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Perturbation of binary phospholipid mixtures by melittin: A fluorescence and Raman spectroscopy study

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The effect of melittin on different binary mixtures of phospholipids has been studied by polarization of DPH fluorescence in order to determine if melittin can induce phase separation. Since the interaction between lipids and melittin is sensitive to both electrostatic and hydrophobic forces, we have studied the effect of the acyl chain length and of the polar head group of the lipids. In spite of the difference of the chain length between dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine (DSPC), no phase separation occurs in an equimolar mixture of these lipids in presence of melittin. However, when the charged lipid dipalmitoylphosphatidylglycerol (DPPG) is mixed with either DPPC or DSPC, the addition of melittin leads to phase separation. The DSPC/DPPG/melittin system, which shows a very complex thermotropism, has also been studied by Raman spectroscopy using DPPG with deuteriated chains in order to monitor each lipid independently. The results suggest that the higher affinity of melittin for DPPG leads to a partial phase separation. We propose the formation of DPPG-rich domains perturbed by melittin and peptide-free regions enriched in DSPC triggered by the head group charge and chain-length differences.

Introduction

Biological membranes are complex lipid mixtures in which proteins are embedded. It is still not clear if the lipid distribution around the proteins is modulated by specific interactions between certain types of lipids and the proteins. The specificity of lipid–protein interactions and phase separation induced by proteins may play an important role in cellular biology [1] and could explain a specific requirement of the lipid composition of the membrane, for the enzymatic activity for example. The goal of this study is to determine to what extent a different affinity between a protein and lipids could lead to the formation of heterogeneities.

The first systems on which such a phase separation has been observed were binary mixtures of a zwitterionic and an acidic lipids in the presence of divalent cations such as Ca^{2+} or Mg^{2+} [2–5]. Even though the electrostatic interaction between the polar head groups and the cations is the driving force for the segregation, some results have shown that the size of the polar head [3] and the lipid chain length [5] also influence these demixings. In a recent paper, Devaux and Seigneuret [1] reviewed the literature regarding phase separations induced by proteins. Results obtained by several techniques have shown that some proteins bind preferentially to a specific type of lipids and may induce the formation of inhomogeneities in the bilayer.

In order to improve the understanding of the factors which govern the specificity of lipid–protein interactions and the phase separation phenomenon, we have studied a model system composed of binary mixtures of phospholipids and melittin, a small basic peptide well known for its interaction with phospholipids [6–8]. Since melittin has a net positive charge at physiological pH, it shows a higher affinity for anionic than for zwitterionic lipids. For example, phosphatidylserine vesicles can bind up to 8 times more melittin than phosphatidylcholine

Abbreviations: DPH, 1,6-diphenyl-1,3,5-hexatriene; DPPC, dipalmitoylphosphatidylcholine; EDTA, ethylenediaminetetraacetic acid; T_m, gel to liquid crystalline phase transition temperature; R_i, lipid to peptide incubation molar ratio; P, fluorescence polarization ratio; DSPC, distearoylphosphatidylcholine; DPPG, dipalmitoylphosphatidylglycerol; DMPC, dimyristoylphosphatidylcholine; DMPG, dimyristoylphosphatidylglycerol; PS, phosphatidylserine.

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vesicles [9]. On the other hand, the long N-terminal segment of the toxin (residues 1 to 20), which is largely composed of apolar residues, can form an amphipatic α helix and consequently interact with the apolar region of the bilayer. The importance of the hydrophobic interaction is shown by the stronger binding of melittin to phosphatidylcholine bilayers in the fluid phase than in the gel phase [7], and by the greater perturbation induced by the toxin on bilayers composed of phospholipids with shorter chains [8,10].

The thermotropism of complexes of melittin with mixtures of lipids with different polar head groups and chain lengths was first studied by fluorescence spectroscopy using 1,6-diphenyl-1,3,5-hexatriene (DPH) as an intrinsic probe. The degree of fluorescence polarization is sensitive to the dynamic of the probe environment and was used to highlight the role of various forces on the phase separation phenomenon. These fluorescence results suggest that melittin can induce a selective lipid demixing. To reinforce this interpretation, the effect of melittin on distearoylphosphatidylcholine (DSPC) and dipalmitoylphosphatidylglycerol (DPPG) mixtures has been studied by Raman spectroscopy using DPPG with perdeuteriated acyl chains. The usefulness of this intrinsic technique for the investigation of multicomponent systems has been put forward in several studies [11–14].

Materials and methods

Materials

All lipids used were purchased from Sigma Chemical (St. Louis, MO) except for DPPG that was obtained from Medmark (München, F.R.G.) or Avanti Polar Lipids (Birmingham, AL). DPPG with perdeuteriated chains (DPPG-d_{62}) was also purchased from Avanti Polar Lipids. Melittin was purified from bee venom (Sigma Chemical, St. Louis, MO) as described previously by Dasseux et al. [6], or obtained directly from Bachem Feinchemikalien (Bubendorf, Switzerland) and used without purification in the presence of EDTA to inhibit phospholipase activity.

Fluorescence measurements

Lipids were weighted to obtain equimolar mixtures, dissolved in benzene/methanol (98:2, v/v), lyophilized, and suspended in 20 mM phosphate buffer (pH 7.6) containing 1 mM EDTA, or 40 mM phosphate buffer (pH 7.6) containing 1 mM EDTA and 1.5 M NaCl for high ionic strength experiments. A few μl of the DPH solution in tetrahydrofuran ($6 \cdot 10^{-3}$ M) were added to obtain 0.75 mol% of the probe compared with total lipids, and samples were incubated at a temperature above their gel to liquid-crystalline phase transition (T_m). Aliquots of the stock solutions were diluted with the same phosphate buffer to obtain a final lipid concentration of $1.2 \cdot 10^{-4}$ M. Melittin in solution was then added to reach the desired melittin to lipid incubation molar ratio (R_i) relative to the total lipids. Lipid/melittin complexes were also incubated at $T > T_m$ for 90 min with several heating and cooling cycles through the transition temperature.

The degree of polarization of DPH fluorescence (P) was measured with a home built entirely computerized spectrometer described elsewhere [10]. The fluorescence intensity of the components with polarization parallel and perpendicular to that of the 360 nm excitation light were measured simultaneously at wavelength greater than 450 nm, and each data point is an average of 10 measurements. The temperature of the samples was controlled with thermoelectric heat pumps and the heating rate was 15C°/h.

Raman measurements

Appropriate amounts of DSPC and DPPG-d_{62} were weighted to obtain equimolar mixtures, dissolved in benzene/methanol (98:2, v/v), lyophilized, and suspended in 100 mM phosphate buffer (pH 7.6) containing 10 mM EDTA to yield a lipid concentration of 10% by weight or approx. 150 mM. Samples were then incubated at 10C° above T_m for 90 min with several heating and cooling cycles through the transition temperature, transferred in glass capillary tubes, and centrifuged in a hematocrit centrifuge. Complexes of DSPC/DPPC-d_{62} with melittin were prepared similarly except that the lyophilized lipids were hydrated with solutions of melittin in the same buffer to yield the desired incubation molar ratio relative to the total lipids.

Raman spectra were recorded with a computerized Spex Model 1400 double monochromator [15] with a spectral resolution of 5 cm^{-1} and an accuracy of ± 2 cm^{-1}. Samples were excited with the 514.5-nm line of a Spectra Physics Model 2020 argon ion laser, the laser power at the sample was approx. 150 mW. Capillaries containing the samples were placed in a thermoelectrically regulated sample holder [16] whose temperature was monitored at ± 0.2°C with a copper-constantan thermocouple. Spectra were recorded digitally with an integrating period of 2 s. For the C-^{2}H stretching mode region (1950–2350 cm^{-1}), the increment was 1 cm^{-1}, and spectra were seven-point-smoothed by using the algorithm of Savitski and Golay [17]. For the C-H stretching mode region, the increment was 2 cm^{-1}, and the spectra were corrected for the spectral contribution of the non-deuteriated head group of DPPG-d_{62} in the 2900 to 2960 cm^{-1} region using the C-^{2}H band of the deuteriated lipid as an internal intensity standard. Spectra of lipid/melittin complexes were also corrected for the melittin contribution using the tryptophan band of the toxin at 1010 cm^{-1} as described previously [8].

Results

DPPC/DSPC mixture

The effect of temperature on the degree of fluorescence polarization of DPH in an equimolar DPPC/DSPC mixture containing melittin at various R_i is shown in Figs. 1 and 2. At low ionic strength (Fig. 1), an augmentation of P with the concentration of melittin is observed in the fluid phase while in the gel phase a small decrease of this parameter is detected. The phase transition of the pure lipid mixture remains fairly cooperative and appears at an intermediate temperature between those of the pure lipid components. Up to $R_i = 5$, this transition is almost unaffected by the presence of melittin, but at $R_i = 1$ the transition broadens and shifts slightly toward low temperatures.

Since the thermotropism of pure phosphatidylcholine/melittin complexes is sensitive to the ionic strength [6], we have verified if complexes of melittin with an equimolar mixture of DPPC and DSPC display the same behavior. As for pure phosphatidylcholines, the effect of melittin at high ionic strength on this mixture

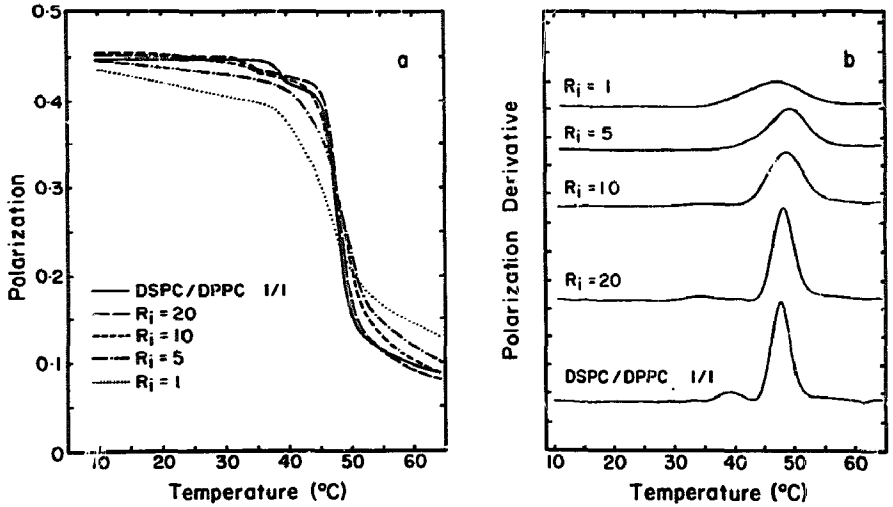

Fig. 1. (a) Effect of melittin on a DPPC/DSPC equimolar mixture followed by DPH fluorescence polarization at different lipid to peptide incubation molar ratios. 20 mM phosphate buffer (pH 7.6), 1 mM EDTA. (b) Derivatives of the curves shown in (a).

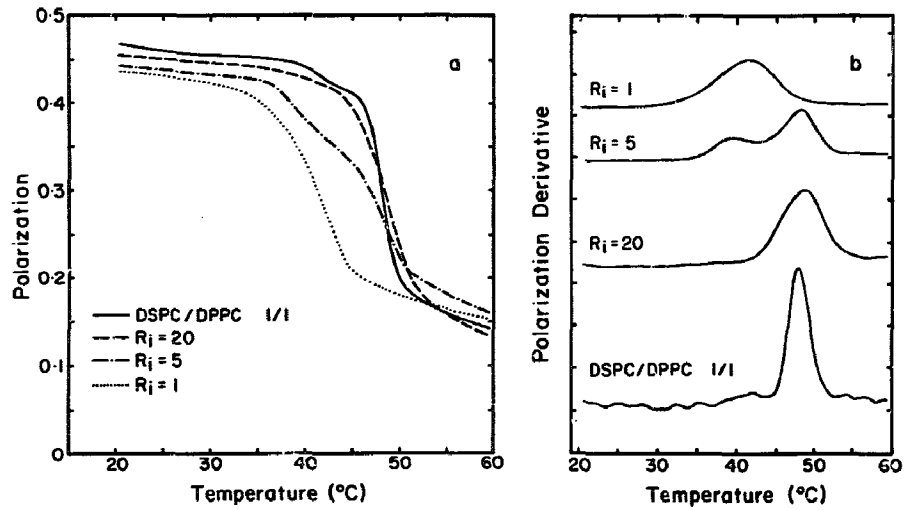

Fig. 2. (a) Effect of melittin on a DPPC/DSPC equimolar mixture followed by DPH fluorescence polarization at different lipid to peptide incubation molar ratios. 40 mM phosphate buffer (pH 7.6), 1 mM EDTA and 1.5 M NaCl. (b) Derivatives of the curves shown in (a).

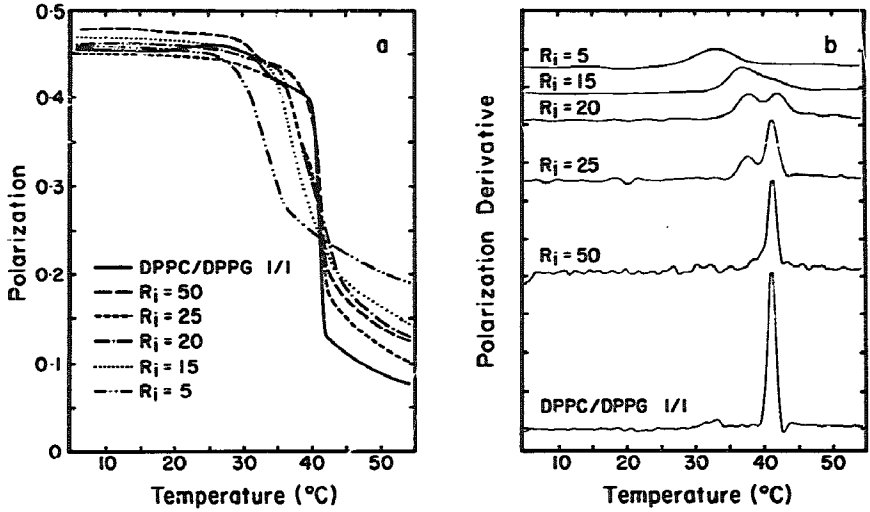

Fig. 3. (a) Effect of melittin on a DPPC/DPPG equimolar mixture followed by DPH fluorescence polarization at different lipid to peptide incubation molar ratios. 20 mM phosphate buffer (pH 7.6), 1 mM EDTA. (b) Derivatives of the curves shown in (a).

is more pronounced (Fig. 2). A large shift of the transition towards low temperature is induced by melittin (from 48°C to 42°C at $R_i = 1$ for example). In addition, at $R_i = 5$, two successive overlapping transitions are observed as previously detected for complexes of melittin with pure DPPC [6] and pure DSPC [18] at $R_i = 3.7$ and 5, respectively.

DPPC/DPPG mixture

Fig. 3 shows the effect of melittin on the thermotropic behavior of an equimolar DPPC/DPPG mixture. In this case, the role of the polar head group is highlighted. As we have observed for phosphatidylcholine/melittin complexes, the toxin induces a slight decrease of P in the gel phase. However, there is a much larger increase of P in the liquid-crystalline phase. For example, at 55°C, it equals 0.18 for the DPPC/DPPG/melittin complex at $R_i = 5$ while its value is around 0.10 for DPPC/melittin complex at the same R_i. The phase transition is also markedly affected by the toxin. At $15 < R_i < 25$, there are two distinct transitions at 41°C and around 37°C. As the amount of melittin increases,

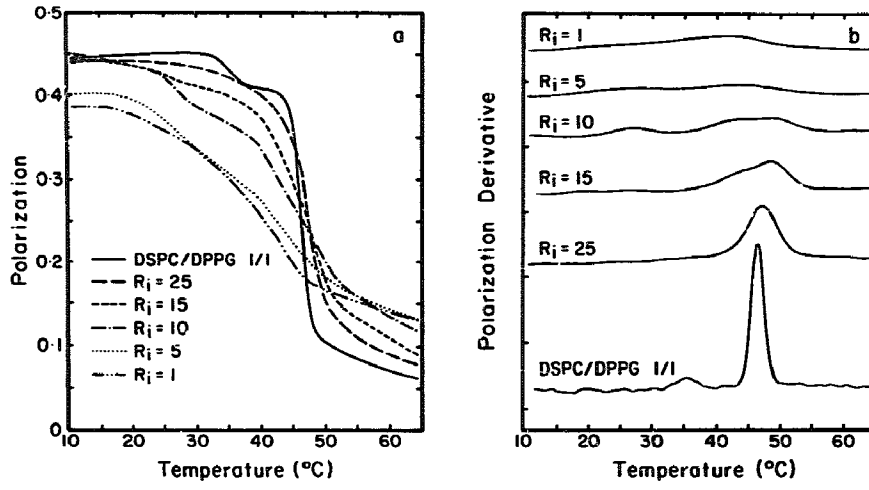

Fig. 4. (a) Effect of melittin on a DSPC/DPPG equimolar mixture followed by DPH fluorescence polarization at different lipid to peptide incubation molar ratios. 20 mM phosphate buffer (pH 7.6), 1 mM EDTA. (b) Derivatives of the curves shown in (a).

the amplitude of the transition at 41°C decreases progressively while that at low temperature increases. For the complex at $R_i = 5$, only the latter transition is detected but shifted down to 33°C. Therefore, as opposed to DPPC/DSPC mixtures, melittin seems efficient to induce phase separation in DPPC/DPPG mixtures.

DSPC/DPPG mixture

The thermotropic behavior of an equimolar DSPC/DPPG mixture in the presence of melittin is represented in Fig. 4. Despite the difference between the two lipids, they are completely miscible at equimolar concentration [5] and the mixture gives a sharp gel to fluid phase transition around 46°C, preceeded by a premelting at 35°C. When melittin is added at $R_i > 15$, there is an important loss of the cooperativity of the transition which gradually shifts towards high temperature. As the quantity of melittin increases, a new transition appears around 25°C and the main transition becomes biphasic with maxima around 43°C and 48°C. The high-temperature component decreases in amplitude as the quantity of melittin increases and is continuously shifted up to reach 49°C at $R_i = 10$. At $R_i \leq 5$, this transition is no longer detectable. Conversely, the relative amplitude of the transition around 44°C increases with the quantity of toxin. Finally, when the peptide is present in large amount, $R_i < 5$, one can observe a very broad transition that occurs over a 30 C° range.

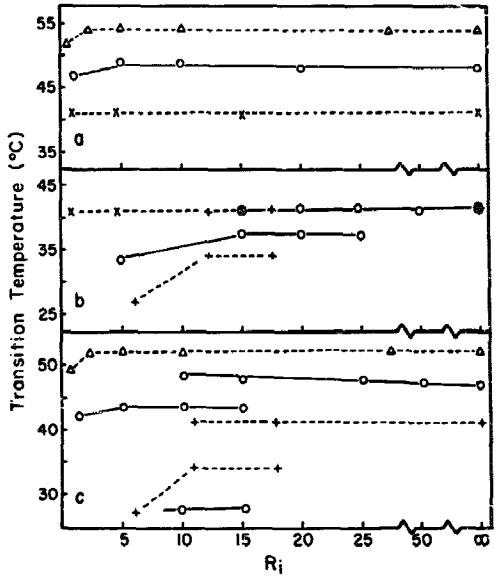

Fig. 5. Summary of the thermotropism behavior followed by DPH fluorescence polarization of (a) DPPC/DSPC, (b) DPPC/DPPG and (c) DSPC/DPPG mixtures (○) and each pure lipid component (DPPC (×), DSPC (△) and DPPG (+) (obtained from Bernard et al. (1982) and Dasseux et al. (1984)) in presence of melittin. 20 mM phosphate buffer, 1 mM EDTA, pH = 7.6.

Discussion

The effect of melittin at low ionic strength on the thermotropic behavior of various equimolar lipid mixtures and on pure lipid components determined by DPH fluorescence polarization is summarized in Fig. 5. The results obtained for the DPPC/DSPC mixture show that these lipids are completely miscible at equimolar concentration since only one cooperative transition is observed (Fig. 1). This is in agreement with previous studies showing that lateral demixing is observed only for phospholipids with a minimum chain length difference of four methylenes [5,19]. In the presence of melittin at low ionic strength, the progressive decrease of the cooperativity of the transition and the small shift of its mid-point temperature suggest that no lipid phase separation occurs. Hydrophobic interactions between melittin and phospholipids are important as revealed by the chain length dependence of the perturbation of phosphatidylcholine [8] or phosphatidylglycerol bilayers [10] by melittin. However, our results suggest that the difference of hydrophobic interactions between the toxin and the palmitoyl and the stearoyl chains is not sufficient to induce phase separation in DPPC/DSPC mixtures. The thermotropism of the DPPC/DSPC/melittin system is in fact very similar to that already observed for pure phosphatidylcholine/melittin complexes [6].

As for complexes of melittin with phosphatidylcholines, the lipid perturbation induced by the toxin is more important at high ionic strength [6]. This effect of the toxin can be explained by the screening of the charges of melittin which allows a better binding of the peptide and by the existence of melittin tetramer at high ionic strength [20]. At $R_i = 5$, two overlapping transitions are clearly detected (Fig. 2) and, at first sight, could be associated to a specific phase separation. On the other hand, such a biphasic transition has already been observed for pure DPPC/melittin [6] and pure DSPC/melittin [18] complexes at $R_i = 3.7$ and 5, respectively. Therefore, it seems unlikely that the two transitions observed are due to domains of different lipid composition specially that at low ionic strength such a phase separation is not detected.

As seen in Fig. 5, the behavior of the DPPC/DPPG/melittin system is rather similar to that of DPPG/melittin complexes where double transitions were also observed [10]. In this case, it was proposed that melittin induces in the gel state a phase separation between unperturbed lipid regions and peptide-rich domains. In DPPC/DPPG/melittin complexes, a similar phenomenon seems to occur. The phosphatidylglycerol molecules assure the existence of strong electrostatic

interactions which facilitate the binding of melittin [9]. For the $15 \leq R_i \leq 25$ range, perturbed and nonperturbed domains are formed which indicates the great affinity of melittin for the surrounding phospholipids. Although this phase separation is characteristic of charged lipids, it is impossible to tell from our results if there is a selectivity in this demixing because, despite the binding of the toxin to the bilayer surface by electrostatic interactions with phosphatidylglycerol polar head group, both lipids could be randomly distributed around the peptide. Since the transition previously observed at 27°C for DPPG/melittin complexes at $R_i = 6.5$ is not detectable in DPPC/DPPG/melittin complexes, the total selectivity of melittin for DPPG is excluded. In DPPC/DPPG/melittin complexes at $R_i = 5$, the results show that all lipids are uniformly perturbed and give rise to a cooperative transition at 33°C. This intermediate transition temperature between those of DPPG/melittin and DPPC/melittin complexes (Fig. 5) favors the idea of low selectivity or absence of specificity.

In the case of DSPC/DPPG mixtures, both electrostatic and hydrophobic interactions favor a higher affinity of melittin for DPPG. The shorter chains of DPPG reduce the cohesion of the bilayer and facilitate the incorporation of melittin. Moreover, the electrostatic forces lead to a particular affinity of the toxin for the charged polar head of DPPG. The first striking feature of the thermotropic behavior of DSPC/DPPG/melittin complexes detected from the fluorescence data (Fig. 5) is the temperature increase of the high temperature component of the main transition by 3 C°. For all complexes of melittin with pure lipids already studied, such an augmentation has never been observed; the toxin always induced a destabilisation of the bilayer as reflected by a lowering of either the amplitude of the transition or its mid-point temperature [6,10]. For DSPC/DPPG/melittin complexes, the increase of T_m towards that of pure DSPC reveals the existence of a more stable lipidic domain and strongly suggests the formation in the gel phase of peptide-free regions enriched in DSPC. The high temperature component of the phase transition for the DSPC/DPPG/melittin system reaches a maximum value of 49°C. According to the phase diagram of the DSPC/DPPG system, this temperature corresponds to the transition of a DSPC/DPPG mixture with a molar ratio of 60:40. Therefore, the selectivity of melittin for DPPG, even in mixtures with DSPC where the conditions for specific interactions are favorable, is only partial and results in a limited increase of the proportion of DSPC in the DSPC-rich domains. The intermediate transition that appears around 43°C can be attributed to the DPPG-rich region perturbed by the toxin. The addition of a larger amount of the toxin could lead to the formation of a more important proportion of these charged lipid rich areas and to a greater enrichment in DSPC of the peptide free domains. For $R_i \leq 5$, the DSPC-rich phase is no longer observed.

In order to further characterize the phase separation phenomenon suggested by the above DPH fluorescence polarization data, the thermotropic behavior of the complexe of melittin with an equimolar mixture of DSPC and DPPG at $R_i = 10$ was studied by Raman spectroscopy. Since these experiments were carried out using DPPG with perdeuteriated chains, it was possible to follow DPPG and DSPC independently in the mixture from the Raman signals arising in the C-^{2}H (1950–2350 cm^{-1}) and C-H (2750–3100 cm^{-1}) stretching mode region, respectively. The detailed assignment of the bands in these regions is well documented in the literature [21–26] and will not be discussed in detail in this report.

To follow the effect of temperature on the molecular order of DSPC in the mixture, the h_{2925}/h_{2880} intensity ratio of the C-H region which monitors essentially the overall disorder of the lipid acyl chains [6,24] was used. The thermotropism of the DPPG-d_{62} component was followed from the bandwidth of the 2103 cm^{-1} feature at 65% of the peak height intensity. This parameter is independent of intermolecular chain interactions and monitors mainly the *gauche* population [13].

The thermotropism of the DSPC/DPPG mixture as determined by these two parameters is shown in Fig. 6. As can be seen, in the absence of melittin, both lipids undergo the gel to liquid-crystalline phase transition at exactly the same temperature, showing the ideality of the mixture. The transition is observed at 44°C which is 2 C° lower than the transition temperature determined by fluorescence spectroscopy (Fig. 4). This effect is due to the difference in the interchain van der Waals interactions caused by the perdeuteriation of the acyl chains of DPPG [13.27]. In the presence of melittin, the two curves do not superimpose anymore and the cooperativity of the transition is decreased (Fig. 5b). The transition of DPPG-d_{62} is clearly shifted to a temperature lower than that of DSPC, showing that in the gel phase, the environment of the toxin is richer in DPPG. These Raman results support the interpretation of the fluorescence data.

The formation of a phosphatidylcholine-enriched phase in which a considerable amount of the charged lipid remains has also already been observed for other systems. According to the deuterium nuclear magnetic resonance spectroscopy study on an equimolar mixture of DMPC/DMPG, polymyxin interacts preferentially with DMPG leading to the formation of phospholipid peptide clusters enriched in DMPG and a phase rich in DMPC in which the quantity of DMPG is estimated at 25% [28]. Similarly, lipophilin which binds preferentially to acidic lipids also induces the formation of phosphatidylcholine-rich phase free of protein [29]. For ex-

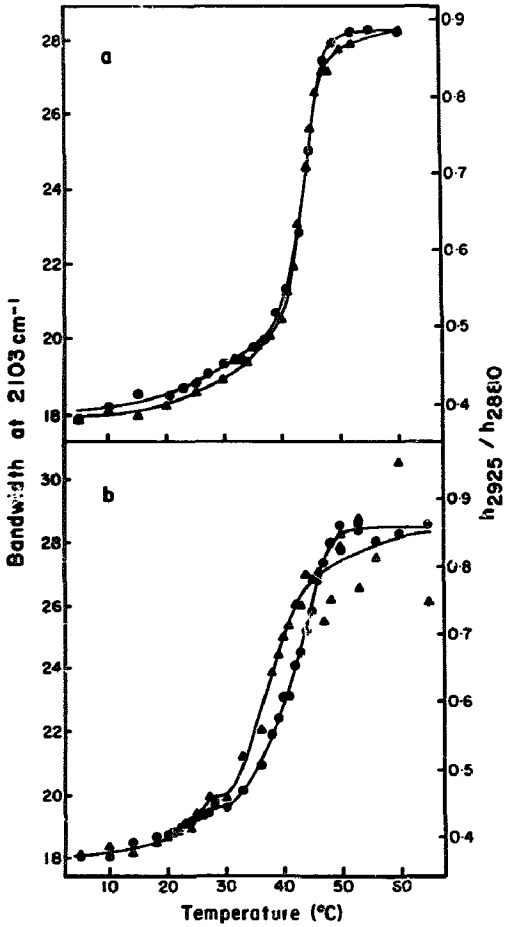

Fig. 6. Temperature profiles derived from the h_{2925}/h_{2880} peak height intensity ratio (●) and from the bandwidth at 65% of the height of the 2103 cm^{-1} band (▲) in the Raman spectra of the DSPC/DPPG-d_{62} equimolar mixture (a) in the absence and (b) in the presence of melittin at $R_i = 10$.

ample, in the case of an equimolar mixture of PS/DPPC, the calorimetric results show that the DPPC content of the lipophilin free domains is increased to 68%.

The small transition detected around 25°C by the DPH fluorescence probe is also seen on the Raman temperature profiles of both components of the mixture. Therefore, it cannot be associated to a phase containing only DPPG and melittin as detected previously for pure DPPG/melittin complexes at $R_i = 6.5$ by fluorescence spectroscopy [10]. Its origin is unknown. It could be associated to a lipid/melittin complex with a particular structure. In this case, since the amplitude of the transition is small, the relative amount of this phase should be most likely very small. On the other hand, a molecular rearrangement of the whole bilayer could also be at the origin of the increased disorder detected at this temperature.

Our interpretation of the thermotropic behavior determined by DPH fluorescence polarization of the DSPC/DPPG/melittin complexes indicates a partial specificity of melittin for DPPG and the formation of heterogeneities in the system in the gel state. The electrostatic forces are determinant in this demixing since the addition of 1.5 M NaCl causes the disappearance of multiple transitions by reducing the net charges of the PG polar head group at the interface. At high ionic strength, complexes of DSPC/DPPG with melittin at $R_i = 2$ still show a cooperative transition which is shifted towards low temperatures by 6 C° (data not shown). The smaller perturbation can be also due to a different state of aggregation of melittin under these conditions [20].

Conclusion

The above results clearly establish that melittin can induce phase separation in binary mixtures of phospholipids. A chain length difference of two carbons is not sufficient to lead to a demixing, even though it has previously been shown that melittin has more affinity for DPPC than for DSPC [6]. This shows that a different affinity between the peptide and different lipids does not necessarily gives rise to specific interactions in lipid mixtures. On the other hand, the presence of DPPG in mixtures with DPPC or DSPC leads to a phase separation. The analysis of the thermotropism of the DSPC/DPPG/melittin system seems to indicate a selectivity of melittin for DPPG which results in the formation of DSPC-rich domains and perturbed DPPG-rich domains. Our results indicate that this specific affinity is governed by both electrostatic and hydrophobic forces. Even though the electrostatic interactions are much stronger and increase the binding of the toxin, the hydrophobic interactions act as an important factor since the DPPC/DPPG/melittin and DSPC/DPPG/melittin systems display a different behavior. The phase separations documented herein from the analysis of the thermotropism exist in the gel phase but can not be, however, directly transposed in the fluid phase where the conditions are different.

Acknowledgements

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References

1 Devaux, P.F. and Seigneuret, M. (1985) Biochim. Biophys. Acta 822, 63–125.
2 Ito, T. and Ohnishi, S.-I. (1974) Biochim. Biophys. Acta 352, 29–37.
3 Papahadjopoulos, D., Poste, G., Schaeffer, B.E. and Vail, W.J. (1974) Biochim. Biophys. Acta 352, 10–28.
4 Galla, H.J. and Sackmann, E. (1975) Biochim. Biophys. Acta 401, 509–529.
5 Findlay, E.J. and Barton, P.G. (1978) Biochemistry 17, 2400–2405.
6 Dasseux, J.-L., Faucon, J.-F., Lafleur, M., Pézolet, M. and Dufourcq, J. (1984) Biochim. Biophys. Acta 775, 37–50.
7 Dufourcq, J., Faucon, J.-F., Fourche, G., Dasseux, J.-L., Le Maire, M. and Gulik-Krzywicki, T. (1986) Biochim. Biophys. Acta 859, 33–48.
8 Lafleur, M., Dasseux, J.-L., Pigeon, M., Dufourcq, J. and Pézolet, M. (1987) Biochemistry 26, 1173–1179.
9 Dufourcq, J. and Faucon, J.-F. (1977) Biochim. Biophys. Acta 467, 1–11.
10 Bernard, E., Faucon, J.-F. and Dufourcq, J. (1982) Biochim. Biophys. Acta 688, 152–162.
11 Mendelsohn, R. and Tarashi, T. (1978) Biochemistry 17, 3944–3949.
12 Mendelsohn, R. and Maisano, J. (1978) Biochim. Biophys. Acta 506, 192–201.
13 Kouaouci, R., Silvius, J.R., Graham, I. and Pézolet, M. (1985) Biochemistry 24, 7132–7140.
14 Laroche, G., Carrier, D. and Pézolet, M. (1988) Biochemistry 27, 6220–6228.
15 Savoie, R., Boulé, B., Genest, G. and Pézolet, M. (1983) Can. J. Spectrosc. 24, 112–117.
16 Pézolet, M., Boulé, B. and Bourque, D. (1983) Rev. Sci. Instrum. 54, 1364–1367.
17 Savitzky, J.E. and Golay, M.J.E. (1964) Anal. Chem. 36, 1627–1639.
18 Faucon, J.-F., Dasseux, J.-L., Dufourcq, J., Lafleur, M., Pézolet, M., Le Maire, M. and Gulik-Krzywicki, T. (1986) in Surfactants in Solution, Vol. 5 (Mittal, K.L. and Bothorel, P., eds.), pp. 873–884.
19 Knoll, W., Schmidt, G., Sackmann, E. and Ibel, K. (1983) J. Chem. Phys. 79, 3439–3442.
20 Talbot, J.C., Dufourcq, J., De Bony, J., Faucon, J.-F. and Lussan, C. (1979) FEBS Lett. 102, 191–193.
21 Spiker, R.C., Jr. and Levin, I.W. (1975) Biochim. Biophys. Acta 388, 361–373.
22 Gaber, B.P. and Peticolas, W.L. (1977) Biochim. Biophys. Acta 465, 260–274.
23 Bunow, M.R. and Levin, I.W. (1977) Biochim. Biophys. Acta 487, 388–394.
24 Hill, I.R. and Levin, I.W. (1979) J. Chem. Phys. 70, 842–851.
25 Mendelsohn, R. and Koch, C.C. (1980) Biochim. Biophys. Acta 598, 260–271.
26 Bryant, G.L., Lavialle, F. and Levin, I.W. (1982) J. Raman Spectrosc. 12, 118–121.
27 Petersen, N.O., Kroon, P.A., Kainosho, M. and Chan, S.I. (1975) Chem. Phys. Lipids 14, 343–349.
28 Sixl, F. and Watts, A. (1985) Biochemistry 24, 7906–7910.
29 Boggs, J.M., Wood, D.D., Moscarello, M.A. and Papahadjopoulos, D. (1977) Biochemistry 16, 2325–2329.