Synergetics of the Membrane Self-Assembly: A Micelle-to-Vesicle Transition

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Abstract. A model approach is developed to study intermediate steps and transient structures in a course of the membrane self-assembly. The approach is based on investigation of mixed lipid/protein-detergent systems capable of the temperature induced transformation from a solubilized micellar state to closed membrane vesicles. We performed a theoretical analysis of self-assembling molecular structures formed in binary mixtures of dimyristoylphosphatidylcholine (DMPC) and sodium cholate (NaC). The theoretical model is based on the Helfrich theory of curvature elasticity, which relates geometrical shapes of the structures to their free energy in the Ginzburg-Landau approximation. The driving force for the shape transformation is spontaneous curvature of amphiphilic aggregates which is nonlinearly dependent on the lipid/detergent composition. An analysis of the free energy in the regular solution approximation shows that the formation of mixed structures of different shapes (discoidal micelles, rod-like micelles, multilayer membrane structures and vesicles) is possible in a certain range of detergent/lipid ratios. A transition from the flat discoidal micelles to the rod-like cylindrical micelles is induced by curvature instabilities resulting from acyl chain melting and insertion of detergent molecules into the lipid phase. Nonideal mixing of the NaC and DMPC molecules results in formation of nonideal cylindrical aggregates with elliptical cross section. Further dissolution of NaC molecules in DMPC may be accompanied with a change of their orientation in the lipid phase and leads to temperature-induced curvature instabilities in the highly curved cylindrical geometry. As a result the rod-like micelles fuse into less curved bilayer structures which transform eventually to the unilamellar and multilamellar membrane vesicles. The theoretical analysis performed shows that a sequence of shape transformations in the DMPC/NaC mixed systems is determined by the synergism of four major factors: detergent/lipid ratio, temperature (acyl chain melting), DMPC and NaC mixing, and reorientation of NaC molecules in mixed aggregates.

Key words: Micelle-to-vesicle transition, mixed lipid-detergent systems, Membrane self-assembly, solubilization and reconstitution of membranes

Introduction

The mixed lipid-detergent systems are currently of great interest because of their wide use in membrane studies and in particular for solubilization and reconstitution of membrane proteins (see, for example, reviews [1–3]). Despite successful reconstitution of a great number of membrane functions, molecular mechanisms of the membrane self-assembly are largely not clear. Of special interest in this

respect is the micelle-to-vesicle transition which represents an essential stage in transformation of solubilized micellar aggregates into closed bilayer vesicles [1, 4, 5].

In earlier studies a dilution technique was used to induce micelle-to-vesicle transition in mixed lipid/detergent systems [6]. Recently a new experimental approach has been developed which allows one to initiate the transition in the phosphatidylcholine-bile salt mixed systems solely by temperature variations without changing the concentration and actual lipid/detergent ration in the sample under investigation [7]. The temperature-induced micelle-to-vesicle transition (TI-MVT) occurs in a narrow range of lipid/detergent ratios and appears to be triggered by the phospholipid acyl chain melting [8]. Similar temperature-dependent transformations have been observed also with other lipid/detergent mixtures [9–11] and seems to be a common property of the mixed systems composed of saturated phosphatidylcholines and some appropriate detergents. The temperature-sensitive systems like these are particularly convenient for studying individual stages of the micelle-to-vesicle transition [7, 12] since the transition is completely reversible and can be arrested at any required step for measurements to be taken in stationary isothermic conditions.

In the present study a theoretical analysis was performed of self-assembling molecular structures formed in binary mixtures of dimyristoylphosphatidylcholine (DMPC) and sodium cholate (NaC). The analysis shows that a sequence of shape transformations in the DMPC/NaC mixed systems is determined by the synergism of four major factors: detergent/lipid ratio, temperature, DMPC and NaC mixing and reorientation of NaC molecules in mixed aggregates. A preliminary theoretical consideration of the system under study was presented elsewhere [13].

1. The Model

In the DMPC-NaC phase diagram the systems competent of TI-MVT fall into the range of R_e (effective detergent/lipid ratio in the mixed aggregates) from 0.14 to 0.33 [8]. The salient feature of the systems is their unusual thermotropic behaviour. On heating, a drastic increase in turbidity occurs over a relatively narrow temperature increment (1–2 °C) which has been assigned to the micelle-to-vesicle transition [7, 8]. The transition starts at temperatures exceeding the lipid main phase transition (23 °C for DMPC), its exact position being dependent on the detergent/lipid ratio in the mixture.

Studies with a variety of physico-chemical techniques (light scattering, electron microscopy, NMR, DSC, fluorescence, and small angle scattering) [7, 8, 12, 14–16] have shown that dramatic structural and morphological changes are induced in the transitional systems during TI-MVT. Thus, whereas micellar or vesicular structures are predominant at the terminal stages of TI-MVT, a number of various intermediate structures (large bilayer fragment, worm-like micelles, reticular aggregates and extended multilayer structures) are observed in the middle of TI-MVT. A general
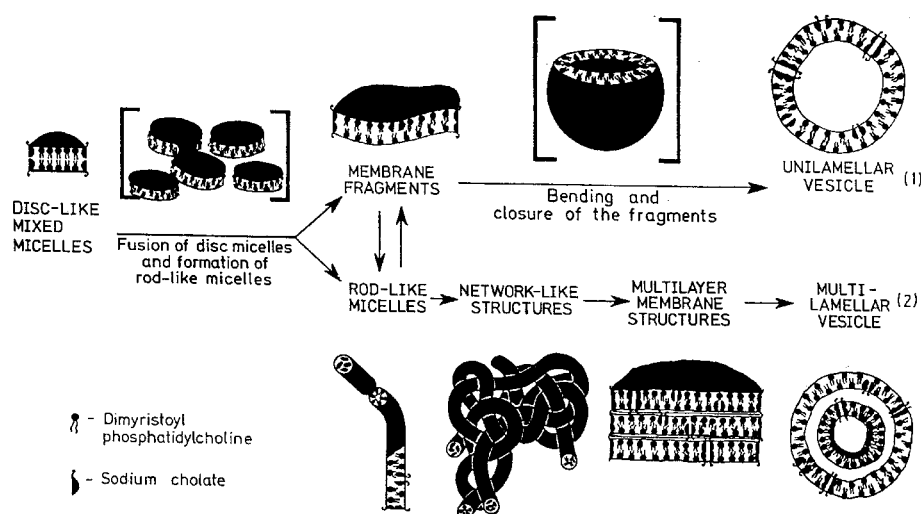
Figure 1. A tentative scheme of the temperature-induced micelle-to-vesicle transition in binary mixture of DMPC an NaC [12].

scheme was proposed of possible sequences of structural rearrangements in the course of TI-MVT in the DMPC-NaC mixed systems (Figure 1) [12]. The scheme makes a basis for the following theoretical consideration.

1.1. GEOMETRICAL APPROACH

Theoretical studies of the micelle-to-vesicle transition [17–21] have shown that critical factors governing of the process are packing characteristics of molecules in the mixture, primarily the mean surface area of polar head groups and the hydrophobic volume of a molecule.

A relation between geometrical parameters of molecules and their aggregate states in the case of pure lipidic systems was analyzed earlier by Israelashvili [22]. The molecular packing parameter proposed

$$\delta = \frac{v}{al},\tag{1}$$

is a function of the hydrophobic volume, v, of acyl chains in the lipid molecule, the mean surface area of its head group, a, and the length of acyl chains, l.

It is assumed [22] that lipid molecules with the packing parameter $\delta < 0.3$ form spherical micelles, with $0.33 < \delta < 0.5$ cylindrical micelles, and with $0.5 < \delta < 1$ bilayers and vesicles.

The packing parameter concept was extended to mixed systems [23]. In this case the effective packing parameter is considered to be dependent on the system composition and thereby can be used to analyze the vesicle-to-micelles transition in mixed lipid/detergent systems.
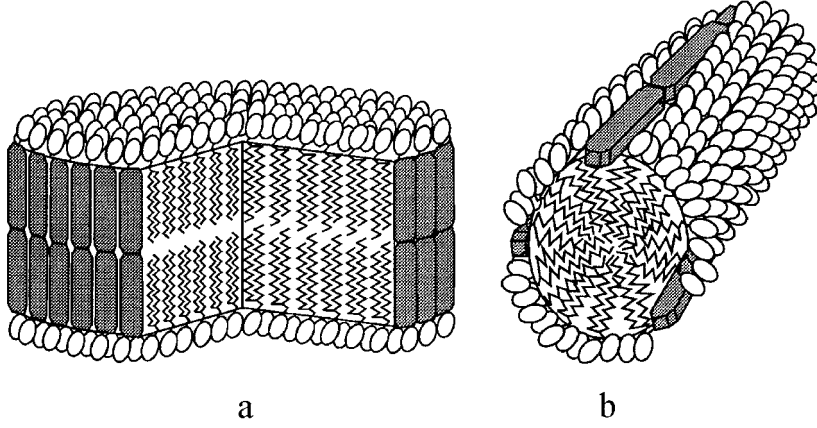
Figure 2. The models of the disc-like (a) and rod-like (b) micelles formed in binary mixtures of DMPC and NaC.

We define the effective packing parameter, δ_{eff}, for the lipid/detergent systems as follows:

$$\delta_{eff} = \frac{N_l v_l + N_d v_d}{(N_l a_l + N_d a_d)h} = \frac{v_l + R_e v_d}{(a_l + R_e a_d)h}, \tag{2}$$

where v_l and v_d are hydrophobic volumes of lipid and detergent, a_l and a_d are mean surface areas of lipid and detergent, h is the thickness of the mixed monolayer, and $R_e = N_d/N_l$ is the effective detergent/lipid molar ratio in mixed aggregates.

The effective packing parameter, δ_{eff}, for the DMPC/NaC mixed system was estimated on the basis of the following geometrical parameters: the hydrophobic volumes of DMPC, $v_l = 0.9$ nm^3, [19] and that of NaC, $v_d = 0.6$ nm^3, the mean surface area of DMPC in fluid phase, $a_l = 0.6$ nm^2, and that of NaC $a_d = 1.2$ nm^2, the value of $h = 1.5$ nm was chosen as the mean length of melted acyl chains. The effective packing parameter, δ_{eff}, calculated with these parameters for effective detergent/lipid molar ratios $R_e = 0.14 \div 0.33$ falls in the range $0.61 \div 0.5$ which lays at the phase boundary between cylindrical micelles and lamellar structures. A presumed packing DMPC and NaC molecules in cylindrical and dish-like micelles is shown in Figure 2.

The conditions for the cylindrical micelle formation were evaluated by analysis of the spontaneous curvature, c_o, for the DMPC/NaC mixtures. Calculation of the spontaneous curvature of monolayers and bilayers is a complex problem and different theoretical approaches are developed for such calculations [17–25]. In the mean-field approximation the spontaneous curvature is determined by a balance of all forces acting in the monolayer: electrostatic and steric interactions between polar head groups, interfacial interactions (surface tension), and interactions between hydrocarbon tails in the hydrophobic part of the monolayer [20]. An application of the full free energy minimization allows one to calculate optimal value of the mean molecular surface area and the spontaneous curvature as a
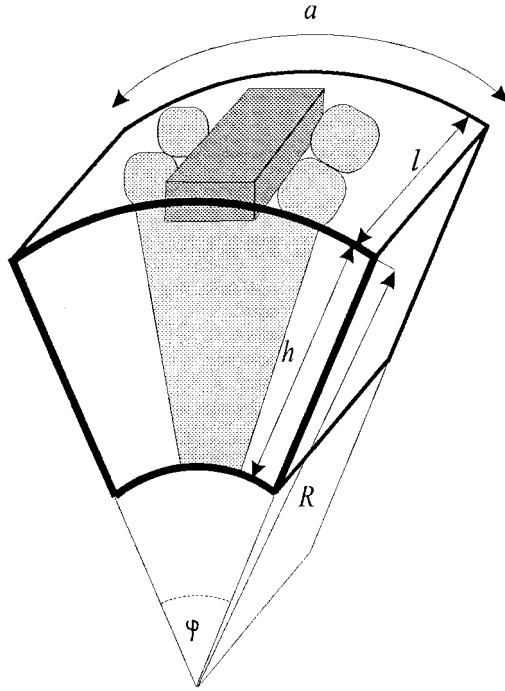
Figure 3. A proposed packing model of DMPC and NaC molecules in the mixed monolayer of cylindrical geometry.

function of the composition of mixed aggregates [19, 20]. In this work, for calculation of the monolayer spontaneous curvature we use the geometrical approach which takes into account only geometrical parameters of interacting molecules with no regard for other factors. As a first approximation, this enables deducing of a role of packing properties in structural transformation of the DMPC/NaC mixed aggregates.

The packing pattern of DMPC and NaC molecules is represented schematically in Figure 3. With this geometry the spontaneous curvature $c_o = 1/R$ of the mixed DMPC/NaC monolayer is given by

$$c_o = \frac{2(ah - v)}{ah^2}.\tag{3}$$

When the total hydrophobic volume, v, and the total mean surface area, a, are expressed in terms of the partial hydrophobic volumes of lipid, v_l, and detergent, v_d, and their mean surface areas, a_l, and a_d, as

$$a = N_l a_l + N_d a_d, \quad v = N_l v_l + N_d v_d,$$

the dependence of the spontaneous curvature of the mixed DMPC/NaC monolayer on the effective detergent/lipid molar ratio, R_e, can be estimated as
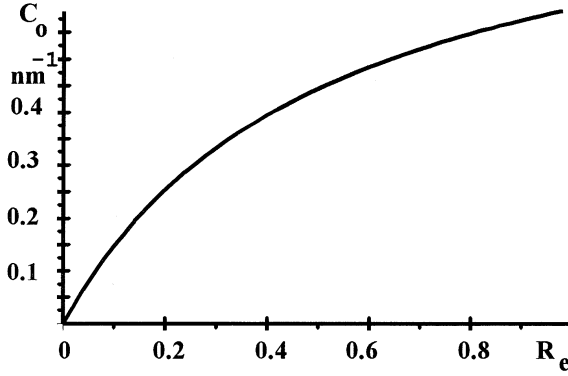
Figure 4. The dependence of the spontaneous curvature, c_0, of the mixed DMPC/NaC monolayer on the effective detergent/lipid molar ratio, R_e.

$$c_o(R_e) = \frac{2}{h^2} \frac{(a_l h - v_l) + R_e(a_d h - v_d)}{(a_l + R_e a_d)}. \tag{4}$$

This relationship is displayed graphically in Figure 4. In case of cylindrical DMPC/NaC micelles shown in Figure 2(b), the minimal curvature radius must be equal to the length of the acyl chains of the lipid, i.e. $\sim$ 1.5 nm with curvature value of about 0.6 nm^{-1}. As seen from Figure 4, in the region of micelle-to-vesicle transition with R_e values of $0.14 \div 0.33$ the spontaneous curvature, c_o, lays in the range of $0.12 \div 0.35$ nm^{-1} which is far lower than the curvature value $c \simeq 0.6$ nm^{-1} evaluated for the cylindrical micelle with regularly shaped circular cross-section. This implies that cylindrical micelles formed by DMPC and NaC are most likely to be of the non-regular shape, probably, with an elliptical cross-section which helps to maintain the lower curvature in most part of the micelle surface. This assumption is in good agreement with recent experimental data obtained on studying the mixed DMPC/NaC systems with small angle neutron scattering [14, 15]. The geometry of rod-like micelles formed by DMPC and NaC in the micellar region of TI-MVT is better described by elliptical cross-section with the axes ratio of about 1.8 at $R_e = 0.23$. Our theoretical estimation for the same R_e value gives the axes ratio of about 1.9. It is worth to note that formation of flattened cylindrical micelles was observed on computer simulation of the self-assembly of model surfactant molecules into bilayer membranes [26].

Variation of the DMPC and NaC molecular parameters in a reasonable range of numerical values do not influence in a crucial respect the results of the theoretical calculation. Moreover, they show that the lipid and detergent can be packed into ideal cylinder with the curvature of about 0.6 nm^{-1} only by assuming that the mean surface area of NaC, a_d, is more then 4 nm^2. This value exceeds more than twice the area (1.2 nm^2) expected for NaC from molecular model estimations and is evidently higher than the limiting surface area (1.5–3 nm^2) determined from monolayer surface tension studies [27–30]. Thus, the phenomenological calculation of

the spontaneous curvature based only on geometrical molecular parameters seems to describe adequately the mixed DMPC/NaC system.

1.2. THERMODYNAMICAL APPROACH

More detailed analysis of transient structures formed in the micelles-to-vesicle transformation requires the geometrical approach to be supplemented by thermodynamic considerations. Our approach has its origins in the thermodynamical models developed earlier to investigate the micelle-to-vesicle transition induced by changing detergent concentration in lipid/detergent mixed systems [17–21]. These models are based on the mean-field calculation of free energies for different shapes taken by mixed aggregates as a function of the detergent concentration. An equilibrium structure with the lowest free energy can be determined by comparing free energies calculated for each of the possible aggregates [17–21]. Another approach to deduce the interface shapes of mixed aggregates is to solve some dynamical equations which describe relations between phase separation and curvature [24, 31–33].

All shapes of mixed aggregates can be represented as different forms of monolayer self-organization [21]. The main parameter controlling monolayer geometry is monolayer curvature or radius of curvature. Consequently, mixed monolayers assemble into distinct structures depending on curvature value. Monolayers with radius of curvature larger than the monolayer thickness, $R \gg h$, form flat bilayer structures (e.g., multilamellar or unilamellar vesicles). Strongly curved monolayers with radius of curvature of order the monolayer thickness, $R \sim h$, exist in water in the form of cylindrical or spherical micelles.

Let us consider the dynamical equations that describe different structures formed by mixed monolayer. In order to derive these equations we consider the free energy as a function of two coupled local fields: the curvature field $c(x, t)$ and the field of concentration $\phi(x, t)$ which gives the area fraction occupied by the detergent [18]

$$\phi = \frac{a_d N_d}{a_d N_d + a_l N_l}.$$

We consider the one-dimensional fields characterized by coordinate, x, and time, t.

The free energy of one-dimensional monolayer (per unit area) can be written in the following form

$$\mathcal{F}[\phi, c] = u + \frac{1}{2} K_1 |\nabla c(x, t)|^2 + \frac{1}{2} K_2 |\nabla \phi(x, t)|^2 - Ts. \tag{5}$$

The internal energy, u, is considered as the Helfrich elastic energy of bending (per unit area) [35]

$$u = \frac{1}{2} k(c(x, t) - c_o)^2, \tag{6}$$

where c_o is the spontaneous curvature, k is the bending elastic modulus.

The second and third contributions in Equation (5) are presented by smoothing terms.

As for the entropy of mixing, s, an approximation for the ideal mixture is used

$$s = -k_B \frac{1}{a_l}[\phi \ln \phi + (1 - \phi) \ln(1 - \phi)], \tag{7}$$

where k_B is Boltzmann's constant and T is absolute temperature of the mixture.

The spontaneous curvature, c_o, is assumed to be a function of mixture composition $\phi(x, t)$. A reasonable approximation of the spontaneous curvature, c_o, can be given by averaging the spontaneous curvature of pure lipid system, c_{ol}, and that of pure detergent, c_{od}, as following [18]

$$c_o(\phi) = \phi c_{od} + (1 - \phi)c_{ol}. \tag{8}$$

A linear dependence of $c_o(\phi)$ (8) on the monolayer composition ϕ has been shown [18] to be a good approximation in the case of common detergents with a flexible hydrocarbone chain, such as octyl glucoside, which are able to form simple spherical micelles with curvature $c_{od} = 1/R$. The spontaneous curvature of the pure NaC system is difficult to determine since the aggregation behaviour of NaC is more complex than that of classical detergents [36]. At low concentrations NaC forms small globular aggregates containing 2–10 monomers which can be converted at higher concentrations into rod-like secondary micelles.

The expression for the spontaneous curvature $c_o(R_e)$ (4) derived above can be applied for the mixed systems if the cylindrical geometry of monolayer is assumed. As can be seen from Equation (4), the cylindrical geometry corresponds to the linear dependence of $c_o(\phi)$. A more weak dependence of $c_o(\phi)$ is expected at low ϕ values due to preferential spherical geometry of monolayer at low ϕ. In the frame of the model the quadratic dependence of $c_o(\phi)$ is chosen as a first approximation:

$$c_o(\phi) = \alpha \phi^2, \tag{9}$$

where constant $\alpha \simeq 0.3$.

With this assumption a correlation can be established between the composition $\phi(x, t)$ and the curvature $c(x, t)$ owing to the Helfrich energy of bending

$$u = \frac{1}{2}k(c - c_o)^2 = \frac{1}{2}k(c^2 - 2\alpha c\phi^2 + \alpha^2 \phi^4). \tag{10}$$

The quadratic approximation can be used also for the entropy, s, (7) [18]

$$s = -k_B \frac{1}{a_l}(2\phi(\phi - 1) - 0.2). \tag{11}$$

Under approximations (9)–(11) the free energy $\mathcal{F}[\phi, c]$ (5) takes the forms of the Ginzburg-Landau functional

$$\mathcal{F}[\phi, c] = \left(\frac{2k_BT}{a_l} - \alpha kc\right)\phi^2 + k\alpha^2\phi^4 + kc^2 - \frac{k_BT}{a_l}(2\phi + 0.2) +$$

$$+ \tfrac{1}{2}K_1|\nabla c|^2 + \tfrac{1}{2}K_2|\nabla\phi|^2. \tag{12}$$

As the equation of motion for the conservative field, $\phi(x, t)$, the Cahn-Hilliard equation [37] is used

$$\frac{\partial\phi(x, t)}{\partial t} = M\nabla^2\frac{\delta\mathcal{F}[\phi, c]}{\delta\phi(x, t)}, \tag{13}$$

where M is a mobility coefficient.

For the curvature field $c(x, t)$ we require

$$\frac{\delta\mathcal{F}[\phi, c]}{\delta c} = 0, \tag{14}$$

i.e. the field $c(x, t)$ follows adiabatically the field, $\phi(x, t)$, with time [33]. The adiabatic approximation implies that the bending modes driven by the Helfrich elastic energy (10) are fast as compared to diffusion modes given by Equation (13). Moreover, as shown [34] the bending modes of bilayer are overdamped with the frequency $\omega_1 \sim kq^3/2\rho$ for a slow modes and $\omega_2 \sim \eta q^2/\rho$ for a fast modes, where η and ρ are viscosity and density of a surrounding fluid. A comparison between these relaxation rates and diffusion rate $\omega \sim Dq^2$, where D is diffusion coefficient, shows that the monolayer has time to adopt its equilibrium form defined by Equation (14) for wave vectors $q \sim 2\pi/L$, where L is length scale. Note that adiabatic approximation is used only at small length scale ($L \sim 100$ Å) on which curvature instability induces MVT.

Substituting the free energy $\mathcal{F}[\phi, c]$ (12) into Equations (13), (14) gives the following nonlinear coupled equations:

$$\frac{\partial\phi(x, t)}{\partial t} = M\nabla^2\left[2\left(\frac{2k_BT}{a_l} - \alpha kc\right)\phi + 4k\alpha^2\phi^3\right] - MK_2\nabla^4\phi, \tag{15}$$

$$K_1\nabla^2 c - kc = -k\alpha\phi^2. \tag{16}$$

For simplicity we substitute the variable $c(x, t)$ in the Equation (15) for constant $\bar{c}$ which is equals to the spontaneous curvature $c_o(\phi_o)$ where

$$\phi_o = \frac{R_e}{R_e + a_l/a_d}.$$

In a linearized approximation [37] a special solution of the Equation (15) can be written as

$$\phi(x, t) = \phi_o + A(q, t)\cos qx,$$

where q is a wave vector. The evolution of the amplitude $A(q, t)$ is subject to the relation:

$$A(q, t) = A(q, 0)e^{R(q)t},$$

where 'enhancement' coefficient $R(q)$ is given by the following equation

$$R(q) = 2Mq^2 \left[2\left(\frac{2k_B T}{a_l} - \alpha k\bar{c}\right) + K_2 q^2 \right].$$

The amplitude of the homogeneous fluctuations grows with time for values of the wave numbers $q < q_c$ and decreases for $q > q_c$, where

$$q_c^2 = -2\left(2\frac{k_B T}{a_l} - \alpha k\bar{c}\right) / K_2. \tag{17}$$

The maximal amplification rate corresponds to $q_m = q_c/\sqrt{2}$.

Substitution of the solution $\phi(x, t)$ in the Equation (16) gives

$$K_1 \nabla^2 c - kc = -k\alpha(\phi_o + A(q_m, t) \cos q_m t)^2.$$

The solution of the equation

$$c(x, t) = \alpha\phi_o^2 + \frac{1}{2}\alpha A^2(q_m, t) + \frac{2k\alpha\phi_o A(q_m, t)}{k + K_1 q_m^2} \cos q_m x$$

$$+ \frac{k\alpha A^2(q_m, t)}{k + 4K_1 q_m^2} \cos 2q_m x, \tag{18}$$

oscillates around the mean curvature over one period

$$\langle c \rangle = \alpha\phi_o^2 + \frac{1}{2}\alpha A^2(q_m, t).$$

Several conclusions can be drawn from analysis of the equations derived in this section. First, the temperature of MVT, T_c, can be obtained from the following condition of curvature instability

$$2\frac{k_B T_c}{a_l} - \alpha k\bar{c} = 0,$$

which is deduced from Equation (17). With the estimation of bending elastic modulus $k \sim (10 - 50)k_B T$ [19] and the values of the curvature $\bar{c} = 0.012 \div 0.035$ for $R_e = 0.14 \div 0.3$, the temperature of MVT lies in the range $20 \div 60\,°C$ which correlates well with the range of experimental values $(20 - 45\,°C)$ measured for the mixed systems formed by NaC with saturated phosphatidylcholines [8].

The second conclusion relates to shape of micelles. As mentioned above, micellar structures are described in our approach by the solution (18) with high amplitude of monolayers curvature $(c \sim 1/h)$. This solution holds for the phase separation region at temperature $T < T_c$. By analogy with oscillatory behaviour of ellipse curvature, the solution (18) can be related to a cylindrical micelle with elliptic-like cross-section as shown schematically in Figure 5.
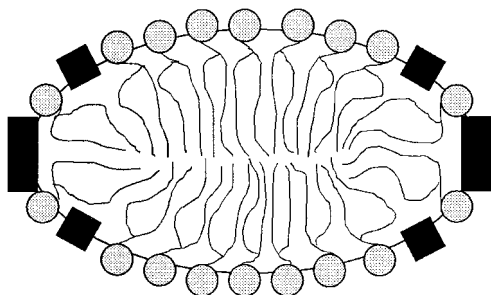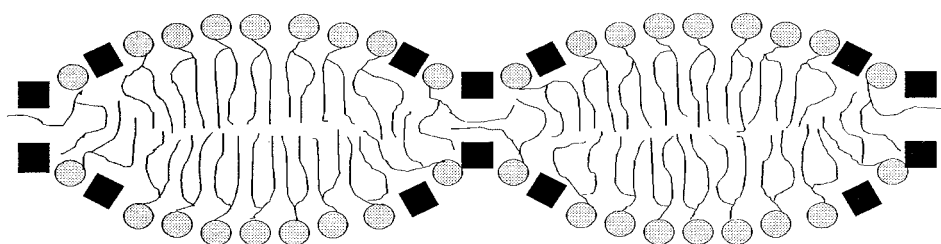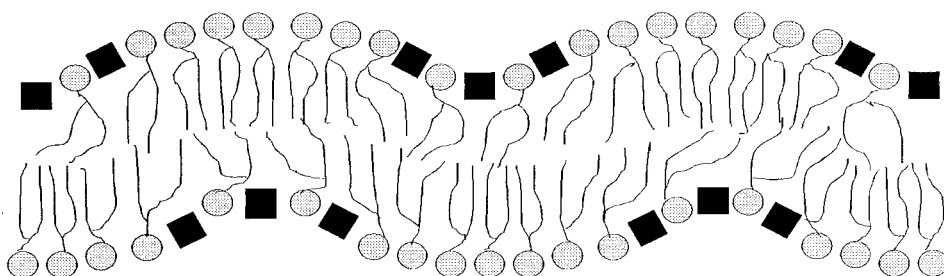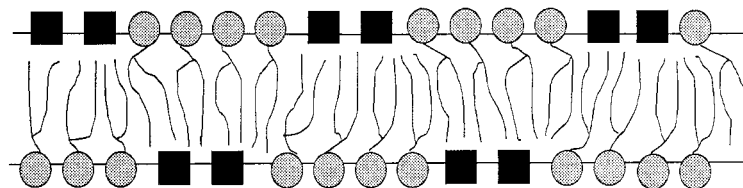
Figure 5. An elliptical cross-section of cylindrical micelle.

a

b

c

Figure 6. Different deformations of a mixed bilayer: a – peristaltic mode, b – undulation mode of deformation, c – an intermediate membrane phase.

And finally, structure of bilayers and their shapes can be deduced from the produced equations. Bilayer structure is described in the model by the solution (18) with small amplitude of monolayer curvature. The bilayer, composed of two deformed monolayers, can be found in two states. The first one corresponds to a peristaltic mode of bilayer deformation shown schematically in Figure 6(a). One can assume that on the cooling regime of the TI-MVT the decrease in the wave length of the membrane structural deformation will lead to fragmentation of bilayers to worm-like micelles with elliptical cross-section as shown in Figure 5.

The second state of the bilayer can be represented by an undulation mode of deformation as shown in Figure 6(b). The shift of deformed monolayers relatively each other may release the structural stress within bilayer by forming an intermediate membrane phase shown in Figure 6(c). This membrane structure is characterized by periodic alternation of regions enriched in the lipid or detergent. The thickness of this structure should be evidently lower than that of the purely lipidic bilayer. The difference can be detected experimentally by physical techniques, such as small angle neutron scattering. That this is actually the case has been confirmed recently by the SANS study of TI-MVT in the mixed DMPC/NaC systems [15]. It was demonstrated that the membrane of the DMPC/NaC vesicles formed by TI-MVT was distinctly thinner than that of the pure DMPC vesicles at the same temperature domain $(40 - 60\ °C)$.

2. Conclusion

In general, the temperature induced micelle-to-vesicle transition can be represented within the framework of the proposed model as follows. At high temperatures detergent is uniformly distributed along the membrane surface so that the membrane can be considered as an isotropic fluid-like phase. With cooling the thermal energy $k_B T$ becomes less than the mean energy of bending, so that the mean deformation of membrane induces the phase separation of lipid and detergent and leads to the domain formation. Further decrease in temperature induces fragmentation of membrane along the domain boundaries. It results in the formation of the network of worm-like micelles or living polymer phase.

When temperature approaches the main lipid phase transition, fragmentation of worm-like micelles occurs. This process seems to be associated with the phase separation in aggregates of cylindrical geometry and with bending instability of worm-like micelles in the range of the lipid crystallization temperature. Probably the worm-like micelles are fragmented to complex-shaped aggregates which are intermediate between bilayer disks with parallel orientation of lipid molecules and classical cylindric micelles with radial distribution of the molecules. Having retained partially the bilayer pattern of molecular packing, these small ellipsoidal particles may be represented as rod-like bilayer fragments, or more simply, bilayer rods. This assumption looks particularly attractive in the light of non-ideal

cylindrical shape of worm-like micelles theoretically substantiated in the present study.

On heating the reverse sequence of structural transitions occurs – the individual bilayer rods fuse to form sequentially long cylindric micelles, then the network of worm-like micelles and finally extended bilayer structures. Of course, the theoretical description of these transitions in the framework of models developed here leaves unexplained many details of molecular interactions in the mixed lipid-detergent aggregates and further information is still required to understand their structure and dynamic behaviour. Nevertheless, such an analysis can form the basis for further investigations and may be helpful in interpretation of experimental data obtained not only for the temperature-induced micelle-to-vesicle transition but for other related structural transitions as well.

Acknowledgements

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