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THE ACTION OF MELITTIN ON PHOSPHATIDE MULTIBILAYERS AS STUDIED BY INFRARED DICHROISM AND SPIN LABELING

A MODEL APPROACH TO LIPID–PROTEIN INTERACTIONS

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SUMMARY

1. We have studied interaction of melittin with phosphatide multibilayers by infrared absorption spectroscopy, infrared dichroism and spin label methods.

2. The ratio of the infrared absorption peaks at 1460 and 1370 cm^{-1} depends upon the hydrocarbon chain mobility, in that it increases as the chain mobility decreases. The ratio increases upon melittin binding. Concomitantly, the CH_2-rocking band at 720 cm^{-1} sharpens, also indicating a decrease in chain mobility.

3. The infrared spectra of phosphatidyl ethanolamine in the region of 1250–800 cm^{-1} depend upon head group orientation.

4. Melittin changes the transition vector for the bands at 1730 cm^{-1} (C=O ester stretch), 1240–1220 cm^{-1} (P=O stretch), 1020 cm^{-1} (P–OH stretch in phosphatidylethanolamine) and 960 cm^{-1} (P–O–C deformation in lecithin). This points to reorganization of the polar head groups.

5. The amide I band of melittin incorporated into phosphatide multibilayers lies at 1650 cm^{-1}, but exhibits no dichroism; this suggests that the peptide chain is in an unordered array. The ESR spectra of a cholestane spin probe, intercalated in phosphatides multibilayers indicate that melittin disrupts the multibilayers but concurrently decreases the mobility of the spin probe.

6. The ESR spectra of a stearate spin probe labelled at C-5 also indicate that melittin disorders the multibilayers and restricts probe mobility.

7. The fact that the amphipathic polypeptide melittin influences both polar and apolar regions of phosphatide multibilayers, suggests a specific type of lipid–protein interaction.

Abbreviations: CSL, 3-spiro-(2′-*N*-oxyl-4′,4′-dimethyl-oxazolidine)cholestane; 5-nitroxy stearate, *N*-oxyl-4′,4′-dimethyloxazolidine derivative of 5-ketostearic acid.

INTRODUCTION

A wealth of evidence indicates that most membrane proteins associate with membrane phosphatides through apolar interactions. In their analysis of this problem, Haydon and Taylor [1] show that apolar lipid–protein interactions via apolar side chains, extending from a surface-located protein into a phosphatide bilayer, are sterically impossible and energetically improbable. One must, therefore, consider structural arrangements in which part or all of the membrane protein penetrates into the membrane core.

A relatively simple model for apolar protein–membrane associations is suggested by the structure of melittin, a membrane-active polypeptide from bee venom in which the apolar residues lie at one end of the polypeptide chain [2]. Here, the first 20 residues from the NH_2 terminal comprise principally apolar entities, while the COO^- terminus consists of the basic sequence –Lys–Arg–Lys–Arg–Glu–Gln. This substance might intercalate its apolar residues among the relatively liquid fatty acid chains of a phosphatide bilayer in a random array. Alternatively, the first 14 residues (up to a proline) could from an apolar α-helical "plug" still permitting the polar segment to protrude to the membrane surface.

We have accordingly examined the interaction of melittin with phosphatide multibilayers using infrared absorption spectroscopy, infrared dichroism and spin label techniques. Our experiments provide detailed insight into the interaction of melittin with the polar and apolar moieties of membrane lipids, as well as the probable conformation of the polypeptide in multibilayers.

EXPERIMENTAL

Materials

We obtained chromatographically pure egg phosphatidylcholine and egg phosphatidylethanolamine, both unsaturated, from Lipid Products (South Nutfield, Great Britain), cholesterol from Sigma (St. Louis, Mo., U.S.A.) and melittin from Nutritional Biochemicals (Cleveland, Ohio, U.S.A.). 3-Spiro-(2′-*N*-oxyl-4′,4′-dimethyloxazolidine) cholestane (CSL) was prepared as in ref. 3 and the *N*-oxyl-4′,4′-dimethyloxazolidine derivative of 5-ketostearate (5-nitroxy stearate) was purchased from Syvar (Palo Alto, Calif., U.S.A.). We obtained AgCl plates for infrared measurements from Harshaw (Solon, Ohio, U.S.A.). All other reagents were of analytical grade.

Procedure

To form multibilayers, we prepare phosphatide solutions (10 mg/ml) in chloroform and deposit these on AgCl plates as described earlier [4]. After drying, we soak the lipid layers first in 0.1 M KCl for about 30 min and then in 0.1 M KCl, 0, 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} molar in melittin for about 30 min. We measure infrared absorption and infrared dichroism at 28 °C (55% relative humidity) with a Perkin–Elmer 621 spectrophotometer equipped with a common beam wire grid polarizer (Model 186-0187).

For electron spin resonance we deposit chloroform solutions of phosphatides (10 mg/ml), 10^{-5} M CSL or 5-nitroxy stearate as films on the walls of flat ESR cells, using a stream of wet N_2 followed by evacuation for 2 h. In some experiments, we

add cholesterol (2 mg) to the chloroform solution of phosphatides. We then soak the films with 5 mM phosphate buffer, pH 8, for about 30 min, followed by the same buffer containing stated levels of melittin for about 30 min. We record ESR spectra on a Varian E-9 spectrometer without removing the buffer and with the magnetic field parallel or perpendicular to the plane of the multibilayer film.

RESULTS

Infrared spectra

Fig. 1 depicts the typical infrared spectrum of a phosphatidylcholine multibilayer with and without melittin between 1600 and 900 cm^{-1}. Previous assignments [5] indicate that the 1460 and 1370 cm^{-1} bands arise from the bending modes of $-CH_2-CH_2$ and, likely, $-CH_2-CH_3$, respectively. We find that the ratio, R (absorbance 1460 cm^{-1}: absorbance 1370 cm^{-1}) serves as a useful, if arbitrary index of acyl chain mobility and use it to assess possible effects of melittin on phosphatide acyl chains.

Table I, showing the effect of temperature on R, shows that this ratio indeed reflects the mobility of these hydrocarbon chains: the higher the temperature, the lower the R value. Table I also shows that exposure to increasing concentrations of melittin, progressively immobilizes phosphatide acyl chains. We also observe that

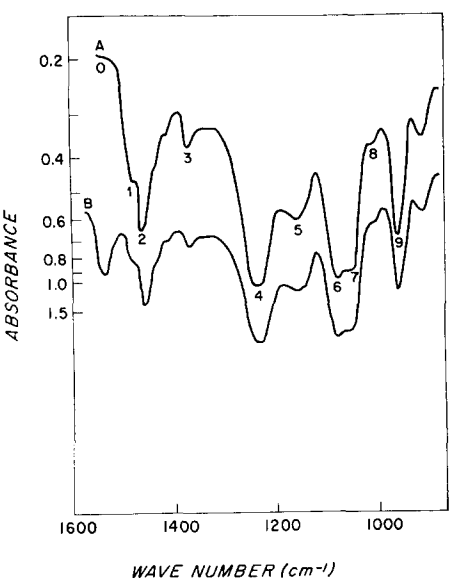

Fig. 1. Infrared absorption spectra of phosphatidylcholine multibilayers deposited on AgCl plates, soaked in 0.1 M KCl. A, phosphatidylcholine alone; B, phosphatidylcholine+melittin (10^{-3} M). Curve B is arbitrarily displaced by 0.1 absorbance at 1600 cm^{-1}. The peaks marked are assigned as in ref. 5. 1, 1475 cm^{-1} (unidentified); 2, 1460 cm^{-1} (δR–CH_2– scissoring); 3, 1370 cm^{-1} (δC–H; R–CH_3, symmetrical bending); 4, 1235 cm^{-1} (υP=O or PO_2^-); 5, 1160 cm^{-1} (υC–O; C–O–C); 6, 1080 cm^{-1} (υC–O– (P)); 7, (Sh) 1050–1060 cm^{-1} ($\upsilon PO_2^{(-)}$ symmetrical); 8, (Sh) 1020–1010 cm^{-1} (υC–$N^{(+)}$ vibrations, or (symmetrical) P–OH [7]); 9, 960 cm^{-1} [υC–$N^{(+)}$ vibrations or P–O–(C) vibrations). In curve B 1535 cm^{-1} band is due to the Amide II band of melittin. υ denotes stretch; δ denotes deformations; Sh, shoulder.

TABLE I

INFRARED PARAMETERS FOR PHOSPHATIDYLCHOLINE WITH AND WITHOUT MELITTIN*

Multibilayers	$[R]_{1460/1370}$**	θ at wave number (cm^{-1})***		
		1730	1240	960
Phosphatidylcholine (28 °C)	5.7	45°	48°45′	60°55′
Phosphatidylcholine (≈ 70 °C)	5.2			
Phosphatidylcholine (< −100 °C)	11.0			
Phosphatidylcholine + melittin (10^{-6} M)(28 °C)	5.3	45°	48°45′	67°35′
Phosphatidylcholine + melittin (10^{-5} M)	6.1	45°	49°29	61°17′
Phosphatidylcholine + melittin (10^{-4} M)	6.0	43°57′	50°43′	62°58′
Phosphatidylcholine + melittin (10^{-3} M)	7.0	43°41′	54°44′	67°35′

* Representative data from one of four experiments.

** R, ratio of absorbances at 1460 cm^{-1} vs 1370 cm^{-1}.

*** θ, a measure of dichroism, is the angle between the transition moment of a given vibration and the polarization y-axis [4].

melittin sharpens the $-CH_2-$ rocking band at 720 cm^{-1} (not illustrated), again incating reduced fatty acid chain mobility [6].

Fig. 2 shows typical infrared spectra obtained from phosphatidylethanolamine multibilayers under specified conditions. Phosphatidylethanolamine, when in the form of multibilayer arrays, displays complex infrared spectra, particularly in the region of polar head group vibrations (1250–800 cm^{-1}). Moreover, as the head group arrangement varies, depending upon the salt, pH, etc., so do the infrared vibrations in the region 1250–800 cm^{-1}. For example, dry films (Fig. 2A) show two bands at 1240 and 1220 cm^{-1}. However, films well soaked in 0.1 M KCl, pH 6.0 (Fig. 2C) display

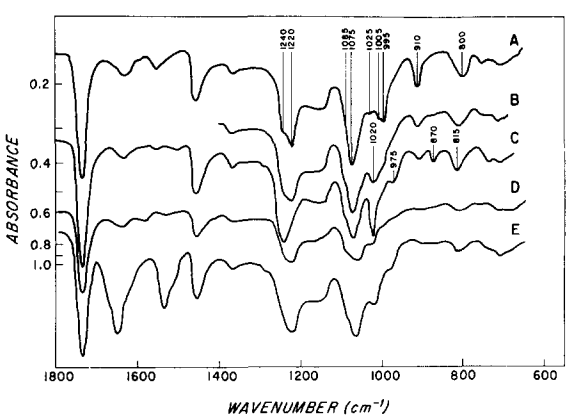

Fig. 2. Infrared absorption spectra of phosphatidylethanolamine multibilayers deposited on AgCl plates. A, dry film; B, soaked in 0.1 M KCl pH ≈ 6 (≈ 10 min); C, soaked in 0.1 M KCl pH ≈ 6.0 for more than 30 min; D, soaked in 0.1 M KCl, pH 11.6, more than 30 min; E, soaked in 10^{-3} M melittin (0.1 M KCl) more than 30 min. Each curve is arbitrarily displaced by 0.1 absorbance units at units at 1200 cm^{-1}.

only one peak at 1240 cm^{-1}. This complex situation is not observed with phosphatidylcholine multibilayers.

A shoulder at 1085 cm^{-1}, apparent in the 0.1 M KCl-soaked multibilayers, cannot be detected in dry films. The band at 1025 cm^{-1} in dry phosphatidylethanolamine films (Fig. 2A) increases in intensity with soaking to produce a sharp band at 1020 cm^{-1} (Figs 2B and 2C). We suspect that this frequency arises from P–OH stretching vibrations [7].

The bands at 1005 and 995 cm^{-1} (Fig. 2A) either disappear with soaking or form a shoulder at 1080–995 cm^{-1} (Fig. 2C). The band at 910 cm^{-1} (Fig. 2A) decreases in intensity with soaking (Fig. 2C). Coincidentally, a new band appears at 870 cm^{-1} (Fig. 2C) and the band at 800 cm^{-1} (Fig. 2A) shifts to 815 cm^{-1}.

The addition of melittin (10^{-3} M) to phosphatidylethanolamine multibilayers produces complex infrared changes (Fig. 2E) resembling those caused by raising pH. Particularly notable is the decreased intensity of the 1020- and 910-cm^{-1} peaks; the latter also seems to shift to lower frequency. As Table II shows, R, while numerically smaller than in the case of phosphatidylcholine, rises with exposure to increasing melittin levels, suggesting progressive acyl chain immobilization. Concordantly the $-CH_2-$ rocking band at 720 cm^{-1} also sharpens [6].

TABLE II

INFRARED PARAMETERS FOR PHOSPHATIDYLETHANOLAMINE WITH AND WITHOUT MELITTIN*

Multibilayers	$[R]_{1460/1370}$**	θ at wave lengths (cm^{-1})***		
		1730	1230	1020
Phosphatidylethanolamine	3.3	41°15′	49°30′	60°7′
Phosphatidylcholine+melittin (10^{-6} M)	3.8	44°25′	52°	63°26′
Phosphatidylcholine+melittin (10^{-5} M)	4.0	44°9′	54°12	68°11′
Phosphatidylcholine+melittin (10^{-4} M)	4.5	45°	52°57′	0
Phosphatidylcholine+melittin (10^{-3} M)	5.0	43°52′	51°34′	0

* Representative data from one of four experiments.

** R, ratio of absorbances at 1460 cm^{-1} vs 1370 cm^{-1}.

*** θ, a measure of dichroism, is the angle between the transition moment of a given vibration and the polarization y-axis [4].

Infrared dichroism

We have reported that, in phosphatidylcholine multibilayers, the C=O stretching band (1730 cm^{-1}), P=O stretching band (1240–1230 cm^{-1}) and P–O–C deformation peak (960 cm^{-1}) exhibit dichroism [4]. As Table I shows, their transition vector θ, changes progressively upon exposure to increasing melittin levels, from 10^{-5} to 10^{-3} M. This reflects a reorganization of the phosphatidylcholine head groups, not unlike that observed upon incorporation of cholesterol into multibilayers [4].

Table II shows the effect of melittin on the dichroism of phosphatidylethanolamine multibilayers. In comparison to phosphatidylcholine films, we observe altered dichroism already at 10^{-6} M melittin. We cannot detect a regular change in θ, with melittin concentration for the 1730- and 1230-cm^{-1} bands. However, $\theta_{1020\ cm^{-1}}$

increases sharply as we raise the melittin level up to 10^{-5} M, but at 10^{-4} M this peak shrinks into a shoulder and θ becomes immeasurable.

The Amide I and Amide II bands for melittin in the phosphatide multibilayers lie at 1650 and 1535 cm^{-1}, respectively. Neither exhibit dichroism.

ESR

Cholestane spin probe (CSL). Detailed descriptions of the ESR spectra of CSL in phospholipid multibilayers have been presented earlier [8]. In a phosphatidylcholine film, the probe's long axis undergoes rapid motion about a perpendicular to the plane of the film. Increasing cholesterol content decreases the range and rate of this motion [8, 9]. Also, in films of lipids from the white matter of ox brain, CSL undergoes rapid motion only about its long axis with an essentially random distribution of long axis orientations (ref. 10 and Neal, M., Butler, K. W. and Smith, I. C. P., unpublished). Increasing proportions of cholesterol decrease the range of the long axis distribution with retention of rapid motion about the long axis. These phenomena have been detailed in a recent review [11].

For a perfectly-ordered film of phosphatidylcholine, the ESR spectrum of CSL (rotating rapidly about is long axis) should consist of three hyperfine lines separated by 6 or 19 G when the applied magnetic field is perpendicular or parallel, respectively, to the plane of the bilayers. The intensity ratio, r, of the lines marked b and c in Fig. 3 approaches 1.0 with perfect ordering of the CSL long axis. Imposing

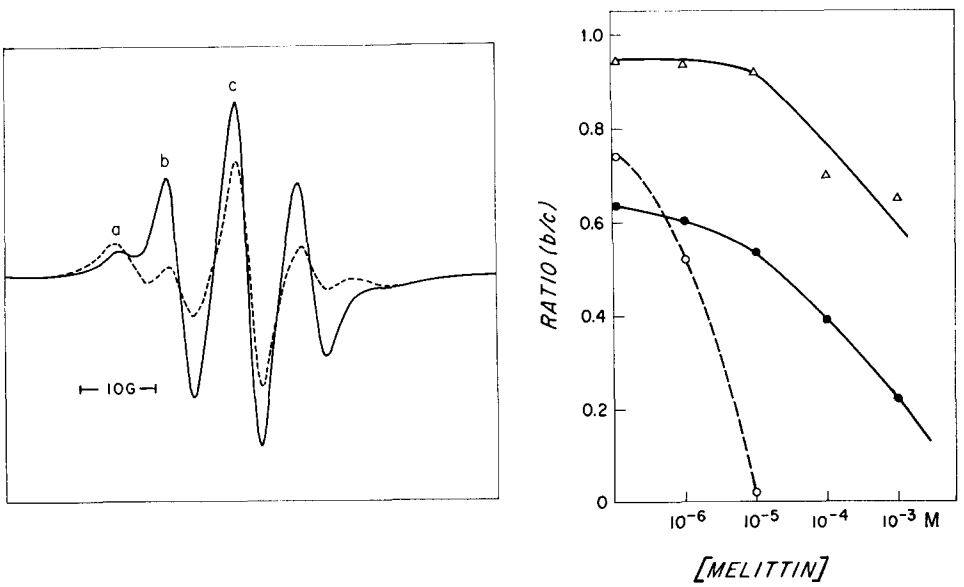

Fig. 3. ESR spectra of CSL (applied magnetic field perpendicular to plane of film) in multibilayers of phosphatidylcholine with and without melittin, hydrated with 5 mM phosphate (pH 8.0). —, phosphatidylcholine multibilayers; - - -, phosphatidylcholine+melittin (10^{-3} M).

Fig. 4. Melittin concentration plotted against the ratio r (b/c) calculated from the ESR spectra ($\perp$) of CSL in phosphatide multibilayers with and without melittin, soaked in 5 mM phosphate buffer (pH 8.0). Δ phosphatidylcholine, cholesterol (1:1 mole ratio) with and without melittin; ●, phosphatidylcholine with and without melittin; ○, phosphatidylethanolamine with and without melittin.

a static distribution of increasing width for the long axis results in a decrease in amplitude of b, and the appearance of a resonance approx. 19 G downfield from the center of the spectrum (a in Fig. 3). As the width of the distribution increases, r decreases, and resonance a develops in intensity (Polnaszek, C., Lapper, R. D., Butler, K. W. and Smith, I. C. P., unpublished). Thus, the appearance of a spectrum such as that in dashes in Fig. 3, corresponds to disordering of the CSL long axes with a decrease in mobility.

The solid ESR spectrum in Fig. 3 is similar to those already reported for CSL in phosphatidylcholine multibilayers [8, 9, 11]. On addition of melittin, resonance a (Fig. 3) increases in intensity and the ratio r decreases (Fig. 4). This is consistent with the model outlined above, where increasing melittin concentrations result in a

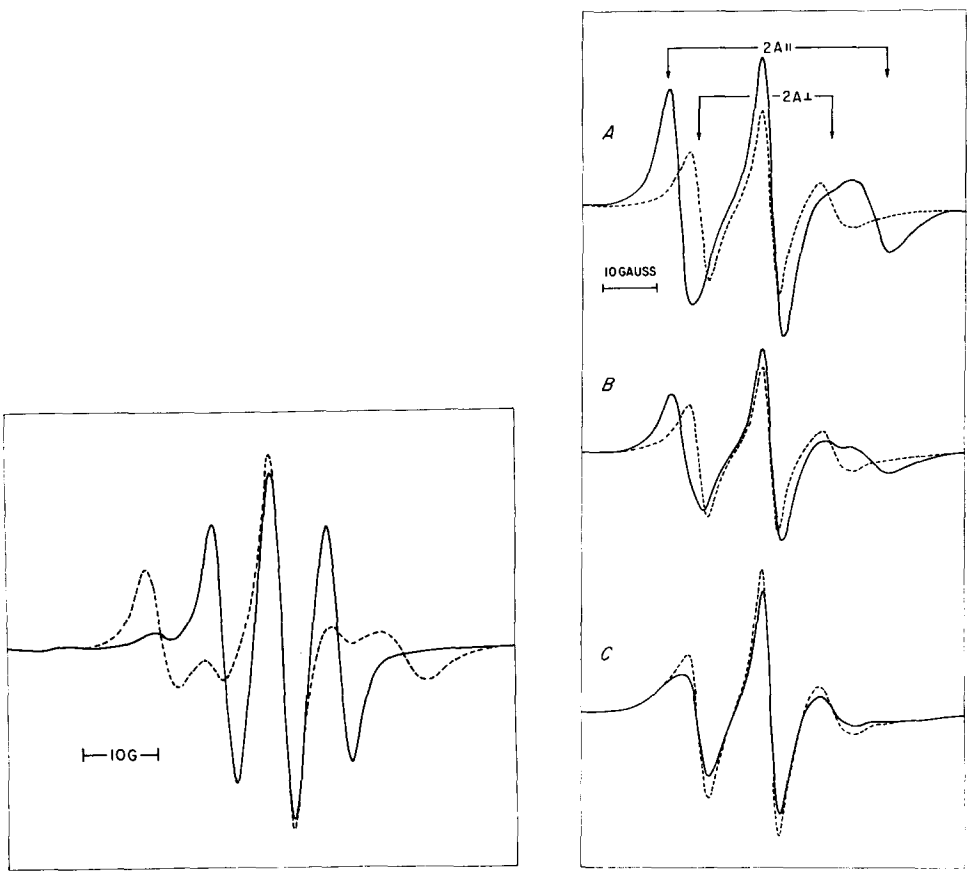

Fig. 5. ESR spectra ($\perp$) of CSL in phosphatidylethanolamine multibilayers with and without melittin, hydrated with 5 mM phosphate buffer (pH 8.0). —, phosphatidylethanolamine multibilayer; - - -, phosphatidylethanolamine + melittin (10^{-4} M).

Fig. 6. ESR spectra of 5-nitroxy stearate in phosphatidylethanolamine multibilayers with and without melittin, hydrated with 5 mM phosphate (pH 8.0) A, phosphatidylethanolamine; B, phosphatidylethanolamine + melittin (10^{-4} M), C, phosphatidylethanolamine + melittin (10^{-3} M), solid and dotted curves identify parallel and perpendicular spectra, respectively.

distribution of CSL long axes of increasing width. Thus, melittin decreases the degree of order and mobility of the phase probed by CSL.

Incorporation of cholesterol (50 moles %) into the phosphatidylcholine multibilayers make them resistant to disordering by melittin (Fig. 4). Disordering occurs only at higher concentrations of melittin and never reaches the same level.

Fig. 5 shows typical ESR spectra of CSL in phosphatidylethanolamine multibilayers with and without melittin. The data indicates that without the polypeptide, phosphatidylethanolamine forms oriented multibilayers, with $r = 0.74$ and a hyperfine separation of 7.5 G. Melittin begins to disorder phosphatidylethanolamine films at 10^{-6} M (Fig. 4), a concentration at which phosphatidylcholine multibilayers are not influenced. At 10^{-3} M melittin, the CSL spectra are identical whether the films are perpendicular or parallel to the applied magnetic field. Thus, as with phosphatidylcholine, rising melittin levels result in an increased distribution width for the CSL probe. At 10^{-3} M melittin, the probes are randomly distributed.

The 5-nitroxy stearate spin probe. Jost et al. [12] and Schreier-Muccillo et al. [13] have analysed the ESR spectra of the stearic acid spin probes in phosphatidylcholine multibilayers. The observed hyperfine splittings were interpreted in terms of rapid anisotropic motion of the probe with respect to a perpendicular to the bilayer plane. Increasing amplitude of the rapid motion resulted in a decreasing difference (ΔA) in the hyperfine splittings observed with the magnetic field parallel ($A_{\parallel}$) and perpendicular ($A_{\perp}$) to plane of the film. In the limit of very wide amplitude for this rapid motion, these splittings become equal, their magnitude being that for the probe under conditions of isotropic motion (14–16 G, depending on polarity of the medium).

We present the ESR spectra and derived parameters for phosphatidylethanolamine films containing the stearic acid probe 5-nitroxy stearate in Fig. 6 and Table III, respectively, and for phosphatidylcholine films in Table IV. Clearly, increasing

TABLE III

ESR PARAMETER OF 5-NITROXY STEARATE IN PHOSPHATIDYLETHANOLAMINE MULTIBILAYERS WITH AND WITHOUT MELITTIN

Multibilayers	$A_{\perp}$ (G)	$A_{\parallel}$ (G)	ΔA (G)
Phosphatidylethanolamine	22.0	11.2	10.8
Phosphatidylethanolamine+melittin (10^{-6} M)	22.0	11.2	10.8
Phosphatidylethanolamine+melittin (10^{-5} M)	20.5	12.0	8.5
Phosphatidylethanolamine+melittin (10^{-4} M)	20.5	11.8	8.7
Phosphatidylethanolamine+melittin (10^{-3} M)	15.0	15.0	0

melittin concentrations increases the amplitude of the probe's rapid anisotropic motion. With 10^{-3} M melittin, the spectra in the parallel and perpendicular directions are almost identical, and the motion of the probe has become pseudo-isotropic. Also, with increasing melittin concentration the ESR lines broaden, indicating a decreased rate of the probe motion. Thus, increasing melittin concentrations increase the range of probe orientations and decrease the rate of probe motion. This is consistent with the probe environment becoming less ordered and less fluid.

TABLE IV

ESR PARAMETERS OF CSL IN PHOSPHATIDYLCHOLINE MULTIBILAYERS WITH AND WITHOUT MELITTIN

Multibilayers	$A_{\parallel}$ (G)	$A_{\perp}$ (G)	ΔA (G)
Phosphatidylcholine	19.8	11.8	8.0
Phosphatidylcholine + melittin (10^{-6} M)	20.0	11.3	8.7
Phosphatidylcholine + melittin (10^{-5} M)	20.0	11.8	8.2
Phosphatidylcholine + melittin (10^{-4} M)	19.5	12.0	7.5
Phosphatidylcholine + melittin (10^{-3} M)	11.8	11.8	0

Interestingly, in phosphatidylethanolamine films, 5-nitroxy stearate responds appreciably to melittin only at concentrations of 10^{-4} M and greater, whereas CSL responds at 10^{-6} M. This indicates that the probes monitor different regions of the lipid–protein complex.

Melittin in aqueous solution interacts strongly with probe 5-nitroxy stearate, producing an ESR spectrum (Fig. 7), close to that of a randomly oriented powder; this demonstrates that a direct probe–melittin interaction results in almost complete immobilization of the probe, in contrast to the situation in multibilayers.

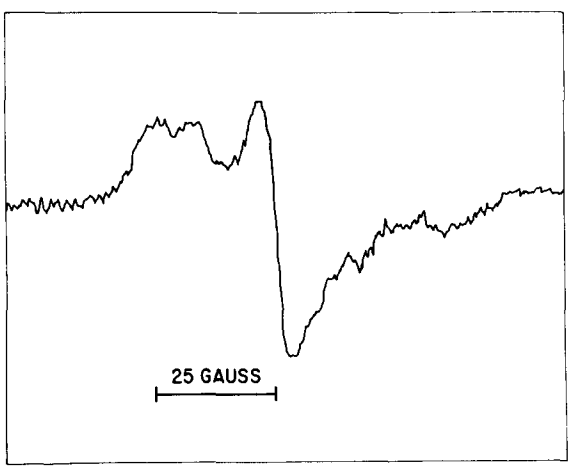

Fig. 7. ESR spectrum of 5-nitroxy stearate (approx. 10^{-5} M) in a solution of melittin (10^{-4} M, 5 mM phosphate buffer, pH 8,0).

DISCUSSION

The infrared spectra of phosphatidylethanolamine multibilayers suggest a complex behavior of the head groups. Such might be expected for at least two reasons: first, above neutrality phosphatidylethanolamine bears net negative charge increasing with pH. The resulting repulsions between like charges tend to disorder the phosphatide array and alter head group orientation. The drastic spectral changes

occurring as the pH is raised from 6.0 to 11.5 (Fig. 2) are best viewed in this light. Soaking in KCl solutions presumably screens Coulombic effects and has been shown to be necessary for the orientation of charged phosphatides in multibilayers [10]. A second complication, which also depends on pH, derives from the fact that phosphatidylethanolamine can form intramolecular $-PO-H-NH_2-$ linkages [7], with as yet poorly defined orientation. It appears that the region of head group vibrations (1250–800 cm^{-1}) has not been adequately investigated in charged phosphatides and, thus, deserves thorough study under defined experimental conditions.

Our data indicate that the amphipathic polypeptide melittin can influence both the polar and apolar regions of phosphatides arranged in multibilayers. The progressive increase of R, as well as the enhancement of the $-CH_2-$ rocking mode at 720 cm^{-1}, with both phosphatidylcholine and phosphatidylethanolamine, suggest that the apolar region tends to immobilize the fatty acid chains of the phosphatides. This conclusion agrees with the observations of William and Bell [14], who found an immobilizing effect of 10^{-4} M melittin, using the spin probe 12-nitroxide stearate as reporter group, and phosphatide liposomes as a membrane model.

Measurement of the infrared dichroism at 1730 cm^{-1} (C=O stretch) 1240–1220 cm^{-1} (P=O stretch) and 960 cm^{-1} (P–O–(C)) deformation demonstrate that melittin also effects a reorganization of the polar head groups of the phosphatides. These effects become apparent at very low melittin levels (10^{-6} M) equivalent to the concentrations which induce premeability alterations in phosphatide liposomes [15].

We suspect that the reorganization seen in phosphatidylcholine multibilayers arises from one or both of two mechanisms: first, head group reorientation secondary to apolar interactions between the melittin and phosphatide fatty acids; second, possible Coulombic interactions between the strongly basic –COO terminal of melittin and phosphatidylcholine phosphate. We consider the former suggestion most reasonable since the choline of phosphatidylcholine is a strong base and phosphatidylcholine is a zwitterion at all accessible pH values. The situation appears to differ in phosphatidylethanolamine multibilayers at pH values near neutrality. First, these appear much more sensitive to melittin (Fig. 4, Table II). Second, they exhibit a strong band at 1020 cm^{-1}, absent in phosphatidylcholine films. Since the last reduces in intensity upon exposure to pH 11.6, one can reasonably relate it to the amino moiety (apparent pK = less than 9.8), which will be fully deprotonated at the higher pH, and its electrostatic interaction with the phosphatidylethanolamine phosphate.

Importantly, addition of melittin to phosphatidylethanolamine films simulates the situation at high pH, and markedly reduces absorption at 1020 cm^{-1} at a concentration of 10^{-4} M (Fig. 2, Table II). We conclude that melittin forms ion pairs with the phosphatidylethanolamine phosphates and, thus, rearranges the phosphatidylethanolamine head group in a drastic fashion; this may be simulated by deprotonating the phosphatidylethanolamine amino group.

The amide I and II bands of melittin in phosphatidylcholine and phosphatidylethanolamine multibilayers (1650 and 1535 cm^{-1}, respectively) give no indication that the polypeptide assumes the β-conformation in its association with phosphatides. Moreover, neither band exhibits infrared dichroism, such as observed with oriented polypeptides in α-helical or β-conformations. Three possibilities exist: (a) the summed transition moments making up the amide I and II bands, yield a composite transition vector with the observed angle, θ of 45°; this explanation appears

unlikely since θ should differ for the amide I and II bands. (b) The melittin exists in α-helical arrays, but these are distributed randomly with respect to the multibilayer plane; we cannot exclude this possibility now, but the association of melittin with oriented phosphatide multibilayers, whose component molecules exhibit infrared dichroism for numerous lipid bands, makes this possibility unlikely. (c) Melittin lies with its basic terminus at aqueous interfaces, but its peptide linkages lack any geometrically fixed order: i.e. the apolar segment of melittin exists in unordered conformation. We consider this third possibility probably, but not established.

Our infrared data indicate that the film phosphatides bind to melittin through both polar and apolar interactions. The former presumably occur at the aqueous interfaces, the latter reduce hydrocarbon chain mobility. The spectra also suggest that the melittin molecules exist in an unordered conformation; hence, the melittin-associated lipids can assume a broader range of hydrocarbon chain orientations than in an unperturbed multibilayer. Both 5-nitroxy stearate and CSL report such a disordering effect. An unordered aggregate of melittin–lipid complexes, unlike a fluid lipid domain, should exhibit hindered mobilities of the spin probes, as we observe experimentally.

Phosphatide multibilayers show less sensitivity to the action of melittin in the presence of cholesterol (Fig. 4). However, with increasing melittin levels, the cholesterol analogue CSL exhibit a progressive decrease in degree of order. We assume that CSL adequately reports the behavior of cholesterol [16], and therefore conclude that melittin, in forming close associations with phosphatide acyl chains, disrupts the association of cholesterol with these residues and interferes with their orderly packing. CSL becomes disordered, but rigid.

We suggest that the melittin–phosphatide multibilayer model represents a type of membrane lipid–protein interaction other than that reported for cytochrome oxidase [17]. An interesting feature is the appearance of unordered lipid–peptide domains. These should exhibit high ion permeability, as is in fact observed with melittin–phosphatide liposomes [15].

Melittin–lipid interactions derive directly upon its amphipathic character, i.e. a polar head group and a long apolar tail. One can envisage other models depending on tertiary and quaternary protein structures, as in the case of murein lipiprotein [18].

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