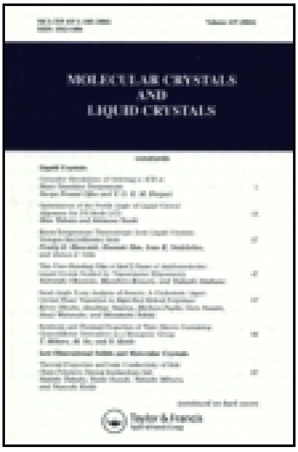

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Electric Field Manipulation of Phospholipid Tubules: Optical Birefringence Measurements

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The polymerizable phospholipid $DC_{23}PC$ forms straight, hollow cylinders in water. Using ac electric fields it is possible to achieve significant orientation of these structures. A birefringence method was used to obtain the effective electric susceptibility anisotropy $\Delta\chi_E$, the value of which is in good agreement with that obtained earlier from light scattering measurements.

Several years ago it was discovered that the lecithin 1,2-bis(10,12,tricosadiynoyl)-*sn*-glycero-3-phosphocholine ($DC_{23}PC$) forms a microstructure in water consisting of bilayers which wrap around a hollow core.[1-5]. These tubules are generally straight, tens of microns in length, and about 0.75 μm in diameter. The thicknesses of the walls range between one and ten bilayers, with two to three bilayers being the most common. Since the tubules can easily be polymerized, a variety of applications may become possible. In order to fully exploit these unique structures, however, it's essential to be able to manipulate the tubules in vitro, as well as to understand

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their interactions. To that end we have embarked on a program of magnetic and electric field alignment using both birefringence and light scattering as probes. Not only do these methods allow us to orient the tubules in water, but they allow us to measure the anisotropic part of the long range tubule-tubule potential[6–8] as well.

In an earlier work we performed a birefringence measurement of partially oriented tubules in a magnetic field.[9] For a suspension sufficiently dilute in which steric interactions were negligible, we found that the order parameter $S > 0.95$ for fields $H \sim 20$ kG, where $S \equiv \langle P_2(\cos\theta)\rangle$. P_2 is the second Legendre polynomial $1/2(3\cos^2\theta - 1)$ and θ is the angle between the tubule axis and the magnetic field. Physically, since the tubules exhibit different magnetic susceptibilities perpendicular and parallel to the tubule axis, an applied magnetic field exerts a torque on the tubule in order to reduce the total magnetostatic enthalpy. Thus, by effectively inducing full orientational order in the experiment, we were able to extract the volume magnetic susceptibility $\Delta\chi_M$. As expected, we found that $\Delta\chi_M$ was simply an orientational average of the individual molecular susceptibility anisotropies. More recently we performed a light scattering measurement in order to ascertain the efficacy of electric field alignment.[10] Using ac fields, we found that the tubules exhibited considerable order for fields as small as 30 V/cm, although at higher fields we were unable to further increase the degree of order to due the presence of turbulence. Nevertheless, by fitting the data for fields less than 30 V/cm, we were able to extract a volume electric susceptibility anisotropy $\Delta\chi_E$. It turns out that $\Delta\chi_E$ was nearly three orders of magnitude larger than what would be expected if the volume electric susceptibility anisotropy were merely the sum of the molecular anisotropies (as in the magnetic case above). Instead, we proposed a model[10] in which the effective susceptibility anisotropy arises from an orientational anisotropy of the total electrostatic enthalpy for a dielectric tubule in an electric field. By calculating the electric field in all space for an isotropic dielectric tubule oriented parallel and perpendicular to an external field, and then calculating the respective enthalpies, we found that the enthalpy difference was of order k_BT in relatively weak fields, thus permitting substantial alignment. Moreover, our calculation resulted in an effective $\Delta\chi_E$, which turned out to be in good agreement with the experimental value. Thus we found that magnetic and electric field orientation of tubules rely on two vastly different mechanisms.

The purpose of this paper is to report on birefringence results for electric field alignment of tubules. Given the unusual result of our earlier light scattering measurement, and its new interpretation, it is important to provide an independent test of that data. Our central result is that the effective volume susceptibility anisotropy $\Delta\chi_E$ is 140 ± 45 cgs, in good quantitative agreement with our light scattering measurement.[10] Thus further evidence is found to support our model for $\Delta\chi_E$.

$DC_{23}PC$ was synthesized according to previously published methods[11]; our sample comes from the same batch as used in both the magnetic field birefringence and electric field light scattering experiments. The added water was deionized and distilled. The tubule length distribution was obtained by photomicroscopy of 103 individual tubules, where the average length $\langle L\rangle$ was found to be 14.8 μm, with an rms deviation of 5.9 μm; the actual distribution can be found in Table I. The distribution of wall thicknesses t, obtained by means of scanning transmission

TABLE I

Tubule length distribution

Length	Number
5 μm	7
10	35
15	30
20	20
25	8
30	3

electron microscopy, is the same as published earlier,[9] where $\langle t \rangle = 0.0125$ μm, corresponding to approximately 2.5 bilayers. Since the average tubule diameter is 0.73 μm, the average area A of the annular cross-section is 2.9×10^{-10} cm^2.

The tubule suspension was diluted to a concentration of 0.20 ± 0.02 mg/ml which, according to the Onsager criterion,[6] is sufficiently dilute so as to avoid excluded volume interactions. The suspension was placed in an epoxy-sealed cell consisting of a pair of microscope slides separated by two parallel wires of diameter $d = 0.0812$ cm and spaced 0.803 cm apart. The wires thus served the dual role of spacer and electrode. Using the formalism of Reference 10, we find that the electric field midway between the two wires is given by $E_o = 0.825$ V, where E_o is in volts/cm (statvolts/cm) and V is in volts (statvolts). Field-induced birefringence was measured by means of an automatically compensating Pockels cell modulated at 2800 Hz. The apparatus is described in detail elsewhere.[12] Although sensitivity for the device is better than $\Delta n = 10^{-8}$ for a sample of this path length, much larger noise fluctuations ($\sim 10^{-7}$) were observed, ostensibly due to the sample itself. Similar problems were encountered in the magnetic birefringence experiment,[9] and thus it is necessary to attach a rather large uncertainty to $\Delta\chi_E$. In addition, a substantial artifact was observed due to non-uniform heating of the cell from the applied field E_o. This arises from the small but non-zero ionic strength of the tubules, which carry no charge at precisely pH = 7. Although the pH was carefully controlled, it was impossible to attain an ionic strength identically equal to zero. It was found that by focusing the beam to a spot of approximately 60 μm on the sample, such that only the uniformly heated part of the sample was being probed, it was nevertheless possible to significantly reduce this artifact.

With the sample in place, the near-zero birefringence was recorded by computer every two seconds for thirty seconds to establish a baseline. A 2-kHz field was then switched on for four minutes, and Δn vs. time was again recorded every two seconds. Owing to the long rotational relaxation times for particles many microns in length, only the last forty seconds of data was used to determine Δn. Finally, the field was turned off, the birefringence allowed to relax back to near zero, and a post-field $\Delta n(E_o = 0)$ was obtained; the pre-field and post-field birefringences, which were always quite close in value, were then averaged to obtain the zero-field baseline. Thus the net birefringence $\Delta n(E_o)$ at field E_o was taken to be the difference between readings at field E_o and the baseline. These results are shown in Figure 1.

As is quite obvious, the birefringence does not asymptotically approach a limiting value, as in the magnetic case, but rather exhibits a significant change in slope vs.

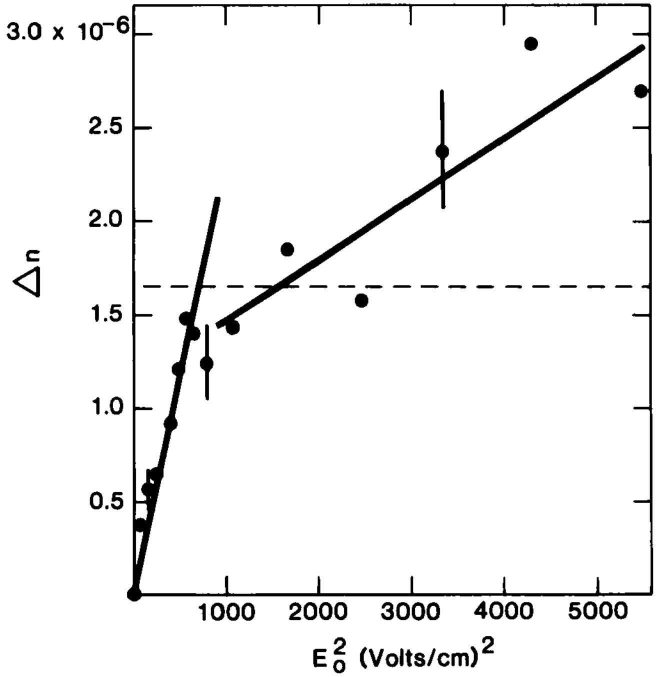

FIGURE 1 Birefringence vs. E_o^2 (rms). Linear fits for the initial and final slopes are shown by the solid lines. The dashed line represents the expected saturated birefringence for this sample. Note typical error bars.

E_o^2 in the neighborhood of E_o = 30 V/cm. Above this field the slope $d\Delta n/dE_o^2$ is dramatically smaller, but still not zero. In most Kerr measurements a short pulsed field is applied, inducing a birefringence in a time period much shorter than the characteristic heating time of the sample. (This assumes, of course, that the aqueous medium has a small but unavoidable conductivity.) The tubules, however, are quite large, and the rotational relaxation times are typically of the order of a minute. Thus it is necessary to apply the fields for long durations, resulting in at first a transient, then ultimately in a non-equilibrium steady-state temperature distribution. Such a condition results in unwanted birefringences in the water and glass cell, which scale as the power dissipated, i.e., E_o^2. Interestingly, the change in slope occurs in the neighborhood of $\Delta n \sim 1.5 - 1.6 \times 10^{-6}$. In the magnetic case[9] the birefringence at a concentration ρ = 0.167 mg/ml asymptotically approached a value 1.38×10^{-6}; for this sample, one would expect the peak birefringence for concentration ρ = 0.20 mg/ml to be 1.65×10^{-6} in the absence of heating. This is, in fact, the approximate region in which the slope change occurs. In addition, it should be noted that these spurious problems had no effect on the earlier light scattering experiment, which measured the form factor for the tubules and was insensitive to small changes in the birefringence.

In order to extract a value for $\Delta\chi_E$ we need a model for the birefringence vs. field. As shown earlier,[9]

$$\Delta n = \Sigma\, \Delta n_o\, V_i\, \langle P_2(\cos\theta_i)\rangle \qquad (1)$$

where V_i is the volume of tubule i, Δn_o is the birefringence per volume of lipid, and the sum is taken over all tubules i illuminated by the laser. Since the tubules are non-interacting at this low concentration, the only component of the orientational part of the free energy for tubule i is given by $\mathcal{F}_i = -(1/2)\Delta\chi_E V_i E_o^2 \cos^2\theta_i$. Thus, we can easily determine $\langle P_2(\cos\theta_i)\rangle$ from the Boltzmann distribution:

$$\langle P_2(\cos\theta_i)\rangle = \frac{\int [(3/2)\cos^2\theta_i - (1/2)] \exp(\Delta\chi_E V_i E_o^2 \cos^2\theta_i/2k_BT)\, d(\cos\theta_i)}{\int \exp(\Delta\chi_E V_i E_o^2 \cos^2\theta_i/2k_BT)\, d(\cos\theta_i)} \tag{2}$$

where k_B is Boltzmann's constant and T is temperature. Inserting Equation 2 into Equation 1 and expanding for small fields, we find

$$\Delta n = \Sigma\, \Delta n_o\, V_i \frac{\Delta\chi_E\, V_i\, E_o^2}{15\, k_B T} \tag{3}$$

If we divide Equation 3 by the saturation birefringence $\Delta N_o = \Sigma\, \Delta n_o V_i$, we obtain the orientational order parameter $\langle S\rangle$ averaged over all the tubules:

$$\langle S\rangle = \frac{\Sigma\, V_i^2\, \Delta\chi_E\, E_o^2}{15\, k_B T\, \Sigma\, V_i} \tag{4}$$

Looking again at Figure 1, we note that the difference between the initial slope (due to tubule alignment + the heating artifact) and the final slope (de to the heating artifact only, since tubule order has saturated) corresponds to $d\Delta n/dE_o^2$ for the tubules alone. Fitting the initial slope to the first eight data points such that the line passes through the origin, we obtain $(d\Delta n/dE_o^2)$ initial $= (2.4 \pm 0.4) \times 10^{-9}$ $(\mathrm{V/cm})^{-2}$; this voltage region not only corresponds to the expected linear Δn vs. E_o^2 domain, but is also below the applied fields for which turbulence became a problem in the light scattering measurements.[10] The slope at high fields, where the orientational order of the tubules is near saturation, is much smaller, approximately $(0.35 \pm 0.06) \times 10^{-9}$. In this region the effect of any turbulence is to cause a slower than expected saturation of the order parameter with E_o^2. The measured high field slope is therefore an upper limiting value. Thus we find that the field-induced birefringence $d\Delta n/dE_o^2$ due to the tubules alone is $(2.0 \pm 0.5) \times 10^{-9}$ $(\mathrm{V/cm})^{-2}$. The order parameter susceptibility $d\langle S\rangle/dE_o^2$ can then be obtained by dividing $d\Delta n/dE_o^2$ by the saturation birefringence $(1.65 \pm 0.2) \times 10^{-6}$ derived from our earlier magnetic results. We thus obtain $d\langle S\rangle/dE_o^2 = (1.2 \pm 0.4) \times 10^{-3}$ $(\mathrm{V/cm})^{-2}$, which corresponds to a cgs value $(1.1 \pm 0.4) \times 10^2$ $(\mathrm{statvolts/cm})^{-2}$. In Equation 4 we note that the tubule volume distribution is needed. Since $V_i = A\, L_i$, where the annulus area $A = 2.9 \times 10^{-10}\ \mathrm{cm}^2$ and L_i is given in Table I, we finally obtain a value $\Delta\chi_E = 140 \pm 45$ cgs. This figure compares nicely to the value 121 ± 25 obtained from the electric field light scattering experiment. As discussed earlier, if $\Delta\chi_E$ were due to the molecular susceptibility anisotropies of the individual molecules which comprise the tubule,[10] one would expect a value $\Delta\chi_E \sim 0.25$, nearly three orders of magnitude smaller than observed. Instead, we have suggested that the susceptibility anisotropy arises from the difference in total electrostatic enthalpy for isotropic tubules aligned parallel and perpendicular to an applied field. Such a mechanism gives rise to values of $\Delta\chi_E$ of order 100 cgs, comparable to that observed by both light scattering and birefringence.

In conclusion, we have performed an independent birefringence experiment on a system of phospholipid tubules, extracting an effective electric susceptibility anisotropy consistent with our earlier light scattering results. Although different mechanisms are involved, both magnetic and electric fields appear to be promising methods for manipulating the tubules.

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