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MAGNETIC-FIELD-INDUCED ORIENTATION AND BENDING OF THE MYELIN FIGURES OF PHOSPHATIDYLCHOLINE

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In a magnetic field of up to 7 kG, myelin figures of the egg-yolk phosphatidylcholine/water system were oriented because of the diamagnetic anisotropy of the molecules through bending at the roots with the long axes parallel to the field. The time interval for an angular change of their axes was nearly in proportion to H^{-2}. From the orientational behaviour, a curvature-elastic modulus κ of lipid-bilayer could be roughly evaluated as $4 \cdot 10^{-13}$ erg.

Several earlier investigations on the magnetic properties of, and the effects of magnetic field on, biological systems treated living organisms as a whole and reported that there were detectable effects of magnetic field on the growth pattern of an individual or on the histological or morphological features of cells [1]. The problem of how and to what extent a magnetic field affects living organisms, however, seems still open. Magnetic properties of lipids, the major component of biomembranes, should afford the most fundamental information concerning this problem. The magneto-orientation and the diamagnetic susceptibility anisotropy have already been reported by the present authors [2,3] for thin-layered single crystals of dipalmitoylphosphatidylcholine. When the crystals with stacked molecular bilayers [4,5] suspended in xylene were placed in a magnetic field of about 5 kG, they showed an orientation whereby the direction of hydrocarbon chains perpendicular to the bilayer and that of the phosphorylcholine group parallel to the optical axis in the bilayer plane were perpendicular to the field. The value of the volume diamagnetic susceptibility anisotropy of the crystal (i.e., the difference between susceptibilities parallel and perpendicular to the direction of the hydrocarbon chains in the phosphatidylcholine crystal), was estimated to be about $-9 \cdot 10^{-8}$ e.m.u./cm^3 from the magneto-orientation behaviour [2].

The myelin figures are cylindrical rods with concentrically stacked mutilamellae of lipid-bilayers in a fully hydrated state [6–8]. The structural feature of the rods is essentially similar to that of the nerve myelin sheath and that of the bilayers bears a resemblance to biomembranes [6,7].

Small lumps of phosphatidylcholine prepared from hen egg by the method of Singleton et al. [8] were placed in a plastic cell 1 mm in depth and 4 mm in diameter. Soon after water had been poured into the cell at room temperature, the rod-like myelin figures began to grow from the lumps of phosphatidylcholine towards the surrounding water, the direction of growth being parallel to the cylindrical axis of the rod. The bilayers of phosphatidylcholine are in a fully hydrated state and contain a considerable amount of free water molecules between them. Strong optical anistropy is observed with the optical axis perpendicular to the rod axis. The growth of a myelin figure in such a simple form as indicated by '*a*' in Fig. 1 was almost saturated within a few minutes under the

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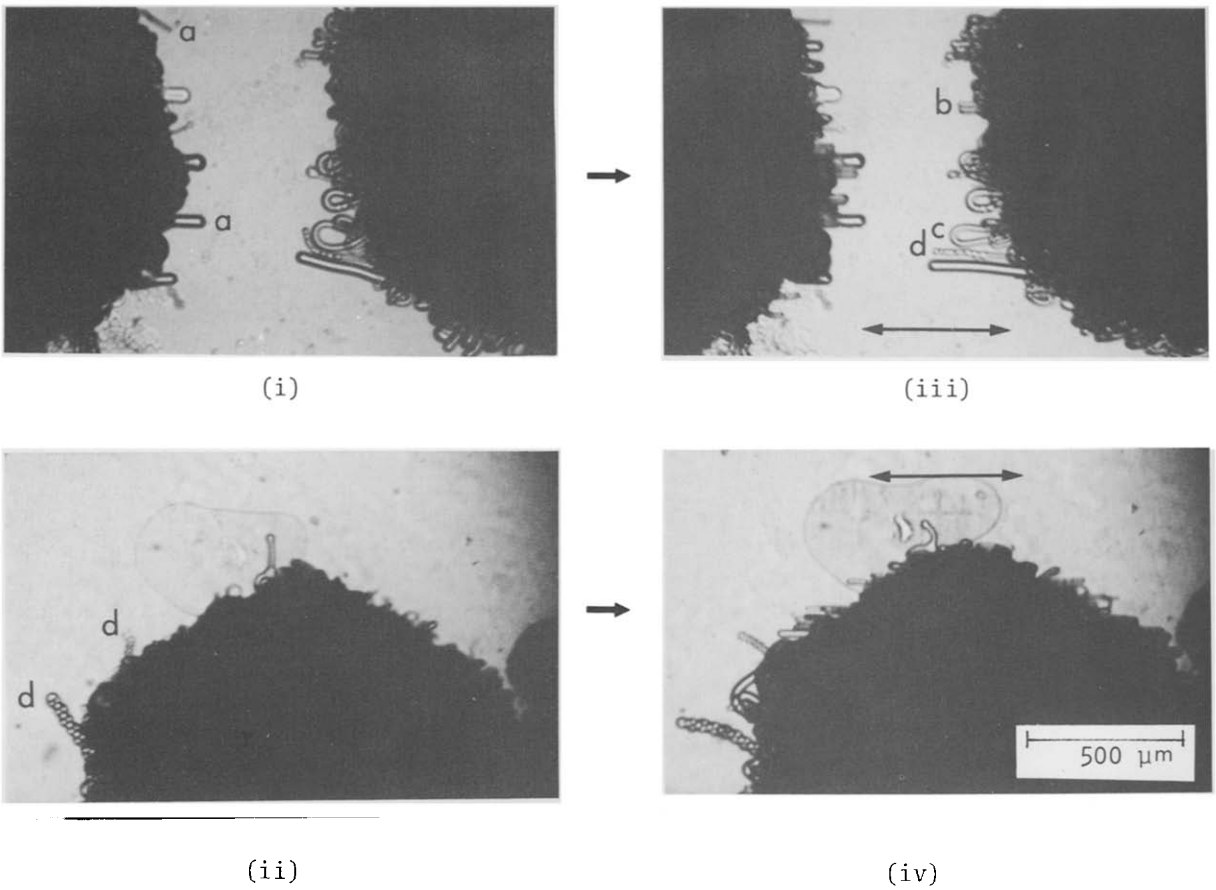

Fig. 1. Photographs of the myelin figures in a plastic cell observed by a polarized microscope from above. The cell, sealed by thin glass plates, was placed between the pole pieces of an electromagnet with the pole pieces of 30 mm in diameter and 7.5 mm in the gap width. (i) and (ii), the myelin figures before the application of the magnetic field (a few minutes after pouring the water); and (iii) and (iv), a minute after the application of the field of 7 kG to the myelin figures in (i) and (ii), respectively. The arrows show the direction of magnetic field. Field-induced orientation of myelin figures are clearly observed in these photographs. The small letters in the photographs distinguish between types of myelin figure. When the magnetic field is applied, the myelin figures of simple form, (a), grown freely into the water phase are quickly oriented with their growth axes parallel to the field, but those which touch with the inner surfaces of cover glasses and/or the other myelin figures, (b), are hardly oriented or, if at all, oriented very slowly. Loop-like myelin figures, (c), are gradually deformed so as to extend along the magnetic field direction. The twisted myelin figure consiting of two simple myelin figures, (d), is also bent and oriented like a simple one but it takes a longer time to be oriented.

present conditions, and myelin figures of 5–30 μm in diameter and typically no more than 200 μm in length were obtained. Further growth resulted in rather complicated forms. The thicker ones took a longer time for growth saturation.

The observation of magnetic field effects on the myelin figures was made through a microscope by putting the cell between the pole pieces of an electro-magnet at room temperature of about 25°C. When a magnetic field of about 7 kG was applied, myelin figures of simple rod-like form which grew free into water without touching the inner surface of the cell had been reoriented with their growth axes parallel to the magnetic field within about 10 s, possibly through partial bending of each rod near the root. The other myelin figures of simple rod-like form but seemingly with partial touching on the inner surface of the cell or those grown up in such a complicated form as to make a helical twist of two rods (see 'd' in Fig. 1) took longer time, of about several tens of seconds, to accomplish the field-induced reorientation. When the magnetic field was turned off within the first ten seconds or so of the application, the initial orientation of axes was almost recovered, but this was not so after a prolonged application of the field – more than several tens of seconds.

The bending deformation of the rod observed was reversible, at least within about ten seconds or so after the application of the magnetic fields as described above. On account of the irregularity of the surface of the lumps of phosphatidylcholine, the bending at the root of rod was not clearly observed. Several rods, however, were also reversibly bent at half their length, resulting in a hairpin-like conformation when they were initially oriented almost to the field direction or $\pi/2$ bending when they were initially oriented nearly perpendicularly to the field directtion. The shape of a reversibly bent rod should be determined by equilibrium between the elastic and the magnetic energy densities, from which the apparent bending modulus of rod can be evaluated. Assuming bilayers as two-dimensional incompressible liquid layers with molecules being perpendicular to the layer plane on the average and also the deformation being small, the bending modulus can be related to an elastic constant κ of such a layer, which corresponds to the splay elastic constant [10]. According to Servuss et al. [11] the elastic energy per unit length of bent tube of lipid is given as $(1/2)\pi\kappa r(1/R^2)$, where r is the radius of tube and $1/R$ is the curvature of bending deformation of tube as a whole. For myelin rod consisting of stacked liquid-like bilayers, the elastic energy per unit length of bent rod of radius r_0 can be approximately given as the sum of elastic contribution of each tube bilayer:

$$F_R^B \approx (1/2)\pi\kappa \cdot \sum_{i=1}^{n} id(1/R^2)$$

$$\approx (1/2)\left(\pi\kappa r_0^2/2d\right)(1/R^2)$$

where i is the number of bilayers counted along the radius of the rod and d is the layer spacing, and $r_0 = nd$, n being the total number of bilayers along the radius of the rod and $n \gg 1$. Thus the apparent bending modulus of rod can be expressed as $K = \pi\kappa r_0^2/2d$. If we consider a uniformly bent rod with a constant curvature of $1/R$ measured at the axis of it. The elastic energy of the rod with $\pi/2$ bending is simply given as

$$F_R^B = (1/2)K(1/R^2)(\pi R/2)$$

On the other hand, the magnetic energy of the rod due to the diamagnetic susceptibility anisotropy is written as

$$F_R^M \simeq -(1/2)\Delta\chi\left(\pi r_0^2\right)H^2 \int_{l_1}^{l_2} \cos^2\theta(l)\,dl$$

where $\Delta\chi$ is the diamagnetic susceptibility anisotropy of the rod, i.e., $\Delta\chi \equiv \chi_r - \chi_\parallel$ and χ_r and $\chi_\parallel$ denote the diagmagnetic susceptibilities perpendicular and parallel to the rod axis respectively, $\theta(l)$ is the angle between $\vec{H}$ and the surface normal of the rod, and l is the distance measured along the contour of rod. If we again consider a uniformly bent rod with the constant curvature of $1/R$ and put $\theta(l_1) = 0$, the magnetic energy for $\pi/2$ bending of rod between l_1 and l_2 (i.e., $\theta(l_2) = \pi/2$) is given as

$$F_R^M \approx -(1/2)\Delta\chi\pi r_0^2 H^2(\pi R/4)$$

As the equilibrium conformation of rod should be

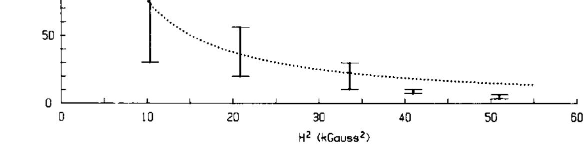

Fig. 2. H^2 dependence of T of the myelin figures of egg-yolk phosphatidylcholine/water system. H is the applied magnetic field and T is the lapsed time for the axis of a simple form myelin (a in Fig. 1) to have been reoriented by about $\pi/2$ from its initial orientation nearly perpendicular to the magnetic field. For each H^2, T is shown by a bar, within which measured values are scattered. The dotted curve is only a guide for the eye. Above 9 kG^2, however, it expresses the relation, $T = A/H^2$, with A compatible with $\Delta\chi$ and dimensions of the rod given in the text.

determined by the balance between elastic and magnetic contributions, κ can be evaluated by equating F_R^B and F_R^M. From the observed field-induced bending of a myelin figure, where the rod initially perpendicular to the field direction is reversibly bent on application of the field by about $\pi/2$, κ or K were evaluated as $4 \cdot 10^{-13}$ erg or $3 \cdot 10^{-13}$ erg · cm, respectively. In evaluation of κ, we used the value of $\Delta\chi$ evaluated from the magneto-orientation behaviour of myelin figures [12], i.e., $\Delta\chi = -2 \cdot 10^{-9}$ e.m.u./cm^3, and d approx 60 Å from X-ray measurement. The obtained value of κ is comparable with that reported for the human red cell membrane [13], but rather smaller than that reported for the bilayer tubes of egg phosphatidylcholine [11].

Fig. 2 shows the lapsed time, T, for the axis of the myelin to have been reoriented by about $\pi/2$ from its initial orientation as a function of H^2, where T is taken for such myelin figures as to have initially grown nearly perpendicularly to the field H and, at the same time, freely into water from the lump of phosphatidylcholine without any contact with each other and the cover glasses of the cell. In lower H region, the observed T was scattered considerably. This may come from the differences in the thickness, shape and the 'age' of the myelin figure. Though below 1 kG, T increases divergently, it essentially changes linearly with H^{-2} in the region between 3 and 7 kG. This indicates that the orinetational change is brought through the anisotropy of diamagnetic susceptibility of myelin figures. For myelin figures having contact with each other or with the surface of the cover glasses of the cell or having a complicated shape, T was increased by more than a order of magnitude compared with that of simple and free myelin figures at the same field strength.

Therefore, it might be considered that the orienting action under magnetic field could have some effect in an actual biological system with sufficient ordering and size. The actual tissue, of course, has a far more complicated shape and has more complicated components than the myelin figure, and is also usually connected with surrounding tissues. However, since an orienting effect on the tissue, though small, can be expected, the effect of the magnetic field on the whole biological system should also be assessed in this context, especially for a prolonged exposure to the field.

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