definitions of other parameters are the same as those of previous ones. The difference is that the rim of the cuplike vesicle does not exist for the long rodlike micelle. Thus, the adsorbed number N in eqs 47−49 means the total adsorbed number to the rod in the system. The independent variables are N and R in this case. The free energy F is minimized with respect to N and $1/R$ as follows:

$$\frac{\partial F}{\partial N} = -kT\log\left(\frac{X-N}{V}\right) + kT\log\left(\frac{N}{b(N_L a + Nb^2)}\right) - xkT - \epsilon_b - \frac{\epsilon_1}{R} + \frac{\partial E_b}{\partial N} = 0 \tag{50}$$

$$\frac{\partial F}{\partial\left(\frac{1}{R}\right)} = -\epsilon_1 N + 4\kappa(N_L a + Nb^2)\left(\frac{1}{R} - \frac{x}{\rho}\right) = 0 \tag{51}$$

By use of

$$\frac{\partial x}{\partial N} = \frac{x(1-x)}{N} \tag{52}$$

the derivative of E_b in eq 50 becomes

$$\frac{\partial E_b}{\partial N} = 2\kappa b^2\left(\frac{1}{R} - \frac{x}{\rho}\right)\left[\frac{1}{R} - \frac{x}{\rho} - \frac{2(1-x)}{\rho}\right] \tag{53}$$

By using eqs 51 and 53, eq 50 becomes

$$\frac{\partial F}{\partial N} = -kT\log\left(\frac{X-N}{V}\right) + kT\log\left(\frac{N}{b(N_L a + Nb^2)}\right) - xkT - \epsilon_b - \epsilon_1 x(2-x)\left(\frac{1}{\rho} + \frac{\epsilon_1}{8\kappa b^2}\right) = 0 \tag{54}$$

Eventually, the minimized free energy, F_{min} turns out to be

$$F_{min} = F_{N=0} + XkT\log\left(\frac{X-N}{N}\right) + Nx\left[kT + (1-x)\epsilon_1\left\{\frac{1}{\rho} + \frac{\epsilon_1}{8\kappa b^2}\right\}\right] \tag{55}$$

The result of this subsection is correct when the bile salt concentration is higher than that of lipid, while the formulas for the disclike mixed micelle and cuplike vesicle are correct in the opposite conditions. The more detailed comparison and discussion will be given in the next section.

Discussion and Conclusion

In the previous section, we have derived the statistical mechanical formulations of disclike mixed micelles, cuplike vesicles and rodlike micelles for the lipid and bile salt system. To compare our theoretical model to the observed data, we will further calculate the physical quantities from the formulas in the previous section. First, the surface coverage, x, is obtained from eq 44 by the similar procedure to obtain eq 15 as

$$x\exp(-x) = x_0\exp(-x_0)\exp\left[-\ln 2 - \frac{\epsilon_1 x}{\rho kT}\left\{1 + \frac{\epsilon_1\rho}{4\kappa b^3}\left(2b - \frac{1}{\alpha}\right)\right\}\right] \tag{56}$$

where x_0 is the surface coverage of the bile salt in the disclike micelle determined by eq 15. When the following condition is satisfied:

$$\alpha \le \frac{\epsilon_1^2 x}{4\kappa b^3}\frac{1}{kT\ln 2 + \frac{\epsilon_1 x}{\rho}\left(1 + \frac{\epsilon_1\rho b}{2\kappa b^3}\right)} \tag{57}$$

the value of x is larger than that of the disc in eq 15. The condition of eq 57 can be fulfilled by the dilution procedure. Next, we examine whether the expected cuplike vesicle system has a lower free energy than that of disclike vesicle or not. If the value of eq 57 is lower than eq 17, the cuplike vesicle is more stable than disclike vesicle. The difference $g - f$ eventually becomes

$$g - f = \frac{c_L a}{cb^2}\left[-x + x_0 + \frac{(x\epsilon_1)^2}{8\kappa b^2 kT}\right] \tag{58}$$

As stated before, the value of x could be larger than x_0. Thus, $g - f$ in eq 58 can become negative at certain condition of dilution process. Quantitatively, however, we cannot specify the precise condition because there are many adjustable parameters and the value x cannot be solved analytically.

Now, we show the qualitative shape change of cuplike vesicles by the dilution procedure. Although x should be determined self-consistently by solving eq 56, we write it down as follows:

$$x = \beta \tag{59}$$

when x does not change largely as a function of the concentration. By use of the definition of x in eq 14, the concentration c_b $(=N_b/V)$ can be rewritten as

$$c_b = \frac{\beta}{1-\beta}c_L \tag{60}$$

Then, the factor α defined in eq 25 is expressed as

$$\alpha = \frac{b}{2(a + \gamma b^2)}\frac{c - c_{th} - \gamma c_L}{c_L} \tag{61}$$

where

$$\gamma = \frac{a\beta}{b^2(1-\beta)} \tag{62}$$

Starting from high concentration of c, we express the dilution procedure as follows: we keep the concentration c_L constant and the ratio P of the concentration c to c_L, P $(P=c/c_L)$ is reduced. Then, eq 61 is reexpressed as

$$\alpha = \frac{b}{2(a + \gamma b^2)}\left(P - \gamma - \frac{c_{th}}{c_L}\right) \tag{63}$$

By reducing c from a high concentration region to lower concentrations, the factor α changes as shown in Figure 3a. From eq 56, we say that the value of α cannot become too small because the value of x cannot be greater than unity. From eq 41, the radius r of the cuplike vesicle changes as shown in Figure 3b. Also from eqs 26 and 41, the surface area $2\pi p^2$ becomes

$$2\pi p^2 = \frac{\pi}{\alpha^2 + C^2} \tag{64}$$

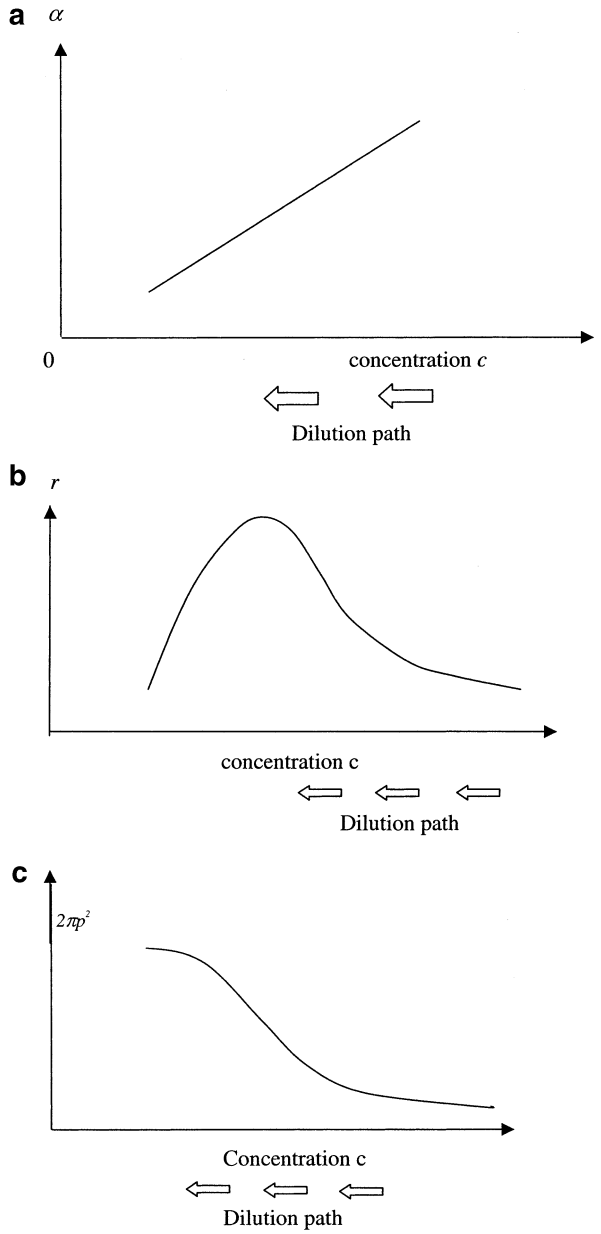

Figure 3. Changes of α, *r*, and $2\pi p^2$ as function of the concentration *c*. a: Change ofα as a function of the concentration *c* of bile salts. b: The radius *r* of the rim of cuplike vesicle. c: Surface area $2\pi p^2$ of the cuplike vesicle.

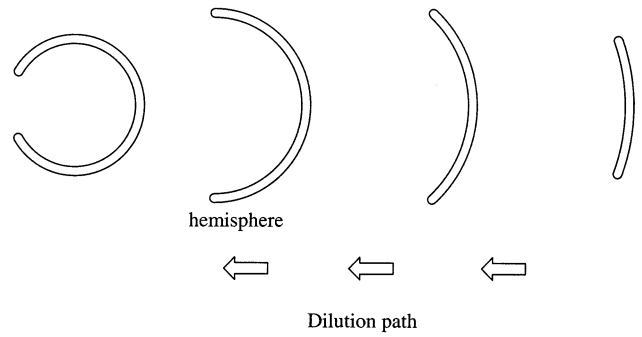

Figure 4. Schematic drawing of the shape change of cuplike vesicles.

Then the surface area $2\pi p^2$ behaves as shown in Figure 3c. The schematic shape change of the cuplike vesicle is shown in Figure 4.

The curvature *1/R* in eq 43 is proportional to *x* and *x* increases when the dilution proceeds as expressed in eq 64. Thus, the size of the cuplike vesicle is expected to possess maximum value at around $\alpha = C$ and this fact is schematically shown in Figure 4.

For the case of rodlike micelles, eq 54 is rewritten as

$$(c - c_N)(c_N + \gamma_0 c_L) = c_0 c_N \tag{65}$$

where

$$c_N = \frac{N}{V} \tag{66}$$

$$c_0 = \frac{1}{b^3}\exp\left[-\frac{1}{kT}\left(xkT + \epsilon_b + \epsilon_1 x(2-x)\left(\frac{1}{\rho} + \frac{\epsilon_1}{8\kappa b^2}\right)\right)\right] \tag{67}$$

$$\gamma_0 = \frac{a}{b^2} \tag{68}$$

Although c_0 in eq 65 is the function of c_N, the value of c_N can be solved formally as

$$c_N = \frac{(c - \gamma_0 c_L - c_0)}{2}\left[1 + \sqrt{1 + \frac{4\gamma_0 c_L c}{(c - \gamma_0 c_L - c_0)^2}}\right] \tag{69}$$

In this case, the threshold concentration does not exist in contrast to the cuplike vesicles.

From the minimized free energy given in eq 55, we obtain

$$h = \frac{F_{min} - F_{N=0}}{XkT} = \log\left(\frac{c - c_N}{c}\right) + \frac{c_N x}{c}\left[1 + \frac{(1-x)\epsilon_1}{\rho kT}\left\{1 + \frac{\epsilon_1 \rho}{8\kappa b^2}\right\}\right] \tag{70}$$

The above function *h* makes sense when the concentration of bile salt is high. By expressing eq 65 as $c - c_N = xc_0$, eq 70 is rewritten as

$$h = \log\left(\frac{xc_0}{c}\right) + 1 - \frac{xc_0}{c} + x_L\left(1 - \frac{xc_0}{c}\right)\left[\frac{\epsilon_1}{kT}\left(\frac{1}{\rho} + \frac{\epsilon_1}{8\kappa b^2}\right) - 1\right] \tag{71}$$

where the value of x_L is small compared to unity. On the other hand, the functions *f* (eq 17) and *g* (eq 46) are applicable only in the low bile salt concentration region. As stated before, assumed disclike vesicle or cuplike vesicle can exist reliably only when the radius of the rim is larger than the molecular size of bile salt. Thus, the energies *f* and *g* terminate at some points as functions of bile salt concentration.

The expected energy profiles and a possible path of shape transformation path is shown in Figure 5. At high concentration of bile salt, the rodlike micelle is stable along the curve *h* in Figure 5. As the dilution proceeds, the path reaches to point *D*, then the coexistence of the rodlike micelle and the disclike mixed micelle realizes between points *D* and *C*. The point *C* is the terminal point where the validity of our theory for disclike micelle comes to an end. From *C* to *B*, the disclike micelle is stable. From *B* to *A*, the disclike micelle and the cuplike vesicle coexist. Finally, by further dilution, the cuplike vesicle becomes stable and the radius *r* and the surface area $2\pi p^2$ change as shown in Figures 3 and 4.

In conclusion, the cuplike vesicle for the lipid and bile salt system has been theoretically shown to be stable by evaluating the free energy of the total system. So far, the evidence of the stable cuplike vesicle has not been reported because the

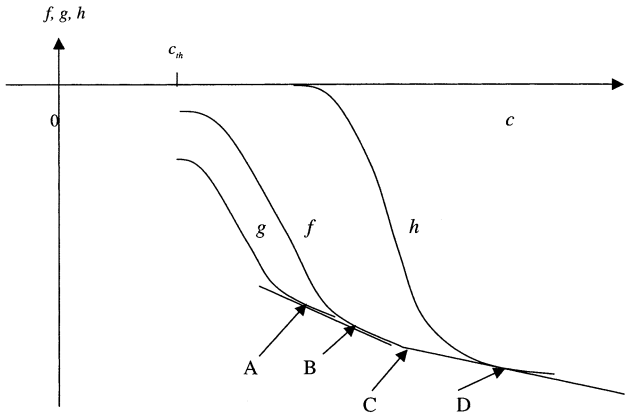

Figure 5. Schematic drawing of the free energies of *f*, *g*, and *h* for disclike micelle, cuplike vesicle an rodlike micelle, respectively. A possible pathway of dilution →D→C→B→A→

aggregate shape cannot be observed by optical microscope due to their too small size. However, the many reported data of light scattering and other methods have room to be reanalyzed. For instance, Egelhaaf and Schurtenberger reported the light scattering data. According to their evaluation,[15] the apparent mass of the aggregate increases monotonically as the dilution proceeds in a certain region, while the hydrodynamic radius shows maximum value in this region. The surface area per cuplike vesicle in Figure 3c corresponds to the molecular weight of the aggregate and it increases monotonically as the dilution proceeds. On the other hand, the radius *r* in Figure 3b possesses a maximum value in this concentration region. The above behaviors of two parameters, $2\pi p^2$ and *r* qualitatively coincide with the measurements made by Egelhaaf and Schurtenberger.[15]

Although the bending modulus κ has been kept constant throughout of this paper, the value of it is expected to change largely as the surface coverage *x* changes in the dilution procedure. In the lipid and melittin system, temperature induced disc to vesicle transition was reported.[19] The observed transition is supposed to be due to the change of the partition coefficient of melittin to the vesicle membrane and also the change of bending modulus induced by the gel to liquid crystalline phase transition. Also in our system, the change of the bending modulus is expected to occur and this fact should be taken into account in future works. The spherical vesicle in high dilution has not yet been written in this paper because our theory cannot reproduce the decreasing trend of vesicle size as the dilution proceeds,[15] thus it will be left for our future study.

Acknowledgment. The author acknowledges to Dr. Y. Murata for his critical discussion on this problem. He also acknowledges Mr. B. Andersen and Mr. H. Ichinose for their assisting in preparing the manuscript.

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