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Frequency dependence of electric-field-induced orientation of myelin tubes

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Abstract

Orientation induced by an alternating electric field was studied for myelin tubes of egg phosphatidylcholine (egg-PC) in water and in KCl electrolyte solution. The orientation was also studied for myelin tubes of egg-PC/cholesterol mixtures in water. The orientation effect was measured by the bending curvature of myelin tubes with hairpin-like deformation at frequencies between 10 kHz and 20 MHz. The bending deformation of egg-PC myelin tubes in water decreased abruptly with a decreasing frequency within the low-frequency range below 100 kHz, and shrinkage of myelin tubes was often observed. The bending deformation decreased as the frequency was increased in a higher frequency range. The profile of the frequency dependence for egg-PC in KCl solution was similar to that in water but shifted towards a higher frequency. At low KCl concentrations below 3 mM, the relaxation frequency increased proportionally with increasing an KCl concentration, which was due to a proportional increase in the conductivity of the surrounding medium. Similar profiles of the frequency dependence were observed for egg-PC/cholesterol mixtures but with no shift in the relaxation frequency. These data in the high-frequency range fitted well with calculations based on theoretical equations for the electric-field-induced orientation of nonspherical particles. The conductivity of myelin tubes was estimated to be in the order of 10^{-4} S/m in water and of 10^{-3} S/m at the low KCl concentrations. The bending modulus of a bilayer membrane was estimated to be $(1.0 \pm 0.5) \cdot 10^{-19}$ J for egg-PC in water. No change in the bending modulus was observed for egg-PC in KCl solution. However, for egg-PC/cholesterol mixtures in water, the bending modulus abruptly increased to $(1.8 \pm 0.5) \cdot 10^{-19}$ J at a cholesterol concentration of 40 mol%.

Key words: Myelin tube; Phosphatidylcholine; Conductivity; Bending elasticity; Bioelectric field effect; Model membrane

1. Introduction

Myelin tubes (or myelin figures) are of a tubular structure composed of concentrically stacked phospholipid bilayers with a considerable amount of water inside the tube. They form at the surface of phospholipid aggregates in excess water and grow into bulk water. The formation of myelin tubes occurs spontaneously at the liquid–crystalline phase transition [1–3]. Myelin tubes are oriented by application of a magnetic field because of the diamagnetic anisotropy of lipid molecules [4,5]. Recently, it was found that orientation of myelin tubes is also induced by an alternating electric field [6]. As a result of the orientation, slightly bent tubes take on a hairpin-like deformation. The electric-field-induced orientation is of significance with respect to the nonthermal effects of alternating electric fields on biological cells, e.g., orientation of the nonspherical cells and pearl-chain formation.

According to a theory provided by Schwarz et al. [7–10], a nonspherical particle suspended in a medium with a different dielectric property is oriented by an alternating electric field, even if the dielectric properties of the particle and the surrounding medium are isotropic. This is in contrast to the orientation induced by a magnetic field. The stable direction of the electric-field-induced-orientation is determined by a minimum of the electrical potential energy of the particle. The orientation depends on the geometry of the

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particle, on the dielectric properties of the particle and medium, and also on the frequency of the applied field.

Previously the electric-field-induced orientation of myelin tubes of egg-yolk phosphatidylcholine (egg-PC) was measured for different strengths of electric field at a constant frequency [6]. In this work, we studied the field strength and also the frequency dependence of the orientation within a wide frequency range of 10 kHz to 20 MHz. The measurement of frequency dependence was done for egg-PC myelin tubes with varying KCl electrolyte concentrations in the surrounding medium, and also for myelin tubes of egg-PC/cholesterol mixtures in water. The orientation effect was measured by the bending curvature of tubes with hairpin-like deformation induced by the orientation. The measured results were analyzed by use of theoretical equations provided by Saito et al. [10] and the bending modulus of a bilayer membrane was determined.

2. Materials and methods

Egg-yolk phosphatidylcholine (egg-PC) and cholesterol were purchased from Sigma and used without further purification. Egg-PC/cholesterol mixtures were made up in an ethanol solution. The cholesterol concentration of the mixture was up to 40 mol%. About 30 μl of the lipid-ethanol solution (lipid concentration: 50

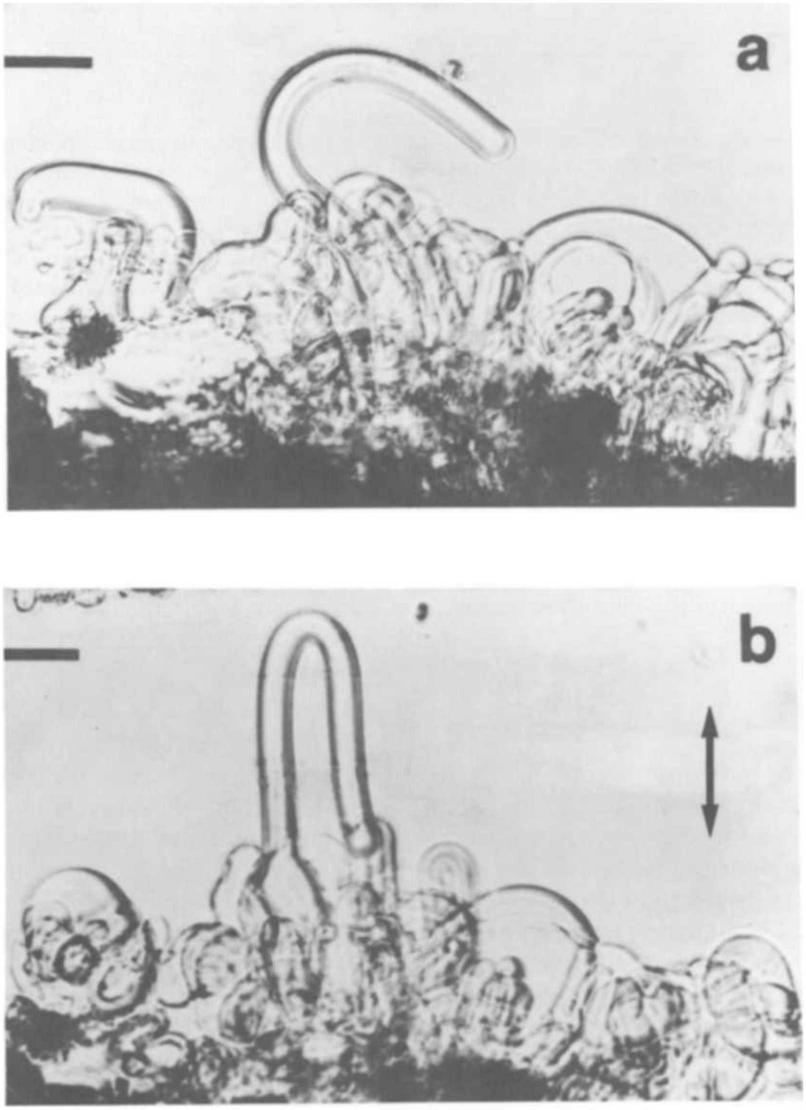

Fig. 1. Hairpin-like bending deformation of myelin tubes resulting from the electric field-induced orientation. Optical micrographs show myelin tubes of egg-PC in water (a) before and (b) after application of an alternating electric field of strength 100 V/cm and frequency 1 MHz. A myelin tube on the left-hand side shrunk on application of the field. The distance between the electrodes is 500 μm. The arrow indicates the direction of the field. Bar: 50 μm.

mg/ml) was dropped into a gap between two built-in electrodes on a glass microscope slide and the solvent was evaporated in vacuum for several hours.

Approx. 30 μl of pure water or aqueous KCl solution was injected to the gap and a cover glass was placed over the gap. The edges of the gap were sealed with silicone grease in order to prevent evaporation and flow of the lipid dispersion. The lipid aggregates stuck to the bottom edges of the gap. After the injection of water or KCl solution, myelin tubes spontaneously formed and began to grow from the surface of the lipid aggregates. This is because the lipid molecules near the surface fell into the liquid–crystalline phase (the transition temperature of egg-PC, approx. -7 to -10°C). The two parallel electrodes of stainless steel (20 mm long, 10 mm wide and 3 mm thick) were positioned at a distance of 500 μm and connected to a function generator (Model 4502, Kikusui Electric) as a sine-wave voltage source for the generation of alternating electric fields. The maximum frequency of the field was 20 MHz. The alternating voltage and the wave shape were monitored on a synchroscope (Model CS-1554, Trio). After the growth of myelin tubes had stopped, an alternating electric field was applied for a minimum of time (about 10 s) in order to avoid temperature increases induced by Joule's heat. Bending curvature of myelin tubes with hairpin-like deformation was measured using a video camera system (Model CTC-5600JS, Ikegami Tsushinki) connected to an optical microscope (Model BHS, Olympus). Conductivity of the lipid dispersions was measured by use of a digital conductometer (Model CM-15A, TOA Electronics). All measurements were made at room temperature.

3. Results and discussion

When an alternating sine-wave voltage was applied, myelin tubes were oriented with the long axis of the tubes parallel to the electric field, and some tubes which were slightly bent took a hairpin-like deformation, as shown in Fig. 1. Tubes with the deformation were oriented with the axes parallel to the field except for the bending position. Shrinkage of tubes induced by the field was sometimes observed. When the electric field was turned off, bent tubes returned to their initial shape. The reversibility of the bending deformation was observed with a short-time application of the field. The straight orientation and the bending deformation were not observed with a low-voltage field, e.g., below about 20 V/cm (maximum amplitude value) at 1 MHz. On the other hand, with a high-voltage field, e.g., above about 350 V/cm at 1 MHz, myelin tubes were enormously elongated. After turning off the field, the tubes shrank and some of them had different shapes from their initial ones, which were spherical. This behaviour may be caused by a breakdown of lipid membranes of both the tube and the lipid aggregate at the root of the tube.

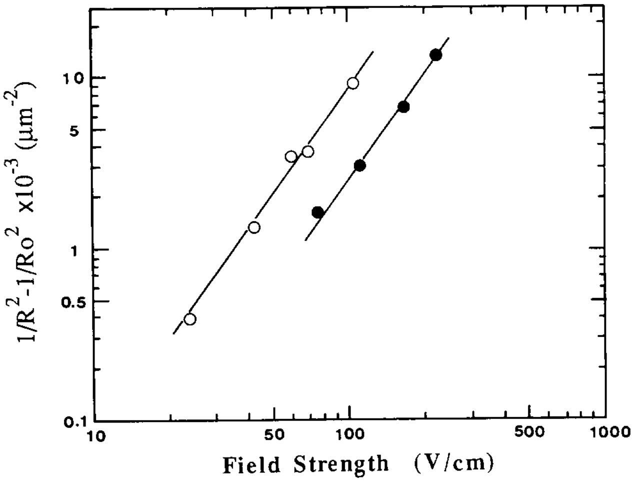

Fig. 2. Field-strength dependence of the hairpin-like bending deformation of egg-PC myelin tubes in water at frequencies of 200 KHz (○) and 5 MHz (●). The degree of bending deformation is represented by the increase of the square of the bending curvature $1/R^2-1/R_o^2$, where R and R_o are the curvature radii of a tube with and without the field, respectively. Solid lines represent a proportional relationship between the degree of bending deformation and the square of the field strength.

Fig. 2 shows the field-strength dependence of the bending deformation of egg-PC myelin tubes in water at frequencies of 200 kHz and 5 MHz. A degree of the bending deformation was represented by an increase of the square of the bending curvature $1/R^2-1/R_o^2$, where R_o and R are the curvature radii of a tube before and after application of electric field, respectively. The curvature radii were measured for a fixed length around the bending position of the tube. This figure reveals that the degree of bending deformation is proportional to the square of the field strength, which is in agreement with the previous result for 1 MHz [6].

We will briefly state a previously described interpretation of the proportional relationship between bending deformation and field strength [6]. The bending elastic energy ΔE_B is related to the degree of bending deformation $1/R^2-1/R_o^2$ as [6]

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

where κ is the bending modulus of a bilayer membrane, d the bilayer repeat distance in the lamellae, r_o the outer radius, r_i the inner radius of the tube and L the tube length. On the other hand, according to the theory of electric-field-induced orientation given by Saito et al. [10], the time mean of the electrical potential energy of an ellipsoidal particle due to an external field of strength E is written as

$$W = \frac{2}{3}\pi abc\epsilon_0 E^2 \sum_{i=1}^{3} u_i n_i^2 \tag{2}$$

where a, b and c are half of the principal axial lengths of the ellipsoid, ϵ_0 the permittivity of the surrounding medium, and n_i the coordinates of the unit vector of the field in a diagonalized system. The dimensionless quantity u_i is a measure of the energy of the ellipsoid oriented along one of the axes.

When u_1 denotes the dimensionless energy for the parallel orientation of a myelin tube, and u_2 is the perpendicular orientation with respect to the field line, the difference Δu $(= u_2 - u_1)$ corresponds to the electric energy of the transition from the perpendicular orientation to the parallel one. When, by the application of an electric field, a myelin tube is bent with the long axis parallel to the field except for the bending position from the initial orientation of the perpendicular, an increase in the elastic energy of the bending deformation should be equal to the difference in the electric energy at an equilibrium conformation of the tube. Therefore, from Eqs. (1) and (2), the degree of bending deformation is given by

$$\frac{1}{R^2} - \frac{1}{R_0^2} = \frac{4\epsilon_0 d}{3\kappa}\Delta u E^2 \tag{3}$$

where the myelin tube was assumed to be a prolate spheroid, i.e., $a = L/2$ and $b = c = r_0$, and $(r_i/r_0)^2$ was neglected because the inner radius r_i is far smaller than the outer radius r_0 for egg-PC myelin tubes. It should be noted that the degree of bending deformation does not depend on the radius r_0, but depends implicitly on the ratio $2r_0/L$ through the quantities u_1 and u_2. This equation indicates that the degree of bending deformation is proportional to E^2 on the assumption of constant values for the repeat distance and bending modulus through bending deformation. This result is consistent with the measured result as shown in Fig. 2.

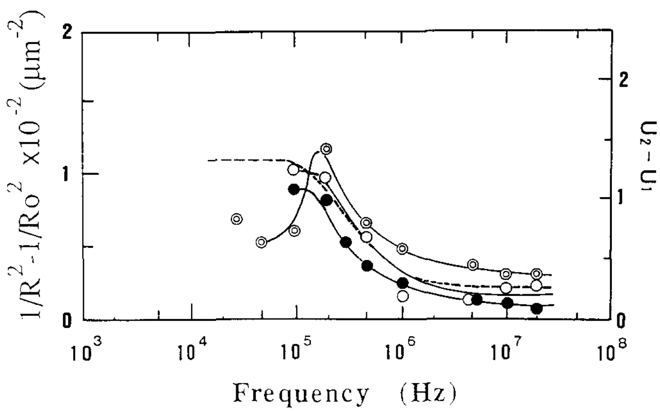

Fig. 3. Frequency dependence of the hairpin-like bending deformation of egg-PC myelin tubes in water at a constant field strength of 120 V/cm. Values of the ratio of the tube diameter to the length $\emptyset/L$ are 0.07 (○), 0.08 (◎) and 0.09 (●). The dotted line shows the electrical energy difference Δu between the parallel and the perpendicular orientation with respect to the field line, which was calculated using values for the permittivity $\epsilon_0 = 80\ \epsilon_r$ and the conductivity $\sigma_0 = 2 \cdot 10^{-3}$ (S/m) for the surrounding medium, and $\epsilon = 30\ \epsilon_r$ and $\sigma = 2 \cdot 10^{-4}$ (S/m) for the myelin tubes.

Next we measured the frequency dependence of the bending deformation at a constant field strength of 120 V/cm. The measurement was done by decreasing the frequency in order to avoid shrinkage of myelin tubes at low frequencies. Fig. 3 shows the result measured for egg-PC myelin tubes in the absence of KCl ions which had similar values for the ratio of the tube diameter to length $2r_0/L$. The bending deformation, as seen in this figure, increased as the frequency decreased down to approx. 100 kHz. However, at frequencies below about 100 kHz, the bending deformation decreased abruptly, and often shrinkage of tubes was observed. No orientation of myelin tubes but motion of lipid aggregates was observed at frequencies below about 10 kHz. This behaviour may be caused by a breakdown of the lipid membranes, which is considered not to be a thermal breakdown but rather an electrical breakdown, since a temperature increase induced by Joule's heat was not observed.

The frequency dependence of the bending deformation in a high-frequency range of 100 kHz to 20 MHz can be also interpreted by use of Eq.(3) as follows. The dimensionless energy u_i is related to the angular frequency of the field ω as [10]

$$u_i = A_i + \frac{B_i}{k_i^2 + e_i^2\omega^2} \tag{4}$$

with

$$k_i = \sigma_o - (\sigma_o - \sigma)L_i \tag{5}$$

$$e_i = \epsilon_o - (\epsilon_o - \epsilon)L_i \tag{6}$$

where σ and σ_0 are the conductivities of the particle and the surrounding medium, respectively, ϵ the permittivity of the particle and L_i the quantity determined by the geometry of the particle. The L_i value depends only on the ratio of the tube diameter and length $2r_0$/L assuming that the myelin tube is a prolate spheroid. A_i and B_i are factors relating to L_i and to the dielectric properties of the particle and the medium. The surrounding medium is not pure water but lipid dispersion containing vesicles and aggregates. The conductivity of the dispersion was measured to be $\sigma_0 = 2 \cdot 10^{-3}$ S/m at a concentration of 50 mg/ml. Redwood et al. [11] have reported that the permittivity of an egg-PC dispersion is about 80 for a frequency range of 1 MHz to 10 MHz at a concentration of 40 mg/ml. Shepherd et al. [12] have reported that for a high lipid concentration of a DPPC/water mixture (75 wt%), the permittivity ranges from 35 to 25 at frequencies from 1 MHz to 10 MHz at 38°C. This high concentration is similar to the lipid concentration of myelin tubes (62 wt%). Then, using these values for the permittivity of the medium and myelin tubes, i.e., $\epsilon_0 = 80\epsilon_r$ and $\epsilon = 30\epsilon_r$ ($\epsilon_r = 8.85 \cdot 10^{-12}$ F/m), we calculated the energy difference Δu for various conductivity values (σ) of myelin the tubes. When the conductivity σ is a tenth part of the outside conductivity σ_0, i.e., $\sigma = 2 \cdot 10^{-4}$ S/m, the calculated curve fitted well with the experimental data points for frequencies above 100 kHz (the dotted line in Fig. 3). The conductivity measurement of egg-PC dispersion showed that the value increased in proportion to the lipid concentration. From this proportional relationship, the conductivity for the same lipid concentration as the myelin tube can be estimated to be in the order of 10^{-2} S/m, which is consistent with a reported value for the DPPC/water mixture above 42°C [13]. However, this high value for the conductivity seems to be unsuitable for myelin tubes because of the well organized membrane system of the tubes. In fact, using this value, the calculation hardly fitted with the data.

The bending deformation above 100 kHz relaxed around a particular frequency. Let the relaxation frequency f_m be represented by a frequency of a middle point between minimum and maximum values of the bending deformation. Values of the relaxation frequency were in the range of 290–380 kHz and the average value was 350 kHz. Theoretically, the relaxation frequency f_m is defined as the frequency of a middle point between two values of Δu at zero and infinite frequencies, i.e., $\Delta u(f_m) = [\Delta u(0) - \Delta u(\infty)]/2$. From this equation, the relaxation frequency is given by

$$f_m = \frac{q}{2\pi} \cdot \frac{\sigma_0}{\epsilon_0} \tag{7}$$

where q is a factor determined by values of σ/σ_0, ϵ/ϵ_0 and geometrical factors L_1 and L_2. For the myelin tubes used here, values of L_1 are in the order of 10^{-2} and values of L_2 are about 0.49. Ignoring L_1, the factor q is calculated to be 0.72 for values of $\sigma/\sigma_0 = 0.1$, $\epsilon/\epsilon_0 = 30/80$. Using this value of q and values of $\sigma_0 = 2 \cdot 10^{-3}$ S/m and $\epsilon_0 = 80\epsilon_r$, the relaxation frequency f_m is estimated to be 320 kHz, which is in good agreement with the observed result.

From Eq. (7), it is expected that the relaxation frequency increases with an increasing conductivity of the medium outside the tubes. As shown in Fig. 4, the conductivity of the lipid dispersion increased proportionally with the addition of KCl. Then we measured the frequency dependence of bending deformation for different concentrations of KCl. A remarkable decrease in the bending deformation was observed. Some myelin tubes hardly deformed. Fig. 5 shows some examples of the measured results and the calculated curves. The curves were calculated using measured values of the conductivity of the lipid dispersion containing KCl for σ_0 and assuming no change in permittivities of the medium and myelin tubes in the presence of KCl ions.

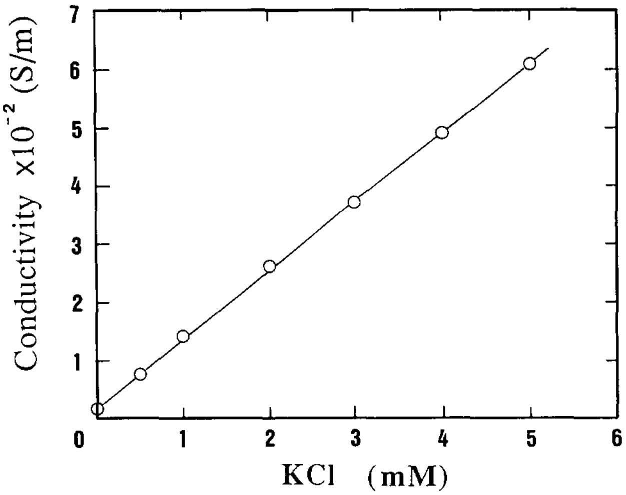

Fig. 4. Dependence of the conductivity of egg-PC dispersion on KCl concentration. The lipid concentration is 40 mg/ml.

Values of the ratio σ/σ_0 are 0.15 for a KCl concentration of 3 mM and 0.2 for 0.6 mM and 1.5 mM KCl concentrations. The increase in the ratio σ/σ_0 is considered to result from ions bound to the membranes. From these values of the ratio, the q value was estimated to be 0.8.

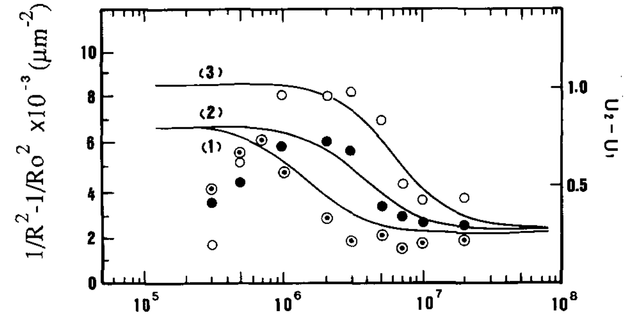

Fig. 5. Frequency dependence of the hairpin-like bending deformation of myelin tubes at different KCl concentrations. (◉): KCl concentration of 0.6 mM, the ratio of the tube diameter and length Ø/L = 0.4, (●): 1.5 mM, Ø/L = 0.1, (●):3 mM, Ø/L = 0.08. Field strength 120 V/cm. The solid lines show the electrical energy difference Δu, calculated using permittivities of $\epsilon_0 = 80\epsilon_r$ and $\epsilon = 30\epsilon_r$, the size ratio of Ø/L = 0.1 and measured values of the conductivity of the surrounding medium (Fig. 4). Values of the conductivity and the ratio of conductivities for the myelin tube (σ) and the surrounding medium (σ_0) are: (1)$\sigma_0 = 8 \cdot 10^{-3}$ S/m, $\sigma/\sigma_0 = 0.2$, (2)$\sigma_0 = 20 \cdot 10^{-3}$ S/m, $\sigma/\sigma_0 = 0.2$ and (3)$\sigma_0 = 37 \cdot 10^{-3}$ S/m, $\sigma/\sigma_0 = 0.15$.

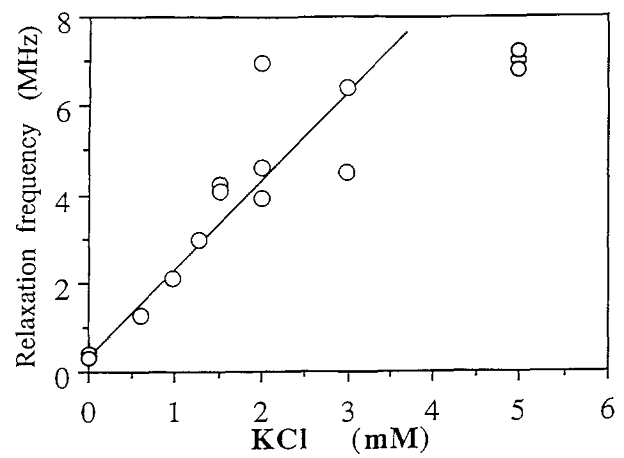

Fig. 6. Dependence of the relaxation frequency of the bending deformation on KCl concentration. A least-squares fit to the data (the solid line) yields a q value of 0.7.

The relaxation frequency shifted towards a higher frequency together with an increase in KCl concentration. As shown in Fig. 6, the increase in the relaxation frequency was in proportion to the KCl concentration below 3 mM. A least-squares fit to the data (the solid line) gives a q value of 0.7, which is in good agreement with the estimated value. However, the relaxation frequency at a concentration of 5 mM was far low with respect to the fitting line. The q value decreases on decreasing the ratio of σ/σ_0 down to a limited value of 0.66. But even the limited value is unsuitable for the low value of the relaxation frequency. The reason why the relaxation frequency is low at high concentrations of KCl remains the subject of further study.

Fig. 7 shows the frequency dependence of the bending deformation measured for myelin tubes of egg-PC/cholesterol mixtures in water. The frequency dependence was similar to that of myelin tubes of pure egg-PC, but the bending deformation could be observed down to a lower frequency without a breakdown of membranes. The frequency of the membrane breakdown was below about 1 kHz at 120 V/cm at cholesterol concentrations above 6 mol%, which was in contrast to that of about 10 kHz for pure egg-PC. This indicates that the lipid membrane is toughened by the addition of cholesterol, which corresponds to an increase in the mechanical toughness observed for large unilamellar vesicles [14]. No change in the relaxation frequency at a high-frequency range above 100 kHz was observed at cholesterol concentrations below 40 mol%, indicating that neither the conductivity nor the permittivity of the myelin tube are changed by the addition of cholesterol. The permittivity of the myelin tube is a linear combination of permittivities of the water layer and the lipid layer in the lamellae. Since volume fractions of water and lipids are nearly constant in the lamellae containing cholesterol, no change in the permittivity occurs. The bending deformation was similar to that of pure egg-PC myelin tubes at cholesterol concentrations below 15 mol%, but decreased remarkably at a cholesterol concentration of 40 mol%. This decrease results from a decrease in the bending elasticity of bilayer membranes because of no change in the dielectric property of the myelin tubes containing cholesterol.

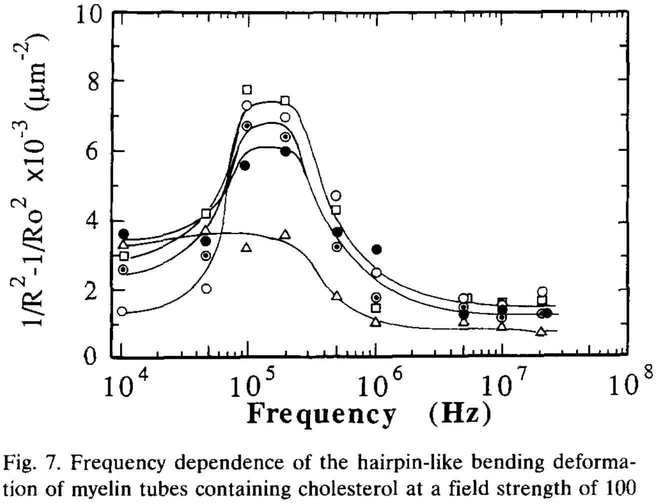

Fig. 7. Frequency dependence of the hairpin-like bending deformation of myelin tubes containing cholesterol at a field strength of 100 V/cm. (○): cholesterol concentration of 3 mol%, the ratio of the tube diameter and length $\emptyset/L = 0.1$, (◉): 10 mol%, $\emptyset/L = 0.16$, (□): 15 mol%, $\emptyset/L = 0.1$, (●): 30 mol%, $\emptyset/L = 0.2$, (△): 40 mol%, $\emptyset/L = 0.2$.

A bending elastic modulus of a bilayer membrane can be calculated from Eq. (3). In the case of bilayer membranes of pure egg-PC, the bending modulus was estimated to be $(1.0 \pm 0.5) \cdot 10^{-19}$ J from the data of the bending deformation of myelin tubes in water and the calculated curve of Δu (Fig. 3). This value is in good agreement with the value of $(1\text{–}2) \cdot 10^{-19}$ J obtained from measurements of thermal shape fluctuations of tubular vesicles for the same lipid [15], but slightly higher than the recently reported values of $(0.4\text{–}0.5) \cdot 10^{-19}$ J [16] and $0.25 \cdot 10^{-19}$ J [17], which were measured for unilamellar vesicles by thermal shape fluctuations and by electric-field-induced deformation, respectively. It took several seconds for the bending deformation of the myelin tubes to reach equilibrium. This slow motion of tubes leads to the occurrence of a lateral rearrangement of lipids. Therefore, the higher value of the bending modulus may be attributed to the fact that the bilayer bending observed here is not only single-layer bending but also bilayer-coupling bending [18]. It is also possible that the simple sum of the elastic contributions of each bilayer does not hold in the bending elasticity of the lamellae because of an interlamellar interaction. The decrease in the bending deformation, observed for myelin tubes in KCl solution (Fig. 5), does not result from an increase in the bending modulus of the membrane but from an increase in the ratio σ/σ_0. The estimated value for the bending modulus is almost the same as that in water within the experimental error.

In the case of egg-PC/cholesterol mixtures, the bending modulus was almost constant at cholesterol concentrations below 15 mol% and the value was also similar to that for pure egg-PC (Fig. 7). A similar value was obtained from the data at frequencies above 500 kHz for a cholesterol concentration of 30 mol%. However, at a cholesterol concentration of 40 mol%, the bending modulus increased to a value of $(1.8 \pm 0.5) \cdot 10^{-19}$ J, which is comparable to a reported value of $(2.5 \pm 0.4) \cdot 10^{-19}$ J measured for a 1:1 mixture of stearoyloleoylphosphatidylcholine (SOPC) and cholesterol [19]. The bending stiffness of a membrane is in proportion to its elastic area compressibility modulus [20,21]. The result of the bending modulus shows no change in the compressibility modulus at cholesterol concentrations up to 30 mol%, but an increase of 80% at a cholesterol concentration of 40 mol%. This result is consistent with direct measurements of elastic area compressibility for SOPC/cholesterol mixtures, where the compressibility is almost constant at cholesterol concentrations below 28 mol% but increases by 70% at 38 mol% and by 200% at 43 mol% [14].

In conclusion, the bending deformation of myelin tubes induced by an alternating electric field depended strongly on the frequency of the field. The relaxation frequency in the high-frequency range increased proportionally at low concentration of KCl, which was due to an increase in the conductivity of the surrounding medium. The results could be interpreted by the theory of Saito et al. [10]. From the analysis based on the theory, the bending moduli obtained are $(1.0 \pm 0.5) \cdot 10^{-19}$ J for the bilayer membrane of pure egg-PC and $(1.8 \pm 0.5) \cdot 10^{-19}$ J for the bilayer membrane containing cholesterol at 40 mol%.

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