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GROWTH MECHANISM OF MYELIN FIGURES OF PHOSPHATIDYLCHOLINE

I. SAKURAI and Y. KAWAMURA

Institute of Physical and Chemical Research, Hirosawa 2-1, Wako, Saitama 351 (Japan)

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The initial growth process of myelin figures, rod-like lyotropic liquid-crystalline structures, formed by phosphatidylcholine in water, ethylene glycol or glycerin, is suggested to be diffusion-limited with an apparent diffusion coefficient D of approx. 10^{-6} cm^2/s. D can be expressed by the sum of two processes. One is considered to describe the diffusion of an aggregate of phosphatidylcholine molecules and the other mainly to describe a lateral diffusion in the bilayer membranes which constitute myelin figures.

In the study of biological systems, it is of great interest how the biological molecules accomplish the self-assembly and self-organization into well-ordered biological tissues in the aqueous medium. As an approach to this problem, we have investigated the growth process of a lyotropic liquid-crystalline structure called myelin figure [1,2] formed by phosphatidylcholine, a major structural component of biomembranes, in water and several hydrophilic organic liquids. The myelin figures are elongated cylindrical rod-like structures composed of many lamellae of phosphatidylcholine bilayers stacked coaxially around the rod axis with hydrophilic liquid between every other bilayer, and are formed at the interface between the bulk phosphatidylcholine in L_α phase [3] and the liquid (Fig. 1). They have a strong optical anisotropy and give both a set of sharp X-ray diffractions giving an approx. 60 Å spacing from the lamellar structure and a diffuse wide-angle diffraction from disordered hydrocarbon chains. The stacked lamellar structure has the symmetry of a smectic A liquid crystal [4]. The structural features of a rod are essentially similar to those of the nerve myelin sheath and the structural features of a bilayer are similar to those of a biomembrane [5]. The myelin figures can also be found in other amphiphilic compounds, such as surfactants [6], in contact with suitable liquids. Little effort, however, has been made to clarify the mechanism of myelin figure formation in the past. We report here, for the first time, quantitative experimental results on the growth of myelin figures of egg-yolk phosphatidylcholine in several liquid media.

The sample of phosphatidylcholine was a commercially available one (Sigma Chem. Co.) which was prepared from hen egg by the method of

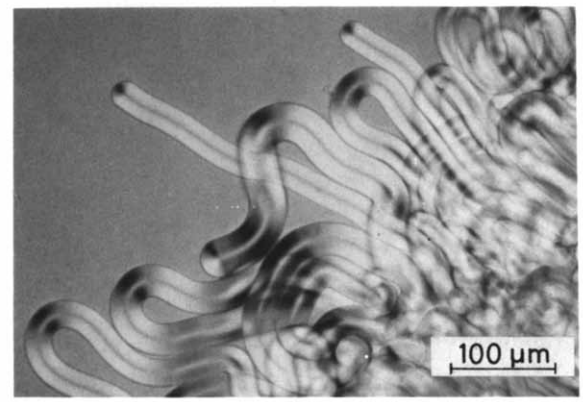

Fig. 1. A photograph of the myelin figures of the phosphatidylcholine/ethylene glycol system observed by a polarizing microscope after 15 min from the beginning of the growth.

Singleton et al. [7], and was used without further purification. Small lumps of phosphatidylcholine were placed on a slide glass. Then a cover glass was put on the lumps and pressed slightly till the thickness of phosphatidylcholine lumps was reduced to about 50 μm. The thickness of the preparation restricts myelin figures in the narrow two-dimensional space so as to facilitate observation of growth along their length. Water and two hydrophilic organic liquids, ethylene glycol and glycerin, were used as the dispersive medium, their viscosity coefficients, reported in Refs. 8 and 9, being 0.890, 16.8 and 954 cP at 25°C, and 0.653, 9.60 and 284 cP at 40°C, respectively. In addition, mixtures of water and glycerin, 80/20 and 85/15 v/v, whose viscosity coefficients at 25°C were 1.54 and 1.33 cP, respectively, were also used. They were chosen to provide a wide range of viscosity in the medium where the myelin figures grow.

The observation of the growth behaviour of myelin figures was made under a polarizing microscope at 25°C and 40°C. A drop of a dispersive liquid medium was brought into contact with the edge of the cover glass of the preparation so that the liquid medium spread over into the gap. As soon as the edge of a lump of phosphatidylcholine was made to come in contact with the dispersive liquid medium, myelin figures of a simple rod-like form [10] were observed simultaneously to start growing. The average diameter of myelin figures in the present preparation method was nearly the same irrespective of the differences in the medium or the temperature, ranging between approx. 20 and approx. 40 μm. For about 5–10 min from the beginning of the growth, those of the simple form growing nearly perpendicular to the interface prevailed. A typical growth pattern in water at 25°C is shown in Fig. 2, where the photographs taken within the first 5 min of the growth process are presented. Further growth of myelin figures tended to produce complicated forms such as a helical twisting of two simple ones, a hair-pin or other bent forms and so on. In Fig. 2d, indications of complicated forms are observed at the growth front. Preliminary observations on the morphological features of myelin figures have been reported elsewhere [10], and a detailed morphology of myelin figures will be presented in the near future. In this paper, only the initial growth stage,

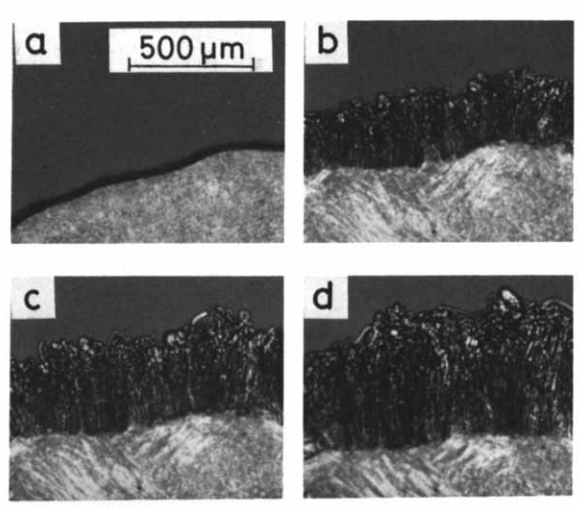

Fig. 2. A series of photographs of the growth pattern of myelin figures in phosphatidylcholine/water system taken by a polarizing microscope; (a) before introducing water, (b) after 1 min, (c) after 2 min, and (d) after 5 min from the contact of water with the edge of the phosphatidylcholine lump.

where the simple rod-like form prevails, will be studied.

From a series of photographs as shown in Fig. 2, the average length of myelin figures, l, was measured as the average distance between the contours of the edge of bulk phosphatidylcholine and the growth front of myelin figures. l thus obtained is shown in Fig. 3 as a function of time t measured from the beginning of the growth for all the systems examined. The results in Fig. 3 are re-plotted against $t^{1/2}$ in Fig. 4, which shows that $l \propto t^{1/2}$,

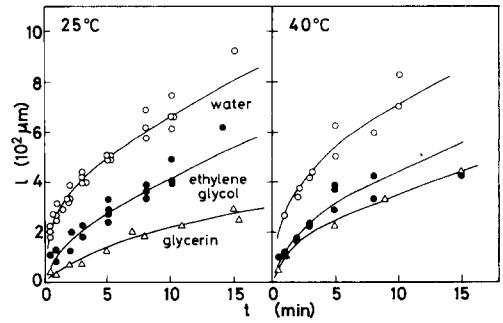

Fig. 3. Average length l of myelin figures in various dispersive media plotted against time t at 25°C and 40°C.

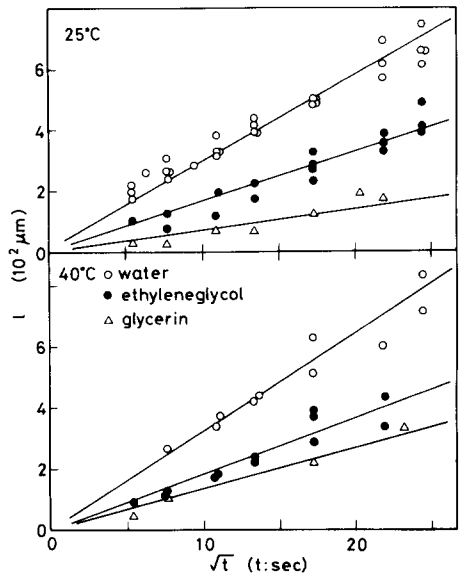

Fig. 4. l plotted against $t^{1/2}$ at 25°C and 40°C.

suggesting that the rate-limiting process of the growth of myelin figures is a diffusion process.

Since a concentration gradient in the system of phosphatidylcholine and a liquid medium should be produced at the interface and the molecules cannot dissolve molecularly into the medium due to their amphiphilic nature, we may expect a transport of aggregated phosphatidylcholine molecules from the less hydrated region in the lump toward the more hydrated myelin figures. Here we will restrict our attention only to the growth process, and the nucleation of myelin figures at the interface will be another problem. Once the growth has begun to proceed steadily, the elastic contraints in stacked bilayers in smectic A phase [4,11–13] will oppose the thickening of the rod along the radial direction. Thickening through the radial diffusion of molecules seems unlikely to occur, since the diffusion coefficient normal to the bilayer is reported to be so low compared with the lateral diffusion constant that the diffusion of molecules across the bilayer would be prohibitively little in a defectless bilayer system [14]. Through this difficulty in diffusion of phosphatidylcholine molecules across the bilayer, together with probable elastic constraints, the growth must be restricted so as to be parallel to the bilayer, resulting in the rod-like form of myelin figures.

According to the conventional treatment of diffusion processes, the mean distance l expected for molecules to be translated by diffusion in one dimension (i.e., along the axis of the myelin figure) during a time t is given by $l = (2Dt)^{1/2}$, where D is the relevant diffusion coefficient. A rough sketch of the relationship between l and t is given as follows. Taking the chemical potential of phosphatidylcholine in the rod state as $\mu = \mu_b - \mu_1$, where μ_b is the chemical potential of phosphatidylcholine in the bulk state and μ_1 is the difference in the chemical potential for the two states, we can write a phenomenological relation between the diffusion flux J and force $(-\nabla\mu)$ as $J = -\Lambda\nabla\mu$, where Λ is a phenomenological coefficient and J expresses the number of phosphatidylcholine molecules flowing through the cross-sectional area of rod per unit time. Assuming that μ_1 is independent of the length l of the rod and that the spatial variations are slow and only along the direction of the rod axis, $\nabla\mu$ is approximated by $-\mu_1/l$, and J is written $J \approx \Lambda\mu_1/l$. On the other hand, J can be related to the growth rate dl/dt as $J = \nu(dl/dt)$, where ν is a concentration that gives the number of phosphatidylcholine molecules per unit length of the rod. We therefore can write $dl/dt \approx (\Lambda\mu_1/\nu)/l$, which gives $l^2 \approx 2Dt$ with $D = \Lambda\mu_1/\nu$. As shown in Fig. 4, the obsreved l values follow the relation $l \propto t^{1/2}$ rather well, at least within 5 min from the beginning of the growth. From the slope of this plot D can be determined as $4.3 \cdot 10^{-6}$, $1.3 \cdot 10^{-6}$ and $0.3 \cdot 10^{-6}$ cm^2/s at 25°C, and $5.3 \cdot 10^{-6}$, $1.5 \cdot 10^{-6}$ and $0.9 \cdot 10^{-6}$ cm^2/s at 40°C for water, ethylene glycol and glycerin, respectively.

As a first step in clarifying physical process involved, we examined the effect of the medium viscosity, η, on D, keeping in mind the Stokes-Einstein relation, $D = kT/f\eta$, where k is the Boltzman constant, T the temperature, and f a factor dependent on the form of a mass of molecules in the diffusion process. Fig. 5 shows D plotted against $1/\eta$, where the difference in temperature between the two sets of data is neglected. Although the points are rather scattered in the higher medium-viscosity region where the medium viscosity becomes comparable with the in-plane viscosity of bilayer (approx. 1 P) [15], it seems that D varies linearly with $1/\eta$ and it does not cross the ordinate

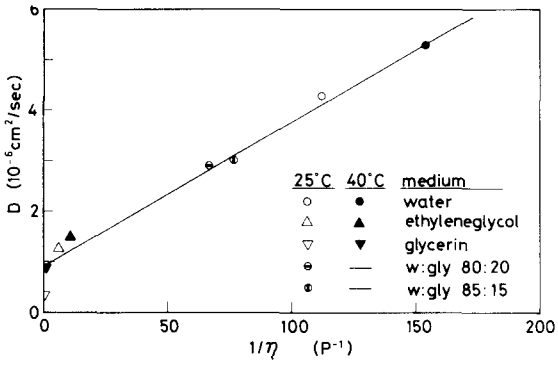

Fig. 5. Apparent diffusion constant D plotted against $1/\eta$.

at the origin when extrapolated to $1/\eta = 0$. Since the Stokes formula is derived for the motion of a regid sphere in a viscous liquid, the scattering might come from the change in the relative magnitudes of relevant viscosities, i.e., the viscosity of the diffusing liquid particles becomes comparable to or lower than that of the surrounding liquid medium. From the linear dependence of D on $1/\eta$ the apparent diffusion coefficient D in this region can be expressed by the sum of two terms: $D \approx D_1 + D_2$, where D_1 is a term inversely proportional to the viscosity of the medium used, a/η, with a a constant with a form like kT/f, and D_2 is a constant term. The first term D_1 enables us to evaluate an effective size of the diffusing mass of phosphatidylcholine molecules in principle, which is implied to be that of several phosphatidylcholine molecules. Since the mass should be supplied from the lump at the root, it is implied that the diffusion of aggregates toward myelin figures begins near the root in the vicinity of the surface of the lump where it has been swollen almost instantaneously by the surrounding dispersive medium. The diffused mass may be deposited on the roots of myelin figures and rapidly rearranged and incorporated into their bilayers and translated toward the top of the myelin figures, during which the second, viscosity-independent, diffusion process should work together with the first one. The value of D_2 obtained from Fig. 5 is approx. $9 \cdot 10^{-7}$ cm^2/s, which is not so large a value compared with the reported values of lateral diffusion constants (approx. 10^{-7} cm^2/s) of planar lipid bilayer membrane [16,17]. Since D_2 corresponds to the diffusion constant at the limiting case of $\eta = \infty$, it may be natural to assign it to the process within the bilayers of myelin figures. During that process, a process similar to the lateral diffusion type within bilayer membranes should occur, rearranging the molecular packing, which will result in such a weak ordering of molecules in L_α phase that the polar headgroups have a tendency to lie on the bilayer plane [18,19] and also that the hydrocarbon chains have a rather small orientational order normal to the bilayer plane so as to give a diamagnetic susceptibility anisotropy of approx. 1/40 of that of a crystalline phosphatidylcholine [10,20]. The process represented by D_2 might be affected by the interaction of polar heads with the surrounding medium. The present result, however, suggests that the interaction is rather weak, since D shows a single linear dependence on $1/\eta$ irrespective of the surrounding medium, and D_2 for $\eta = \infty$ is rather comparable with the lateral diffusion constant.

In conclusion, the present results suggest that the initial growth stage of myelin figures in water and other hydrophilic organic liquids is a diffusion-limited process along the rod axis, in which two processes are implied to operate: one is the diffusion of phosphatidylcholine molecules from the less hydrated lump of phosphatidylcholine to the more hydrated myelin figure, which may occur both in the rod and in the bulk near the root of the myelin figures, and the other is probably the lateral diffusion type of process within the phosphatidylcholine bilayers, by which the molecules may be transported toward the top of the myelin figures.

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