

Effect of electric fields on the structure of phosphatidyl choline in a multibilayer system

Israel R. Miller*

Department of Biological Chemistry, Weizmann Institute of Sciences, P.O. Box 26, 76100 Rehovot, Israel

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Abstract

Polarized attenuated total reflection (ATR)-FTIR measurements were carried out on aligned multibilayers of dipalmitoyl phosphatidyl choline (DPPC) under the influence of high electric fields. The electric fields varied from 0 up to 5.5×10^6 V/cm in the hydrocarbon layer and up to 1.1×10^6 V/cm in the polar layer of the aligned multibilayer, when the applied potential across the 1- μ m-thick multibilayer plus the 0.5- μ m-thick air gap reached the value of 1000 V. At relatively low applied potentials of less than 100 V, when the electric fields in the hydrocarbon and in the polar layer were below 5.5×10^5 and 1.1×10^5 V/cm, respectively, the inhomogeneous field between the two layers is adequate to start driving the polar groups into the hydrocarbon layer, exerting a pressure and penetrating them. This results in distortion of the orientation of the hydrocarbon chains. Only at much higher potentials above 600 and 700 V starts the direct reorientation of the dipoles of the different polar residues by the electric field. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Lipid monolayers, adsorbed on electrodes, are stable around the zero charge point, when the electric field across the lipid layer is low. At higher polarizations, either positive or negative, a potential is eventually reached when the monolayer is desorbed, giving rise to a desorption pseudo-capacitance peak. This peak results from the flow of charge across the interface when the low dielectric lipid phase is displaced by the high dielectric aqueous one. Consequently, the square of the desorption potential can be correlated with the free energy of adsorption [1]. Also the contribution of the electric fields to the pore formation energy in lipid bilayers and in biological membranes is assumed to result from its effect on the displacement of a low capacitance by a high capacitance domain [2,3].

The thermodynamic consideration does not provide a sufficient clue for the mechanism of the displacement process. In order to elucidate the role of the reorientation of the dipoles in the electric field or of their migration in an inhomogeneous electric field, exerting pressure on the low dielectric hydrocarbon domain, I determined the dichroic

ratios of the transition moments of the different vibration bands in oriented multibilayers of phosphatidyl choline (PC), using polarised attenuated total reflection (ATR)-FTIR, as a function of an applied electric field strength.

A similar study has been published recently on multilayers of dioleoyl phosphatidylcholine and melittin [4]. However, instead of dichroic ratios they measured the effect of electric field intensity on the changes in the differences between parallel and perpendicular polarised spectral bands, cancelling by arbitrary choice the area of the C=O band. This approach does not yield any information on the effect of the electric field on the orientation of the different residues of the aligned phospholipid molecules. To obtain this kind of information I preferred to determine the effect of the electric field on the dichroic ratio of the vibration bands of the different residues as they are related to the orientation of their transition dipoles.

I believe that this method renders a better way to elucidate the effect of electric fields on the phospholipid layer structure and stability.

2. Experimental

Dipalmitoyl phosphatidyl choline (DPPC) was purchased from Sigma (St. Louis, MO). Aligned multibilayers of PC

* Tel.: +972-8-9344059/2413; fax: +972-8-9344112.

E-mail address: bmiller@wicc.weizmann.ac.il (I.R. Miller).

were prepared by spreading a suspension of 0.8 mg PC in 0.1 ml water on an area of 8 cm² (10 cm long and 0.8 cm wide) on the two sides of a trapezoidal germanium crystal and aligning it with an Eppendorf tip, while it dried. Germanium served as one electrode, while a 100-nm-thick gold layer sputtered on microscope glass plate was the counter electrode. To eliminate Faradaic current with subsequent corroding of the Ge surface, the counter electrode was not allowed to contact the PS layer, but was separated from it by a ~ 0.5 - μm -thick air gap. This gap was formed by spreading on the glass plate a 1.6- μm -thick formvar layer which secured a 1.5- μm distance between the gold and the Ge surfaces, and a 0.5- μm distance between the gold electrode and the PS layer, as shown in Fig. 1. The range of the homemade DC power supply was 1500 V. Above 1100 V sparking discharge was observed, which ceased after a while, leaving a smooth strip on the slightly corroded surface of the Ge crystal underneath the electrode after the field evaporation of the protrusions in course of sparking. The dichroic ratios undergo above this potential an irreversible change. The electric field between the germanium and the gold electrode surfaces is not uniform, but varies with the dielectric coefficients of the different domains ($D=1$ for the air gap, $D=2$ for the hydrocarbon layers, and $D=10$ for the polar layer) see inset of Fig. 1. The value of $D=10$ for the polar layer is a mean estimate [5,6] and it can be lower (~ 8) in a completely dry state and higher (~ 12) in a hydrated state with > 12 H₂O molecules per PS [7]. In the present experiments, the number of water molecules per PC varied between 6 and 8. Under these conditions, every 100 V on the power supply produces field strengths of about 11×10^5 V/cm in air, 5.5×10^5 V/cm in the hydrocarbon layer and 1.1×10^5 V/cm in the polar layer.

The hydration state or the number of water molecules retained per molecule of PC in the multilayer was calculated from the ratio of the intensities of the water OH stretching peaks at 3250 cm⁻¹ to the asymmetric CH₂ stretching peaks at 2920 cm⁻¹ [7].

3. Results

In Fig. 2A, the dichroic ratios of the spectral bands of the different polar and nonpolar residues are given as a function of applied potential. The dichroic ratios measured when the potentials were increasing were the same as when measured in course of decreasing potentials, unless an irreversible change occurred at very high potentials. From the dichroic ratios, the angle β between the fiber axis of the transition moments of the specific vibrations and the normal to the crystal surface plane can be calculated. (Note that the crystal surface plane is nearly parallel to the lipid bilayer plane.)

The dichroic ratios of CH₂ wagging are not presented in the figures as even its largest amplitude at 1375 cm⁻¹ is low in comparison with the neighboring bands and their accuracy is relatively low (+8%). Moreover, the values are affected by the corresponding changes in the strong neighbor bonds. Nevertheless, the dichroic ratio at 1375 cm⁻¹ changes from ~ 1.17 at 0 to V to ~ 1.52 at very high potentials (>500 V), which corresponds to a change in orientation angle (Eqs. (1)–(3)) from $\sim 70^\circ$ at no electric field to $\sim 61^\circ$ at high electric fields, if the wagging is strictly parallel to the chain. Neither of these angles is keeping up with the angles derived from the stretching and bending bands. This eliminates the possibility that the wagging vibration giving rise to the band at 1375 cm⁻¹ is parallel to the hydrocarbon chain.

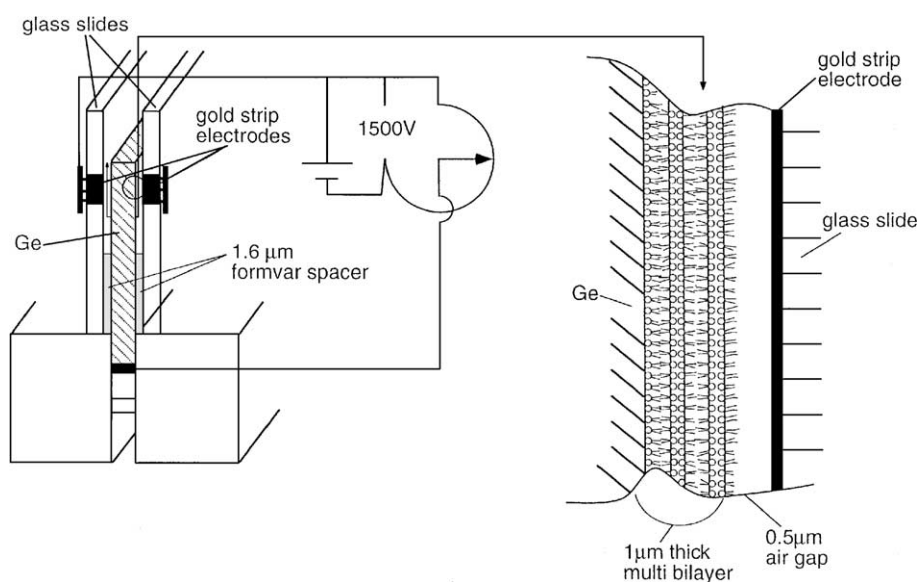


Fig. 1. Experimental setup for the polarized ATR-FTIR measurements on phospholipid multibilayers under the influence of electric fields. Inset: Blown up view of the multibilayer with the air gap and the gold strip electrodes.

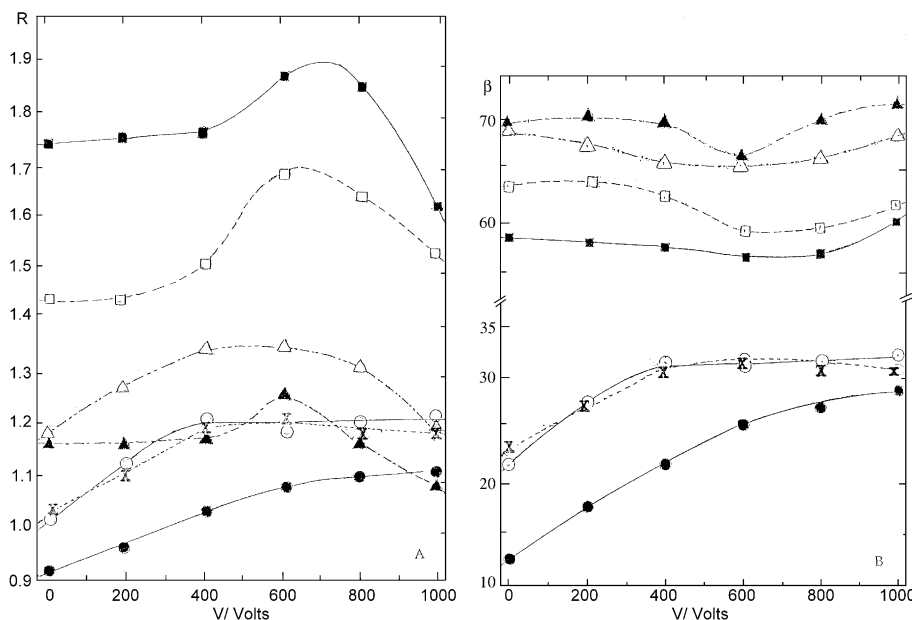


Fig. 2. Effect of an electric field on the orientation of different polar and nonpolar residues of DPPC as demonstrated by its influence on their vibration bands: \circ asymmetric stretching of CH_2 , \bullet symmetric stretching of CH_2 , \times bending of CH_2 , \triangle stretching of $\text{C}=\text{O}$, \blacktriangle asymmetric stretching of PO_2 , \square symmetric stretching of PO_2 , \blacksquare stretching of CO[P] . (A) The effect on the dichroic ratio R . (B) The effect on the angle β .

The relation between the order parameter $f(\beta)$, and the angle β is given by [8]:

$$f(\beta) = 1/2(3\cos^2\beta - 1) \quad (1)$$

and $f(\beta)$ is related to the dichroic ratio R , in the case of a Ge crystal, for the polar residues assumed to be oriented along the fiber axis by:

$$f(\beta) = (R - 2)/(R + 1.45) \quad (2)$$

and for the CH_2 stretching or bending transition dipoles, assumed to be oriented perpendicular to the fiber axis:

$$f(\beta) = -2(R - 2)/(R + 1.45) \quad (3)$$

The values of the transition moment angles β calculated in this way are presented in Fig. 2B as a function of the applied potential.

It is evident from Fig. 2A and B that the dichroic ratios and thus also the angles of the transition moments of the stretching and bending vibrations of CH_2 and to some extent also that of the transition moment of the vibration of CO are affected by lower applied electric potentials than those of the other polar residues.

4. Discussion

These results can be explained as follows. In the polar region, the electric fields are relatively low, e.g. at an applied potential of 100 V between two electrodes at a

distance of 1.5 μm the electric field is only about 1.1×10^5 V/cm. The interaction energy of this field with a fairly large dipole moment of 2 Debye units is less than 0.075 kT. In the case of interlinked dipoles like those of the different residues in the polar headgroup, every dipole can orient independently in a uniform electric field. However in a nonuniform electric field, there is a translational movement along the field gradient and the interlinked dipoles move in concert. At an applied potential of 100 V, the electric field varies between $\sim 1.1 \times 10^5$ V/cm in the polar region and 5.5×10^5 V/cm in the nonpolar region over a fraction of a nanometer. The dipoles of all the residues in a polar headgroup sum up to at least 6 Debye units and are driven across this inhomogeneous region by a force of the order of 5×10^{-7} dyn and the transfer energy of a whole polar group from the polar to the nonpolar region would amount to about 0.9 kT. This energy is probably considerably lower as the transferred polar groups affect their new environment, increasing its dielectric coefficient. If the dielectric coefficient of the initially nonpolar phase should rise from 2 to 4 by the influence of the incorporated polar residues, the transfer energy would be only about 0.44 kT and it would reach 1 kT only at around 230 V. In any event the headgroups are exerting a pressure on the hydrocarbon layer of the same molecules distorting their orientation. The change in the orientation of the hydrocarbon chains affects the orientation of the transition dipoles of CH_2 also, and to a lesser extent also that of CO . Thus, the dichroic ratios of the vibration bands of CH_2 and also of CO are more affected by lower applied potentials than those of the residues in the more polar region. The effect of the potential on the dichroic

ratio and on the orientation of CH_2 seems to reach saturation above 400 V.

At this potential, the distorted orientation of the molecule also starts affecting the dichroic ratios of the vibration bands of the polar residues. At still higher potentials, the direct effect of the electric field on the orientation of the polar residues starts. This happens only after their penetration into the lower dielectric environment, starting between 600 and 700 V for the different vibrations of the polar residues having different dipole moments. If this occurs when the interaction energy between the uniform electric field and the vibrating dipole is around 1 kT and the dipole moment about 2 Debye units, then the estimated dielectric coefficient varies between 3 and 4. These values may be somewhat higher if the reorientation of the dipoles can be discerned at lower interaction energies than 1 kT. In any event, they are keeping up with the measured dielectric constants of long chain phospholipids [9]. The present discussion is only of qualitative value as neither the local dielectric coefficients nor the dipole moments of the different polar residues are known exactly. It helps however to understand the nature of the driving forces acting on the different parts of a lipid membrane or surface layer under the influence of an electric field.

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