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ELECTRIC FIELD EFFECTS ON LIPID MEMBRANE STRUCTURE

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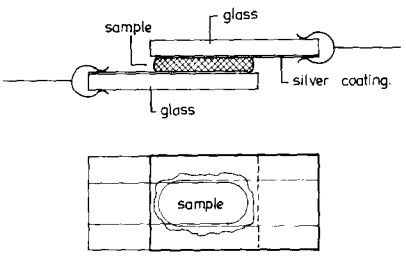
Summary

Multiple bilayers of dimyristoyl phosphatidylcholine and potassium oleate were macroscopically oriented between silver-coated glass slides. These model membranes were subjected to an electric field of up to 10^5 V · cm^{-1}. The influence of the field on the molecular structure was monitored by ESR of cholestane and stearic acid spin labels and by NMR of the phosphorus atom in the phosphatidylcholine headgroup.

It is concluded that the conformation of the headgroup is greatly affected while no influence on the structure and dynamics of the hydrocarbon chains can be detected. At electric fields above 10^4 V · cm^{-1}, where structural effects are still reversible, spontaneous current fluctuations are observed. At fields above 10^5 V · cm^{-1}, irreversible breakdown of the bilayer structure occurs.

Introduction

Smectic lipid/water model membranes are a useful stepping-stone towards the understanding of the more complicated biological membranes and have been investigated with numerous physical techniques. The molecular organization of lipid bilayers is rather well understood and many efforts are now being undertaken to clarify the interaction between lipids and proteins in model systems. In this communication we shall focus our attention on a feature of pure lipid membranes which has been scarcely investigated, but which is intimately connected with a physiological membrane property: the influence of a potential difference across a lipid bilayer on its molecular structure. It is easy to generate a potential difference across a black lipid membrane [1] consisting of a single lipid bilayer separating two aqueous compartments, but here the tiny amount of membrane material prohibits the use of detection tech-



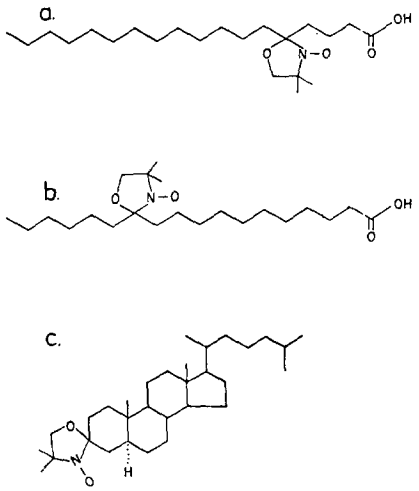


Fig. 1. Experimental set-up for the application of a potential difference across oriented lipid bilayers.

Fig. 2. Three lipid spin labels. (a) 12,3-stearic acid label; (b) 5,10-stearic acid label; (c) cholesterol label.

niques which yield detailed information on the molecular level. We therefore exploited the planar multilayer system, developed in our laboratory [2], for this purpose. The experimental set-up consisted of a thousand parallel bilayers, oriented between two silver-coated coverglasses, the membrane area being about 0.1 cm^2. This is enough material to apply ESR and NMR methods during the action of an external electric field. To our knowledge this has never been done before; there are, however, reports of an electric field effect on lipid films, measured by infrared techniques [3,4].

Of course we have in mind a comparison with the situation in a nerve membrane, where there can exist a potential difference of up to 70 mV across a 50 Å thick bilayer resulting in an electric field strength of the order of 10^5 V · cm^{-1}. It is very probable that the magnitude and polarity of this potential difference are closely connected with the molecular configuration of the membrane constituents and consequently with the permeability of the membrane to ions. Although it is likely that specific proteins play a role in the process of nerve excitation, this has never been established with certainty. It is the purpose of this communication to test the hypothesis that there can be a voltage-induced change in the conformation of membrane lipids, especially in that of their polar headgroups, which might be responsible for changes in ion permeability [5]. We used the model systems dimyristoyl phosphatidylcholine/water and potassium oleate/water. The influence of the applied electric field on the lipid structure was monitored by ESR, using added lipid spin labels [6], and ^{31}P-NMR [7].

Experimental Procedure

Dimyristoyl phosphatidylcholine and oleic acid were purchased from Fluka and used without purification. Potassium oleate was prepared by refluxing

stoichiometric amounts of oleic acid and KOH in ethanolic solution. The product was recrystallized from ethanol, washed with cold ethanol and diethyl ether and dried under reduced pressure.

Smectic mesophases were prepared by equilibration of the powdered lipids at constant relative humidity (r.h.), produced by a 10% H_2SO_4 solution (r.h. 95%) in the case of dimyristoyl phosphatidylcholine and a saturated KCl solution (r.h. 87%) with potassium oleate. These conditions resulted in a water content of 22 ± 1% (w/w) for both samples, which are then within their smectic lamellar range [8]. Oriented lipid multilayers were prepared as described by de Vries and Berendsen [2], using coverglasses provided with a transparant silver coating. Sharp Bertrand crosses, invariant under sample rotation, were observed in the polarizing microscope, indicating that orientation is not affected by the silver surface. Fig. 1 shows the detailed experimental set-up. The thickness of the multilayer sample was measured with a micrometer.

Electrical measurements. To get some impression of the electrical properties of the multilayer samples, their admittance was measured as a function of frequency with a Wayne-Kerr B 224 universal bridge.

ESR measurements. Three lipid spin labels (Fig. 2) were used as monitors of the lipid structure: the cholestane label (prepared according to the method of Keana et al. [9]) and two derivatives of stearic acid (purchased from Syva). The molar ratio of lipid to spin label was 100 : 1. ESR spectra were recorded on a Varian E_3 X-band spectrometer at room temperature. During these experiments the voltage across the multilayers was not increased step-wise, but continuously, at a rate of about 20 V per h. This was done to avoid the occurrence of transient current peaks which could result in destruction of the sample. Using this procedure, the current through the sample was kept below 10^{-7} A throughout the voltage range (0—100 V), thus avoiding any significant Joule heating.

The ^{31}P-NMR measurement was performed on a Bruker HX-360 spectrometer operating at 145.7 MHz.

Results

Electrical measurements. Fig. 3 shows a typical example of the frequency-dependent behavior of the admittance, expressed as the parallel capacity and resistance, for a dimyristoyl phosphatidylcholine sample. The high-frequency capacitance is determined by the series capacitance of the multilayers. From this value we arrive at a bilayer capacity of 0.4 ± 0.2 $\mu F \cdot cm^{-2}$, the same order of magnitude as that observed for natural and black lipid membranes [10].

At low frequency both resistance and capacity reach high values. This is most likely related to the slow migration of ions, which are present in trace amounts, resulting in the build-up of a space charge. This is consistent with the observation that a sample, after being subjected to a prolonged d.c. voltage, retains considerable polarization after the d.c. source has been disconnected. Thus, the lipid multilayers behave as an electret.

An interesting phenomenon was observed during the ESR measurements. Above a d.c. electric field strength of about 10^4 $V \cdot cm^{-1}$ transient changes in

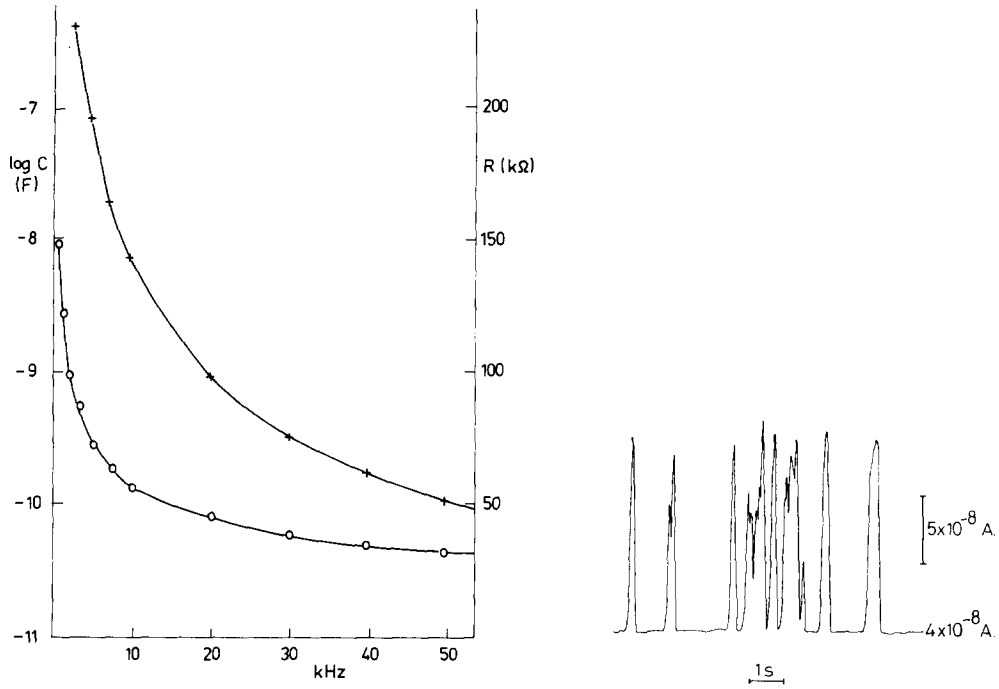


Fig. 3. R and C as a function of frequency for a dimyristoyl phosphatidylcholine multilayer sample of 8 μm thickness. The membrane area was 0.07 cm^2.

Fig. 4. Spontaneous current fluctuations in oriented dimyristoyl phosphatidylcholine multilayers. A constant d.c. voltage of 5 V was applied across the 10 μm thick membrane.

the current occur (Fig. 4), comparable to the spontaneous conductance changes in black lipid membranes [11].

ESR results. In Fig. 5a, ESR spectra of a dimyristoyl phosphatidylcholine sample oriented parallel and perpendicular to the magnetic field are shown. These spectra also indicate that the multilayers are well oriented [6]. The ESR results are easily summarized. Up to a certain break-through value of the electric field strength, which is about 2.10^5 V · cm^{-1} for dimyristoyl phosphatidylcholine multilayers and 10^5 V · cm^{-1} for potassium oleate samples, there is absolutely no effect on the ESR spectra, provided the voltage is increased slowly and continuously. Also, during the current fluctuations, no change in the ESR spectra could be observed. After the breakdown the sample is irreversibly damaged, indicated by changes in the ESR spectrum (Fig. 5b) which point to the creation of randomly oriented lamellae. Sometimes an intermediate stage is encountered where this change appears to be slowly reversible (of the order of minutes) after switching off the applied voltage. So, the multilayers probably have a limited resealing capacity.

NMR results. The ^{31}P-NMR spectrum of a dimyristoyl phosphatidylcholine sample was recorded with and without an applied voltage of 50 V ($E = 10^5$ V · cm^{-1}). The result is shown in Fig. 6. The voltage-induced change, consisting

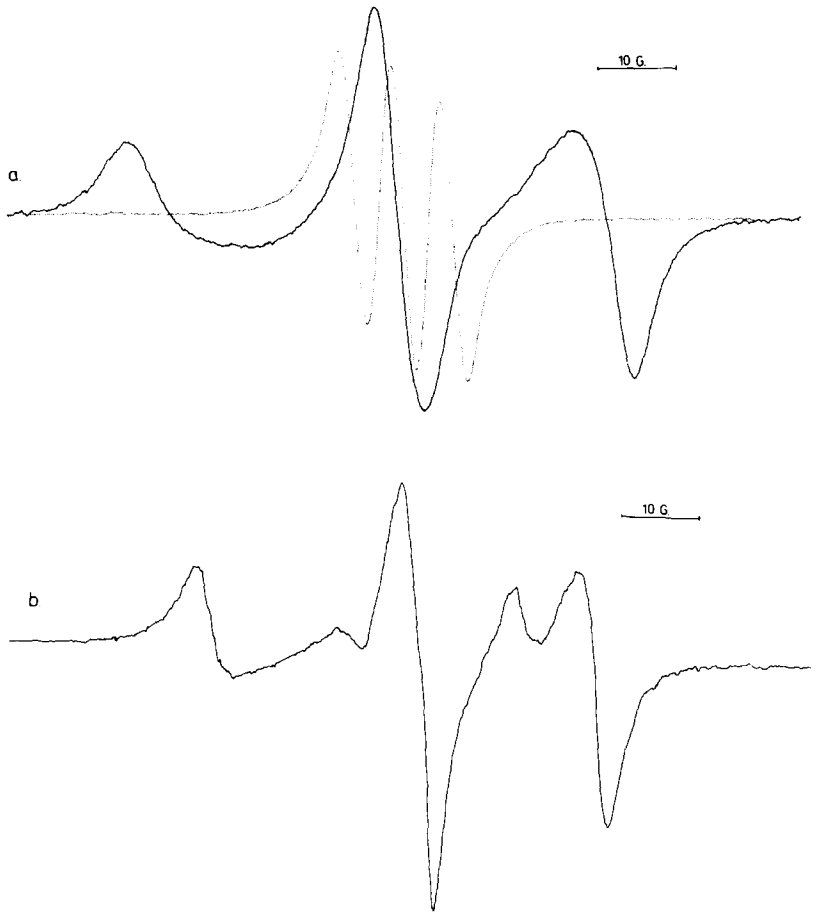


Fig. 5. ESR spectra of oriented dimyristoyl phosphatidylcholine multilayers, using the cholestane label. (a) Spectra observed before the breakdown under the influence of the applied field. (———) Magnetic field H_0 perpendicular to bilayer normal; (· · ·) magnetic field parallel to bilayer normal. (b) Spectrum observed when the bilayers break down, taken in the stage where this process is slowly reversible. H_0 perpendicular to bilayer normal.

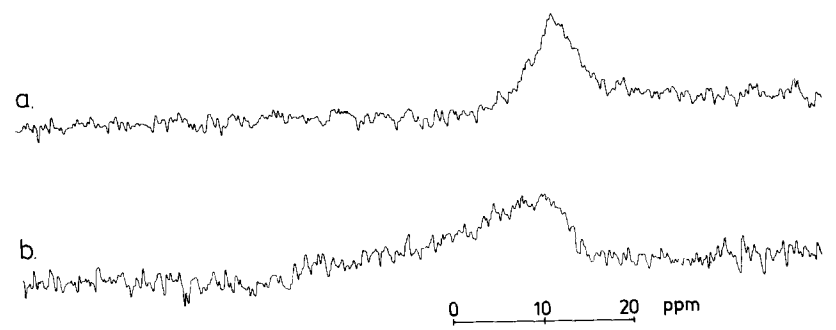


Fig. 6. Proton-decoupled. ^{31}P-NMR spectra of oriented dimyristoyl phosphatidylcholine multilayers at 145.7 MHz. Magnetic field H_0 perpendicular to bilayer normal, ppm values relative to H_3PO_4. (a) No voltage applied; (b) 50 V d.c. applied across the 6 μm thick membrane. 40 000 free induction decays were accumulated.

of an asymmetric broadening and a down-field shift, was completely reversible and could be repeated several times.

Discussion

What can we expect from the action of an electric field of the order of 10^5 V · cm^{-1}? First of all, it must be noted that the field acts differently on both halves of the bilayer. In the case of dimyristoyl phosphatidylcholine, we can expect some orientation of the static dipole, since the magnitude of this dipole is about 10 debye, so $\mu E = 3 \cdot 10^{-22}$ J whereas $kT = 4 \cdot 10^{-21}$ J at room temperature. In a potassium oleate sample the field acts on discrete ions. In both cases, the result could be a more or less pronounced disruption of the headgroup region, with consequent impact on the structure of the hydrocarbon layer. The magnetic resonance labels are chosen such that they cover the whole bilayer. The stearic acid labels monitor the hydrocarbon part at different depths; the rigid cholestane label is sensitive to structural changes anywhere in the hydrocarbon region. The ^{31}P-NMR measurement gives a direct indication of the headgroup orientation in the dimyristoyl phosphatidylcholine membrane.

It is clear from the negative ESR result that the hydrocarbon part of the lipid bilayer is not distorted by the applied field (at least to the extent that the ESR spectrum could sense such distortions), whereas the ^{31}P-NMR result shows that the polar headgroup orientation is affected. This is a very interesting result, which is compatible with the finding of McLaughlin et al. [12], that the headgroup in phosphatidylcholine membranes is capable of independent rotation.

We shall now discuss the ^{31}P-NMR result in more detail [7]. The value of this probe lies in the chemical shift anisotropy of the phosphorus nucleus. The chemical shift tensor relative to the phosphate segment is known from single-crystal experiments and can be used to deduce the probable conformation of the headgroup in phosphatidylcholine membranes. Because the bilayer normal is an axis of rotational averaging, a single ^{31}P resonance is observed in planar-oriented bilayers. If the bilayer normal is oriented perpendicular to the magnetic field, as was the case in our experiment, this resonance position is denoted by $\sigma_\perp$, which can be related to the order parameters, S_{ii}, defined as time averages, $\frac{1}{2}(3\cos^2\theta_i - 1)$, where θ_i is the angle between the bilayer normal and a specified molecular axis. The following expression for $\sigma_\perp$ can be deduced:

$$\sigma_\perp = 3.3 + 20.0\, S_{11} - 43.3\, S_{33} \text{ (in ppm units relative to } H_3PO_4) \quad (1)$$

where S_{11} is the order parameter of the normal to the plane containing the phosphorus nucleus and the two non-esterified oxygen atoms and S_{33} that of the perpendicular to the plane containing the phosphorus nucleus and the two esterified oxygen atoms.

This equation is underdetermined because there are two unknown order parameters and only one experimental result. Without the electric field we indeed observed a single resonance at 11 ppm (Fig. 6a). On applying the field the resonance broadens and now covers a shift range from −2 to 11 ppm. It is not surprising that there is not just a shifted, sharp resonance because

(i) the field is not homogeneous across the sample because of the inhomogeneous space charge, built up by the d.c. voltage during the NMR experiment, (ii) the field acts differently on both halves of the bilayer and (iii) part of the sample is not affected by the field, since the membrane area is not completely covered by the electrodes (Fig. 1). The spectrum of Fig. 6b is consistent with this view as it partly overlaps with the zero-field situation. Unfortunately, it is not possible to resolve these different contributions to the voltage-induced effect and together with the underdetermination of Eqn. 1 this implies that we cannot give a detailed description of the change in headgroup orientation. We do, however, note that the effect is quite large and must involve an appreciable change in the order parameters.

In conclusion, we can say that an electric field across a lipid bilayer, comparable to that existing across nerve membranes, can affect the conformation of the headgroup without influencing the structure and dynamics of the hydrocarbon chains.

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