J. Membrane Biol. 2, 192–200 (1970)

The Effect of Electric Fields on Brain Cephalin and Lecithin Films*

LEOPOLD MAY, ARVIND B. KAMBLE, and IDALIA P. ACOSTA

Department of Chemistry, The Catholic University of America, Washington, D.C. 20017

Received 29 October 1969

Summary. The effect of a direct-current electric field on cephalin and lecithin films was measured using infrared spectral techniques. The intensities of the spectral bands assigned to the vibrations of the phosphate and the fatty acid chain increased to a maximum as the applied potential was increased. These changes were observed only with brain cephalin and brain lecithin films and not with synthetic lipid films. These observations may be due to changes in the alignments of the phosphate and base dipoles in the lipid molecule as the applied field is changed. The electric field strengths at which the maximum intensities of the spectral bands are observed increase as the thickness decreases. Extrapolation to the thickness of the nerve membrane yields a value of the field strength that is much larger than is to be expected in the neuron. This suggests that only the phosphate group and the hydrocarbon chain change conformation during the passage of the nerve impulse.

With the passage of an electric impulse along the neuronal membrane, changes in the permeability of the membrane to ions occur. These changes are probably associated with alterations in the structures of the membrane constituents, some of which are phospholipids. Although the exact nature of the function of these lipids is not known, the behavior of films of these substances in an electric field may shed light upon the actual behavior of the membrane during the passage of an impulse along the neuron. Phospholipids such as cephalin have been implicated in the excitation process of a neuron [5].

Chapman and his co-workers [1, 2] found that the polymorphic forms of 2,3-diacetyl-DL-phosphatidyl ethanolamine could be determined using infrared spectral techniques. The spectral variations of the band near 720 cm^{-1} with a decrease in temperature were interpreted as reflecting the change from a liquid to a crystalline structure. Their results suggest that similar spectral changes might be observed if the application of an electric

* Presented in part at the First Meeting of the International Society for Neurochemistry, 1967, and the 156th Meeting of the American Chemical Society, 1968.

field across cephalin films caused similar configurational changes. Films were used because they can be considered as models for the lipid structures within the neuronal membrane. The results of such experiments with cephalin and lecithin are described in this paper.

Materials and Methods

Commercial sheep brain cephalin (Sigma Chemical Co.) was purified by dissolving a sample in ether and precipitating the cephalin with acetone. This sample showed two peaks upon separation with thin layer chromatography (TLC) with the systems described below. Bovine brain lecithin (Sigma Chemical Co.) and phosphatidyl ethanolamine (Pierce Chemical Co.) were purified in the same manner. A purer sample of the phosphatidyl ethanolamine was prepared by separation on a column of diethylaminoethanol cellulose by the procedure of Rouser, Kritchevsky, Heller and Lieber [8]. The purified fraction gave one spot only on TLC (Silica gel G) with three different solvent systems: $R_F = 0.30$ in chloroform-methanol-water (65:25:4, v/v/v) [10]; $R_F = 0.23$ in chloroform-methanol-35% ammonia (14:6:1, v/v/v) [6]; and $R_F = 0.44$ in *n*-butanol-acetic acid-water (3:1:1, v/v/v) [7]. To ensure further that the lipid was pure, a sample was hydrolyzed and tested for the base present following the procedure of Chargaff, Levine and Green [3]. Only ethanolamine was present. Synthetic cephalins [phosphatidyl-L-serine (General Biochemicals) and DL-α-cephalin (ethanolamine) (Fluka AG)] and synthetic phosphatidyl choline (LaMotte Chemical Products Co.) were used without further purification.

The films were prepared by dropping concentrated chloroform or chloroform-methanol (1:1, v/v) solutions of the lipid onto silver chloride plates (1 inch × 1 inch × 1 mm thick). The average thickness of the film was evaluated from the weight of lipid deposited on the silver chloride plate and the measured areas of the lipid film. The areas of the film were reproducible to about 0.5%. The following measured densities (g/ml) were used: 1.07 for brain cephalin and brain lecithin and 1.06 for the synthetic lipids.

The direct-current voltage was applied across a cell consisting of two silver chloride plates with the phospholipid film between the plates. Voltages up to 300 V from a variable power supply were applied to the silver chloride plates through silver wires heat-sealed to the plates (Fig. 1). An ammeter was inserted in series with the cell to monitor the

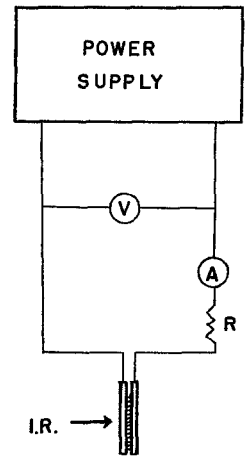

Fig. 1. Circuit for application of voltage across lipid films

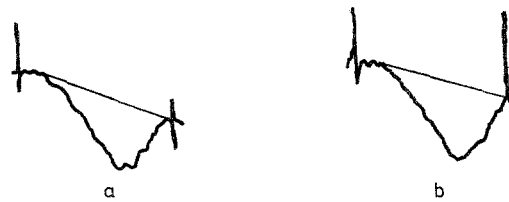

Fig. 2a and b. Measurement of a typical spectral band: the 820 cm^{-1} band from the spectrum of a film (0.011 mm thickness) of brain phosphatidyl ethanolamine. Applied direct current potentials were *a*) 0 V and *b*) 110 V

current passing through the cell. In all experiments, no current was observed (<1 μamp). Since there was no detectable current flowing, no heating took place at the electrodes.

The infrared spectra were recorded on the Perkin-Elmer Models 13 and 21 infrared spectrophotometers with a screen in the reference beam to balance the spectral beams. The spectrophotometers were equipped with sodium chloride optics and calibrated with polystyrene, water vapor and carbon dioxide. The Model 13 spectrophotometer was used in the voltage studies.

The intensities of the spectral bands were evaluated by measuring the areas under the band with a planimeter. The areas of a band were reproducible to 2% (2.80 inches 2 ±0.056) measured on five separately run spectra of the same film. The change in the area is illustrated in Fig. 2 for the 820 cm^{-1} band in the spectrum of chromatographically purified brain phosphatidyl ethanolamine after the application of 0 and 110 V.

Results

It was observed that only four of the nineteen bands in the spectrum of brain cephalin and brain phosphatidyl ethanolamine and three bands in the spectrum of brain lecithin changed their areas when the electric field was applied (Table). The infrared spectra of typical phospholipid films used are shown in Fig. 3 with the bands that changed marked by arrows. No bands in the infrared spectra of the synthetic cephalins or lecithins were affected by the electric field.

The areas of the spectral bands increased as the applied field increased reaching a maximum near 80 to 90 V of the applied field. The graph in Fig. 4 is one example that illustrates the change in the area of one band. The ordinate (A/A_0) is the ratio of the area of the band at an applied voltage to the area of the band when no voltage was applied. As the electric field was increased above the voltage for the maximal change, the ratio of the areas decreased to one, which is observed with no applied field. The rate of return to the initial area varied with the film. This variation of the ratio could be reproduced several times using the same film. The same variation was also found when the potential on silver chloride plates was reversed. To confirm that the spectral measurements represented equilibrium values, a

Table. *Infrared absorption bands of brain cephalin and lecithin affected by an electric field*

Band maximum (cm^{-1})	Absorption	Lipid
1,070	P–O–C asym. stretch	Cephalin
820	P–O–C sym. stretch	Cephalin, lecithin
760	P–O–C sym. stretch	Cephalin, lecithin
725	$-(CH_2)_n-$ rock	Cephalin, lecithin

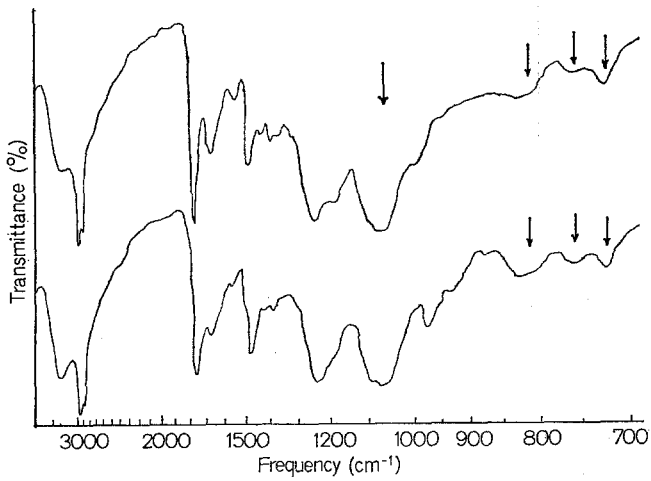

Fig. 3. Infrared spectra of brain phospholipid films. Arrows indicate the bands that change in an electric field. *Top*, cephalin; *bottom*, lecithin

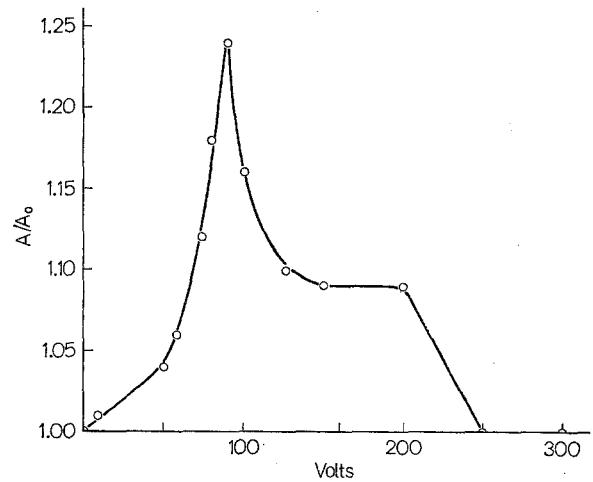

Fig. 4. Variation of the intensity of a typical spectra band with the applied electric field. A/A_0 is ratio of area of band to the area with no field

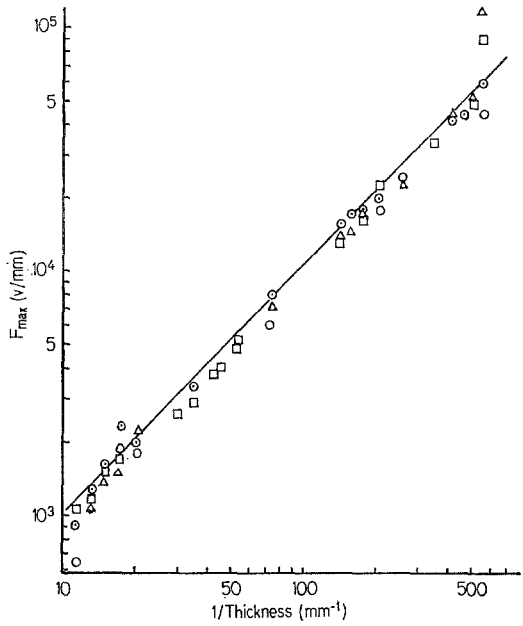

Fig. 5. Variation of the electric field strength at the maxima of change of intensities of spectral bands, F_{max}, with the thickness of brain cephalin films. ⊙ 1,070, ▫ 820, ▵ 760, ○ 725 cm^{-1}

band was scanned four times during a period of 1 hr using a film across which a voltage of 100 V was applied. No significant differences were observed in the areas. The ratio of the areas at the maximum voltage generally varied between 1.03 and 1.80.

The effect of the electric field as a function of the thickness of the lipid films was also examined. The results are plotted in Figs. 5 and 6 with the electric field strength (V/mm of film) at which the maximum change of the intensity of the absorption band occurs, F_{max}, as the ordinate and the inverse of the thickness of the film, 1/t (mm^{-1}), as the abscissa. F_{max} increases as the thickness decreases for all bands examined. The lines were fitted by the method of least squares, and it was found that the values of the slopes for all bands in both brain cephalin and lecithin were not different. The equations of the lines were $F_{max} = 101.1$ (1/t) with a SD of 20.6. There were between 17 and 23 measurements on each line.

To eliminate the possibility that an impurity was present in the brain cephalin and lecithin that was changing conformation in an electric field, a preparation of brain phosphatidyl ethanolamine was purified by column chromatography (*see* Methods). The results of the electric field on this lipid-film spectrum were consistent with the previous observations made with brain cephalin.

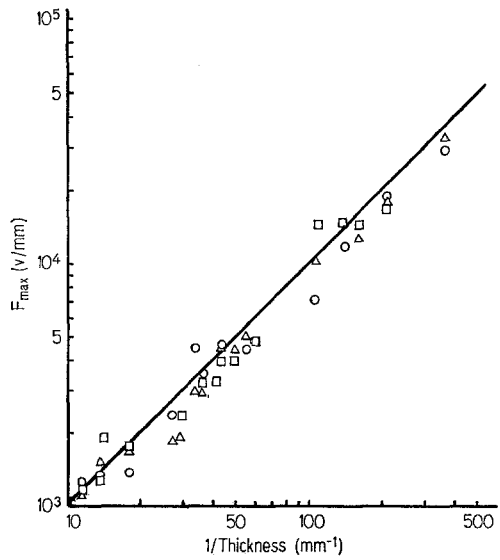

Fig. 6. Variation of the electric field strength at the maxima of change of intensities of spectral bands, F_{max}, with the thickness of brain lecithin films. ▫ 820, ▵ 760, ○ 725 cm^{-1}

Discussion

The bands in the spectra of brain cephalin and the lecithin that change with the application of a direct current electric field have been assigned to vibrations of the phosphate and $-(CH_2)_n-$ groups (Table). The assignments of the bands have been made in accordance with those published previously [2]. Chatt and Heaton [4] have presented evidence that the band near 1,070 cm^{-1} should be assigned to the C–O–P stretching mode of the P–O–C group. Hence, the phosphate group and $-(CH_2)_n-$ chain in the fatty acid of the lipid are altered in the direct current electric field.

The variation in the intensity of the band with the electric field (Fig. 4) can be explained as resulting from the change in the two dipoles within the phospholipid molecule, the phosphate and the base. The phosphate is the stronger dipole and tends to align itself with the applied field at a low applied voltage. This causes a change in the spatial relationship between the atoms in the P–O–C group. As the applied potential increases, the second dipole, the base [for example, $NH_3^+(CH_2)_2-O^-$ in phosphatidyl ethanolamine] begins to align with the field. This causes the P–O–C group to return to its original conformation. The change in the conformation of the $-(CH_2)_n-$ groups follows the changes in the phosphate group. It is interesting to note that the shape of the intensity-electric field curve is very similar to that of the ion-flux concentrations with the change in the voltage of the neuronal impulse.

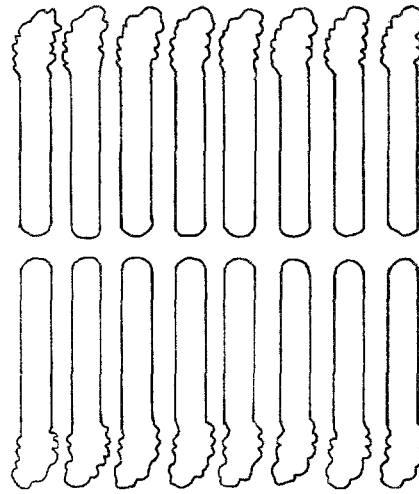

Fig. 7. Hypothetical structure of phospholipid film

This phenomenon is observed only with cephalin and lecithin isolated from brain and not with the synthetic phospholipids. This suggests that the nature of the fatty acid constituents of the lipids may be very important in determining the spatial relationships between the phosphate and base groups that change conformation in the electric field. In the brain lipids, the two fatty acid constituents are different, whereas in the synthetic molecules, they are identical. This permits the hydrocarbon chains to be more closely packed in the synthetic lipid films than are the chains in the film of tissue lipids (Fig. 7). This difference has been observed in measurements using film spreading techniques. If a lecithin contained one mono-unsaturated chain, its film could be expanded more than a film of a lecithin with two saturated hydrocarbon chains under the same conditions [9]. Watkins [11] found that the area/molecule ratio was smaller for films of synthetic lecithins than for films of egg lecithin. Thus, at the levels of the electric field used in our experiments, the configuration of the hydrocarbon chains in the synthetic phospholipids cannot be altered because they are so closely packed. However, in the tissue phospholipids, the chains are not as closely packed which leaves sufficient space for the molecules to change their configuration in the electric field.

The variation in the electric field at which the maximal change in intensity of the spectral bands, F_{max}, occurs is a linear function of the inverse of the thickness of the lipid film (Figs. 5 & 6). At film thicknesses less than about 0.009 mm, the area under the spectral band is too small to measure accurately. At the other limit, the bands become extremely broad when the

thickness is too large. Extrapolation of our data to the thickness that one postulates to be that of the neuronal membrane (100 A) yields a value of about 10^6 V/mm for F_{max}. If it is assumed that the nerve impulse is about 100 mV, the electric field strength in the membrane is 100 mV/100 A or 10^4 V/mm. This suggests that the effect of the field in the membrane is much less than the maximal values (F_{max}) observed in these *in vitro* studies of phospholipid films. Hence, only the phosphate group would undergo a conformational change. Since the $-(CH_2)_n-$ group in the fatty acid constituents follows the phosphate group changes, we would expect that to change also. These should be considered only speculations, however, since extrapolations from properties of the thick films are not always valid when measurements are made on extremely thin films.

These changes in the spectral bands reflect a change in the structure of the phospholipid under the influence of the electric field. One of the bands is related to the vibration in a $-(CH_2)_n-$ group within the hydrocarbon chain of the fatty acid in the phospholipids. Hence, this suggests that as the voltage is applied, the hydrocarbon chain changes its orientation. This change in orientation could, therefore, change the relationship between the hydrocarbon chain of the lipids in the structure of the membrane. If the hydrocarbon chains change their conformation, there would be a change in the distance between the chains. To accommodate this change, it would be necessary for the protein to undergo a slight change in its structure. This will then change the effective pore size of the protein and the permeability of the membrane to various ions (*e.g.*, Na^+ and K^+). Since the changes in the lipid in these experiments are reversible, the lipid would return to its original structure after the nerve impulse has passed.

This investigation was supported by Contract Nonr-2249-(07) (NR 101-586) between the Office of Naval Research, Department of the Navy, and the Catholic University of America.

We thank Dr. G. W. Castellan for helpful discussions and Miss Evelyn Cuesta for technical assistance.

References

1. Byrne, P., Chapman, D. 1964. Liquid crystalline nature of phospholipids. *Nature* **202:**987.
2. Chapman, D., Byrne, P., Shipley, G. G. 1966. The physical properties of phospholipids. I. Solid state and mesomorphic properties of some 2,3-diacyl-DL-phosphatidylethanolamines. *Proc. Roy. Soc.* (*London*) *A* **290:**115.
3. Chargaff, E., Levine, C., Green, C. 1948. Techniques for the demonstration by chromatography of nitrogenous lipid constituents, sulfur-containing amino acids, and reducing sugars. *J. Biol. Chem.* **175:**67.

4. Chatt, J., Heaton, B. T. 1967. Assignment of infra-red absorption frequencies associated with the P–O–CH_3 group. *Spectrochim. Acta* **23A**:2220.
5. Goldman, D. E. 1964. Molecular structural basis for the excitation properties of axons. *Biophys. J.* **4**:167.
6. Horrocks, L. 1963. Thin-layer chromatography of brain phospholipids. *J. Amer. Oil Chem. Soc.* **40**:235.
7. Rouser, G., Galli, C., Lieber, E., Blank, M. L., Privett, O. S. 1964. Analytical fractionation of complex lipid mixtures: DEAE cellulose column chromatography combined with quantitative thin-layer chromatography. *J. Amer. Oil Chem. Soc.* **41**:836.
8. — Kritchevsky, G., Heller, D., Lieber, E. 1964. Lipid composition of beef brain, beef liver and the sea anemone. Two approaches to quantitative fractionation of complex lipid mixtures. *J. Amer. Oil Chem. Soc.* **40**:425.
9. Van Deenen, L. L., Houtsmuller, U. M., De Haas, G. H., Mulder, E. 1962. Monomolecular layers of synthetic phosphatides. *J. Pharm. Pharmacol.* **14**:429.
10. Wagner, H., Horhammer, L., Wolff, D. 1961. Dünnschicht-Chromatographie von Phosphatiden und Glycolipiden. *Biochem. Z.* **334**:175.
11. Watkins, J. C. 1968. The surface properties of pure phospholipids in relation to those of lung extracts. *Biochem. Biophys. Acta* **152**:293.