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Electric field-induced orientation of myelin figures of phosphatidylcholine

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Orientation of myelin figures of egg phosphatidylcholine was observed by application of an alternating electric field. The time constant of the orientation was inversely proportional to the square of the field strength. For myelin figures with the bending deformation, the increase of the elastic energy was in proportion to the square of the field strength. A bending modulus of a bilayer could be roughly estimated as $1.8 \cdot 10^{-19}$ (J). The field-induced orientation is considered to be caused by charge separation and also by orientation of the polar groups of the lipid molecules.

In excess water tubular protrusions, so-called myelin figures or myelin tubes, grow from hydrated phospholipid lumps resulting from swelling [1,2]. This phenomenon is important to understand the mechanism of the self-assembly and the self-organization in biological membranes. The myelin figures are cylindrical rod-like structures composed of concentrically stacked bilayers with a considerable amount of water inside the tube. The myelin figure formation occurs spontaneously at the phospholipid phase transition [3-5] and the tubes formed are considerably flexible. Sakurai et al. have reported that myelin figures are oriented in a magnetic field because of the diamagnetic anisotropy of the lipid molecules [6,7]. As is well known, orientation and pearl-chain formation of biological cells are induced by an alternating electric field [8-10]. Harbich et al. [11] have observed that nonspherical phospholipid vesicles are orientated in an alternating field, and that thin-walled vesicles are ruptured at both ends and become cylinders by fairly low fields. In this paper we investigate the effects of alternating electric field on orientation of myelin figures of egg phosphatidylcholine.

Egg-yolk phosphatidylcholine was purchased from Sigma Chemical Co. and used without further purification. An appropriate amount of the chloroform solution (50 mg/ml) was dropped on a glass microscopic slide with the built-in two parallel electrodes and the solvent was removed by evaporation in vacuum. The two electrodes of stainless steel (10 mm long, 20 mm wide and 1 mm thick) were positioned at a distance of 500 μm and connected to a function generator (JASCO, CET200) as a voltage source for generation of alternating electric fields. The frequency range of the field was from 0.5 to 2 MHz. The frequency and the voltage were monitored on an oscilloscope. A video camera system (Olympus FCD 720) connected with a optical microscopy was used. All experiments were done at room temperature. Temperature increase induced by Joule's heat was not observed because of a high specific resistance of the lipid solution (10^4-10^5 $\Omega \cdot$ cm).

When water (double-distilled) was injected into the gap between the electrodes, myelin figures spontaneously formed and began to grow from the lipid lumps which were stuck on the slide glass, because the lipids fell into the liquid-crystalline phase (the transition temperature of egg-PC, -7 to -10°C). Some of myelin figures separated from the lumps at once. After several minutes, myelin figures linked with the lumps almost stopped the growth. An alternating voltage of sine wave was then applied. It was observed that myelin figures were oriented with their long axes of tube parallel to the electric field. Fig. 1 shows typical photographs of the orientation which was induced by an alternating field of 1 MHz and 200 V/cm (maximum value). Isolated tubes with short lengths were oriented in a straight line (Fig. 1a), which is consistent with the observation by Harbich et al. [11]. Bent tubes with long lengths took a hairpin-like conformation and tubes linked with the lumps were

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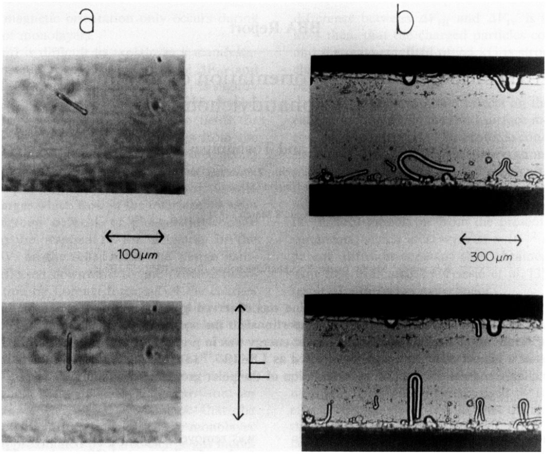

Fig. 1. Optical micrographs of myelin figures of egg phosphatidylcholine/water system in the absence of an alternating electric field (upper photographs) and in the presence of the field of 200 V/cm, 1 MHz (lower ones). The distance between the electrodes is 500 μm. The arrow indicates the direction of the field. (a) Simple orientation of isolated myelin tube, and (b) the orientation with bending deformation of tubes linked with the lipid lump and the hairpin-like conformation of bent tube.

oriented through the bending at the roots (Fig. 1b). Similar results were observed for the frequencies of 0.5 MHz and 2 MHz.

When the electric field was turned off, isolated tubes with straight orientation maintained their direction for a while, but bent tubes returned to the initial orientation of each tube. The reversibility of the bending deformation was observed under a short-time application of the field strength below about 250 V/cm. The electric-induced orientation was not observed at an alternating field below about 20 V/cm which is in good agreement with the result for nonspherical vesicles reported [11]. On the other hand, above about 350 V/cm, some myelin tubes were more elongated and also lipid lumps around the electrodes moved in the direction of the opposite electrodes. This behaviour may be caused by a breakdown of the lipid membranes. Such elongation of tube was observed for the tube being growing at the electric field below the breakdown voltage. This suggests that the electric field enhances the bilayer rearrangement from a confused packing in the lumps to more ordered assembly in the myelin figure membranes.

Fig. 2 shows the field strength dependence on the bending deformation of a hairpin-like tube. The curvature radius R was measured for a fixed length (52 μm) around the bending position of the tube (the whole length of 125 μm, and the diameter of 20 μm). This figure reveals that the increase in the square of the bending curvature is proportional to the square of the field strength. This result can be explained as follows. The elastic energy per unit length of bent tube with a lipid bilayer is given as $(1/2)\pi\kappa r(1/R^2)$, where κ is the bending modulus of the bilayer, r the radius of the tube [7,12]. For myelin tube consisting of stacked bilayers, since its elastic energy is the sum of elastic contribution of each bilayer, r should be replaced by $(r_0^2 - r_i^2)/2d$, where r_0 is the outer radius of tube, r_i the inner one and d the bilayer spacing. The increase of the elastic energy ΔE_B is given as

$$\Delta E_B = \tfrac{1}{2}\pi\kappa \frac{r_0^2 - r_i^2}{2d}\left(\frac{1}{R^2} - \frac{1}{R_0^2}\right)L \qquad (1)$$

where L is the tube length and $1/R_0$ the initial curvature of the tube in the absence of the electric field. On the other hand, for any nonspherical particle, the time

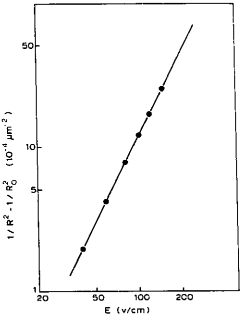

Fig. 2. Field strength dependence of the increase in the elastic energy of bent tube ΔE_B. R_0 and R are the curvature radii of the tube in the absence and in the presence of the electric field E of 1 MHz, respectively. The solid line represents the relation $\Delta E_B \propto E^2$.

mean of the electrical potential energy due to an external field is written as [13–15],

$$W = \sum_{i=1}^{3} \lambda_i e_i^2 E^2 \tag{2}$$

where the λ_i are factors determined by the geometry of the particle and by the dielectric properties of the material involved, and the e_i the coordinates of the diagonalizing coordinate system. Since the increase of the elastic energy should be equal to the electrical energy at the equilibrium conformation of tube, $(1/R^2) - (1/R_0^2)$ is proportional to E^2 assuming that the radii and the spacing are constant through the bending deformation.

The bending modulus κ has been estimated according to $\Delta E_B = \Delta W$, where ΔW is the difference in the electric energy between two directions of tube. In estimation, the tube was assumed to be a prolate spheroid with a uniform composition and to be oriented with the long axis parallel except the bending position from a perpendicular orientation with respect to the field line. Since the lipid concentration of tubes is estimated to be a high value of 62 wt%, we used reported values of permittivity ε and conductivity σ of DPPC/water mixture (75 wt%) above T_c, i.e., $\varepsilon = 30$ and $\sigma = 1.2 \cdot 10^{-2}$ (S/m) [16]. A permittivity and conductivity of $\epsilon_0 = 80$ and $\sigma_0 = 4 \cdot 10^{-3}$ (S/m) were used for the medium used here. Using values of $a = L/2 = 62$ μm, $b = c = r_0 = 10$ μm, for Eqn. 18 of Ref. 15, $r_i = 2$ μm and $d = 6$ nm, we obtained a bending modulus of $1.8 \cdot 10^{-19}$ (J), which is in good agreement with a reported value of $2.3 \cdot 10^{-19}$ (J) [12].

Fig. 3 shows the time course of orientation of an isolated tube without bend for different field strengths where the orientation was measured by an angle θ between the long axis of tube and the electric field line. It is seen that the change of the orientation is nearly exponential with time. The insert shows the time constants as a function of the field strength. This figure indicates that the time constant is inversely proportional to the square of the field strength. Similar results were obtained for the orientation of tubes linked with the lump through the bending at the roots, but the time constants became longer because of an internal friction around the roots. It is interesting to note that the field strength dependence of the time constant is similar to that of the formation time of pearl-chain [17]. However, the time constant was not strongly dependent on the diameter of tube in contrast to the pearl-chain formation time.

The myelin tube behaves like a large dipole in the alternating field in a way similar to biological cells. The field-induced dipole of cells arises from charge separation at the membrane interface [10]. For the myelin tube, it seems that the orientation of polar groups of the lipid molecule contributes to the induced dipole as well as to the charge separation because of so many bilayers stacked, i.e. the thickness is about 60–80% of the tube

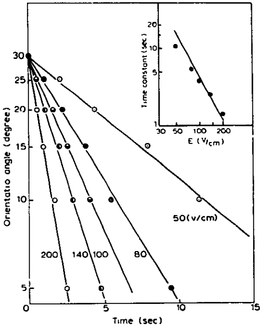

Fig. 3. Time courses of the orientation of isolated tube for different electric field strength indicating exponential changes with time. The orientation is measured by an angle between the long axis of tube and the field line. The insert shows the time constant τ as a function of the field strength E. The solid line represents the relation $\tau \propto E^{-2}$. Similar results were obtained for tubes with different diameters (eight experiments). The square-low could be better fitted to most of their other data.

radius. An almost free rotation of polar groups of phosphatidylcholine about the glycerol bond has been observed [18]. In the presence of an alternating electric field, not all the polar groups can orient in the direction to the field lines because of the barrier of the hydrocarbon chains. There is parallel orientation of the polar group with respect to the bilayer plane. Moreover, parallel orientation with respect to the tube axis occurs in lipid molecules where the direction of the molecule is in a plane made by the tube axis and the field line, and just the orientation of these polar groups can contribute to the field-induced orientation of the tube. The field-induced dipole moment may be proportional to the field strength E. Since a torque induced by an electric field is a vector product of the field and the induced dipole moment, the torque which acts on the tube is proportional to $E^2 \sin\theta$. Assuming that a term of the moment of inertia may be neglected and that the hydrodynamical friction term is a linear function of the angular velocity, $d\theta/dt = -aE^2 \sin\theta$, where a is a constant being inversely proportional to friction coefficient of the tube in a medium. When the orientation angle is small, $d\theta/dt = -aE^2\theta$. This relation leads to the time course of orientation and the field strength dependence of the time constant observed.

In this work, orientation of myelin tubes induced by an alternating electric field was qualitatively explained in cosideration of the field-induced dipole. Further, a strict calculation of the field-induced dipole should be performed. Finally, we should like to mention that direction of myelin tubes can be easily controlled by application of an alternating electric field. This method is useful in studing the growth mechanism and probably in the technological applications of myelin tubes.

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