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Double helix formation of phosphatidylcholine myelin figures

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Double helix formation of phosphatidylcholine myelin figures has been studied by use of optical microscopy. The winding of the double helices was looser than a geometrically possible one and the pitch was related proportionally to the outer radii of helical myelin figures. The regularity in the winding was explained in terms of the intermembrane binding energy and the bending elastic energy.

Introduction

When phospholipid bilayers change into the liquid-crystalline phase, a tube-like structure, so-called myelin figure grows from the surface of the lipid aggregates into bulk water resulting from a swell [1–5]. The structure of myelin figures is composed of concentrically stacked multi-lamellar bilayer membranes with a considerable amount of water inside the tube. The myelin figures grow straight in the initial growth stage. However, morphological changes as hairpin-like deformation and helix formation occur after the initial growth stage. The formation and structure of helices are important to understand morphological changes in biological cells and organs.

Helical myelin figures, together with elongate myelin figures, were observed since olden times. Two types of helical myelin figures, i.e., a double-helical myelin figure and a myelin figure itself wound around the tube, were described in 1908 [6]. However, there are few reports regarding the formation mechanism. Lin et al. have reported that binary mixtures of cardiolipin and phosphatidylcholine form tightly folded single- and double-helical myelin figures in the presence of calcium ions [7]. They interpreted the helix formation in terms of the Ca^{2+}-mediated intermembrane interaction. The curvature elastic energy for bending the helical myelin tube, which opposes the helix formation, was considered to be negligible because of a small number of bilayer membranes of the myelin figure.

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Although myelin figures of egg-yolk phosphatidylcholine (egg-PC) have a large number of bilayer membranes (from several hundreds to several thousands), they form easily double helices without calcium ions. The double helices are looser in the winding than the Ca^{2+}-mediated double helices. This suggests that the helix formation is related to not only the intermembrane interactions but also the bending elastic force. In this paper, we report measurements of the pitch of egg-PC double helices using an optical microscopy and give an explanation of the helix formation taking into account the effect of the membrane bending elasticity.

Materials and Methods

Egg-yolk phosphatidylcholine was purchased from Sigma Chemical Co. and used without further purification. Water was ion-depleted by an ion exchanger (Yamato Co.) and double-distilled. An appropriate amount of the methanol solution of the lipid (50–100 mg/ml) was dropped on a glass microscopic slide and the solvent was removed by evaporation in vacuum. A glass coverslip was put on the lipid aggregate spacing with spacers of 50–100 μm thickness and pressed down slightly in order to prevent a drift of the aggregate induced by addition of water. Then water was injected into the gap between the glass plates and the gap was sealed with silicon grease against evaporation.

Myelin figures were formed immediately and began to grow in crowds from the surface of the aggregate into bulk water because the lipids around the surface changes into the liquid-crystalline phase (the phase transition temperature of the fully hydrated lipid: -7°C to -10°C). After several minutes, more elongated myelin figures bent and some of them began to form

double helices. The pitch and the tube size of helical myelin figures were measured on the optical micrographs. All experiments were carried out at temperature range of 20°C to 25°C. No effects of the temperature fluctuations on the formation and structure of helices were observed.

Results and Discussion

Myelin figures of egg-PC tended to form double helices by themselves. Sometimes, it was observed that two myelin figures formed a double helix. Double helix formation was initiated at a tip of a hairpin loop, or at a constricted part in regions of membrane-membrane contact. The helix formation progressed at a slow rate of about 10 s per pitch. It looked like the helices grew in order to increase the area of membrane-membrane contact. Irregularity in the helix formation was sometimes observed, e.g., a double helix with both right- and left-handed windings and an asymmetrical double helix formed by two myelin tubes with different diameters. Fig. 1 shows typical micrographs of regular double helices. It is seen that they are symmetrical in the winding, and that the outer diameters of the helices are twice the diameters of the tubes indicating no large distortion of the circularity of the tubes.

Most of double helices grew with a constant pitch from the initiation. However, some helices were loose at the initiation and became tight together with the growth. The pitch of them got to a final constant value. As shown in Fig. 2, the final pitch was related proportionally to the outer radius of the tube for regular double helices. The ratio of the outer radius to the pitch had a constant value of 0.175 ± 0.015. This value contrasts to the ratio (0.214) for the tightly wound double helix observed in phosphatidylcholine/cardiolipin mixture in the presence of calcium ions, indicating looser helix formation of egg-PC myelin figures.

We now consider a mechanism of double helix formation. The driving force for helix formation is considered to be the intermembrane interaction between helical tubes, and the forces opposing helix formation arise from the elasticity of the membrane. The tube length per unit pitch l for a double-helical tube is given geometrically as

$$l = \left(l_p^2 + 4(\pi r_o)^2\right)^{1/2} \tag{1}$$

where l_p is the pitch and r_o the other radius of the helical tube. Assuming no twist of the helical tube around its own long axis, the length per unit pitch along the contact area between helical tubes, l', is also given as

$$l' = \left(l_p^2 + 8(\pi r_o)^2\right)^{1/2} \tag{2}$$

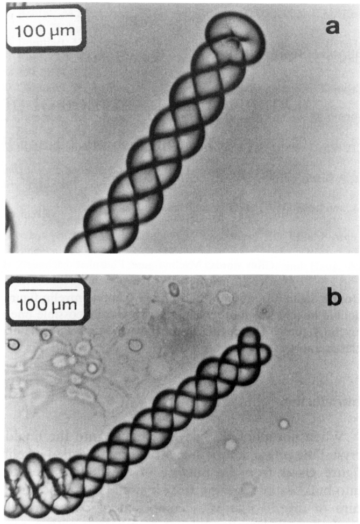

Fig. 1. Optical micrographs of double helical myelin figures of egg-yolk phosphatidylcholine. Double helices formed by one myelin tube (a) and by two tubes (b).

The energy due to the intermembrane interaction per unit length g_{in} can be written as

$$g_{in} = -A\omega l'/l \tag{3}$$

where A is the interaction energy per unit area and ω the width of the contact area.

The forces opposing the helix formation arise from the elasticity of multi-lamellar bilayer membranes. The

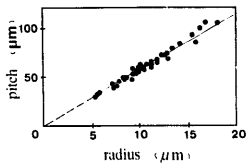

Fig. 2. Dependence of the pitch on the outer tube radius for helical myelin figures with regular winding. The pitch and the tube radii were measured on the optical micrographs. The ratio of the outer radius to the pitch was 0.175 ± 0.015.

shear elasticity of the bilayer membranes is essentially negligible because of fluid membranes. Also, the stretch or compression elasticity can be negligible because of no large distortion in the circularity of the helical tube. Therefore the bending elasticity of the bilayer membranes is dominant. According to Helfrich et al. [8,9], the bending elasticity of the bilayer membranes is dominant. According to Helfrich et al. [8,9], the bending elastic energy per unit length is $\pi\kappa r/2R^2$ for a tube of radius r with one bilayer membrane, where κ is the bending modulus of the bilayer and R the curvature radius of the tube. For a myelin tube with multilamellar bilayer membranes, the bending energy per unit length g_{el} is the sum of the elastic contribution. Therefore,

$$g_{el} = \frac{\pi\kappa\Sigma r_j}{2R^2} = \pi\kappa\frac{r_o^2 - r_i^2}{4dR^2} \tag{4}$$

where r_j is the radius of the j bilayer, r_i the radius of the inmost bilayer and d the bilayer repeat distance.

The curvature radius of the bending tube R is approximately given as

$$R = \frac{r_o}{2}\left(\frac{1}{16}\left(\frac{l_p}{r_o}\right)^2 + 1\right) \tag{5}$$

The inmost radii of egg-PC myelin figures were far smaller than the outer radii, i.e., the ratio of r_i to r_o is less than about 0.3. For this case of $(r_i/r_o)^2 \ll 1$, the total energy per unit length g ($= g_{in} + g_{el}$) is written as

$$g = -A\omega\left(\frac{1+8\pi^2 x}{1+4\pi^2 x}\right)^{1/2} + \frac{\pi\kappa}{d}\left(\frac{16x}{1+16x}\right)^2 \tag{6}$$

where $x = (r_o/l_p)^2$. Values of x are geometrically ranged from 0 (no helix formation but simple contact) to 1/16 (= 0.0625, tightest helix formation).

We calculated the total energy for different values of $A\omega$ using reported values of the bending modulus $\kappa = 1 \cdot 10^{-19}$ J [9,10], and of the repeat distance $d = 6$ nm for the lamellae of phosphatidylcholine/water systems [11]. For a smaller value of $A\omega$ than about $0.05 \cdot 10^{-10}$ J/m, the total energy increased with x indicating no helix formation. On the other hand, for a larger value of $A\omega$ than about $1.6 \cdot 10^{-10}$ J/m, the total energy decreased up to the geometrical limit of x. This is the case of tightest helix formation. As shown in Fig. 3(c), when $A\omega$ is $1.2 \cdot 10^{-10}$ J/m, the total energy has a minimum at $x = 0.046$. This case corresponds to the Ca^{2+}-mediated helix formation observed in phosphatidylcholine/cardiolipin mixture. When $A\omega$ is $0.7 \cdot 10^{-10}$ J/m (Fig. 3(b)), a value of x to minimize the energy agrees with the observed value for egg-PC helices (0.031 ± 0.007).

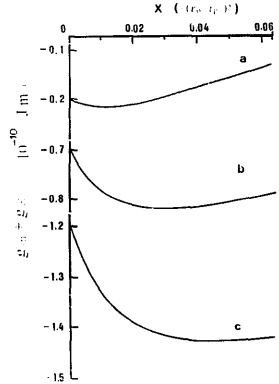

Fig. 3. The total energy of a helical myelin figure as a function of $x[=(r_o/l_p)^2]$ for different values of the intermembrane interaction energy per unit length, $A\omega$. (a) $A\omega = 0.2 \cdot 10^{-10}$ J/m, (b) $A\omega = 0.7 \cdot 10^{-10}$ J/m and (c) $A\omega = 1.2 \cdot 10^{-10}$ J/m.

Now we examine whether this value of $A\omega$ is reasonable or not. The intermembrane interaction is considered to arise mainly from van der Waals interaction as an attractive force, which is related with a distance between membranes [12]. Recently Sakurai, I. et al. observed a frozen double helix of egg-PC myelin figures by use of a scanning electron microscope (SEM) [13]. Unfortunately, it is impossible to determine the distance between the outmost bilayer membranes and the contact width of the helical tubes since the micrograph is a side view. They also observed cross sections of frozen egg-PC myelin figures which were in close contact [13,14]. The distance between the outmost membranes is not known from the micrographs because of low magnification. However, it is seen that the contact widths are several micron meters. Assuming that the distance between the outmost bilayer membranes is similar to a thickness of the water layer in the multi-lamellar structure, and using values of a Hamaker constant of $7.5 \cdot 10^{-21}$ J [15], the lipid layer thickness of 3.5 nm and of the water layer thickness of 2.5 nm for the lamellae of phosphatidylcholine/water systems [11], the van der Waals interaction energy per unit area is calculated to be $2.3 \cdot 10^{-5}$ J/m^2. Using this value for A, the contact width ω is estimated to be 3 μm in the case of $A\omega = 0.7 \cdot 10^{-10}$ J/m. The estimated values of ω is consistent with the observation of the cross sections by SEM. However, measurements of the contact width and also the distance for non-frozen helical

tubes remain the subject of further study. In conclusion, double helices of egg-PC myelin figures have regularity in the winding so as to minimize the sum of the intermembrane binding energy and the bending elastic energy.

References

1 Mishima, K. and Satoh, K. (1977) Membrane 2, 387–394 (in Japanese).
2 Sandermann, Jr., H., Falk, H. and Schumacher, G. (1977) Anal. Biochem. 82, 583–585.
3 Mishima, K., Satoh, K. and Ogihara, T. (1984) Chem. Phys. Lett. 106, 513–516.
4 Mishima, K. and Yoshiyama, K. (1987) Biochim. Biophys. Acta 904, 149–153.
5 Mishima, K. and Satoh, K. (1989) Forma 4, 103–121.
6 Kelker, H. (1973) Mol. Cryst. Liquid Cryst. 21, 1–48.
7 Lin, K.-C., Weis, R.M. and McConnell, H.M. (1982) Nature 296, 164–165.
8 Heifrich, W. (1973) Z. Naturforsch. 28c, 693–703.
9 Servuss, R.M., Harbich, W. and Helfrich, W. (1976) Biochim. Biophys. Acta 436, 900–903.
10 Mishima, K. and Morimoto, T. (1989) Biochim. Biophys. Acta 985, 351–354.
11 Janiak, J.M., Small, D.M. and Shipley, G.G. (1976) Biochemistry 15, 4575–4580.
12 Ninham, B.W. and Parsegian, V.A. (1970) J. Chem. Phys. 52, 4578–4587.
13 Sakurai, I., Suzuki, T. and Sakurai, S. (1989) Biochim. Biophys. Acta 985, 101–105.
14 Sakurai, I., Suzuki, T. and Sakurai, S. (1990) Mol. Cryst. Liq. Cryst. 180B, 305–311.
15 Marra, J. (1985) J. Coll. Interface Sci. 107, 446–458.