Biochimica et Biophysica Acta, 510 (1978) 124—139

BBA 78052

POLYMYXIN BINDING TO CHARGED LIPID MEMBRANES

AN EXAMPLE OF COOPERATIVE LIPID-PROTEIN INTERACTION

WOLFGANG HARTMANN, HANS-JOACHIM GALLA and ERICH SACKMANN

Abteilung Experimentelle Physik III, Universität Ulm, Oberer Eselsberg, D-7900 Ulm (Donau) (G.F.R.)

(Received November 21st, 1977)

Summary

The binding of polymyxin-B to lipid bilayer vesicles of synthetic phosphatidic acid was studied using fluorescence, ESR spectroscopy and electron microscopy. 1,6-Diphenylhexatriene (which exhibits polarized fluorescence) and pyrene decanoic acid (which forms excimers) were used as fluorescence probes to study the lipid phase transition.

The polymyxin binds strongly to negatively charged lipid layers. As a result of lipid/polymyxin chain-chain interactions, the transition temperature of the lipid. This can be explained in terms of a slight expansion of the crystalline lipid lattice (Lindeman's rule). Upon addition of polymyxin to phosphatidic acid vesicles two rather sharp phase transitions (width $\Delta T = 5^\circ C$) are observed. The upper transition (at T_u) is that of the pure lipid and the lower transition (at T_l) concerns the lipid bound to the peptide. The sharpness of these transitions strongly indicates that the bilayer is characterized by a heterogeneous lateral distribution of free and bound lipid regions, one in the crystalline and the other in the fluid state. Such a domain structure was directly observed by electron microscopy (freeze etching technique). In (1 : 1) mixtures of dipalmitoyl phosphatidic acid and egg lecithin, polymyxin induces the formation of domains of charged lipid within the fluid regions of egg lecithin.

With both fluorescence methods the fraction of lipid bound to polymyxin-B as a function of the peptide concentration was determined. S-shaped binding curves were obtained. The same type of binding curve is obtained for the interaction of Ca^{2+} with phosphatidic acid lamellae, while the binding of polylysine to such membranes is characterized by a linear or Langmuir type binding curve. The S-shaped binding curve can be explained in terms of a cooperative lipid-ligand (Ca^{2+}, polymyxin) interaction.

A model is proposed which explains the association of polymyxin within the

membrane plane in terms of elastic forces caused by the elastic distortion of the (liquid crystalline) lipid layer by this highly asymmetric peptide.

Introduction

The particular roles played by charged phospholipids in biological membranes are probably related to the fact that layers of such lipids are greatly influenced by external charges: (1) Transitions between different liquid-crystalline states may be triggered by changes in the proton concentration (pH) at the membrane surface [1—4]. (2) As shown by different methods such as the spin label technique [2], the fluorescence spectroscopy [5] and the electron microscopy [6] external charges may trigger lateral phase separation which can lead to a mosaic-like organization (domain structure) [7]. (3) Domains of charged lipids may strongly bind charged cytoplasmic proteins which thus become "membrane-bound" proteins. (4) Charged lipids may also play an important role in the binding of certain drugs to membranes.

One example of the last point could be the action of the antibiotic peptide polymyxin-B on gram-negative bacteria. Under physiological conditions this peptide carries five residues of diaminobutyric acid each carrying a positive charge. The mechanism of action of polymyxin has been suggested to be related to its electrostatic interaction with the anionic cell envelope and with negatively charged lipids [8]. The preferential binding of polymyxin to charged lipids has recently been established experimentally by Teuber and Miller [9]. The present work was stimulated by these studies as well as by our previous work on ion-induced domain formation in model membranes containing charged lipids [5,6]. Moreover, polymyxin-B may be considered to be a very simple model protein with amphiphilic properties. In addition to its charged hydrophilic headgroup it possesses a hydrophobic side chain which may act to anchor it to the hydrophobic region of the membrane. From a physical point of view this antibiotic is similear to natural amphiphilic membrane proteins.

In the present paper it is shown that polymyxin binds strongly to charged lipid but may not interact with zwitterionic lipid. This leads to a pronounced decrease in the main phase transition of the charged lipid. The ratio of lipid bound to peptide to free lipid can thus be measured as a function of the molar fraction of the peptide.

Materials and Methods

Lipids. Dipalmitoyl phosphatidylcholine, dipalmitoyl phosphatidic acid and dimyristoyl phosphatidylcholine were purchased from Fluka. All lipids were checked for purity by thin-layer chromatography before use.

Probes. Pyrene-decanoic acid was synthesized in our laboratory. Diphenylhexatriene was a product of Aldrich. I(12,3) fatty acid spin label was purchased from Syva. Polymyxin was delivered from Sigma.

ESR measurements were performed with a Bruker ESR spectrometer and fluorescence measurements were made with a Schoeffel instrument. The latter was equipped with an analog computer wich allowed continuous recording of

the degree of polarization and the excimer to monomer intensity ratios. The temperature was controlled to an accuracy of 0.2 degrees. For the ESR measurements multilamellar liposomes were prepared by vortexing a lipid film at a concentration of 12 mg per ml in excess buffer. For the fluorescence studies sonicated small vesicles were used (lipid concentration 1 mg/ml). The antibiotic drug was applied after sonication. For the electron microscopy, giant vesicles were prepared as described elsewhere [11]. After addition of the drug, the vesicle preparation was centrifuged at 15 000 rev./min. The pellet was freeze etched on a Balzers device. Electron micrographs were taken with a Zeiss EM 10.

Experiments and their Interpretation

Fluorescence polarization of 1,6-diphenylhexatriene

Polymyxin-induced phase separation. Sonicated vesicles were studied. The antibiotic was added after the sonication procedure. Some typical results are given in Fig. 1. The temperature dependence of the degree of fluorescence polarization, P, of the probe is recorded for dipalmitoyl phosphatidic acid vesicles at different concentrations of polymyxin. At pH 9 the lipid has two negative charges. The pure phospholipid bilayers exhibit a sharp phase transition (width $\Delta T = 2.5°C$) at $T_u = 55°C$, as indicated by a sharp change in the degree of polarization. Addition of polymyxin leads to the appearance of a new phase transition at a lower temperature $T_l = 33°C$. With increasing peptide concentration, the height (h) of the jump P at T_l increases while the transition temperature does not change appreciably. At 28.5 mol % polymyxin content the transition of the pure lipid has nearly vanished, i.e. all the lipid interacts with polymyxin. This indicates that 1 mol of polymyxin may bind about 3 mol of

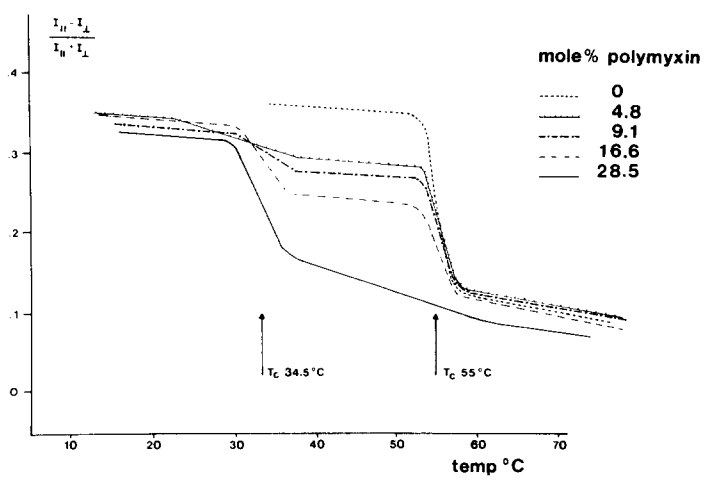

Fig. 1. Degree of polarization, P, of 1,6-diphenylhexatriene (2%) in bilayer vesicles of pure dipalmitoyl phosphatidic acid at pH 9 and at different concentrations of polymyxin. The pH was adjusted with borate buffer which has an ionic strength of 0.1. $I_{\parallel}$ and $I_{\perp}$ are the fluorescence intensities observed at parallel and perpendicular orientation of polarizer and analyzer.

phosphatidic acid carrying two negative charges. The transition of the lipid bound to polymyxin is rather sharp and has a width $\Delta T = 5°C$.

The appearance of a sharp new phase transition upon addition of polymyxin provides very strong evidence (1) that the peptide incorporates spontaneously into the charged lipid layer and (2) that extended regions composed of lipid and polymyxin in the ratio of 3 : 1 are formed at pH 9. The P versus temperature curves are nearly identical if the antibiotic is added to the sonicated vesicle preparation or if the preparation is sonicated once more after addition of polymyxin. In the first case the vesicles are not disrupted by concentrations of the antibiotic below 20 mol %. This has been clearly shown by electron microscopy (cf. Fig. 6). Since the vesicles remain stable at low polymyxin concentrations it follows that, in these cases at least, a mosaic-like lateral organization of regions of free phosphatidic acid and of phosphatidic acid bound to polymyxin is formed within the plane of the membrane. Such a structure is clearly present below the transition temperature (T_u) of the pure lipid. But the sharpness of the transition of the pure lipid ($\Delta T = 2.5°C$ at all polymyxin concentrations up to 20 mol %) indicates that a mosaic-like pattern of regions of free lipid and lipid bound to polymyxin is also present above T_u. Otherwise, one would expect a very broad transition around T_u due to the process of phase separation when the region of pure lipid are formed upon cooling.

Further evidence for such a fluid heterogeneous structure is presented in Fig. 2. Here the experiment shown in Fig. 1 has been repeated for bilayers of a 1 : 1 mixture of egg lecithin and dipalmitoyl phosphatidic acid. In the absence of polymyxin one observes a nearly continuous temperature change in the degree of polarization, which is characteristic for a random mixture of the two components. Upon addition of polymyxin a relatively sharp transition ($\Delta T = 8°C$) appears at a temperature of $T_1 = 26°C$. Since polymyxin does not bind appreciably to lecithin * it follows that agglomerates of a polymyxin/phosphatidic acid mixture must exist within the egg lecithin lamellae. The somewhat lower transition temperature, T_1, as compared to that for pure phosphatidic acid membranes ($T_1 \simeq 33°C$), may be due to limited solubility of the unsaturated compound in the clusters of lipid bound to peptide.

Determination of the polymyxin binding curve. The degree of polarization of a fluorescence probe is related to the time scale of its tumbling motion according to the Perrin equation [12,13].

$$\left(\frac{1}{P}-\frac{1}{3}\right)=\left(\frac{1}{P_0}-\frac{1}{3}\right)\left(1+\frac{3\tau}{\tau_c}\right) \tag{1}$$

τ_c is the rotational correlation time of the probe while τ is the lifetime of its excited state. (Note that τ_c is directly related to the local viscosity, η_{loc}, of the hydrophobic bilayer region.). If a membrane exhibits a heterogeneous lateral distribution of two different lipid phases, A and B, the observed degree of polarization, $\langle P \rangle$, may be expressed as

$$\langle P \rangle = x \cdot P_A + (1-x)\, P_B \tag{2}$$

* Up to polymyxin concentrations of 50 mol % no appreciable change in the phase transition of dipalmitoyl phosphatidylcholine could be observed.

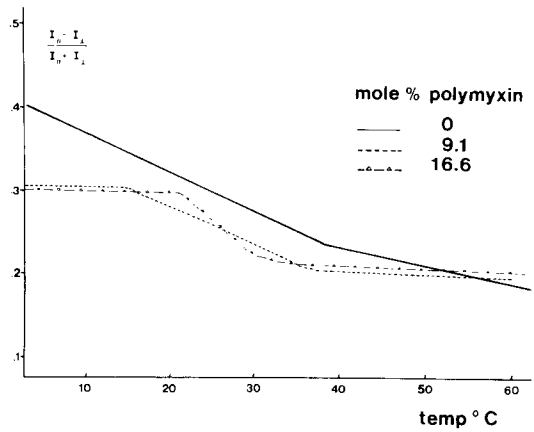

Fig. 2. Temperature dependence of degree of polarization of 1% diphenylhexatriene in 1 : 1 mixed membrane of egg lecithin and dipalmitoyl phosphatidic acid in absence and in presence of polymyxin at pH 9.0.

x and $(1-x)$ denote the lipid fraction in phases A and B, respectively. P_A and P_B are the values of P characteristic for phases A and B, respectively. This approximation holds (1) if the lifetime in the two phases are equal and (2) if the fluorescence probe does not exchange between the two phases during its lifetime. In dimyristoyl phosphatidylcholine for instance, the lifetime of diphenylhexatriene varies from $\tau = 10.4$ ns in the rigid state ($T \approx 15°C$) to $\tau \approx 9$ ns in the fluid state [14]. Therefore, the lifetimes in the two phases are the same to within 10%. The average distance (δ_x) a probe may diffuse during its lifetime τ is $\delta_x \simeq \sqrt{2D \cdot \tau}$. The lateral diffusion constant of the hydrophobic probe is

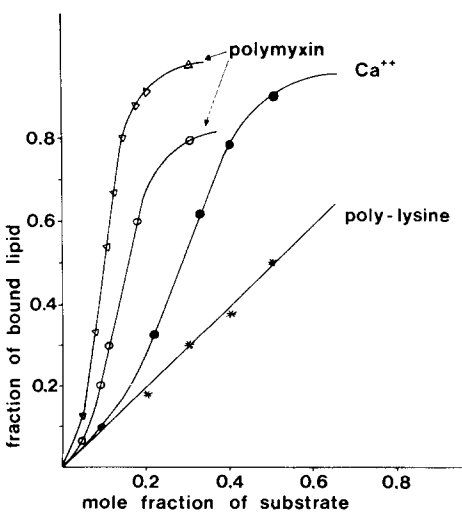

Fig. 3. Binding curves of Ca^{2+}, polymyxin and polylysine to bilayers of dipalmitoyl phosphatidic acid. The fraction, ρ, of lipid interacting with the external substrate is plotted as a function of the substrate concentration. The latter is given as molar fraction with respect to the lipid. The curves for Ca^{2+} and polylysine were obtained from studies with pyrene decanoic acid. For polymyxin pyrene decanoic acid (△) as well as diphenylhexatriene (○) have been used as optical probes.

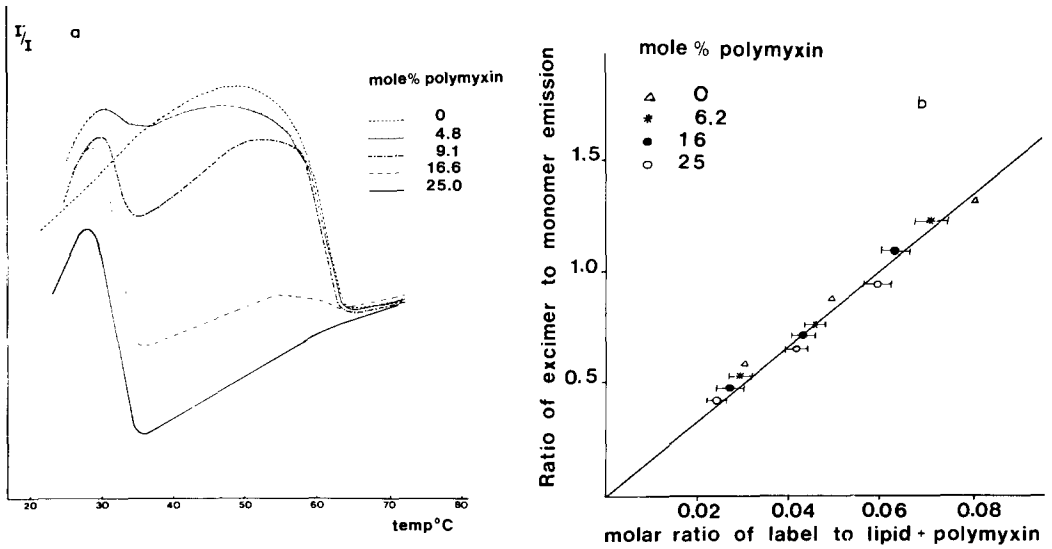

Fig. 4. (a) Temperature dependence of ratio I'/I of excimer (I') to monomer (I) fluorescence intensities of pyrene decanoic acid (3 mol %) in dipalmitoyl phosphatidic acid in absence and presence of different amounts of polymyxin at pH 7.0. (b) Effect of polymyxin on lateral mobility within dipalmitoyl phosphatidic acid bilayers at $T = 60°C$. I'/I is a measure for the collision rate of pyrene decanoic acid probes. △, values for pure lipid; ★, 6 mol %; ●, 16 mol % and ○, 25 mol % polymyxin.

expected to be of the order of $D \approx 10^{-7}$ cm^2/s in fluid membranes at about 50° C [15]. This leads to a diffusion length of $\delta_x \simeq 15$ Å with $\tau \approx 10$ ns. Due to its relatively short lifetime the diphenylhexatriene probe is thus very well suited for studying the local environment of laterally heterogeneous artifical and natural membranes. This outstanding property of the fluorescence polarization probe allows one to determine the fraction ρ of lipid bound to polymyxin from the P versus temperature plots of Fig. 1 according to the relationship $\rho = h_l/(h_l + h_u)$. In Fig. 3 the values of ρ thus determined are plotted as a function of polymyxin concentration. An S-shaped binding curve is clearly obtained.

Excimer formation by a fluorescence probe

Phase separation. Diphenylhexatriene is a probe which is mainly sensitive to the local membrane viscosity. In order to learn something about the effect of polymyxin on lateral mobility within the membrane, we performed a parallel study with the excimer forming fluorescence probe pyrene decanoic acid [5,16]. This amphiphilic label is also highly sensitive to membrane phase transitions. The parameter which is sensitive to membrane structure is the ratio of the intensity of the excimer emission (I') to the intensity of the monomer emission (I). In Fig. 4a I'/I is recorded as a function of temperature for dipalmitoyl phosphatidic acid membranes in the absence and in the presence of different amounts of polymyxin at pH 7. At this lower pH the transition temperature of the free lipid ($T_u = 60°C$) is higher compared to pH 9 ($T_u = 55°C$). The phase transition temperature of the lipid interacting with the antibiotic ($T_l = 33°C$) is nearly unaffected. At polymyxin concentrations above 5 mol % the

lipid/antibiotic mixture again shows a rather sharp phase transitions with a width $\Delta T \approx 5°C$.

Lateral diffusion. As shown previously [15,16], the intensity ratio I'/I is directly related to the coefficient of lateral diffusion of the probe according to

$$D_L = \frac{\text{constant}}{c} \frac{I'}{I} \tag{3}$$

where c is the concentration of label per unit area. A plot of I'/I versus c should thus give a straight line if excimer formation is diffusion controlled which is always the case in fluid lipid layers. By this technique the effect of polymyxin on the lateral mobility within dipalmitoyl phosphatidic acid had been studied. The result is summarized in Fig. 4b. I'/I is given as a function of the molar ratio of label to lipid + polymyxin. Taking into account the hydrophobic tail of polymyxin which has an area equal to a lipid molecule, a straight line is obtained upon plotting I'/I versus the ratio of label to lipid + polymyxin at 60° C, where the whole bilayer is in the fluid state. This gives strong evidence for partial incorporation of the polymyxin molecule into the lipid matrix. The microviscosity seems to be unaffected.

Binding curves for polymyxin, Ca^{2+} and polylysine. As shown above, incorporation of polymyxin does not affect the lateral mobility of lipid above the phase transition. The heights of the abrupt changes in I'/I at the phase transitions of the free lipid (at T_u) and the polymyxin bound lipid (at T_l) from the fluid to the rigid states, are proportional to the amounts of lipid in these two states (see Fig. 4a). The ratio, $\rho = h_l/(h_l + h_u)$, is thus again an approximate measure of the fraction of lipid bound to polymyxin. The values of ρ that have been determined by this method are given in Fig. 3 as a function of the polymyxin concentration. As in the case of the fluorescence polarization study, a S-shaped binding curve is obtained. The values of ρ obtained with the excimer-forming probe are somewhat higher. This can be attributed to the fact that some of the label is squeezed out of the free lipid regions into the bound lipid regions when the former start to crystallize. However, the S-shaped character of the binding curve should not be affected by this phenomenon. The same type of S-shaped binding curves has been observed previously for the binding of Ca^{2+} to phosphatidic acid membranes [5]. For comparison such a curve for a membrane containing 90% dipalmitoyl phosphatidic acid and 10% dipalmitoyl lecithin is also given in Fig. 3. The latter component was added in order to stabilize the vesicles. A different type of binding curve is observed for the interaction of polylysine with phosphatidic acid. This is also illustrated in Fig. 3. Polylysine binds strongly to the surface of negatively charged lipid layers. The binding leads to an increase in the transition temperature and to the formation of regions covered by the polypeptide [6]. As shown in Fig. 3 the fraction of bound lipid increases linearly with increasing polylysine concentration.

Effect of polymyxin on lipid order

Fig. 5a shows some typical ESR spectra of the I (12,3) fatty acid spin label in dipalmitoyl phosphatidic acid lamellae in the presence of polymyxin. In Fig. 5b the order parameter $S = <3(\cos^2\nu - 1)>$, as determined from these spectra, is given as a function of temperature for different polymyxin concen-

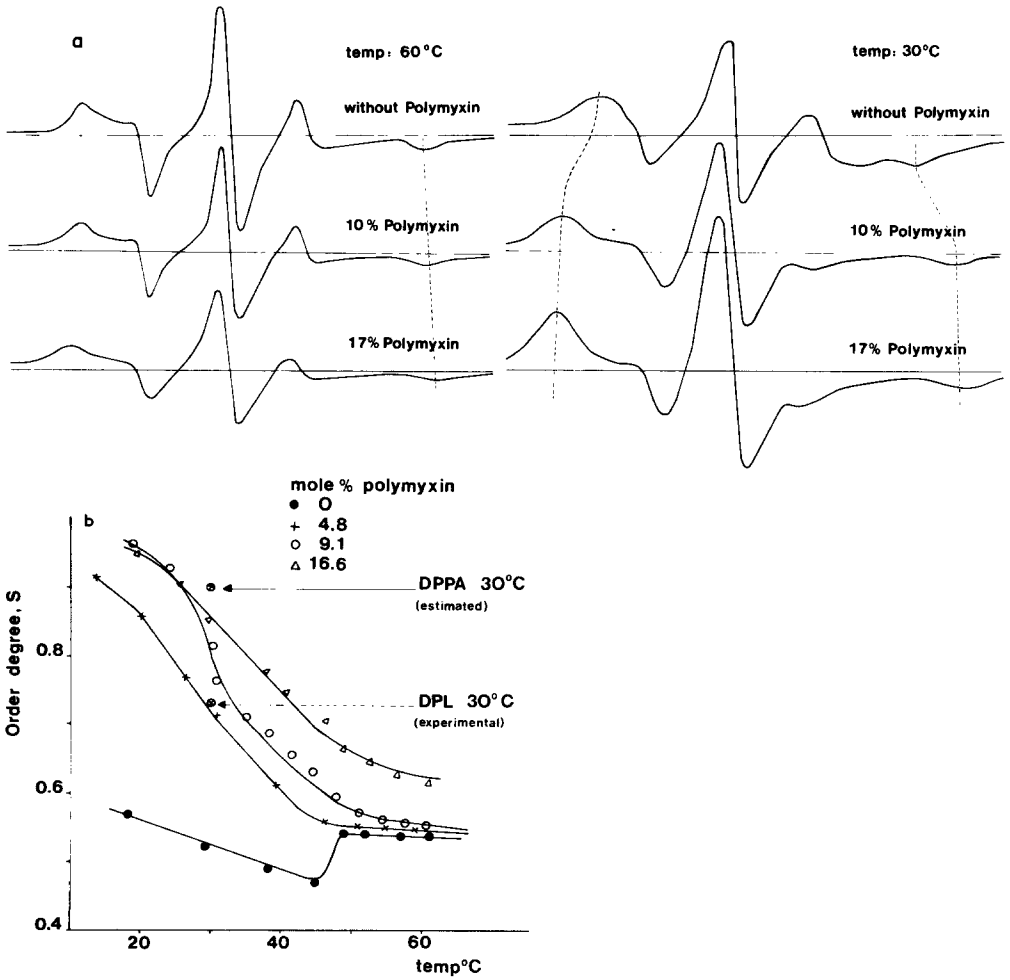

Fig. 5. (a) ESR spectra of I (12.3) fatty acid label in vesicles of dipalmitoyl phosphatidic acid in absence and after addition of polymyxin-B. (b) Order parameter $S = <3\ (\cos^2\nu - 1) >$ of I (12.3) stearic acid spin label in dipalmitoyl phosphatidic acid membranes and effect of polymyxin. Arrow (⇠ -), experimental value of S for dipalmitoyl lecithin in crystalline state. Arrow (←), value of S for dipalmitoyl phosphatidic acid as estimated from monolayer study.

trations. The order parameter for the crystalline phase of the pure lipid ($T < T_u$) is surprisingly low. The value of S for dipalmitoyl phosphatidylcholine (cf. Fig. 5b) is much higher. From monolayer studies it follows, however, that the lipid packing density in crystalline phosphatidic acid ($\gamma = 2.22$ molecules/nm^2) is considerably higher than in crystalline phosphatidylcholine layers ($\gamma = 2.0$ molecules/nm^2) [17]. The order parameter must thus also be higher in the membrane of the charged lipid. Indeed, it is this very high packing of the phosphatidic acid molecules which prevents the proper incorporation of the label into the crystal lattice. In addition, S is proportional to the square of the lipid packing density [18,19]. Accordingly, the value of $S = 0.72$ for crystalline dipalmitoyl phosphatidylcholine at 30°C leads to a value of $S = 0.93$ for the dipalmitoyl phosphatidic acid. This value is also indicated in the Fig. 5b (→).


a

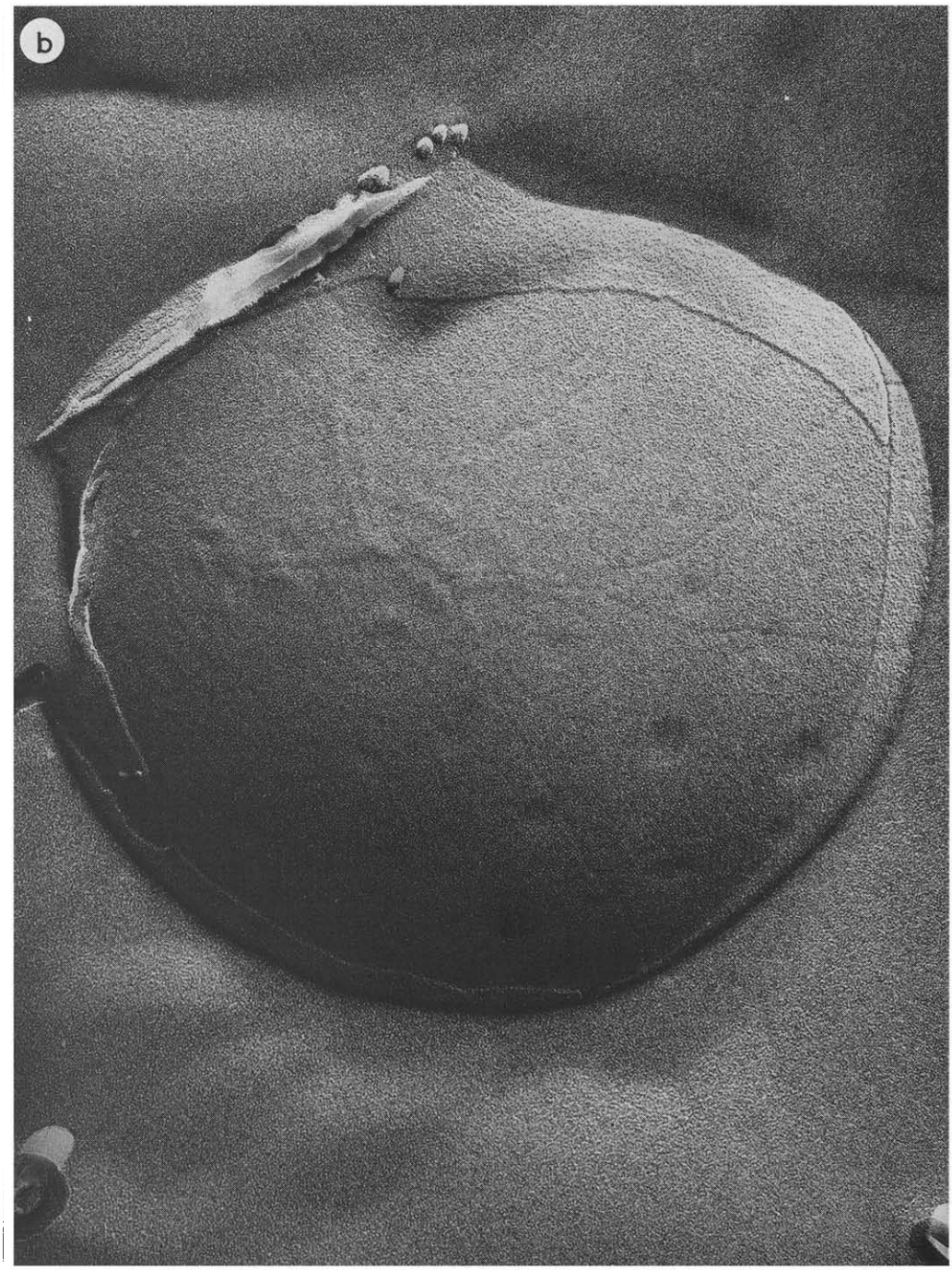

Fig. 6. Electron micrograph of giant vesicles of a 1 : 1 mixture of dioleyl phosphatidic acid and dioleyl lecithin. (a) In the absence of polymyxin. (b) Vesicle from the same preparation after addition of 20 mol % polymyxin. The small bulbs at the surface are attributed to lipid-polymyxin domains.

Addition of polymyxin clearly causes a strong increase in the apparent value of the order parameter of the crystalline state. This shows that the peptide causes an expansion of the crystalline phase and thus creates the free space for label incorporation. Surprisingly, the order parameter in the presence of only 10% polymyxin is nearly as large as in the case where all lipid interacts with the peptide (≈ 25 mol %). The fatty acid molecules thus exhibit a strong tendency to attach to the membrane-bound macromolecules if the bulk lipid phase is rigid.

Above the phase transition of both the free and the bound lipid, the order parameter S increases with increasing polymyxin concentration. The value of S obtained for 20 mol % polymyxin represents the situation where all lipid is bound.

Electron microscopy

In a previous paper [6] we showed that the domain formation induced in charged membranes by external charges (e.g. polylysine, Ca^{2+}) may be directly observed by freeze-etching electron microscopy. The polymyxin-induced domain structure may be observed in the same way. In Fig. 6 this is shown for vesicles composed of a 1 : 1 mixture of dioleyl lecithin and dioleyl phosphatidic acid. The pure lipid bilayer exhibits a completely smooth surface. Addition of polymyxin leads to the formation of circular domains which protrude from the membrane surface. These domains are attributed to the lipid/polymyxin regions.

Discussion

Polymyxin-B has the typical properties of amphiphilic membrane-bound proteins. Although its hydrophobic tail consists of a simple paraffin chain, studies of polymyxin-lipid model membranes are expected to reveal some of the basic features of lipid-protein interactions. Polymyxin is distinguished from natural membrane proteins by its high solubility in water and by the concentration of five positive charges in its head group. Due to the latter property, this antibiotic may strongly bind to membranes containing negatively charged lipids. As shown by Teuber and Miller [9] the binding to such membranes is so strong that the polymyxin becomes a true membrane-bound particle. The most important results presented in the previous section may be summarized as follows:

(1) Polymyxin-B binds to lipid lamellae of negatively charged lipids but not to layers of zwitterionic lipids, such as lecithin. (Polymyxin does not interact with zwitterionic phosphatidyl ethanolamine. However, at a pH larger than 9, where the lipid becomes negatively charged, polymyxin is strongly bound.)

(2) The transition temperature of the lipid bound to polymyxin is shifted to lower temperatures by about 20° C. The average orientation of the lipid hydrocarbon chains in the fluid state is considerably increased by polymyxin binding. The lateral diffusion of the lipid is, however, not influenced appreciably by the peptide. In the rigid state the orientation is somewhat decreased due to an expansion of the crystalline lattice.

(3) The binding of polymyxin to phosphatidic acid layers causes phase

separation leading to a mosaic-like lateral organization of regions of free lipid and of polymyxin-bound lipid. Most probably this heterogeneous distribution is also present if both "phases" are fluid. In mixtures of uncharged egg lecithin and charged phosphatidic acid the polymyxin binds to the charged component. The lipid/antibiotic mixture then forms domains within the fluid layer of uncharged lipid.

(4) The fraction ρ of charged lipid bound to polymyxin increases in a S-shaped fashion as a function of polymyxin concentration. An analogous S-shaped binding curve is observed for Ca^{2+}, while for polylysine ρ increases linearly with polypeptide concentration.

Change in transition temperature

The most obvious effect of polymyxin is the decrease of about 20°C in the transition temperature for dipalmitoyl phosphatidic acid. In contrast, polylysine (or Ca^{2+}) increases the transition temperature of phosphatidic acid. This latter effect is caused by the neutralization of the lipid charges by the positive external charges followed by a tightening of the lipid lattice. This shows that the interaction of polymyxin with the lipid is not purely electrostatic. Clearly, the amphiphilic tail of the peptide sticks into the membrane, which leads to a strong chain-chain interaction. The final result of electrostatic and chain-chain interaction is an expansion of the lipid film as suggested by the lowering in transition temperature. This follows from Lindeman's rule of melting, which predicts that melting is observed if the lattice displacement of the chains which is caused by the vibrational motion exceeds a certain fraction, f, of the lattice spacing, a. According to Marcelja et al. [20], the melting temperature may be approximately expressed as $T_m \propto f^2 \cdot U(a)$ where $U(a)$ is the repulsive interaction potential ($U(a) \propto a^{-n}$). f is a nearly universal constant ($f \approx 0.1$). For tightly packed lipid layers, such as phosphatidic acid bilayers, which are characterized by a hard repulsive potential, a small expansion of the chain lattice will decrease T_m considerably. According to Salem [21] the chain-chain repulsion potential decreases with the chain distance, a, according to $U(a) \approx a^{-6}$. An increase in the chain distance of the crystal lattice by 1.0% would therefore decrease the transition temperature by 6% or by about 18°C. As judged from the change in the order parameter by polymyxin (Fig. 5b) an expansion in the lipid distance of 1% is reasonable.

Polymyxin (and Ca^{2+}) binding as a cooperative process

The incorporation of polymyxin into negatively charged phosphatidic acid layers cannot be understood as a simple solution process. Similarly, the adsorption of Ca^{2+} to this lipid is not of the simple Langmuir type. The sigmoidal binding curves of Fig. 3 rather suggest that the interaction of these ligands with the lipid layer is a cooperative process. The binding process is thus an example of the "cooperativity in membranes" as described about 10 years ago by Changeux et al. [10].

Such a cooperative binding process may be most simply described by the Bragg-Williams theory (cf. ref. 22, Chapter 14). Upon binding of polymyxin (or Ca^{2+}) the lipid is changed from a state A (e.g. crystalline) to a state B (e.g. fluid) which may be understood as a reaction A → B between two isomeric

states A and B. A reaction energy ϵ is needed to promote a lipid molecule from state A to B (If polymyxin is added at a temperature between T_l and T_U, ϵ is of the order of the heat of melting.). If η_B and η_A denote the number of molecules present in state B and A, respectively, the fraction of bound lipid is $\rho = \eta_B/(\eta_A + \eta_B) = \eta_A/N$. The average promotion energy is then $E = N\rho\epsilon$. The isomeric change (A → B) is an endothermic process. It becomes cooperative if an attractive interaction energy, w, is introduced between the lipid molecules bound to polymyxin. In the Bragg-Williams theory the total average interaction energy, W, in state B is assumed to be proportional to the fraction of lipip already in state B: $W = zw\rho^2$, where z is the number of nearest neighbours (for a hexagonal lipid lattice $z \approx 6$). The lipid layer consists of regions of free (A) and of bound (B) lipid. The free lipid may be considered as a reservoir from which molecules can go over into the phase B after addition of the ligand. This situation is described by the "grand partition function", ξ, for the system of bound lipid [22]:

$$\xi = 1 + \lambda \exp\{(\epsilon - zw\rho)/kT\} \tag{4}$$

where $\lambda = e^{\mu/kT}$ is the absolute activity of the reaction A ⇌ B. It is proportional to the concentration of ligand, c_L (e.g. polymyxin or Ca^{2+}). The average fraction of bound lipid is then given by [22]:

$$\rho = \lambda\left(\frac{\partial \ln \xi}{\partial \lambda}\right) = \frac{\lambda \exp\{(\epsilon - zw\rho)/kT\}}{1 + \lambda \exp\{(\epsilon - zw\rho)/kT\}} \tag{5}$$

This transcendental equation for ρ may be easily solved numerically. By plotting ρ as a function of λ, that is of the concentration of ligand, an S-shaped curve is found if the ratio ϵ/w does not exceed a certain limit. Such a plot is given in Fig. 7. The curve has been fitted to the experimental binding curve of polymyxin of dipalmitoyl phosphatidic acid. A possible explanation for the cooperative lipid-polymyxin binding and domain formation as based on the elasticity of the lipid layer is given below.

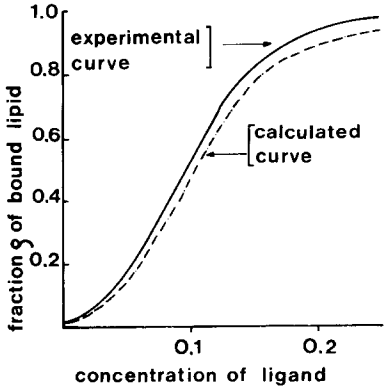

Fig. 7. Comparison of calculated and experimental binding curve of polymyxin attachment of phosphatidic acid membranes. The calculated curve was obtained from Eqn. 5 for $\epsilon = \frac{1}{2}\,kT$, $W = \frac{7}{2}\,kT$.

Possible mechanism of polymyxin-lipid association

Fig. 8 shows a model of the polymyxin molecule. Its polar head group has the shape of an elliptical disc. At its rim a charged short chain is attached to the end of which the hydrophobic paraffin tail is fixed. When the paraffin tail sticks into the hydrophobic membrane region the charged chain will anchor within the polar head group region, while the flat disc rather will lay upon the membrane surface. The whole molecule is completely asymmetric and will thus cause a strong asymmetric distortion of the lipid matrix. For topological reasons the distortion may be described as a tilt of the lipid chains as indicated

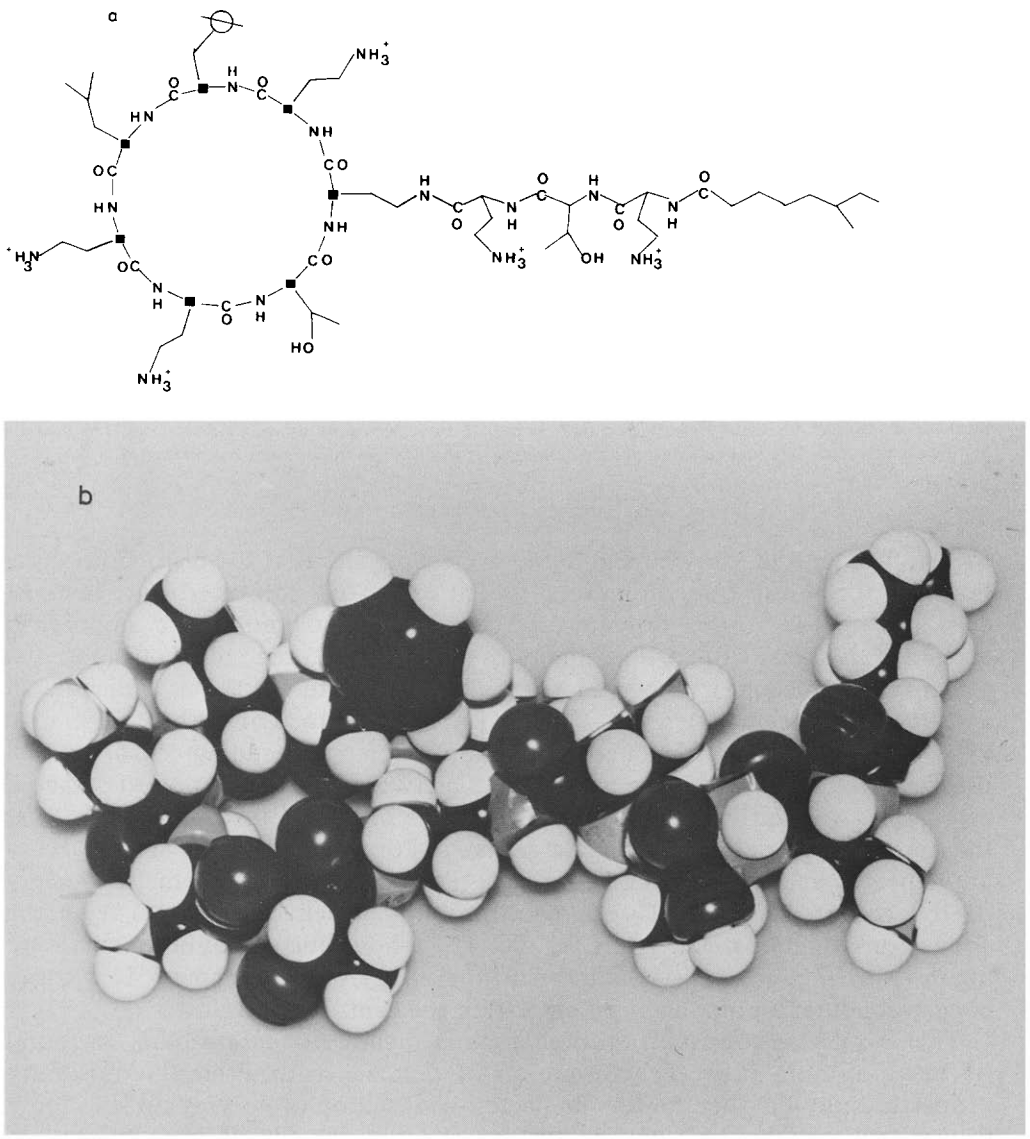

Fig. 8. Structure (a) and molecular model (b) of polymyxin-B molecule.

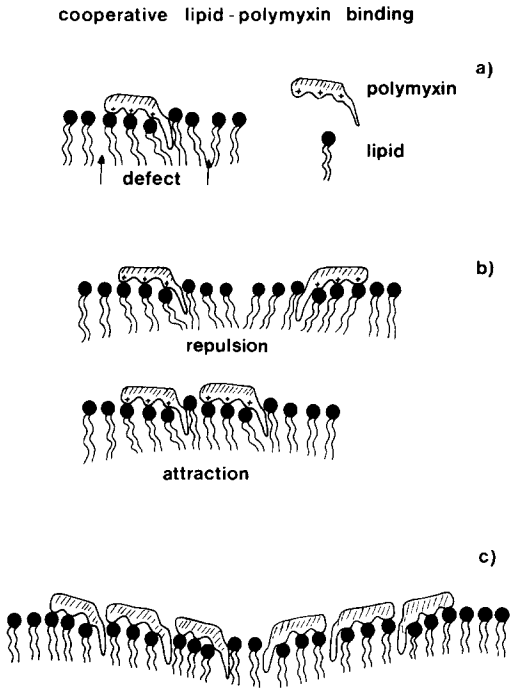

Fig. 9. (a) Possible distortion of lipid monolayer by polymyxin. The bound lipids are tilted with respect to the free lipid molecules. (b) Formation of repulsive or attractive elastic forces depends on the mutual orientation. (c) Schematic representation (side view) of domain of polymyxin/lipid mixture. The domain exhibits a curvature in order to minimize the splaying of the lipid at the domain boundary (cf. a).

in Fig. 9. It is well known from liquid crystal physics that the distortion of the lipid matrix at the transition from the tilted to the non-tilted state involves elastic energy due to the orientational elasticity of the lipid matrix. In order to minimize this elastic distortion energy the lipid layer in the neighbourhood of a polymyxin molecule is expected to exhibit a curvature [23] as indicated in Fig. 9c. It has been shown recently [24] that the distortion of the lipid matrix will lead to attractive or repulsive mechanical forces depending on the mutual orientation of the two solute molecules. The two situations have been depicted in Fig. 9b. A quantitative analysis of this type of elastic force has shown that they are characterized by a very large range. Forces of the order of 10^{-7} dynes are expected at a distance of 100 Å. This corresponds to an interaction energy of the order of 0.1 eV. The domain structure which one expects from the above considerations is given in Fig. 9c. The whole cluster exhibits a curvature in order to minimize the elastic distortion energy at its boundary. It is easily conceivable that a pore might be created in the centre of the cluster.

This would be consistent with changes in membrane permeability as found by other authors [25]. The formation of domains with altered permeability properties may be essential for the bacteriocide action of polymyxin since only distinct types of bacteria may possess target molecules for effective polymyxin in their outer membrane.

Acknowledgements

The authors wish to thank Mr. U. Theilen and Mrs. B. Kast for their excellent technical assistance. This work was supported by Deutsche Forschungsgemeinschaft under contract No. Sa 246.

References

1 Träuble, H. and Eibl, H. (1974) Proc. Natl. Acad. Sci. U.S. 71, 214—219
2 Gallo, H.J. and Sackmann, E. (1975) Biochim. Biophys. Acta 401, 509—529
3 Jacobson, K. and Papahadjopoulos, D. (1975) Biochemistry 14, 152—161
4 MacDonald, R.C. Simon, S.A. and Baer, E. (1976) Biochemistry 15, 885—891
5 Galla, H.J. and Sackmann, E. (1975) J. Am. Chem. Soc. 97, 4114—4120
6 Hartmann, W., Galla, H.J. and Sackmann, E. (1977) FEBS Lett. 78, 169—172
7 Gebhardt, C., Gruler, H. and Sackmann, E. (1977) Z. Naurforsch. 32c, 581—594
8 Teuber, M. (1974) Arch. Microbiol. 100, 131—144
9 Teuber, M. and Miller, I.R. (1977) Biochim. Biophys. Acta 467, 280—289
10 Changeux, J.-P., Thiery, J., Tung, Y. and Kittel, C. (1966) Proc. Natl. Acad. Sci. U.S. 57, 335—341
11 Reeves, J.P. and Dowben, R.M. (1969) J. Cell. Physiol. 73, 49—60
12 Dörr, F. (1971) in Creation and Detection of the excited state (Tamala, A.A. ed.), Vol. I, pp. 53—122, Academic Press, New York
13 Shinitzky, M. and Inbar, M. (1976) Biochim. Biophys. Acta 433, 133—149
14 Andrich, M.P. and Vanderkooi, J.M., (1976) Biochemistry 15, 1257—1261
15 Galla, H.J. and Sackmann, E. (1974) Biochim. Biophys. Acta 339, 103—115
16 Sackmann, E. (1976) Phys. Chem. N.F. 101, 391—416
17 Albrecht, O., Gruler, H. and Sackmann, E. (1977) to be published in J. Phys.
18 Jähnig, F. (1977) Habilitationsschrift Göttingen
19 DeGennes, P.G. (1974) Phys. Lett. 47A, 123
20 Marcelja, S., Mitchell, D.I. and Ninham, B.W. (1976) Chem. Phys. Lett. 43, 353—357
21 Salem, L. (1962) J. Chem. Phys. 37, 2100—2113
22 Hill, T.L. (1962) An Introduction to Statistical Thermodynamics, Addison-Wesley Publ. Co., London
23 Gruler, H. (1975) Z. Naturforsch. 30c, 608—612
24 Gruler, H. and Sackmann, E. (1977) Croat. Chem. Acta 49, 379
25 Imai, M., Inone, K. and Nojima. S. (1975) Biochim. Biophys. Acta 375, 130—137