Automatic Formation of Tube Structure

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1. Introduction

Lipid tubes called "myelin figures" are formed automatically from lumps of phospholipids in water above phase transition temperature. This is an example of self-organization by hydrophobic effect. Single helix of a tube also appeares.

Living cell is wrapped by biomembrane, and also many small organelles in the cell are wrapped by the biomembranes. The thickness of the biomembranes is about 10 nm (=0.01 μm). The thin membranes keep the cell to be alive by their functions. Though the biomembranes are ingeniously designed depending on the cells, their fundamental structure is simple and common in all creatures. In short, functional proteins are embedded in lipid membrane with fluidity as shown in Fig. 1.

The main component of the lipids is phospholipids, which form lipid bilayer membranes automatically in excess water as explained in this paper.

2. Formation of Lipid Membrane Tubes

Among phospholipids, lecithin is common in creatures.

As shown in Fig. 2, when lumps of synthesized lecithin (dipalmitoylphosphatidylcholine) in water are heated to a temperature above the specific temperature (41.8°C for the lecithin), the tubes are automatically formed from the lumps. These tubes are called "myelin figures". The morphological changes shown in Figs. 2(2)–(4) suggest metastability of the tubes.

The myelin figures has been found to be composed of concentrically stacked with several hundreds of lipid bilayer membranes shown in Fig. 3(a). As shown in Fig. 3(b), a sheet of the membrane has the structure in which the hydrophilic groups of the phospholipids make contact with water on the surface of the membrane and the hydrophobic groups (two hydrocarbon chains) face each other.

As a preliminary stage the lumps absorb water a bit and the lipid bilayers are formed locally near their surface.

When the temperature of the lumps increases to a value above the specific temperature, water infiltrates the lumps in which the bilayers are formed. At the same time the surface of the lumps bulges in a half sphere locally and the tubes begin to grow. In the growth process fragments of bilayers are thought to be connected to the end of tubes from next to next in the lumps while water infiltrates the lumps. When water stops to flux into the lumps the end of the tubes is closed and the growth of myelin figures stop.

When the myelin figures are teared by mechanical vibration with a ultrasonic generator above the specific temperature, the teared places will be restored and the teared fragments become to be small sphere vesicles which are energetically stable. Those lipid vesicles are called liposomes. In the liposomes the long axes of molecules are directed radially, and therefore they have strong optical anisotropy which produce double refraction (Mishima, 2006). The liposomes have been studied as a biomembrane model and used as containers for drugs in drug delivery system (DDS).

3. Phase Transition of the Lipid Bilayer Membranes

Below the specific temperature the hydrocarbon chains of the lipid molecules are straight and stiff like rods. Above the temperature the chains melt to be flexible like whips and the lipid molecules diffuse freely within the lipid bilayer membrane while rotating about their long axes. The former state of lipid molecules is called crystalline phase (or gel phase), and the latter is fluid phase (or liquid crystalline phase). The myelin figures are formed in the fluid phase. In Fig. 3(c) the zigzag hydrocarbon chains show that the lipids are in the fluid phase. The change between the two phases is called phase transition and the specific temperature is called phase transition temperature. The transition is essentially the same as the transition between ice and water at the transition temperature of 0°C (the endothermic first-order phase transition). Melting of ice corresponds to the melting of the hydrocarbon chains at the transition.

The hydrocarbon chains of natural phospholipids have some double bonds which has lower hydrophobicity than normal bonds. The lower the hydrophobicity of phospholipid is, the lower the phase transition temperature of the phopholipid membrane is. Natural phospholipids has a phase transition temperature lower than room temperature.

For example egg lecithin has a phase transition temperature lower than 0°C. Therefore, when the lump of the egg lecithin only touches water, the myelin figures are formed automatically. For living cells the fluidity of their biomembranes is so important that biochemical reactions advance only at fluid biomembranes. Creatures in cold environment

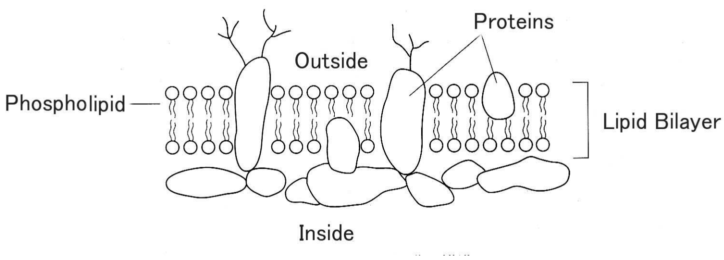

Fig. 1. Model of biomembrane (Satoh and Fujishiro, 2007).

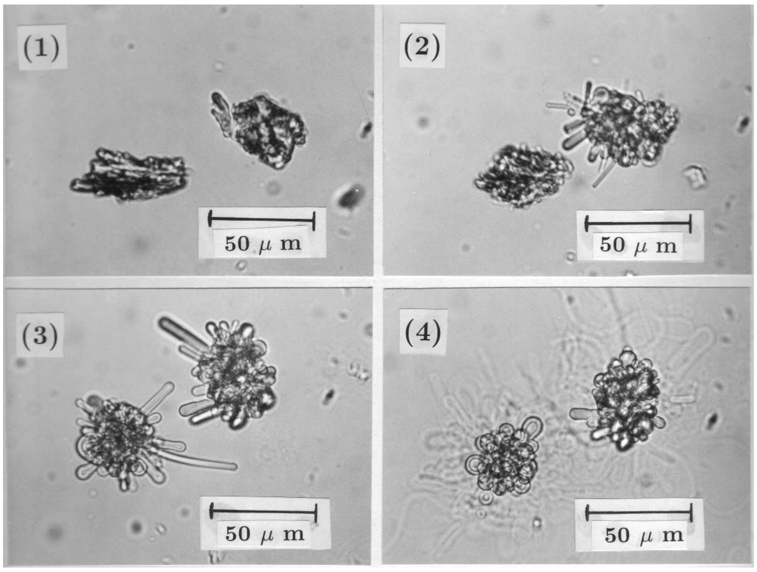

Fig. 2. Myelin figures formation from lumps of synthesized lecithin (Mishima and Satoh, 1989). (1) 28°C, (2) 42°C, (3) 44°C, (4) 54°C.

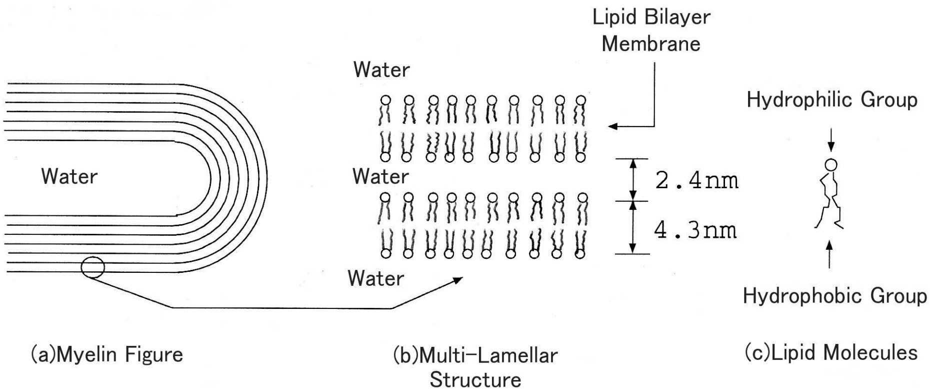

Fig. 3. Structure of myelin figure.

have phospholipids with lower hydrophobicity than those in hot environment in order to keep the fluidity.

4. Wonders in Myelin Figures Formation

Why do lipid molecules take the ordered structure like myelin figures above the transition temperature? Water takes the ordered structure of ice below the transition temperature (0°C) and the order of crystalline is destroyed and water molecules move freely above the transition temperature. In those transitions the degree of disorder (entropy) increases. It might be wonder for us that the myelin figures formation is seemingly unnatural change to get the order above the transition temperature.

If one hydrophobic molecule like hydrocarbon is set in water it will be surrounded by water molecules which take ordered structure by hydrogen bond. Therefore, if many hydrophobic molecules are set separately in water, they will assemble automatically to form aggregates in order to de-

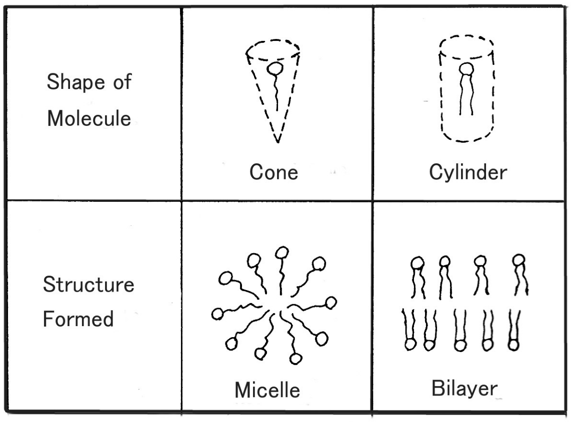

Fig. 4. Shape of molecule and structure formed (Israelachvili, 1991).

Fig. 5. Helical myelin figures formation from lumps of phosphatidylethanolamine at 71°C (Mishima and Satoh, 1991).

crease the space occupied by the order structure around the hydrophobic molecules. This is called as hydrophobic effect (Tanford, 1980). The phospholipids have both hydrophilic and hydrophobic groups, which are called amphiphilic molecules or amphiphiles, and therefore the hydrophobic effect happens at the hydrophobic group. In the process of assembling, the whole entropy of the system should increase. The hydrophobic effect is not interaction between hydrophobic molecules nor simple exclusion of water from the hydrophobic molecules.

The myelin figures grows from the lumps where the phospholipid molecules are packed compactly. When hydrocarbons are melted above the transition, water infiltrates the lumps. The hydrophobic effect prevents the molecules from separating each other and the molecules have strong tendency to be set side by side. As a result the molecules form the lipid bilayers. The degree of disorder (entropy) increases significantly by the melting of the hydrocarbon. Therefore, the myelin figures formation is said to be an example of self-organization.

Why do phospholipid molecules form bilayer in water while soap molecules form micelle? Both molecules are amphiphiles. The structure formed by the amphiphilic molecules depend on shape of the molecules as shown in Fig. 4. The phopholipid molecules have the shape near to cylinder.

Why are these thin membranes stacked at regular interval in the myelin figures? The membranes next to each other attract each other mainly by van der Waals forces. But as the distance between membranes decreases, repulsive steric force increases. The repulsive force comes from thermal fluctuation (Israelachvili, 1991). In water the regular interval is determined by the length of hydrocarbon and the balance between the attractive and the repulsive forces. In electrolyte the interval depends also on electrostatic force and shielding effect by ions. Though the lecithin is electrically neutral in water as a whole, some divalent cations like Ca^{2+} and Mg^{2+} bind strongly to the polar part of the hydrophilic group inducing electrostatic repulsive force.

5. Helix Formation of Myelin Figures

Diameter and length of the myelin figures in water depends on the kinds of phospholipids. Figure 5 shows long and stable myelin figures are formed from the lump of phosphatidylethanolamine. This phospholipid has the same hydrophobic group as the lecithin shown in Fig. 2 but has smaller hydrophilic group than the lecithin. By some chance some tubes begin to fold by itself from its root forming a single helix. The helix is in metastable state and begin to unfold suddenly. This suggests that the attractive force between the tube surfaces overcomes bending elastic force a little bit.

The myelin figures of the egg lecithin often form stable double helices by themselves. The bouble helices have regularity in the winding so as to minimize the sum of the intermembrane binding energy and the bending elastic energy (Mishima *et al.*, 1992).

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