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# Growth rate of myelin figures of egg-yolk phosphatidylcholine

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**The growth length of myelin figures was measured in detail using a video tape recording system of optical microscopy. In the beginning stage of growth, the growth process was not adapted to the diffusion-limited process of lipid molecules, which has been recently proposed. Another interpretation for the growth mechanism was proposed, where the growth results from swelling. The initial growth rate measured was in good agreement with the estimated value in consideration with the water flux in the first approximation of the lipid concentration gradient.**

The elongated tubular structure, called myelin figure or myelin tube, grows from hydrated phospholipid lumps to water. The myelin figure is composed of concentrically stacked multilamellae of lipid bilayers with a considerable amount of water inside the tube. The myelin figure formation is important as a model for the self-assembly and self-organization in biological membranes. The medical and technological applications will be developed in recent future.

Myelin figures, which were formed from natural phospholipids, are found in the middle of the last century [1]. However, there are few reports that study the myelin figure formation. To our knowledge, Chapmann and Fluck [2] were the first to suggest that myelin figure formation occurs above the phase transition temperature ($T_c$). Mishima et al. [3,4] and Sandermann et al. [5] showed that myelin figures are formed spontaneously at $T_c$. In this journal, Sakurai et al. [6,7] have reported results of growth measurement of myelin figure for egg-yolk phosphatidylcholine. They concluded that the growth is limited by self-diffusion of lipid molecules, i.e., the growth length $l$ at time $t$ is given by $l = (2Dt)^{1/2}$, where $D$ is the relevant diffusion coefficient of lipid molecules. However, they could not measure in detail with regard to time because they were using photographs. This report presents detailed measurements of myelin figure growth using a video tape recording system of optical microscopy, and another interpretation of the growth mechanism.

Egg-yolk phosphatidylcholine (egg-PC) was purchased from Sigma and used without further purification. We used an experimental method proposed by Sakurai et al. [6] as follows. An appropriate amount of the chloroform solution was dropped on a slide glass and the solvent was evaporated in vacuum. When water (double-distilled) was added to the lump from all quarters, the lump got out of shape rapidly by forming myelin figures. Therefore, a cover slip was put on the lump spacing with spacers of about 50 $\mu$m thickness and pressed down in order to prevent the lump to get out of shape. When a drop of water was added to the lump edge, at once, myelin

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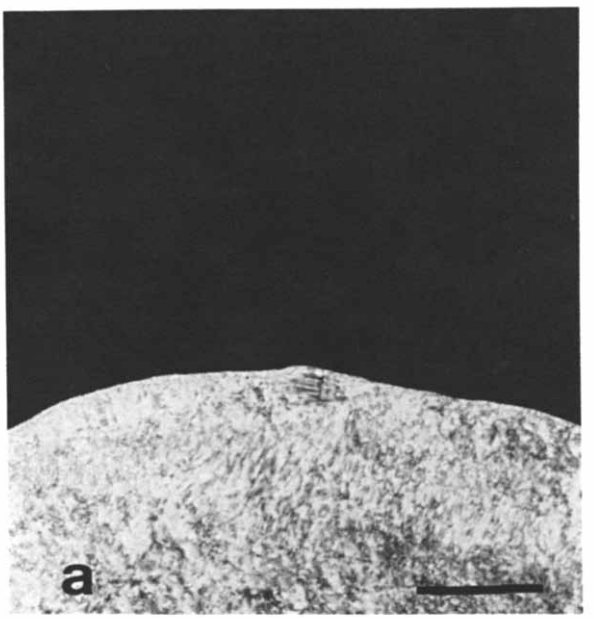
figures were formed at the interface between the lump and water, and began to grow in crowds. As shown in Fig. 1, myelin tubes grew nearly perpendicular to the lump-water interface in the initial growth stage. However, it was not long before the myelin tubes tended to strand each other or to bend around the growth front. Also the tube diameter increased in the top part of tubes. These morphological variations were more complicated in comparison with myelin tubes of synthetic phospholipids [3]. When samples of myelin tubes were hand shaken or incubated for a long time, most of all tubes changed to liposomes. Since liposomes are thermodynamically stable, myelin tubes represent a quasi stable form of higher free energy.

The length of the myelin tubes was measured by use of a video tape recording system (Hamamatsu Photonics Co: C-2400), in which time is resolved into 1/30 s in maximum. Some tubes grew irregularly with time. There were some cases where the growth stopped temporally. The irregular growth may be caused by a structural change inside the lump and the morphological changes in the tubes. It is noted that both the length grown up and the growth rate were depended on the tube thickness and the structural situation of the lump. Furthermore, the starting time differed slightly with place of the lump edge. Then we measured the growth length from the edge of the lump to the top of the tube for several points along the lump edge and averaged them. Fig. 2 shows a typical result of the average length for regularly growing tubes as a function of time. To make clear whether the length $l$ changes with $t^{1/2}$ or not, the time course of length is re-plotted with logarithmic scales in Fig. 3. In comparison with a broken line which shows that $l \propto t^{1/2}$, measured points are far away from the line in the beginning stage of growth. This suggests that the growth process is not limited by self-diffusion of lipid molecules.

We now consider the growth mechanism. Polarizing microscopic observations show that egg-PC lumps before growth of myelin tubes are composed of optical anisotropic regions with different direction of optical axis (Fig. 1(a)). This means that bilayers are already formed, and clusters of stacked bilayers are packed randomly in




the lumps. In fact, egg-PC is highly hygroscopic. When the dried sample was left at room temperature, the water content increased rapidly to about 34% after 4 min and to 57% of the maximum value after 50 min. Then the water content of the lump, which used after 10–30 min for growth measurement, is considered to be about 50 wt% before the addition of excess water. When water is added, confusingly stacked bilayers are favored with water. Since almost all lipid molecules do not dissolve one by one into the outer water, the packed bilayers swell with rearranging to more ordered assemblies around the lump surface, and then to construct myelin tubes.

Since the assembly of bilayers around the surface is a complicated structure, we will consider this assembly, which contained the membrane at the lump/water interface, as a membrane of apparent thickness of $d'$. Then we consider a system which is composed of bulky bilayers packed inside the lump, the membrane of a more ordered bilayer assembly around the lump surface and a myelin tube. Here, we will focus our attention on the water flux into this system. Water flows through the lump/water interface, and also through the inside of the myelin tube because of the concentration gradient at the interface between the lump and the water inside the tube. The total

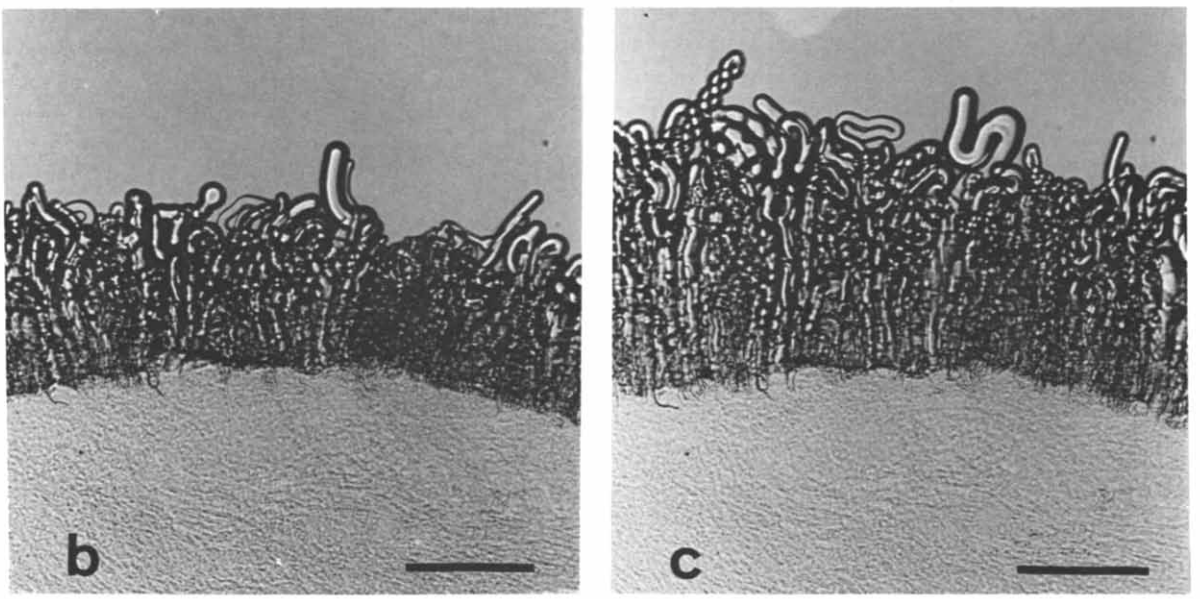



Fig. 1. Optical micrographs of myelin figures of the egg phosphatidylcholine/water system (a) taken with crossed-Nicols before addition of water, (b) without crossed-Nicols after 2 min and (c) after 5 min from the beginning of growth. Bar = 100 $\mu$m.

volume flux of water per unit time is written by $\tilde{v}_w \cdot (J \cdot S + J' \cdot S')$, where $\tilde{v}_w$ is the molar volume of water, $J$ the water flux per unit time and unit cross-sectional area through the tube of inner cross-sectional area of $S$, and $J'$ the water flux through the lump/water interface of relevant surface area of $S'$ around the tube. We assume that the difference in the chemical potential of water between the inside and outside of the lump is dominated only by a first order of the lipid mole fraction. Since the water content of the lump is large, the water flux is proportional to the lipid mole concentration of $C$ inside the lump. Thus, $J = P \cdot C$ and $J' = P' \cdot C$ where $P$ is the permeability for the membrane of apparent thickness $d$ at the interface between the lump and the water inside the tube and $P'$ for the membrane of apparent thickness $d'$ around the lump surface.

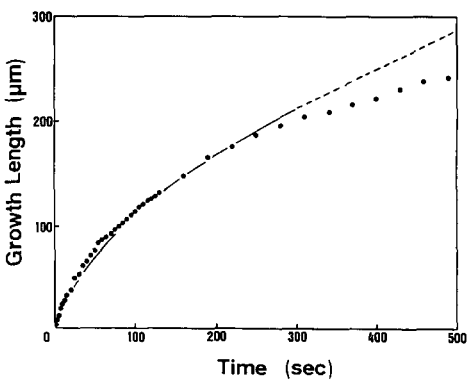



Fig. 2. Plots of typical time course of average length of regularly growing myelin figures at 22° C and a fitting curve (solid line) obtained from Eqn. 4 for measured points before 300 s. After this time, the growth rate decreased markedly because of the morphological changes of the myelin figures.

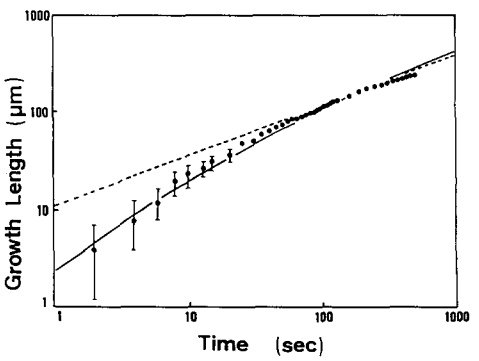



Fig. 3. Re-plots of the length in Fig. 2 with logarithmic scales. The solid line represents the fitting curve of Eqn. 4 and the broken line represents the relation $l \propto t^{1/2}$.

Since it can be approximated to $d = d'$, then $P = P'$. We also assume rapid rearrangements of bilayers to the membrane around the lump surface and to the tube membrane. When $\alpha$ is the part of the total volume flux that is distributed to the inside of the tube at a time interval $\Delta t$

$$S \cdot \Delta l = \alpha \cdot \tilde{v}_w \cdot J \cdot (S + S') \cdot \Delta t \tag{1}$$

where $\Delta l$ is the increase of tube length and assuming that the tube form and diameter are maintained. This equation gives the growth rate of the myelin tube, $v = \Delta l / \Delta t$. On the other hand, $(1 - \alpha)$ is the part of the total volume flux that is distributed to the lump and the membrane around the lump surface. This water may lead to the construction of the membrane. If $\beta$ is the part of this water that is distributed to the membrane, the increase in the average thickness of the membrane at a time interval $\Delta t$ is written by $\Delta d = \beta \cdot (1 - \alpha) \cdot \tilde{v}_w \cdot J \cdot \Delta t + \Delta\gamma$ where $\Delta\gamma$ is a contribution of lipid to the increase of the thickness. When the flux of the bulky bilayers to the membrane around the surface from the inside of the lump is equal to the flux of more ordered bilayers from the membrane around the surface to the tube membrane, $\Delta\gamma = 0$. It may be considered that this is the case for tubes grown in crowds as egg-PC. Combining Eqn. 1, the thickness is given as

$$d = d_o + \beta \cdot \left(\frac{1-\alpha}{\alpha}\right)\left(\frac{S}{S+S'}\right) \cdot l \tag{2}$$

where $d_o$ is the initial thickness of the membrane around the lump surface, which is approximately equal to the thickness of tube membrane. Therefore the growth rate is given by

$$v = \frac{v_o}{1 + k \cdot l} \tag{3}$$

where $v_o = \tilde{v}_w \cdot P_o \cdot C \cdot \alpha \cdot (S + S')/S$, $k = \beta \cdot (1 - \alpha) \cdot S/(\alpha \cdot (S + S') \cdot d_o)$ and $P_o$ the permeability for the initial thickness of $d_o$. The growth length is written as

$$l = \left\{ -1 + (1 + 2v_o \cdot k \cdot t)^{1/2} \right\}/k \tag{4}$$

This equation shows that the growth length changes with $t^{1/2}$ for $t \gg 1/2v_o \cdot k$. This change was observed in studies by Sakurai et al. and also in our studies. However, for a short time of the initial growth stage, the time course of the length is different from $t^{1/2}$ as shown in Fig. 3. The measured length is well fitted to Eqn. 4 as shown by the solid lines of Figs. 2 and 3. This fitting dealt with measured points before 300 s because morphological changes of tubes (bend and helix) were observed after this time. It is noted that the above model should be applied only to obedient tubes. The values of the initial growth rate $v_o$ and the dumping factor $k$ for the results of Fig. 2 are obtained from this fitting are $v_o = 2.5$ ($\mu$m/s) and $k = 0.024$ ($1/\mu$m). Similar values were obtained in repeated experiments.

Let's make an estimate of $v_o$ and $k$ values. Permeabilities of egg-PC to water have been reported by several authors for black membranes and unilamellar vesicle membranes to be in the range from $17 \cdot 10^{-4}$ to $100 \cdot 10^{-4}$ (cm/s) [8]. However, these values may be inapplicable because of the far lower order of the membrane around the lump surface than these membranes. More realistically, the diffusion coefficient of water $D_w$ for similar egg-PC/water systems used here should be applied. In this case the permeability may be given as $P_o = D_w/d_o$. Radioactive measurements of $^3$HHO in egg-PC/water systems by Rigaud et al. [9] who that $D_w$ depends extensively on the water content above 32%. $D_w$ increases about 300–400% with an increase in water content of several %. Then, since the value of $D_w$ reported by them is $8.2 \cdot 10^{-6}$ cm$^2$/s at 22° C for 40% water content, we will use a value of the order of $1 \cdot 10^{-5}$ cm$^2$/s for the water content of 50%. Using a mean molecular weight of 810 [10], the lipid concentration of lump, $C$ is $6.2 \cdot 10^{-4}$ mol/cm$^3$ for the water content of 50%. The tube thickness of the myelin figures could not be measured accurately in video images because of the low contrast inside the tube. Then the tube thickness was estimated from photographs. The outer diameters of tubes ranged from about 8 $\mu$m to 12 $\mu$m in the initial growth stage. Then the average tube thickness is considered to be about 4–5 $\mu$m. As seen in the photograph of Fig. 1, myelin tubes grow in crowds. Therefore the surface area at the lump/water interface $S'$ can be neglected. Also, remarkable changes inside the lump were not seen in the polarizing microscopic observation. This implies

that high values were taken in the water distribution factors $\alpha$ and $\beta$. When $\alpha = 0.9$ and $\beta = 1$, the initial growth rate $v_o$ is estimated to be 2.5 ($\mu$m/s) for $d_o = 4$ $\mu$m and 2.0 ($\mu$m/s) for $d_o = 5$ $\mu$m. Further, the dumping factor $k$ is 0.028 and 0.022 ($1/\mu$m) for $d_o = 4$ $\mu$m and 5 $\mu$m, respectively. These estimated values are in good agreement with the values obtained from measurements. In the data reported by Sakurai et al., the initial growth rate is not known. However, the ratio $v_o/k$ corresponds with the relevant diffusion coefficient of lipid $D$ in their theory. Further, although the diameter of myelin tube prepared by them was larger, this ratio is independent on the myelin tube thickness as seen Eqn. 3. Therefore, our measured value of $v_o/k$, $1 \cdot 10^{-6}$ cm$^2$/s can be compared with their value of $D$, $4.3 \cdot 10^{-6}$ cm$^2$/s at 25 °C. This discrepancy in the value is not serious. Because, if the distribution factor $\alpha$ increases only by several percent or the surface area $S'$ can not be neglected, the ratio $v_o/k$ increases to about $4 \cdot 10^{-6}$ cm$^2$/s for $\alpha = 0.97$ or for $S' = S$.

We conclude that the myelin tube does not grow with $t^{1/2}$ in the begin stage of the growth. The time course of growth can be explained by the water flow resulting from the swelling of the lipid lumps. In this work the change in the growth rate was attributed to the increase in the apparent thickness of the membrane at the lump/water interface inside and outside of the tube. Further, the effect of the ordering in the membrane structure should be considered.

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