ELSEVIER

Biochimica et Biophysica Acta 1195 (1994) 197–204

BBA Biochimica et Biophysica Acta

The effect of pulsed electric fields on the phosphorus-31 spectra of lipid bilayers

Peter Osman [a,*], Bruce Cornell [b]

[a] CSIRO, Bradfield Rd., West Lindfield, NSW 2070, Australia
[b] CSIRO, Delhi Rd., North Ryde, NSW 2070, Australia

Received 21 December 1993; revised 6 June 1994

Abstract

A technique is described for measuring the effect of electric fields on the conformation of lipid bilayer membranes by solid state nuclear magnetic resonance. An apparatus was devised to obtain spectra from samples of aligned phospholipid dispersions at varying electric field strengths up to 100 MV/m. Measurements were carried out on membranes made from dioleoylphosphatidylethanolamine and dioleoylphosphatidylcholine, which resulted in electric field induced phase changes. Calibration experiments were performed using bilayers formed from dimyristoylphosphatidylcholine with glycerol and with a nematic liquid crystal. An electric field induced change, from L_α to H_{II}, was also seen in a dimyristoylphosphatidylcholine/alamethicin bilayer.

Keywords: NMR, ^{31}P-; Lipid bilayer; Pulsed electric field

1. Introduction

It is not clear whether long range electric fields, for example those produced by action potentials, have a direct effect on lipid bilayers. Such fields may possibly influence transport across bilayers via a mediating process such as the modulation of ion concentration, membrane thickness, or lipid conformation. It is, therefore, of interest to measure the effects due to electric fields associated with externally applied potentials. The approach we take here is to acquire the phosphorus-31 nuclear magnetic resonance (^{31}P-NMR) spectra from aligned and powdered lipid bilayers, which are subject to biphasically pulsed fields of from 2 MV/m to 16 MV/m, with pulse widths ranging from 5 ms to 100 ms. A primary consideration is the form of electrical excitation. Difficulties exist with the use of constant (dc) excitation which include, polarisation, electrolysis of the electrode, ohmic heating, and dehydration of the sample. Sinusoidal excitation also presents problems because the sample response to a low frequency sine wave during data acquisition, typically an 10 ms free induction decay, would be difficult to interpret [1].

Much has been done to investigate the effect of electric fields on cholesteric and nematic liquid crystals [2–7]. Such studies have generally employed ^{1}H or ^{2}H nuclei and samples which are highly insulating and of low dielectric constant.

^{31}P-NMR has been used to observe the effect of constant electric field on aligned lipid bilayers [8]. In particular, the application of dc potentials resulted in a sample spectrum consistent with the conversion of a partially aligned sample to a powder.

Several investigators have studied systems in which Donnan equilibrium potentials have been established across vesicle membranes and their effects measured by electron paramagnetic resonance (EPR). With this approach the rapid decay of the ion gradient with time prevents the observation of effects due to a sustained electric field across the membrane.

Many reports are available of the effect of incorporating highly polarisable or charged amphiphiles into lipid membranes and of the resultant alterations in the phospholipid headgroup conformation. EPR has been used to report on the effect on phloretin of the binding of trinitrophenol and phosphonium probes to vesicles and on the transmembrane flow rates of these hydrophobic ions. The increase in binding induced by phloretin and the polarity dependence of the phloretin induced perturbation in ion flow, have

* Corresponding author. Fax: +61 2 4137202.

SSDI 0005-2736(94)00186-3

been interpreted as due to the modulation of the dipole potential, which in turn was shown to affect the ion binding and transmembrane ion current [9,10]. Additionally deuterium NMR has been used to assess the alteration in headgroup conformation from measurements of changes in quadrupolar splitting in response to: the addition of phloretin, changes in cation and anion concentration, or the addition of charged phospholipids. Quadrupole splittings from −11.6 kHz (+ve charge) to +10 kHz (-ve charge) have been observed in response to such lipids [11–13]. This was interpreted as arising in part, from the alteration of the membrane dipole potential which can take values between 200 mV–300 mV within the polar headgroup region of a bilayer. However, the addition of a Hofmeister series of lipophilic anions shows that alterations of the headgroup are not great until strongly chaotropic anions are added [14] and the addition of phloretin has been shown to perturb the membrane dipole potential and headgroup orientation [15] and is consistent with changes in the structure of membrane bound water or alterations in lipid hydration.

It is evident from these studies that the incorporation of polarisable or charged amphiphiles into bilayers causes changes in the region of the headgroup which may be due to the combined effects of alterations in the dipole potential and alterations in hydration of the headgroup region. In the present work we report on the effects of directly applied external potential gradients on the properties of lipid multilayers.

2. Materials and methods

2.1. Instrumentation

NMR measurements were made with CXP300 and MSL400 spectrometers (Bruker, Karlsruhe, Germany), using static solid state probes, types Z32DR and HPWB73A,. The Z32DR probe was fitted with custom made solenoidal coils and modified to permit the application of bipolar electric field pulses to aligned lipid bilayer samples, synchronised with the NMR acquisition (Fig. 1).

The electric field generator had a maximum output of 400 V and a peak power limit of 1 W, to avoid excessive current which could significantly heat the sample. Cross polarisation [16] was used for ^{13}C studies, while a spin locking pulse sequence was used for ^{31}P and ^{2}H studies. The ^{31}P spectra were proton decoupled. The number of electric field pulses, their amplitude, repetition rate and duration prior to the NMR 90° pulse, could be independently varied. As seen in Fig. 1, following the NMR acquisition, a further electric field pulse is applied of reverse polarity to maintain the charge balance.

Sample electrical impedance measurements were carried out before and after the NMR measurements, using impedance spectroscopy, sweeping from 1 kHz to 0.1 Hz

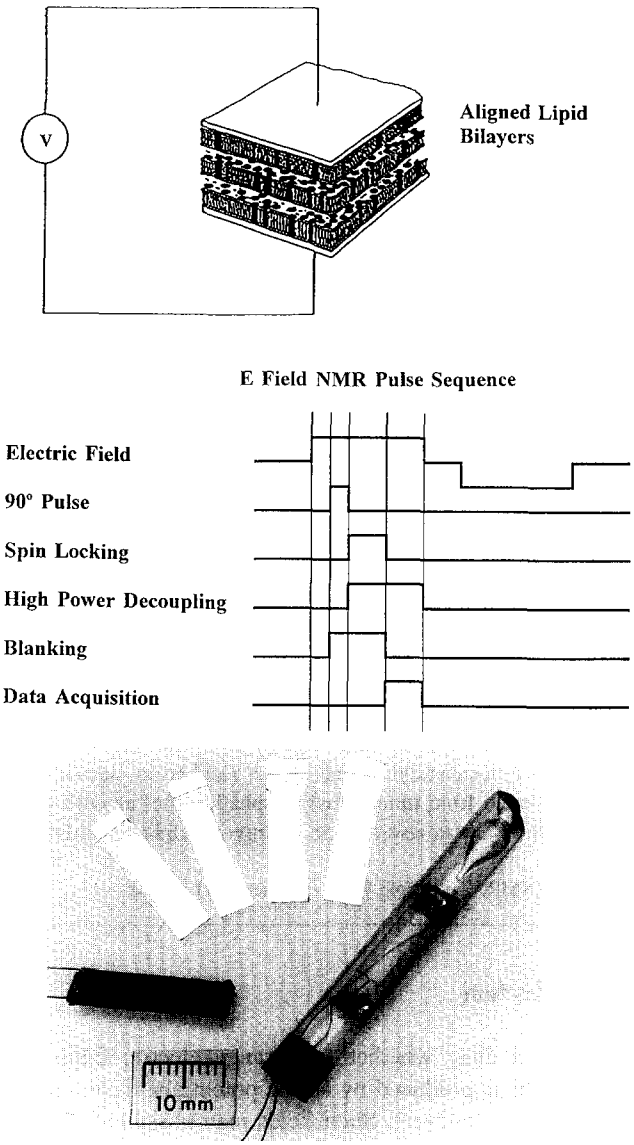

Fig. 1. The lipid is sandwiched between gold coated slides so that about 1500 bilayers are formed (top). The electric field and NMR pulse programs are shown (centre). The electric field was applied several milliseconds before the 90° pulse. The duration of the 90° pulse is exaggerated for clarity and is not to scale. Three items are shown in the bottom photograph: on the right is an assembled NMR sample tube containing an electrode stack and a bottle of saturated salt solution, in the top centre are four 5 mm×20 mm electrode strips and to the left is a stack of electrode strips which have been assembled and which have connecting wires attached.

(Associative Measurement-Sydney, Australia). Temperature measurements were carried out using a 'Numatron 934' thermocouple bridge (Leeds and Northrup-Sydney, Australia).

2.2. Sample preparation

The following lipids were used in this investigation: dioleoylphosphatidylethanolamine (DOPE), dioleoylphosphatidylcholine (DOPC) and dimyristoylphosphatidyl-

choline (DMPC). These lipids were obtained from Avanti (Alabaster, AL, USA) or Sigma (St. Louis, MO, USA). Alamethicin was obtained from Sigma. All material was used without further purification.

The electrodes were made from glass microscope coverslips which were initially cut to 70×20 mm. A 10 nm chromium adhesion layer followed by a 200 nm gold electrode layer was sputtered onto the coverslip using a shadow mask. Fig. 1 shows the electrode pattern. Both sides of the glass and the long edges were metallised in this manner. The glass slide was then cut into strips 5 mm wide and 20 mm long. The strips cut from each end of the glass slide were discarded to avoid short circuits caused by spurious metallisation around the slide at its short edges.

The samples were assembled as a set of interleaved capacitor plates with the lipid acting as the dielectric. Spacings of from 3 to 100 μm were achievable using a variety of spacer techniques. In the DOPE experiments double sided tape (Cat.136-3M, Sydney, Australia), was used to provide a spacing of 100μm. Once the slides had been cut and the spacer material applied, the lipid was deposited from chloroform onto the gold between the spacers, allowed to dry and pumped under vacuum in a desiccator for between 12 to 24 h to remove the solvent. The sample was then hydrated and finally assembled. The amount of lipid used varied proportionally with the spacer thickness. For 100 μm spacing, we used 750 μl of 10 mg/ml DOPE in $CHCl_3$. This gave a surplus of material which was squeezed out during assembly so that the cavity between the electrodes was completely filled. Several methods for hydrating DOPE were tried. The low impedance samples, (typically 50 kΩ at 100 Hz), were hydrated by heating under nitrogen to 333 K for up to 24 h. These samples, once assembled, were again heated at 100% relative humidity for 30 min. The higher impedance samples, (typically 200 kΩ at 100 Hz), were prepared by covering the lipid with approximately 5μl water and leaving the sample to equilibrate at 275 K in a 100% relative humidity atmosphere for 72 h. The surplus water was then removed. Following hydration, from two to twelve slides were stacked and connected in parallel. Most of the DOPE experiments were performed with a single slide pair, i.e two slides, the first covered with lipid and a second which was pressed onto the sample to make the electrical connection. If three or more lipid coated slides were used they were stacked in subgroups of three and the electrical connections between subgroups were made with appropriately metallised slides. Prior to the final assembly, the slides in each subgroup were gently compressed, first at points immediately over the spacer and then uniformly over the whole slide area. This assisted the formation of aligned bilayers, reduced the quantity of lipid which might cover a spacer and minimised the risk of cracking slides. Soldered wire connections were made to each end of the top slide before assembly. When multiple slides were used, the sets of three slides were assembled into stacks which were electrically interconnected using about 0.5 μl silver epoxy resin applied to the shorter and metallised edge of the slide. Migration of this resin into the sample area was prevented by the spacer material. The slide stacks were left for 24 h for the silver resin to cure and then sealed into 7 mm or 9 mm glass NMR sample tubes. If two slides only were used then a single wire was soldered to each slide of the pair. Defects in the lipid bilayer structure were further reduced by annealing the assembly according to the protocol of Powers and Clarke [17]. Where 100% relative humidity was required, a sponge saturated with water was enclosed with the lipid slide stack in the sealed glass tube. For lesser humidities, a narrow necked bottle containing the appropriate saturated salt solution was substituted for the sponge inside the sample tube. Fig. 1 shows a photograph of a typical sample assembly.

Spacers other than double sided tape may be used. For 25 μm spacing, 920 'Scotch' adhesive transfer tape is suitable (GPA Industrial Supplies, Sydney, Australia). Between 3 μm and 25 μm the glass slide may be etched with hydrofluoric acid using a mask of a material such as 'photoresist 732' (KTI, Sunnyvale, CA, USA). This procedure is best carried out by a silicon foundry or personnel experienced in the accurate etching of glass. For 3 μm an additional chromium layer may be introduced as the spacing element.

The error in the separation of the glass slides, over the 100 μm spaced samples, was 10%. The greatest single source of error in the estimation of electric field strength was believed to be due to non uniform field distribution as described in Section 3.3. Another source of error is the variation in sample impedance during phase transitions, which can typically be a factor of $\times 10$. However, the pulse generator source impedance was held at 39 kΩ, to avoid excess current, and samples of lower than 39 kΩ were not used so that the calculable error due to variation in the sample impedance during the phase transition was no more than $\times 2$. All field strengths quoted are based on the applied voltage and the sample spacing.

3. Results and discussion

3.1. Ohmic heating

The extent of ohmic heating was estimated using three methods. First a calculation on a thermal model of the sample gave a value of 0.33°C/mW. A second estimate was to measure the temperature rise in a model sample in which a sheet of carbon impregnated polymer sponge was substituted for the lipid. An 80 μm thermocouple wire was used to measure the temperature while the sample was assembled into an NMR coil. A temperature rise of 0.44 K/mW was recorded in response to dc electrical excitation.

A third approach used an NMR sample as an intrinsic

thermometer by following the temperature dependence of the ^2H quadrupolar splitting from the terminal methyls of lipid bilayers [18] formed from perdeuterated DMPC. In these experiments, glycerol was substituted for water in the lipid bilayer sample, to minimise drifts in impedance due to evaporation. Glycerol has been found to form well aligned bilayers with phospholipids such as DOPC and DMPC [19]. The low saturation vapour pressure of glycerol compared with water resulted in these bilayers being less susceptible to dehydration. All samples were prepared as single slide pairs with 100 μm spacers. The temperature dependencies for a number of materials were tested including: water/lipid, glycerol/lipid, glycerol-NaCl/lipid and glycerol/lipid-gramicidin. The optimal stability and linearity were obtained in two samples of 2:1 glycerol:perdeuterated DMPC. These had linear correlation coefficients of 0.95 and 0.91 against 20 and 22 temperature measurements respectively and were used for measuring the ohmic heating effect. The temperature coefficients for quadrupolar splitting for these samples were 0.022 K/Hz and 0.014 K/Hz. The temperature rise due to ohmic heating was determined from a series of measurements at pulsed electric field power dissipations from 0.5 mW to 26 mW. Below 6 mW no temperature rise was detectable. From 9 to 12 mW the temperature rise per unit power dissipation was 0.11 ± 0.05 K mW^{-1} measured using seven data points at 95% confidence intervals and from 15 to 26 mW it was 0.21 ± 0.06 K mW^{-1} measured from five data points at 95% confidence intervals.

These estimates indicated that the greatest heating effect obtainable in the present series of measurements would be: 3.3°C by calculation, 4.4°C by direct measurement on an artificial sample and 2.7°C by indirect measurement using ^2H-NMR.

An additional control was to test a number of samples: either by changing the applied potential and maintaining constant the electrical power associated with the electric field, or by maintaining the electrical field constant and varying the power dissipation. Similar tests were carried out on samples in which the ohmic heating was negligible due to high electrical resistance caused by reduced water content. These measurements are reported in more detail in Section 3.3 and indicate that the phenomena reported were not primarily caused by ohmic heating effects.

3.2. Testing the presence of electric field: nematic liquid crystal responses

Fig. 2 shows ^{13}C and ^2H spectra obtained from a commercially available eutectic mixture of three phenylcyclohexane nematic liquid crystals, Licristal ZL1-1083 (Merck, Darmstadt, Germany), with and without dissolved nitroanisole (4-methoxy-d_3-nitrobenzene). This demonstrated that the electric field induced changes in long range molecular order could be observed in the substantial chemical shift changes of the natural abundance ^{13}C spectra

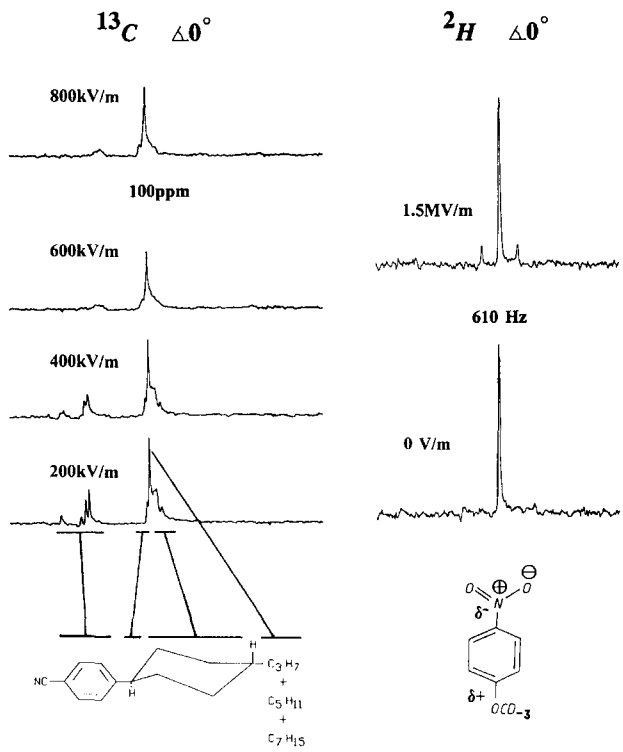

Fig. 2. These ^{13}C and ^2H spectra show the effect of electric field on a eutectic mixture of three phenylcyclohexane nematic liquid crystals, Licristal ZL1-1083 (Merck, Darmstadt, Germany), with and without dissolved nitroanisole (4-methoxy-d_3-nitrobenzene). Natural abundance solid state ^{13}C-NMR spectra were obtained using cross polarisation. Two-thousand transients were acquired with a 12 μs 90° pulse, 2 ms contact time and 2 s recycle time. The ^2H spectra were taken using a solid echo sequence. Twenty-six-thousand transients were acquired with a 30 μs 90° pulse, 0.1 ms echo time and 1 s recycle time. For both nuclei the sweep width was 62.5 kHz and the slides were oriented with their normal perpendicular to the magnetic field. A single biphasic electric field pulse with a period of 1 s was applied for each transient. The line broadening was 100 Hz.

obtained from the liquid crystal and also as alterations in the quadrupolar splitting from a ^2H-labelled nitroanisole solute species which appeared on the application of the electric field. The ^{13}C spectrum reported on the change in orientation of the aromatic and cyclohexane rings in response to the electric field, while the effect of the electric field on the nitroanisole was shown by the appearance of quadrupolar splitting in the ^2H spectrum. From the integral under each line it is possible to account for only about 30% of the nitroanisole. The remainder was thought to be intercalated amongst the unoriented aliphatic chains of the liquid crystal. The energy available to cause electrostatic rotation of the nitroanisole was calculated as 0.25 kT, therefore the changes in long range order were due to cooperative movement of the liquid crystals.

3.3. L_α to H_{II} conversion in DOPE

The limits of the H_{II} to L_α transition, for well hydrated DOPE assembled between glass slides, was found by

Table 1
A comparison of the effect of varying the electric field, while maintaining the power dissipation constant, on the relative proportions of aligned L_α, powdered L_α and H_{II} phases in DOPE samples

Experiment	Power (mW)	Electric field (MV/m)	Initial ratio (0 V/m) H_{II}/L_α (%)	Final ratio H_{II}/L_α (%)
A1	2.2	1.4 (pulsed)	0	0
A2	1.8	4 (pulsed)	0	15
B1	4.8	0.42 (continuous)	20	20
B2	4.5	2 (pulsed)	15	27

This comparison was carried out twice, first with a power dissipation of 2 mW (A) and then with 5 mW (B). The temperature for all measurements was 273 K.

^{31}P-NMR to be between 275 K (completely L_α) and 284 K (completely H_{II}), during heating, and 270 K to 277 K for cooling. Equilibration times of 10 min, 15 min and 20 min were used and the results were within one degree for each equilibration period with a hysteresis of up to seven degrees.

Four sets of measurements were also made, in which an electric field was applied to hydrated samples. These experiments were conducted by first cooling and allowing the sample to equilibrate for approx. 3 h, before applying the electric field. In one sample the power dissipation was estimated as 19 mW and the consequent temperature rise as 5 to 8 K. Although 100% conversion from the L_α to H_{II} phase was observed in response to the electric field, this result is regarded as equivocal because of the excessive power dissipation. In three other measurements the power dissipation was 10 mW and the consequent ohmic heating was estimated as between 3 to 4 K. However, with application of the electric field the percentage composition of H_{II} phase in these three samples increased by an amount that would otherwise have required a temperature change greater than respectively: 10 K, 10 K and 8 K.

Fig. 3 shows an example of this effect. The sequence of spectra, from top to bottom, describe a time series of experiments in which the electric field was altered. The changing line shapes result from variations in the phase composition of the sample and have been simulated using four lipid structures: aligned L_α, powdered L_α, aligned H_{II} and an isotropic component. A pulsed electric field of 4 MV/m increased the proportion of H_{II} at 275 K (Fig 3 C) to levels which were greater than those observed at 283 K, either before or after application of the field (Fig. 3A and Fig. 3E). The last two scans, at 275 K and 283 K, were taken following elevation of the temperature to 300 K, then cooling to 275 K for several hours.

The difference between the upper and lower limits of the L_α to H_{II} transition, in this sample was greater than 8 K. Thus the temperature changes required to cause phase alterations equivalent to those of the electric field were greater than eight degrees, which is at least a factor of two greater than any ohmic heating caused by application of the electric field.

The change in the DOPE phase due to electric field was also measured using partially hydrated samples with much higher impedance. These tests provided additional evidence that the phase changes were not primarily due to ohmic heating. Tables 1 and 2 summarise the measurements.

Table 1 shows data for which the applied electric field was altered while the power dissipation was maintained constant at 2 mW. Sample A1 at 273 K, and an applied field of 1.4 MV/m dissipated 2 mW and showed no H_{II} phase component. By contrast sample A2 at 273 K, dissipated 2 mW and with the application of 4 MV/m showed a H_{II} content of 15%.

Table 1, B1 and B2 gives a similar comparison between two samples each held at 5 mW, but with different electric field strengths. B1 shows that if a field of 0.42 MV/m at 5 mW power dissipation was applied, its H_{II} phase content remained fixed at 20%. However, B2 shows that when a field of 2 MV/m at 5 mW power dissipation was applied, the H_{II} content increased from 15% to 27%.

Table 2
A comparison of the effect of varying the electric field with the power constant (B and C), or varying power with the electric field constant (C and D), on the relative proportions of L_α and H_{II} in a DOPE sample

Experiment	Power (mW)	Electric field (MV/m)	Pulse rate (Hz)	L_α Aligned (%)	H_{II} (%)	L_α Powder (%)
A	0	0	0.1	31	23	46
B	1.3	2	2	29	25	46
C	1.3	4	0.5	25	29	46
D	5.2	4	2	25	29	46
E	0	0	0.5	31	19	50

'E' shows that removing the electric field reverses the phase transition. The sample temperature was 272 K.

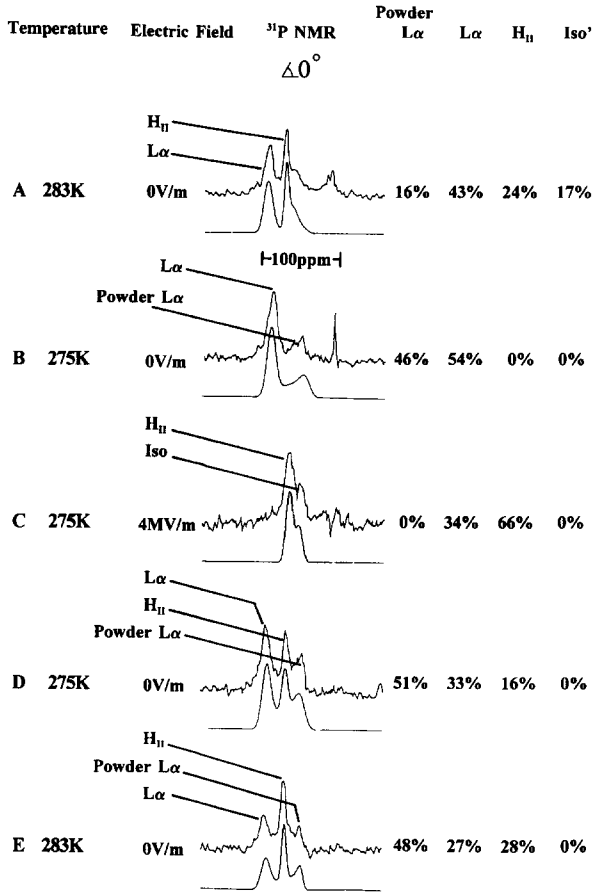

Fig. 3. These ^{31}P spectra show the electric field induced increase in the H_{II} component of a DOPE L_{α}-H_{II} composite. The original spectra are shown together with simulations and the calculated H_{II} components of the lipid structure. Natural abundance ^{31}P spectra were obtained with the bilayer normal parallel to the magnetic field direction. Three thousand transients were acquired using an 8 μs 90° pulse, 1ms spin lock time and 4 s recycle time. A single biphasic electric field pulse with a period of 38.6 ms was applied for each transient. The line broadening was 100 Hz.

Table 2, shows the results of testing; first with a variable field and constant power and then with a constant field while varying the power. In 'A' and 'B' an electric field of up to 2 MV/m with a power dissipation of 1.3 mW was applied and the percentage of H_{II} phase then increased by 2%. In 'B' and 'C' the field was altered to 4 MV/m while maintaining the power at 1.3 mW and the H_{II} phase increased by a further 4%. With 'C' and 'D' the electric field was unaltered and the power raised by $\times 4$ to 5.2 mW by altering the pulse repetition rate. The field was maintained constant at 4 MV/m and the proportion of H_{II} phase did not change. Line 'E' shows that on removing the electric field the percentage of H_{II} phase reduced by 10%. Thus, in Table 2, 'B' and 'C' show that a transition dependent on electric field occurs, while the power dissipation was held constant. In the same table 'C' and 'D' show that increasing the power input by $\times 4$, while maintaining the field constant at a value which induces a partial phase transition, has no further effect on the phase and 'E' shows that the phase transition reverses when the field is removed.

3.4. Theoretical considerations

Estimates of the torque applied by the electric field to the lipid samples indicates that a homogeneous field distribution would result in too small a value to influence the lipid alignment. One mechanism that could amplify the effect of the electric field could be accentuation by focussing in the powder component of well-hydrated samples. The extent of alteration in the phase composition by the electric field was, therefore, thought to be reduced in the drier samples because of the removal of effects due to ohmic heating and because of a reduction in field focussing effects and in sample fluidity.

One possible explanation for the electric field induced phase transition is that electroporation causes a series of pinhole breakdown sites across adjacent bilayers and along the field lines and that these then fuse into tubules which become progressively misaligned with additional field pulses and the perturbation of adjacent lipid regions. Another mechanism may involve successive breakdowns and fusions in the aligned lipid bilayer stack at points of highly focussed electric field strength, followed by rotation or curling of the lipid bilayer edges and fragments. It is evident that aligned L_{α} membranes are not energetically favoured when the normal to their plane lies in the direction of an electric field [20–25]. Further it can be shown that in the L_{α} powder phase, in a fully hydrated sample, the field lines may be focussed by as much as $\times 7$. Fig. 4 gives a plot of equipotentials, calculated as a finite difference solution to the Poisson equation, for two adjacent

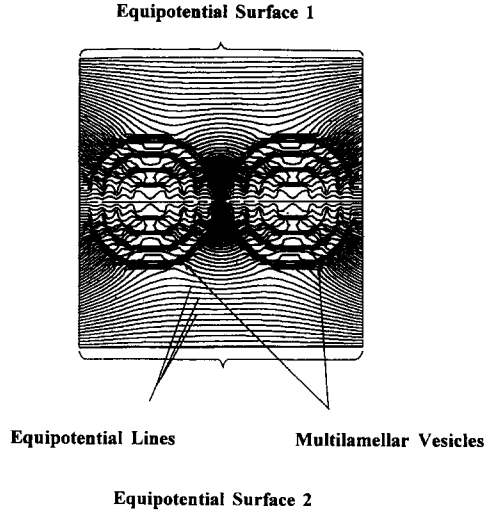

Fig. 4. This is a numerical finite difference estimate of the electric potential distribution associated with two adjacent multilamellar vesicles. In the L_{α} powder phase the field lines may be focussed by as much as $\times 7$. This would exceed the breakdown potential of the bilayer and allow electrofusion of adjacent bilayers and rotation of bilayer fragments. These processes may be a mechanism for electric field induced phase change.

multilamellar vesicles. The field can be seen to be intensified in the lipid bilayer shells and between the adjacent vesicles. If the electroporation potential of such bilayers is estimated to be 200 mV, because of the relatively long duration of the pulsed electric field, then it is possible that field focussing would cause such a potential to be exceeded. Similarly this field strength is sufficient to cause electrofusion of adjacent bilayers at point sites in the sample [26,27]. In addition to this the energy to rotate a fragment of lipid bilayer away from the region of such a focussed electric field is of the order of 50 J mol^{-1} [28,29]. A succession of such breakdowns, fusions and field induced rotations is, therefore, another possible mechanism for the electric field induced phase change.

We concluded from these tests, and the controls described in Section 3.1, that ohmic heating was insufficient to explain the electric field induced changes reported here and that electroporation, electrofusion and dielectrophoretic rotation might also have a role.

3.5. Transition from the L_α to isotropic phase in mixed lipid systems

A DOPE/DOPC mixture is reported not to phase separate where there is less than 33% DOPC present [30]. Introducing DOPC also increases the difference between the gel and the L_α/H_{II} phase transition temperatures. Using a molar ratio of DOPE:DOPC of 10:1, a composite lipid bilayer sample was made. The L_α phase component was found by ^{31}P-NMR to remain between 60% and 70% for temperatures between 277 K and 284 K. The temperature was then set to 279 K and the phase was measured again, with and without the application of 4 MV/m electric field. The sample power dissipation was less then 10 mW. The response to electric field was found to

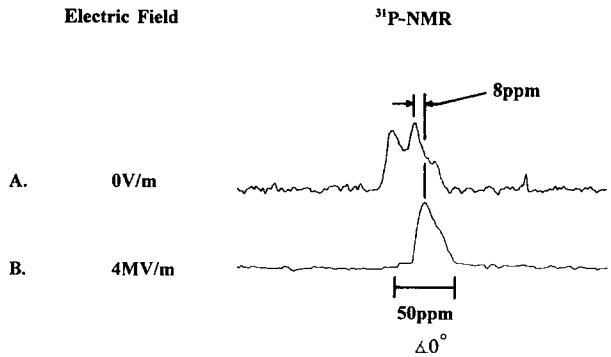

Fig. 5. A composite lipid bilayer sample was made using DOPE:DOPC at a molar ratio of 10:1. The temperature was maintained at 279 K. (A) and (B) show the spectra before and after applying the electric field. The 'isotropic' phase resulting from application of the field did not readily reverse after repeated heating and cooling. Natural abundance ^{31}P spectra were obtained with the bilayer normal parallel to the magnetic field direction and with an 8 μs 90° pulse, 0.1 ms spin lock time and 4 s recycle time. A single biphasic electric field pulse with a period of 38.6 ms was applied for each transient. The line broadening was 100 Hz.

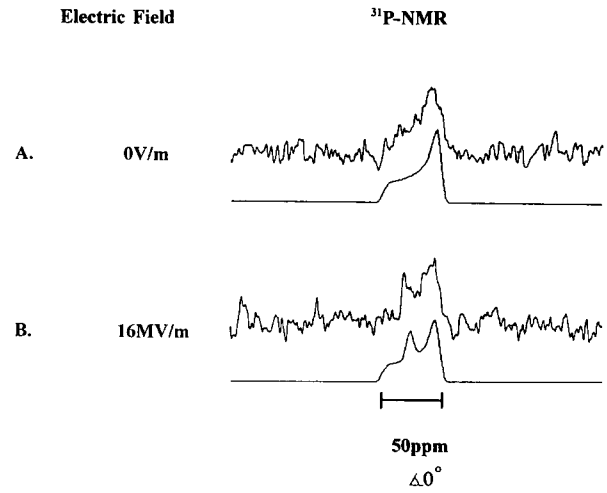

Fig. 6. Reversible electric field induced H_{II} phase components are shown in the above spectra, taken from a sample of alamethicin incorporated into DMPC bilayers in the molar ratio 1:20. The temperature was 308 K. Spectra with and without field were acquired by interlacing scans in which 16 MV/m and 0 V/m were applied to the sample. With applied electric field their is some indication of a H_{II} phase component. The natural abundance ^{31}P spectra were obtained with the bilayer normal parallel to the magnetic field direction. Twenty thousand transients were acquired using an 8 μs 90° pulse, 10ms spin lock time and 4 s recycle time. A single biphasic electric field pulse with a period of 36.5 ms was applied for each transient. The line broadening was 200 Hz.

increase, compared with a 100% DOPE sample, although the transition was to a broad isotropic signal rather than an H_{II} phase [31]. Fig. 5 shows spectra before and after application of the field. The 'isotropic' line had a chemical shift difference of 8 ppm compared with the original H_{II} line and did not reverse after repeated heating and cooling.

3.6. Electric field induced H_{II} phase in DMPC / alamethicin

A further study was carried out of the effect of electric fields on aligned lipid bilayers in which the membrane associated polypeptide alamethicin was incorporated into DMPC bilayers in the molar ratio alamethicin:DMPC of 1:20. A sample of 4 mg of DMPC was prepared on three gold/glass electrodes as described previously. A solution of 0.6 mg of alamethicin in 10 μl methanol and 20 μl of water was then added to the sample which was dried under vacuum. The sample was assembled as a single slide pair with a 25 μm spacer and the bilayers were maintained at 100% relative humidity [32–34] and 308 K. The dipole moment of alamethicin has been reported as between 67 D and 75 D [35,36]. Fig. 6 shows that applying 16 MV/m to these samples appeared to induce a component of H_{II} phase. These spectral changes were reversible. The power dissipation due to electric field was no greater than 10 mW and the ohmic heating contribution to this effect was estimated at less than 3 K. ^{31}P spectra were acquired by interlacing scans with the electric field switched on and those in which no electric field was applied.

4. Conclusions

An apparatus has been demonstrated in which intense fields, up to 100 MV/m, can be applied to water-lipid and glycerol-lipid bilayer structures within an NMR probe.

Using this apparatus an increase in the proportion of H_{II} phase in DOPE lipid bilayers has been observed with the application of pulsed fields up to 4 MV/m. Drier samples did not elicit as great an effect, probably due to the contribution of a reduction in field focussing and ohmic heating effects and a higher sample viscosity. Ohmic heating was not sufficient to explain the electric field induced changes.

In addition a change in phase was induced in a sample with a 10:1 molar ratio of DOPE:DOPC in response to a pulsed excitation of 4 MV/m.

Finally the application of electric field pulses of 16 MV/m to samples of DMPC:alamethicin, in the molar ratio of 20:1, appeared to induce a reversible H_{II} phase change. The response of model membranes to applied electric fields has been demonstrated using NMR spectroscopy.

Acknowledgements

We gratefully acknowledge the support of the Cooperative Research Centre for Molecular Engineering and Technology which has provided access to the NMR facilities used in this paper and also Dr. Frances Separovic for her many helpful comments and discussions.

References

[1] Astumian, R.D. and Robertson, B. (1992) Biochemistry 31, 138–141.
[2] Hilbers, C.W. and MacLean, C. (1972) in NMR Basic Principles and Progress (Diehl, P., Fluck, E. and Kosfield, R., eds.), pp. 1–52, Springer-Verlag, Berlin.
[3] Diehl, P., Khetrapal, C.L., Kellerhals, H.P., Lienhard, U. and Niederberger, W. (1969) J. Magn. Reson. 1, 527–533.
[4] Iizuka, E. (1973) J. Phys. Soc. Jpn. 34, 1054–1058.
[5] Ruessink, B.H., Barnhoorn, J.B.S. and MacLean. C. (1984) Mol. Phys. 52, 939–946.
[6] Ruessink, B.H., Barnhoorn, J.B.S., Bulthuis, J. and MacLean, C. (1988) Liq. Cryst. 3, 31–41.
[7] Huis, L., De Kanter, F.J.J. and MacLean, C. (1991) Mol. Phys. 73, 1077–1083.
[8] Stulen, G. (1981) Biochim. Biophys. Acta. 640, 621–627.
[9] Franklin, J.C. and Cafiso, D.S. (1993) Biophys. J. 65, 289–299.
[10] Perkins, W. and Cafiso, D.S. (1987) J. Membr. Biol. 96, 165–173.
[11] Seelig, J., Macdonald, P.M. and Scherer, P.G. (1987) Biochemistry 26, 7536–7541.
[12] Marassi, F.M. and Macdonald, P.M. (1992) Biochemistry 31, 10031–10036.
[13] Macdonald, P.M., Leisen, J. and Marassi, F.M. (1991) Biochemistry 30, 3558–3566.
[14] Macdonald, P.M. and Seelig, J. (1988) Biochemistry 27, 6769–6775.
[15] Bechinger, B. and Seelig, J. (1991) Biochemistry 30, 3923–3929.
[16] Pines, A., Gibby, M.G. and Waugh, J.S. (1973) J. Chem. Phys. 59, 569–590.
[17] Powers, L. and Clark, N.A. (1975) Proc. Natl. Acad. Sci. USA 72, 840–843.
[18] Marsh, D., Watts, A. and Smith, I.C.P. (1983) Biochemistry 22, 3023– 3026.
[19] Osman, P. and Cornell, B.A. (1992) Biophys. J. 61, A463.
[20] Li, Z., Rosenblatt, C., Yager, P. and Schoen, P.E. (1988) Biophys. J. 54, 289–294.
[21] Gawrisch, K., Parsegian, V.A., Hajduk, D.A., Tate, M.W., Gruner, S.M., Fuller, N.L. and Rand, R.P. (1992) Biochemistry 31, 2856–2864.
[22] Ellens, H., Bentz, J. and Szoka, F.C. (1986) Biochemistry 25, 4141–4147.
[23] Borovjagin, V.L., Vergara, J.A. and McIntosh, T.J. (1982) J. Membr. Biol. 69, 199–212.
[24] Thurk, M. and Porschke, D. (1991) Biochim. Biophys. Acta 1067, 153–158.
[25] Deamer, D.W. and Cornell, B.A. (1992) Biophys. J. 61, 1454–1461.
[26] Sowers, A.E. (1989) in Charge and Field Effects in Biosystems-2 (Allen, M.J., Cleary, S.F. and Hawkridge, F.M., eds.), pp. 229–256, Plenum Press, New York.
[27] Sowers, A.E. (1989) in Charge and Field Effects in Biosystems-2 (Allen, M.J., Cleary, S.F. and Hawkridge, F.M., eds.), pp. 315–337, Plenum, New York.
[28] Osman, P.D. and Cornell, B.A. (1994) Biophys. J. 66, A124.
[29] Pohl, H.A. (1978) in Dielectrophoresis (Woolfson, M.M., Ziman, J.M., eds.), pp. 106–135, Cambridge University Press, Melbourne.
[30] Webb, M.S., Hui, S.W. and Steponkus, P.L. (1993) Biochim. Biophys. Acta 1145, 93–104.
[31] Shyamsunder, E., Gruner, S.M., Tate, M.W., Turner, D.C., So, P.T.C. and Tilcock, C.P.S. (1988) Biochemistry. 27, 2332–2336.
[32] Banerjee, U., Zidovetski, R., Birge, R.R. and Chan, S.I. (1985) Biochemistry 24, 7621–7627.
[33] Brumfield, V. and Miller, I.R. (1990) Biochim. Biophys. Acta, 1024, 49–53.
[34] Yantorno, R.E. Takashima, S. and Mueller, P. (1982) Biophys. J. 38, 105–110.
[35] Schwarz, G. and Savko, P. (1982) Biophys. J. 39, 211–219.
[36] Huang, H.W. and Wu, Y. (1991) Biophys. J. 60, 1079–1087.