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BINDING OF POLYLYSINE TO CHARGED BILAYER MEMBRANES

MOLECULAR ORGANIZATION OF A LIPID · PEPTIDE COMPLEX

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Summary

The interaction between a positively charged peptide (poly-L-lysine) and model membranes containing charged lipids has been investigated. Conformational changes of the polypeptide as well as changes in the membrane lipid distribution were observed upon lipid-protein agglutination:

1. The strong binding of polylysine is shown directly by the use of spin-labelled polypeptide. Upon binding to phosphatidic acid a shift in the hyperfine coupling constant from 16.5 to 14.6 Oe is observed. The spectrum of the lipid-bound peptide is superimposed on the spectrum of polylysine in solution. Half of the lysine groups are bound to the charged membranes. A change in the conformation of polylysine from a random coil to a partially ordered configuration is suggested.

2. Spin labelling of the lipid component gives evidence concerning the molecular organization of a lipid mixture containing charged phosphatidic acid. Addition of polylysine induces the formation of crystalline patches of bound phosphatidic acid.

3. Excimer forming pyrene decanoic acid has been employed. Addition of positively charged polylysine (pH 9.0) to phosphatidic acid membranes increases the transition temperature of the lipid from T_t = 50 to T_t = 62°C. Thus, a lipid segregation of lipid into regions of phosphatidic acid bound to the peptide which differ in their microviscosity from the surrounding membrane is induced. One lysine group binds one phosphatidic acid molecule, but only half of the phosphatidic acid is bound.

4. Direct evidence for charge-induced domain formation in lipid mixtures containing phosphatidic acid is given by electron microscopy. Addition of polylysine leads to a change in the surface curvature of the bound charged lipid. The domain size is estimated from the electron micrographs. The number of domains present is dependent on both the ratio of charged to uncharged lipids as well as on the amount of polylysine added to the vesicles. The size of the

domains is not dependent on membrane composition. However, the size seems to increase in a stepwise manner that is correlated with a multiple of the area covered by one polylysine molecule.

Introduction

It is now well accepted that the function of biological membranes is strongly dependent on their lipid composition. The lipids of microorganisms are generally in a fluid state at normal growth temperature, indicating a requirement for fluidity in some aspects of membrane function [1]. The fluid mosaic model proposed by Singer and Nicolson [2] attempts to account for the dynamic aspects of membrane organization. Kleeman et al. [3] and Kleeman and McConnell [4] have shown the formation of fluid and solid domains in bacterial membranes from fatty acid auxotrophs. More recently several groups have reported a lateral phase separation in the neighbourhood of membrane-bound proteins [5,6]. The cytochrome *P*-450 · *P*-450 reductase complex, for example, is considered to be embedded into rigid patches that "swim" in a highly fluid lipid matrix [7]. Thermally- [8,9] and chemically- [10—12] induced phase separation have been reported by several groups during the last few years. A phase separation in a multicomponent system is expected: (a) at a temperature where one component is in the fluid state while the second one is converted to the quasi crystalline configuration (thermally induced); (b) or upon binding of a charged lipid component to external ions or charged surface proteins that results in an increase in the transition temperature. Rigid lipid patches covered by external charges are supposed to be distributed like a mosaic in a fluid non-bound matrix.

The purpose of the present study was to examine the interaction between external charges and charged lipids. EPR measurements using spin-labelled polylysine give further insight into the binding of this peptide to charged lipids. Results from our EPR experiments using spin-labelled lipids as well as optical measurements using pyrene decanoic acid as fluorescent probe lead to the conclusion that the lipid is organized in domains. A direct approach to the problem of chemically-induced domain structures is provided by electron microscopy. The elastic forces in the plane of the membrane lead to a change in the spontaneous curvature of the membrane. The expected undulation of the surface curvature required by the charge-induced phase separation is observed by electron microscopy of membranes containing dioleyl phosphatidic acid upon addition of polylysine.

Materials and Methods

Lipids and proteins. Dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidic acid from Fluka were checked for purity by thin-layer chromatography and used without further purification. Dioleyl phosphatidylcholine and dioleyl phosphatidic acid were from Serdary (London, Ontario, Canada). The bromide salt of poly-L-lysine with a molecular weight of 30 000 was purchased from Miles Biochemicals.

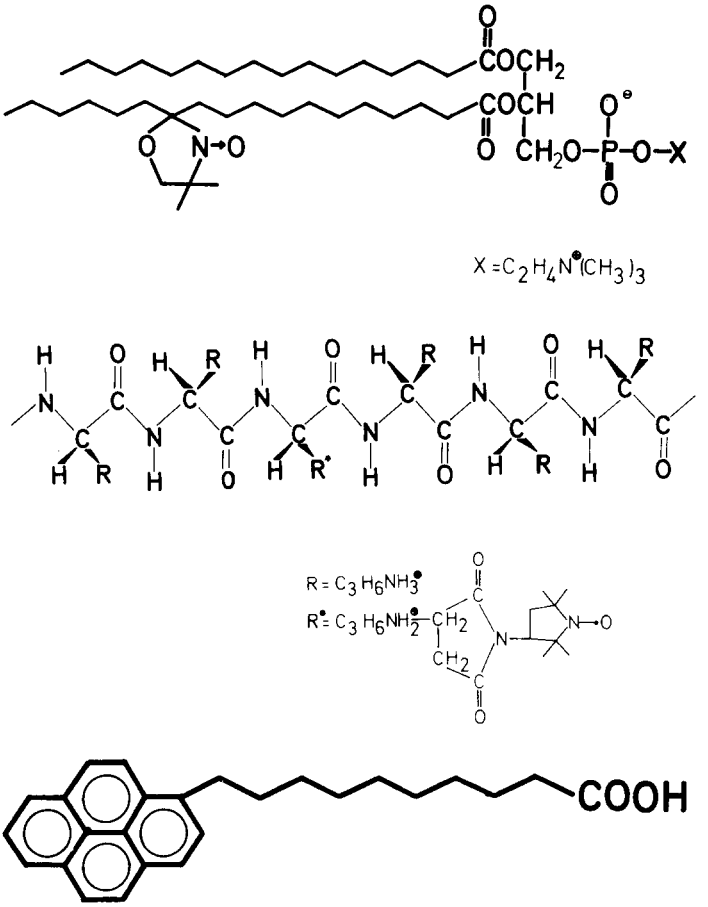


Fig. 1. Molecular structure of labels used to probe the domain formation of charged lipids upon binding to external charges. (I) 1-Palmitoyl-2-stearoyl-phosphatidylcholine labelled with a nitroxid radical in position 12 of the C_{18}-chain. (II) Part of a polylysine spin label in the protonated form at pH 9.0. The ratio of labelled to unlabelled lysine groups is 1 : 30. (III) An excimer-forming probe molecule: pyrenedecanoic acid.

Spin-labelled phosphatidylcholine (Fig. 1, I) was synthesized by acylating 1-palmitoyl lysophosphatidylcholine from Fluka with spin-labelled stearic acid anhydride according to the method of Hubbell and McConnell [13]. Synthesis of the 4,4'-dimethyl oxazolidine-*N*-oxyl derivative of 12-keto stearic acid was performed in our own laboratory. Polylysine has been labelled following the method of Stone et al. [14]: Solid *N*-(1-oxyl)-2,2,5,5-tetramethyl pyrolidinyl)-maleimide (9.5 mg) was added to a solution of 42 mg polylysine (mol. wt. 30 000) in 1 ml phosphate buffer (0.1 M, pH 7.4) at 0°C. After stirring for 2 h at 0°C and another 10 h at 5°C the solution was dialyzed against water. From the observed resonance intensities it is estimated that there are five label groups attached to each polypeptide molecule. Pyrene decanoic acid has been synthesized in our laboratory.

Preparation of vesicles. (a) Bilayer vesicles for EPR and optical measurements were prepared by sonicating a film containing both the lipids and the

label molecules. Sonication time was about 5 min at a temperature above the phase transition. The final lipid concentration was 1 mg/ml. The preparative procedures were performed under a nitrogen atmosphere. All vesicle preparations containing charged lipids were dispersed in a 0.01 M sodium borate buffer adjusted to pH 9.0. Samples containing only phosphatidylcholines were prepared in a 2 mM solution of CsCl. After addition of Ca^{2+} or polylysine the samples were again sonicated for 1 min.

(b) Giant vesicles for electron microscopy have been prepared by the method of Reeves and Dowben [16] following a description given elsewhere [19]. The final lipid concentration was $3 \cdot 10^{-4}$ M. The external charges (e.g. Ca^{2+} or polylysine) were added after preparation. Therefore, they could attach only to the outer monolayer.

Spin label method. ESR spectra were taken using a Bruker spectrometer equipped with a B-NC 12 computer. The samples were sealed in capillaries with an inner diameter of about 1.1 mm and recorded at the given temperature controlled to an accuracy of 0.2°C. The spin label method has been used previously to study the lipid organization in mixed membranes containing steroids [8] or charged phospholipids [10]. The line shape of the EPR spectra was determined both by dipole-dipole and by spin-exchange interaction of the radical molecules. The so-called exchange frequency, W_{ex}, is a direct measure of the degree of interaction of the labelled lipids. Analysis of the EPR spectra has been performed by computer simulation of their line shapes (for a recent review see ref. 15).

Fluorescence method. Pyrene and pyrene decanoic acid have been used to examine the lateral diffusion coefficient [17,18] and phase separation phenomena [11]. The method is based on the formation of excited complexes (PP*) (excimers) between molecules in the ground state (P) and in the first excited singlet state (P*). The monomer fluorescence I is well separated from the excimer emission I'. In the fluid state of the membrane the excimer forming process is diffusion controlled. The intensity ratio I'/I is a direct measure of the collision rate k_a and can therefore be used to calculate the diffusion coefficient of the probe molecules in the membrane. Going from a fluid to a more rigid state of the lipid layers (e.g. by decreasing the temperature or adding Ca^{2+} or polylysine to charged lipids) the probe molecules are squeezed out of rigidified regions. An increase in the intensity ratio I'/I is observed going through the phase transition temperature. If rigidified regions coexist beside more fluid regions, the phase transition of both will be observable. The ratio of the amplitude of each single phase transition detected by plotting I'/I against temperature is a direct measure of the areas of rigidified to fluid regions. Therefore, excimer probes are useful for the quantitative investigation of lateral phase separation in membranes containing regions of different microviscosities.

By measuring the excimer and monomer intensities simultaneous on a Schoeffel fluorescence spectrometer, we could record I'/I continuously as a function of temperature by analog computation.

Electron microscopy technique. Giant vesicles were prepared as described above and concentrated by centrifugation. The vesicles were freeze-etched and platinum shadowed on a Balzers freeze-etching device.

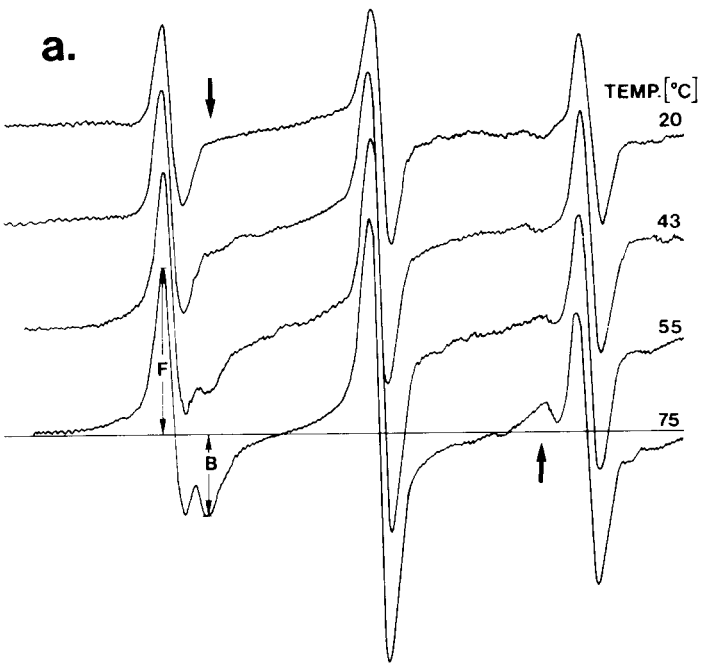
The freeze-etching technique is a well established technique for investigating

the surface curvature of model membrane systems [19,20,22,23]. Domain formation in the plane of the membrane leads to elastic stresses. Therefore, addition of polylysine to mixed membranes containing charged and uncharged components should induce a domain-like undulation of the surface curvature as a consequence of the chemically-induced phase separation.

Results

Binding of polylysine to phosphatidic acid membranes

At pH 9.0 polylysine is reported to bind one-to-one to phosphatidic acid with every second lysine group being bound to the charged vesicle [11]. This finding was confirmed by our EPR data using spin-labelled poly-L-lysine of molecular weight 30 000 containing five label groups per polypeptide chain. The EPR spectra of this polypeptide label added to dipalmitoyl phosphatidic acid vesicles are shown in Figs. 2a—2c. At 20°C a sharp spectrum similar to the label spectrum in buffered solution at pH 9.0 dominates. The hyperfine splitting constant of $a_H = 16$ Oe and the rotational correlation time of $\tau_c = 3.2 \cdot 10^{-10}$ s are calculated following the method of Stone et al. [14] and found to be in good agreement with their values. These data are characteristic for polylysine in a random coil configuration (upper spectrum in Fig. 2d). This sharp spectrum is superimposed on a broad spectrum (dotted line in Fig. 2b) that must be attributed to lysine bound to phosphatidic acid. The line shape is characteristic for an immobilized spin label group. Increasing the temperature increases the mobility of the bound polypeptide side chains. Therefore, the spectrum of bound label groups becomes sharper and separates well from that of the groups in solution. This is clearly seen in Figs. 2a and 2b



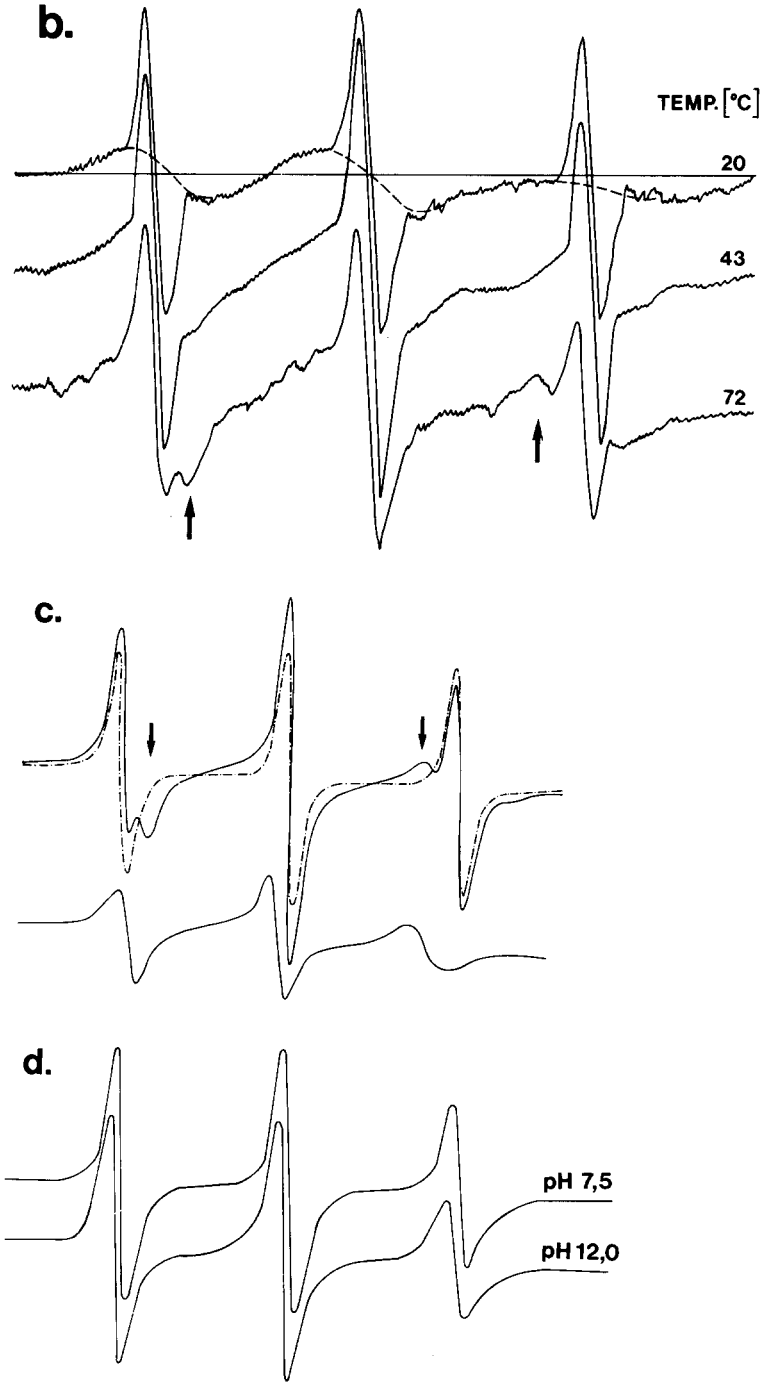


Fig. 2. (a) EPR spectra of spin-labelled polylysine added to dipalmitoyl phosphatidic acid membranes in a 1 : 4 molar ratio of lysine groups to charged lipid molecules. The spectrum attached to bound lysine groups appears at well separated peaks (see arrows). (b) The concentration of lysine groups related to phosphatidic acid molecules is increased to a 1 : 2 molar ratio. The immobilized spectrum (dashed line at 20° C) is seen much more clearly. (c) The experimental spectrum taken at 75° C given in (a) (———, in the upper part of the figure) is opposed to the solution spectrum (- - - - -, free labelled lysine groups). The difference spectrum (lower one of c) is obtained by subtracting the sharp component from the experimental spectrum. (d) Solution spectra of spin-labelled polylysine. At pH 7.5 polylysine is considered to be in a random coil configuration. Upon increasing the pH to 12.0 a transition to an α-helix is obtained.

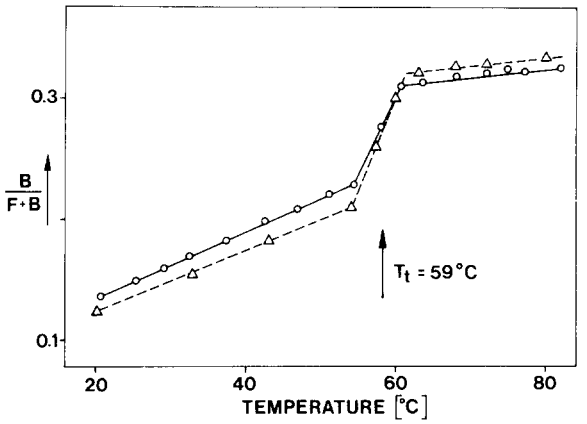


Fig. 3. The intensity ratio $B/(F + B)$ projecting the bound proportion of the polylysine label (e.g. arrows in Fig. 2a) is used as a parameter indicating the lipid phase transition.

at temperatures above the lipid phase transition. Separation of the immobilized spectrum by spectral subtraction leads to an EPR spectrum with a hyperfine coupling constant of $a_H = 14.1$ Oe. Disregarding the anisotropy of this spectrum a corresponding correlation time $\tau_c = 1.3 \cdot 10^{-9}$ s can be calculated. This value differs strongly from $\tau_c = 6.5 \cdot 10^{-10}$ s obtained from the spectrum at pH 12.0 (Fig. 2d) corresponding to polylysine in a helix coil conformation. The difference spectrum is shown in Fig. 2c. Comparing the areas of the integrated signals arising from bound and free labelled lysine groups, we found that half of the lysine groups are attached to the phosphatidic acid membrane. This is also valid at lower temperatures within the limits of the uncertainty arising from the broadness of the underlying immobilized spectrum. The polypeptide label is also sensitive to the phase transition of the lipid matrix. In Fig. 3 the ratio $B/(F + B)$ is plotted against temperature. B and F are the signal intensities of the bound and free lysine groups *, respectively (e.g. Fig. 2a). Due to signal overlapping this ratio is only a qualitative measure for the lysine binding.

A phase transition temperature of 60°C is observed for a 1 : 4 and a 1 : 2 addition of polylysine. This is consistent with the phase transition temperature of polylysine-bound phosphatidic acid determined by the fluorescence method.

EPR spectra of spin-labelled phosphatidylcholine incorporated in phosphatidylcholine and phosphatidic acid membranes

The effect of polylysine compared to that of Ca^{2+}. A characteristic EPR spectrum of spin-labelled phosphatidylcholine incorporated into a dipalmitoyl phosphatidylcholine matrix is shown in Fig. 4a. Even at the given concentration (20 mol % labelled molecules) a sharp three line spectrum is observed at 59°C, which is above the lipid phase transition temperature. The spectrum is only slightly broadened, which is characteristic for a randomized distribution

* The molar ratio of bound to free lysine groups calculated from the areas under the integrated signals is about 0.5.

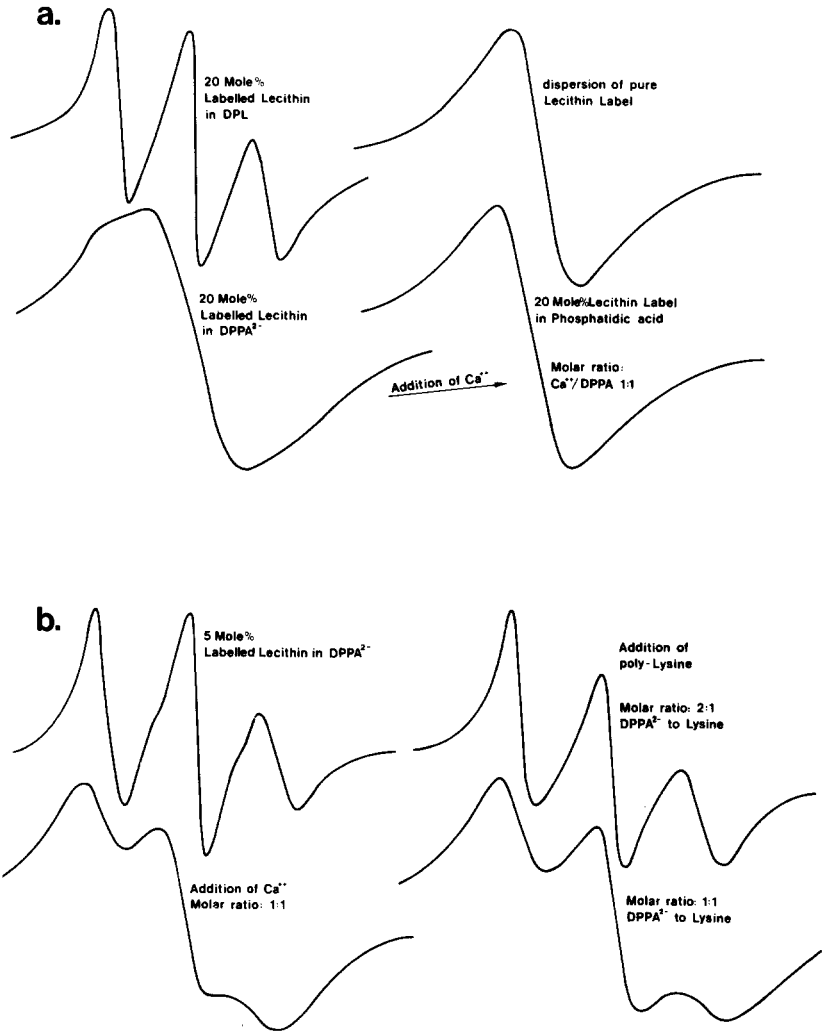


Fig. 4. (a) The EPR spectra of phosphatidic acid and phosphatidylcholine membranes containing both 20 mol% spin-labelled phosphatidylcholine are opposed. Completely different spectra are obtained indicating a random distribution of labelled lipid in a phosphatidylcholine membrane and a phase separation in a phosphatidic acid membrane. Upon addition of Ca^{2+} in equimolar ratio to the charged lipid the resulting spectrum resembles that one of a pure phosphatidylcholine label membrane. (b) The spectrum of a phosphatidic acid membrane containing 5 mol% phosphatidylcholine label is shown. Both addition of Ca^{2+} and polylysine leads to a broadened spectrum indicating an increase of the extent of the lipid segregation. DPL, dipalmitoyl phosphatidylcholine; DPPA, dipalmitoyl phosphatic acid.

of the two lipid components. This is shown much more clearly in Fig. 5a, where the exchange frequency W_{ex} is plotted as a function of the label concentration. A straight line indicating a diffusion-controlled interaction process is obtained.

A completely different spectrum is given in Fig. 4a for a phosphatidic acid membrane again containing 20% spin-labelled phosphatidylcholine. The three lines are nearly 'smeared out' by exchange interaction. The exchange frequency $W_{ex} = 10$ MHz is larger by a factor of 2.5 than in dipalmitoyl phosphatidyl-

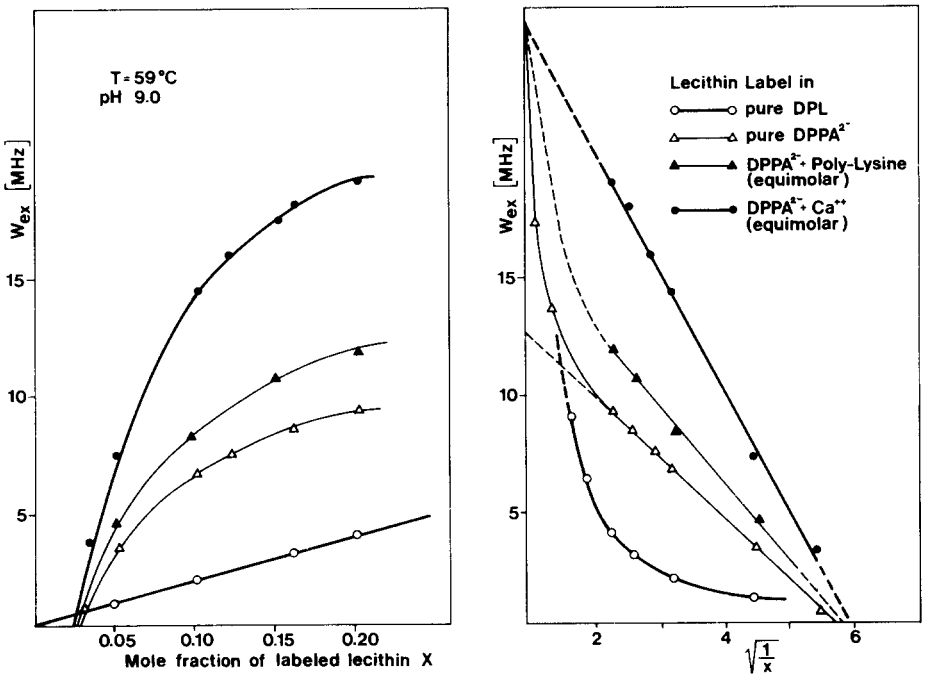


Fig. 5. (a) Concentration dependence of the exchange frequency W_{ex} calculated by computer simulation of the EPR spectra of labelled phosphatidylcholine in lipid membranes of different surface properties. ○———○, Dipalmitoyl phosphatidylcholine (DPL) membrane; △———△, dipalmitoyl phosphatidic acid (DPPA) membrane in the absence of external charges and (●———●) upon addition of Ca^{2+} (▲———▲) or polylysine. (b) The exchange frequency W_{ex} is plotted against the square root of the inverse molar fraction of labelled phosphatidylcholine.

choline (W_{ex} = 4 MHz). Plotting W_{ex} as function of label concentration does not give a straight line (Fig. 5a), indicating a non-random lipid distribution. Addition of Ca^{2+} to a phosphatidic acid membrane containing spin-labeled phosphatidylcholine at pH 9 increases the extent of the lateral phase separation [10]. The EPR spectrum is converted into a one line spectrum due to an increase in the exchange interaction. This spectrum is very similar to that obtained from vesicles of pure phosphatidylcholine label. A comparison between the effects of Ca^{2+} and polylysine is given in Fig. 4b. The EPR spectrum of a phosphatidic acid preparation containing 5 mol% phosphatidylcholine label is presented. Addition of Ca^{2+} in a concentration equimolar to that of the phosphatidic acid leads to a broadened EPR spectrum. The exchange frequency increases from W_{ex} = 3 MHz in the absence of Ca^{2+} to W_{ex} = 7.5 MHz. A comparable increase is observed upon addition of polylysine. For a concentration of lysine groups equimolar to that of phosphatidic acid molecules an exchange frequency of 4.8 MHz is obtained. The change in the line shape of the corresponding EPR spectrum is shown in Fig. 4b for two lysine concentrations. A large increase in the exchange interaction is observed even at a 1 : 2 molar ratio of lysine groups to charged lipid molecules. The change in the EPR line shape upon addition of external charges is an indication of a chemically-induced phase separation. This has also been demonstrated

by Ito and Ohnishi [24,25] in a mixed phosphatidylserine/phosphatidylcholine system.

Quantitative analysis of the EPR spectra in terms of the exchange frequency. Lipid segregation in membranes containing a spin-labelled lipid can be detected very sensitively by plotting the exchange frequency W_{ex} of the spin probe as a function of the mol fraction of labelled component [8]. A linear function characteristic for a random distribution is found for a dipalmitoyl phosphatidylcholine mixture of phosphatidylcholine label in dipalmitoyl phosphatidylcholine (Fig. 5a). A completely different behaviour is observed for phosphatidylcholine label in phosphatidic acid membranes, even in the absence of external charges. W_{ex} is no longer proportional to the molar fraction, X_L, of labelled molecules but to $\sqrt{1/X_L}$. as described in refs. 8 and 10, this is characteristic for the formation of domains of the labelled component. If the concentration of labelled phosphatidylcholine exceeds 20 mol % the predicted upward deflection (see ref. 10) is observed. This is characteristic for the formation of pure phosphatidylcholine clusters (more than 20% label), in addition to clusters containing both phosphatidylcholine and phosphatidic acid (less than 20 mol % label). The clusters are considered to be embedded in a phosphatidic acid matrix. For a 1 : 1 addition of Ca^{2+} a straight line is obtained which could be extrapolated to W_{ex}^0 = 27 MHz for X_L = 1. This value agrees very well with the value W_{ex} = 30 MHz for membranes of pure phosphatidylcholine label, demonstrating that pure phosphatidylcholine domains are obtained upon equimolar addition of Ca^{2+} to mixed phosphatidic acid/phosphatidylcholine membranes. Addition of polylysine leads to a straight line with

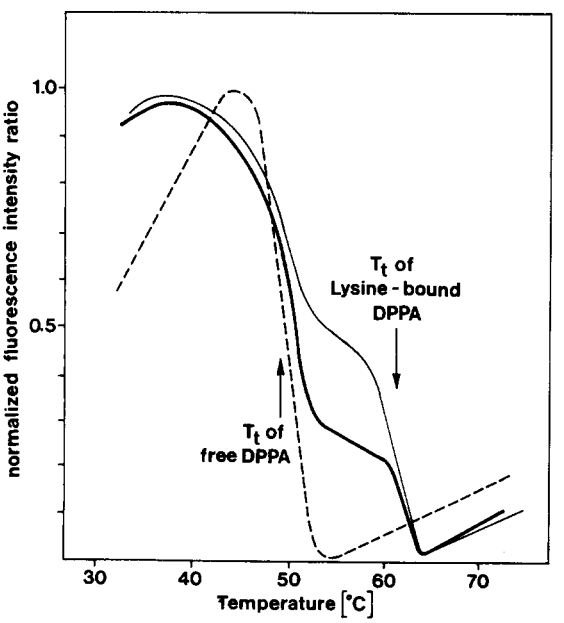


Fig. 6. Temperature dependence of the excimer to monomer ratio I'/I of pyrene decanoic acid incorporated into phosphatidic acid membranes at pH 9.0. ------, Pure dipalmitoyl phosphatidic acid (DDPA); ———, addition of polylysine in a 1 : 2 molar ratio; ———, addition of polylysine in a 1 : 1 molar ratio.

a somewhat smaller slope extrapolating to about 15 MHz. This can be explained by the lower number of charges available at the membrane surface, meaning that not all lysine groups are bound to the phosphatidic acid. Again, an upward deflection is obtained for concentrations of more than 20 mol % labelled phosphatidylcholine. The two cluster model of Galla and Sackmann [18] can be applied indicating that non-bound phosphatidic acid forms clusters mixed together with phosphatidylcholine. Therefore, a lower exchange interaction is expected in comparison to pure phosphatidylcholine label clusters that are obtained with an equimolar Ca^{2+} addition.

Change in transition temperature of phosphatidic acid and phase separation upon addition of polylysine. The ratio of the excimer to monomer emission of pyrene decanoic acid in dipalmitoyl phosphatidic acid vesicles (probe concentration 3%) shows a sharp decrease at 50°C indicating the lipid phase transition. Addition of polylysine leads to a splitting of the transition region into two steps. Part of the lipid now exhibits a phase transition at T_t = 62°C, which is attributed to membrane regions bound to polylysine. The amplitude of the normalized phase transition curve given in Fig. 6 is a direct measure of the fraction of bound lipid. The samples have been sonicated after peptide addition. Therefore, both the inner and the outer layer were accessible to the charges. For a 1 : 1 ratio of lysine groups to phosphatidic acid the two steps of the phase transition curve indicating bound and non-bound phosphatidic acid are of equal height indicating that half of the lipid is bound. Resulting from an equimolar ratio of lysine groups, this means that every second lysine group is involved in binding. This is in good agreement with our EPR results using spin-labelled polylysine.

Electron microscopy: Change in surface curvature brought about by polylysine and Ca^{2+}

From our EPR and fluorescence measurements we made the following conclusions: (a) Polylysine [26] and Ca^{2+} bind strongly to negatively charged membranes. (b) Addition of external charges to mixed membranes containing both charged and uncharged lipids leads to a chemically-induced phase separation. (c) Each monolayer exhibits a mosaic-like pattern containing rigid domains of bound phosphatidic acid beside more fluid patches composed of phosphatidylcholine and free phosphatidic acid. The results from the spectroscopic studies were directly verified by freeze-etching electron microscopy.

Addition of polylysine to mixtures of dioleyl phosphatidylcholine and dioleyl phosphatidic acid

The effect of adding polylysine to giant vesicles containing phosphatidylcholine and phosphatidic acid is demonstrated in Fig. 7. The membrane is cut through the plane of the bilayer. A number of circular domains are obtained. These differ in their curvature from the rest of the membrane, since only differences in the surface are visualized by the freeze-etching procedure. Giant vesicles from the same preparation in the absence of the peptide show a completely smooth membrane surface. All preparations were quenched from room temperature, which for the oleyl derivatives is well above the lipid phase transition temperature.

Furthermore, in Fig. 7 the influence of the concentration of the charged lipid and the peptide is demonstrated. The number of domains decreases with decreasing content of charged lipid (phosphatidic acid) and with decreasing amount of polylysine. The size of the domains, however, seems to remain constant. In light of these relationships the circular plates standing out of the plane of the membrane have to be attributed to phosphatidic acid bound to polylysine. The average size of the domains varies continuously along the direction of shadowing. This is expected for a curved surface of the vesicle itself for geometrical reasons. An estimation of the cluster size is only possible in the region near the top of the vesicles.

We estimated the average domain size from several vesicle preparations differing in the amount of charged lipids as well as in the content of polylysine. Characteristic diameters of about 120, 220, 330, 460 and 600 Å are measured with an uncertainity of about ±10 Å. The smallest clusters were between 100 and 150 Å in diameter. These were not so well defined because of the difficulty in distinguishing between the domain boundary and the shadow of the protruding domains.

The direction of the curvatures is the same as the direction of the shadow. In the outer monolayer the domains are curved to the outside, whereas in the inner monolayer the domains are curved to the inside of the vesicle. However, polylysine was added after the preparation of the vesicles and could therefore only attach to the outer monolayer. From this finding we have to conclude that formation of domains in the outer layer is affecting the inner layer in a similar way. This is very clear in Figs. 7b and 7c, where larger parts of the outer layer are to be seen.

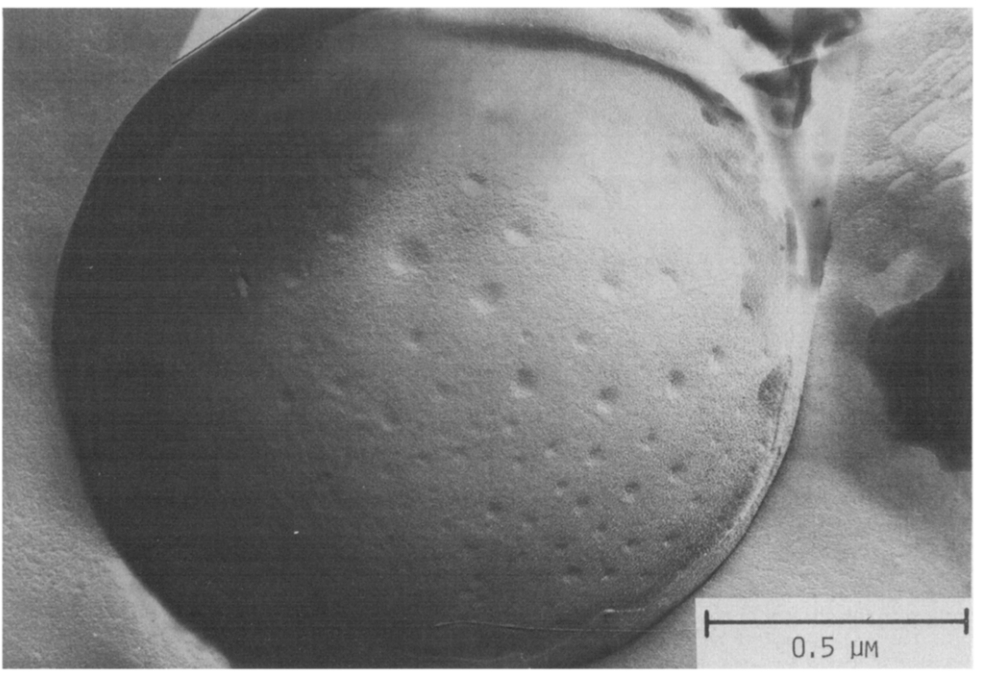


Fig. 7a. For legend see next page.

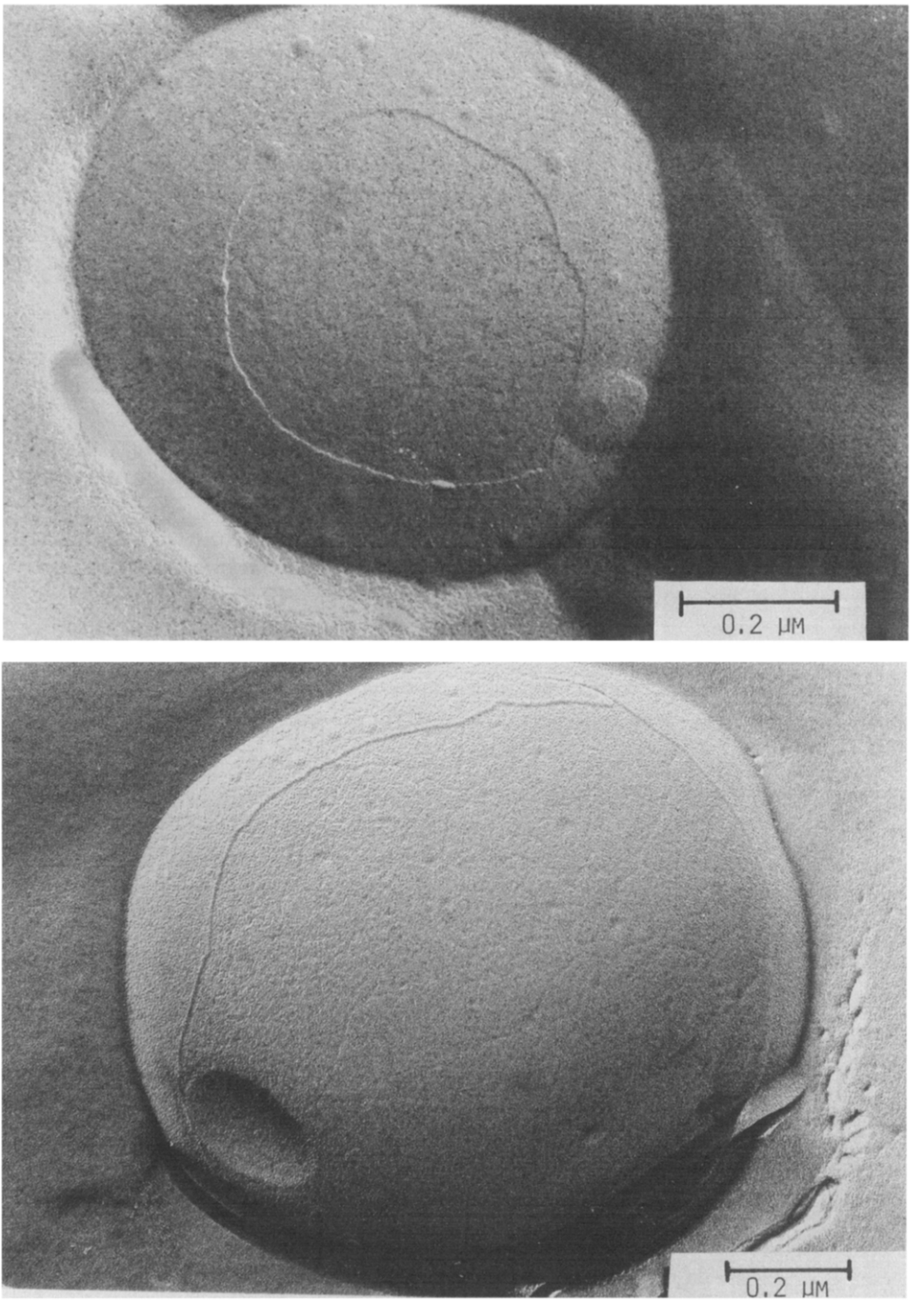


Fig. 7. (a) Electron micrograph of a mixed giant vesicle containing dioleyl phosphatidylcholine and dioleyl phosphatidic acid in equimolar proportions after addition of polylysine (molar ratio 1 : 2 according to the charged lipid). Magnification: approx. X72 000. (b) Electron micrograph of a mixed giant vesicle that is composed of 70 mol% dioleyl phosphatidylcholine and 30 mol% dioleyl phosphatidic acid. Polylysine is added to come to 50% of the charged lipid. Magnification: approx. X112 000. (c) Same vesicle preparation as above but the polylysine content is reduced to 20% according to the phosphatidic acid component. Magnification approx. 88 000X.

Discussion

An interesting question is the conformation of membrane-bound polylysine. At pH 9 free polylysine is in a random coil configuration. From circular dichroism measurements Hammes and Shullerey [21] concluded that polylysine is, to a large extent, transformed from a random coil to an ordered configuration upon binding to phosphatidylserine membranes. This might be an α-helix or a configuration partially ordered in such a way that a maximum number of lysine groups can bind to the membrane surface. According to our results, the second type of conformation is the one present. From the EPR spectra of spin-labelled polylysine dissolved in a buffer solution at pH 9.0 we obtained a correlation time of $\tau_r = 3.2 \cdot 10^{-10}$ s for the tumbling motion of the labelled groups. This value corresponds to the charged peptide in a random coil configuration [14]. Upon changing the pH to 11.0 (i.e., above the pK value of the lysine groups) where the peptide is not protonated, a change in the correlation time to $\tau_h = 6.5 \cdot 10^{-10}$ s is found. This is brought about by the transition from a random-coil to a helix-coil configuration.

Labelled polylysine added to a charged phosphatidic acid membrane at pH 9.0 exhibits a new spectral component with a hyperfine coupling constant of $a_H = 14.1$ Oe ($a_H = 16$ Oe for polylysine in buffer solution). The corresponding value of the correlation time is estimated to be about $\tau_b = 1.3 \cdot 10^{-9}$ s at 70°C. Due to the larger value of τ we attribute this spectral component to the bound polylysine. The decrease in the hyperfine coupling constant is characteristic for a less polar environment. This indicates that the label group 'dips into' the membrane. Because of this change in line position it is possible to distinguish between free and bound parts of the peptide chain. In addition, the relative height of the bound spectrum is affected by the fluidity of the lipid matrix. The temperature dependence of this ratio reflects the lipid phase transition of the bound phosphatidic acid.

From the area under the EPR spectral lines corresponding to bound and non-bound polypeptide we estimated that half of the lysine groups are bound to the charged lipids. This has also been confirmed by our fluorescence experiments. There we could observe the appearance of a new phase transition at higher temperatures due to phosphatidic acid bound to peptide. The amplitudes of the phase transition of free and bound lipid are equal for an equimolar ratio of

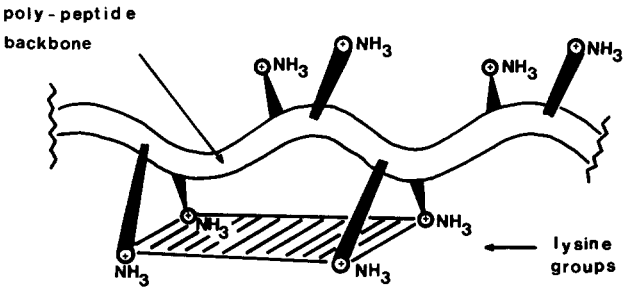


Fig. 8. Configuration of polylysine supposed by our experimental results. Half of the lysine groups are oriented to the membrane surface whilst the other half is extended into the water phase.

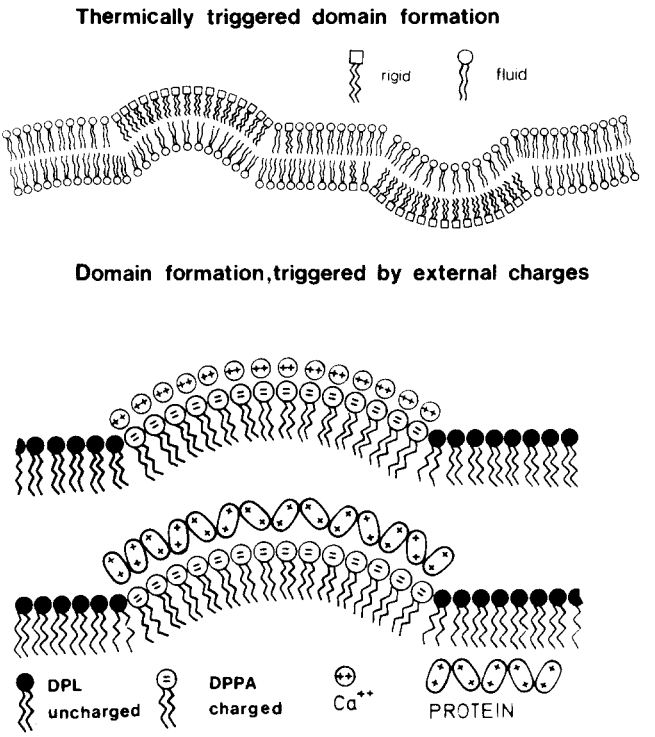


Fig. 9. Schematic presentation of a phase separation followed by a change in the surface curvature of the membrane as visualized by electron microscopy. (a) A domain formation may be triggered thermally if one component undergoes a transition to a structural state that differ in its symmetry from the second component. (b) External charges may trigger a phase separation even if the two components are of the same symmetry but differ in their surface charge. DPL, Dipalmitoyl phosphatidylcholine; DPPA, dipalmitoyl phosphatic acid.

peptide to charged lipid. This indicates that only half of the peptide side chains are involved in the binding process.

Judging from the molecular model of polylysine (shown in Fig. 8) the area covered by a pair and the next but one pair of lysine groups is found to be 140 $Å^2$. The molecular weight of the bromide salt of polylysine used was 30 000 corresponding to about 150 positively charged groups per polypeptide. Assume that every second lysine group binds to the negatively charged membrane surface according to the model given in Fig. 9, then each macromolecule covers an area of about 5000 $Å^2$ containing 75 binding sites for negatively charged lipids. The smallest domains observed in the electron micrograph given in Fig. 6 have a diameter of about 100 Å corresponding to an area of 7800 $Å^2$. This is an upper limit of the domain size, since both the domain and its shadow were taken into account. The smallest domains observed thus correspond to the binding of one macromolecule in a configuration where every second lysine group extends into the aqueous phase. These groups induce coagulation of small vesicles to some extent. The size of the domains increases in a stepwise manner related to multiples of the area covered by one polylysine molecule. No differences in the cluster diameters are obtained by varying the lysine concentration or the lipid composition.

Obviously, the domain dimensions are influenced only by the size of the domain-forming agent.

Our spectroscopic experiments combined with electron microscopy lead us to the conclusion that polylysine may be transformed from a random coil to a partially ordered configuration upon binding to phosphatidic acid. This configuration should not be an α-helix. In an α-helix configuration only every 7th lysine group could attach to the membrane surface. This is not consistent with our spectroscopic findings. Moreover, we found circular clusters induced by polylysine in the electron micrographs. Since an α-helix configuration leads to a rod-like molecular shape, one would expect to see elongated clusters in this case. From the sharpness of the phase transitions in the fluorescence study large cooperative units have to be postulated.

Domain formation was demonstrated by a more direct spectroscopic approach using spin-labelled phosphatidylcholine. In a mixture with phosphatidic acid phosphatidylcholine is squeezed out of the polylysine · phosphatidic acid complexes. This is recorded by an increase in the magnetic interactions between the labelled phosphatidylcholine molecules. In comparison with our previous study with Ca^{2+} [10] a smaller value for the spin exchange frequency is observed here. This means that the phase separation is less pronounced. The spacing of the phosphatidic acid molecules in the peptide complex is mainly determined by the distance of the peptide side chains. The packing density in the phosphatidic acid · lysine complex is smaller than the corresponding complex induced by Ca^{2+}. Therefore, better solubility of phosphatidylcholine in the lysine-induced domains is expected.

Domain structures of lipid alloys have to be considered as a mestastable state of the membrane [27]. The stability of this domain pattern has been explained in terms of a balance between a chemical and an elastic force field. The first type of force tries to increase the domain size to infinity while the elastic interfacial energy yields a restoring force tending to minimize the domain size [19].

A lateral phase separation may be induced in two different ways, as demonstrated in Fig. 9.

(1) A thermally-induced lipid segregation is obtained if the two types of lipids are in structural states of different symmetries [9,19]. This is symbolized in Fig. 9 for a lipid mixture containing a component A in a smectic B_A phase (non-tilted, quasi-crystalline state) and a component B in a smectic A phase (non-tilted but the hydrocarbon chains are melted). The phase separation is followed by a change in the spontaneous curvature of the membrane.

(2) A chemically-induced phase segregation may be induced by external charges if one lipid component is charged while the second one is in a quasi-neutral (i.e., zwitterionic) state. Again a difference in the spontaneous curvature of the membrane is obtained.

This theoretically predicted consequence of peptide binding to the membrane surface could be demonstrated by electron microscopy.

The present study gives information on a simultaneous change in peptide conformation and rearrangement of the lipid matrix. In this way sterical conditions for electrostatic binding between extrinsic peptides and charged lipids could be obtained.

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