Reconstruction of vesicles due to phase separation of lipids

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An amphiphile forms a self-assembly such as a micelle, cylinder, and lamella in water, and its packing parameter – the cross section ratio of the hydrophobic part to the hydrophilic part – governs which structure it prefers. On the other hand, a self-assembly of an amphiphile mixture is more various and complex than that of a single one. For example, an anionic- and cationic-surfactant mixture forms a bilayer in the shape of a disk, punctuated plane, icosahedra vesicle with nanopores on the apexes. Generally speaking, the edge of a surfactant bilayer, where the hydrophobic part is in touch with water, is quite unstable. The edge of the disk and pores in this system is, however, relatively stable because it is covered by one "excess" component, whereas the bilayer part consists of the surfactant mixture. This indicates that phase separation of the amphiphiles plays a key role in the structural formation of the multicomponent system, as well as the amphiphile shapes. The purpose of this study is to apply this idea to a simple biomimetic membrane, that is a phospholipid mixture.

A mixture of dimyristoylphosphatidylcholine (DMPC; 14 carbons/chain) and dihexanoylphosphatidylcholine (DHPC; 6 carbons/chain) form uni-lamellar vesicles (ULVs) above chain melting temperature of DMPC T_c (24°C), whereas small discoidal micelles (bicelles) appeared below T_c. I reported destabilization and reconstruction of ULVs at a temperature jump from 50° to slightly above T_c. Since temperature quench from T_c governs the phase separation between lipids, microdomain formation of short-chain lipids would result in the nanopore formation as in the case of surfactant mixtures. The temperature effect on the phase separation has, however, not been clarified so far. In this study, I have investigated the temperature effect on the phase separation and structural formation in DMPC and DHPC mixture near T_c using SANS-U at the C1-2 port of JRR-3 at Japan Atomic Energy Agency (JAEA), Tokai. A lipid mixtures in the molar ratio of [DHPC]:[DMPC] = 1:3.2 was dissolved in a D_2O solution of 3 mM $CaCl_2$, and a concentration of lipid was controlled to be 0.3 vol%.

Figure the obtained SANS profiles depending on the different temperature paths. After the temperature jump from 0°C to 50°C (path A), fringes reflecting ULVs were observed as previous reports. Next, temperature was jumped from 50°C to 30°C, 27°C, and 25°C (paths B, C, and D). In case of path B, the obtained profile was almost the same as that before temperature jump. Whereas, the period of fringes drastically changed in cases of paths C and D (small ULVs coexist for path C). This change indicates the enlargement of ULVs because the period of fringes is inversely proportional to the radius of ULVs. The size of enlarged ULVs was almost the same as that after the temperature jump from 0°C (paths C' and D'). Whereas, the size of ULVs of path B was smaller than that after the temperature jump from 0°C (path B').

According my previous result, the enlargement of ULVs passes through following processes:

- transformation from ULVs to bicelles,
- growth of bicelles,
- transformation from bicelles to ULVs.

Once small ULVs transform into bicelles, the remaining pathway is the same as the bicelle-ULV transition at the temperature jump from 0°C. For the transformation from bicelles to ULVs and vice versa, their energy balance is important and the radius of ULVs, R, can be described as

$$R = \frac{2\kappa + \bar{\kappa}}{2\gamma}, \tag{1}$$

where κ is bending modulus, $\bar{\kappa}$ is suddle-splay bending modulus, and γ is line tension. Since κ of DMPC bilayers is known and the radius of ULVs can be evaluated by fitting, I evaluated γ with this equation.

path	R [nm]	κ [$k_B T$]	γ [$k_B T$/nm]
path A	12.07	28	4.6
paths B′	20.44	31	3.0
paths C′	28.64	37	2.58
paths D′	48.0	19	0.8

The result clearly indicates that γ decreases with approaching T_c, that is, the rim of bilayers become stable due to enhancement of the phase separation. This is the reason why the reconstruction of ULVs was enhanced with approaching T_c.

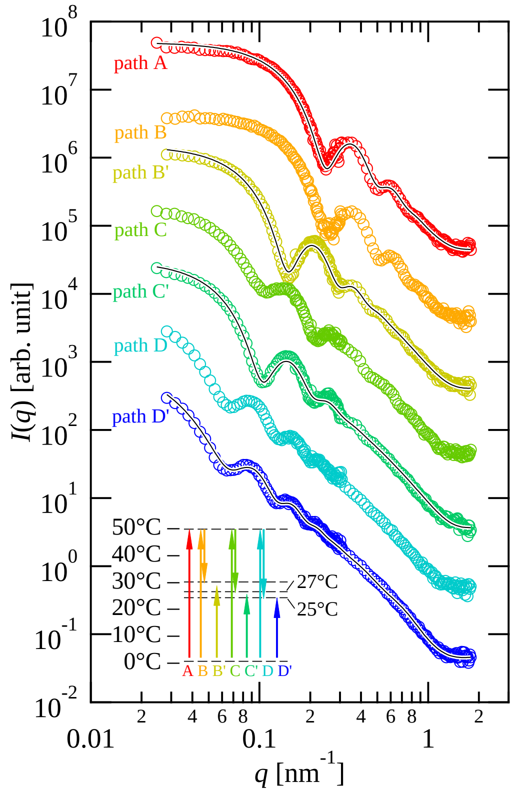

Fig. 1. SANS profiles after the temperature jumps for different paths.