Influence of Lipid Chain Unsaturation on Melittin-Induced Micellization

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ABSTRACT It is well known that melittin, an amphipathic helical peptide, causes the micellization of phosphatidylcholine vesicles. In the present work, we conclude that the extent of micellization is dependent on the level of unsaturation of the lipid acyl chains. We report the results obtained on two systems: dipalmitoylphosphatidylcholine (DPPC), containing 10(mol)% saturated or unsaturated fatty acid (palmitic, oleic, or linoleic), and DPPC, containing 10(mol)% positively charged diacyloxy-3-(trimethylammonio)propane bearing palmitic or oleic acyl chains. For both systems, the presence of unsaturation in the lipid acyl chains inhibits melittin-induced micellization. Conversely, the addition of saturated palmitic acid to the DPPC matrix enhances the micellization. This modulation is proposed to be associated with the cohesion of the hydrophobic core. When the lipid chain packing of the gel-phase bilayer is already perturbed by the presence of unsaturation, it seems easier for the membrane to accommodate melittin at the interface, and the distribution of the peptide in the bilayer could be the origin of the inhibition of the micellization. The cohesion of the apolar core is shown to play an unquestionable role in melittin-induced micellization; however, this contribution does not appear to be as important as the electrostatic interactions between melittin and positively or negatively charged lipids.

INTRODUCTION

Melittin is a small peptide of 26 amino acids, isolated from bee venom (Habermann, 1972). This highly basic peptide interacts spontaneously with membranes, binding as an amphipathic α-helix (Lauterwein et al., 1979; Vogel and Jähnig, 1986; Lafleur et al., 1991). Because of its various modes of action with natural and model membranes (for a general review, see Dempsey, 1990), melittin constitutes an interesting model for studying the interaction between amphipathic peptides such as signal peptides and apolipoproteins, and natural membranes.

When melittin interacts with bilayers composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), it induces the fragmentation of the multilamellar vesicles into small lipid/melittin comicelles (Dufourcq et al., 1986; Dufourc et al., 1986). These comicelles were described as small gel-phase bilayers surrounded by a monomolecular layer of melittin (Dufourcq et al., 1986; Lafleur et al., 1987). The micellization of DPPC multilamellar vesicles is complete when the incubation lipid-to-peptide molar ratio (R_i) is equal to or less than 20. When the temperature is raised above the gel-to-liquid crystalline phase transition temperature (T_m) of the lipid, fusion of the comicelles into extended bilayers is observed for R_i greater than 5. The polymorphism described here is completely reversible: the gel-to-liquid crystalline phase transition is accompanied by a morphological change from small discs to extended bilayers (Dufourc et al., 1986).

In our efforts to determine how the physicochemical properties of model membranes affect melittin-induced micellization, we have investigated the influence of several components of natural occurrence in biomembranes. It has been shown that the presence of a small amount of negatively charged phospholipid or unprotonated fatty acid in DPPC bilayers reduces severely the proportion of disc micellization (Dempsey et al., 1989; Monette and Lafleur, 1995). The electrostatic anchoring of the peptide at the bilayer interface is proposed to be responsible for this inhibition. On the other hand, similar concentrations of positively charged lipids, 1,2-dipalmitoyloxy-3-(trimethylammonio)propane (DPTAP) and 1,2-dioleoyloxy-3-(trimethylammonio)propane (DOTAP), strongly promote melittin-induced micellization compared to pure DPPC vesicles. In that case, the promotion of melittin-induced micellization was rationalized on the basis of electrostatic repulsion between the positively charged bilayer interface and the peptide, favoring the peptide transfer from the surface into the lipid core (Monette and Lafleur, 1995).

It was shown recently that the properties of the bilayer at the apolar core level also influence the activity of melittin. Studies of the effect of cholesterol on the melittin-induced micellization (Monette et al., 1993; Pott and Dufourc, 1995) showed that high concentrations of cholesterol (>30(mol)%) in DPPC bilayers lead to a complete inhibition of the micellization, because of the lack of interaction between the rigid membrane and the toxin. The addition of a high concentration of cholesterol in DPPC bilayers induces the formation of the liquid ordered phase (Vist and Davis, 1990), which is resistant to the micellization induced by melittin; this indicates that the interactions between the chains in the hydrocarbon

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core play an important role in the membrane susceptibility to form comicelles in the presence of melittin. Along the same lines, a correlation between phosphatidylcholine chain length and the stability of the comicelles formed with melittin has been reported (Faucon et al., 1995). The leakage induced by melittin is also dependent on the lipid chain composition of the bilayer (Subbarao and MacDonald, 1994).

To gain more insight into the role of interchain interactions, two systems were investigated. First, we have examined the influence of various fatty acids in DPPC bilayers on the power of melittin to disrupt membranes. These fatty acids (palmitic acid (PA) ($C_{16:0}$), oleic acid (OA) ($C_{18:1\ (9\text{-cis})}$), and linoleic acid (LA) ($C_{18:2(9\text{-cis, }12\text{-cis})}$)) were used to modify the level of unsaturation in the hydrophobic core of the membrane. Second, we have characterized melittin-induced micellization of DPPC membranes containing a positively charged lipid bearing saturated and unsaturated chains. Solid-state ^{31}P nuclear magnetic resonance (NMR) spectroscopy was used to characterize the lipid polymorphism induced by melittin. Slow reorienting bilayer assemblies lead to nonaveraged chemical shift anisotropy. As a result, a typical powder pattern is observed (Seelig, 1978). The small comicelles resulting from the micellization have shorter correlation times and averaged chemical shifts; an isotropic signal is therefore observed. The different spectroscopic features of each phase allowed us to characterize quantitatively the proportion of phospholipids existing under the comicellar form, referred to in this paper as the percentage of micellization.

MATERIALS AND METHODS

DPPC, 1,2-bis(perdeuteriopalmitoyl)-sn-glycero-3-phosphocholine (DPPC-d_{62}), and DOTAP were obtained from Avanti Polar Lipids (Birmingham, AL). DPTAP was synthesized according to the procedure described by Stamatatos et al. (1988). The fatty acids were purchased from Sigma (St. Louis, MO). Melittin was purified from bee venom (Sigma) by ion exchange chromatography on SP-Sephadex C-25. A high-performance chromatography procedure was used to remove the large amount of salt (Lafleur et al., 1987). A typical sample was prepared by hydrating 30 mg of lipids (or lipid mixture) with 1 ml of 10 mM HEPES buffer containing 5 mM EDTA and 100 mM sodium chloride, at pH = 7.5 or 9.5. The sample was heated above T_m and vortexed. In the case of lipid mixtures, lipids were weighted in the desired ratio, dissolved in benzene-methanol mixture (90/10 v/v), and then lyophilized. For lipid/melittin complexes, an aliquot of melittin solution in water was added to the hydrated bilayers in the appropriate molar ratio, R_i. After the addition of melittin, the sample was vortexed, heated, and cooled several times through T_m before data acquisition, because an incubation above the pretransition temperature is required to observe micellization in the gel phase (Monette et al., 1993). When necessary, the pH was adjusted by adding small aliquots of dilute hydrochloride acid or sodium hydroxide aqueous solutions to the sample. Deuterium oxide was added to the samples to maintain a deuterium lock.

A Bruker ARX-400 spectrometer was used to record the ^{31}P NMR spectra. After a single pulse of 20 μs (35°), 2048 points were acquired in quadrature with a dwell time of 15 μs. The relaxation delay was set to 1 s, and proton decoupling was achieved with composite pulse decoupling (GARP pulse sequence). The number of scans was typically 5400. The sample temperature was regulated with a Bruker variable temperature controller.

Differential scanning calorimetry (DSC) was performed on mixtures of DPPC-d_{62} containing 10(mol)% palmitic, oleic, and linoleic acid. These mixtures were prepared as described above and analyzed, using a Microcal MC-1 high-sensitivity DSC, with a scan rate of 10°C/h. We have used a deuterated phosphatidylcholine because these samples were utilized, in parallel, in a ^2H NMR study (Monette, 1994).

RESULTS

First, melittin-induced micellization is examined at different pHs for DPPC/fatty acids mixtures. The ^{31}P NMR spectra recorded at 26°C from DPPC/fatty acid mixtures containing melittin (R_i = 40) were obtained at pH 7.5 and 9.5 (Fig. 1). For purposes of comparison, the spectrum of DPPC/melittin complexes at the same R_i is also illustrated for both pHs. At pH 9.5, all samples containing 10(mol)% fatty acid show a complete inhibition of micellization, as previously shown (Monette and Lafleur, 1995). When the pH is decreased to 7.5, the apparition of an isotropic signal indicates that some micellization has occurred for all samples. The proportion of the isotropic signal is different according to the nature of the fatty acid: the spectra of the samples containing oleic and linoleic acid show a reduced proportion of micellization compared to pure DPPC, whereas the spectrum of the sample containing palmitic acid indicates a promotion of the micellization with respect to DPPC. The variation of pH has a much more reduced effect on the zwitterionic DPPC bilayers because there is no variation in surface charge density during that pH change.

The DPPC/fatty acid samples were characterized in the liquid crystalline phase as well as in the gel phase. Fig. 2 shows the ^{31}P NMR spectra obtained at pH 7.5 at 26°C, 35°C, and 45°C for the DPPC/fatty acid/melittin mixtures. As shown previously, these mixtures give rise to an isotropic signal at low temperature. An increase in temperature leads to the fusion of the small comicelles into extended bilayers, as inferred from the powder pattern obtained at 45°C. At 35°C, a narrow isotropic line is shown for the DPPC and DPPC/PA samples. In comparison, a broader and complex signal is obtained for the sample containing oleic acid. The coexistence of an isotropic signal and a powder pattern is observed when the bilayers contain 10(mol)% linoleic acid. According to these spectra, the fusion of the small comicelles does not occur at the same temperature for all samples and depends on the lipid composition. Nevertheless, the fusion of the small DPPC/fatty acid/melittin comicelles occurs in a fashion similar to that for the DPPC/melittin complexes (Fig. 2), indicating that the nature of the lipid polymorphism induced by melittin is not altered by the presence of fatty acids in the bilayers.

So far, the results indicate that the proportion of melittin-induced micellization depends on the nature of the fatty acids present in the DPPC bilayers. To highlight the importance of acyl chain unsaturation in membrane disruption induced by melittin, we have measured, at 26°C, the proportion of micellization with respect to the peptide concentration for these mixtures and compared these with the micellization observed with pure DPPC (Fig. 3). The percentage micellization is estimated from the ratio of the area

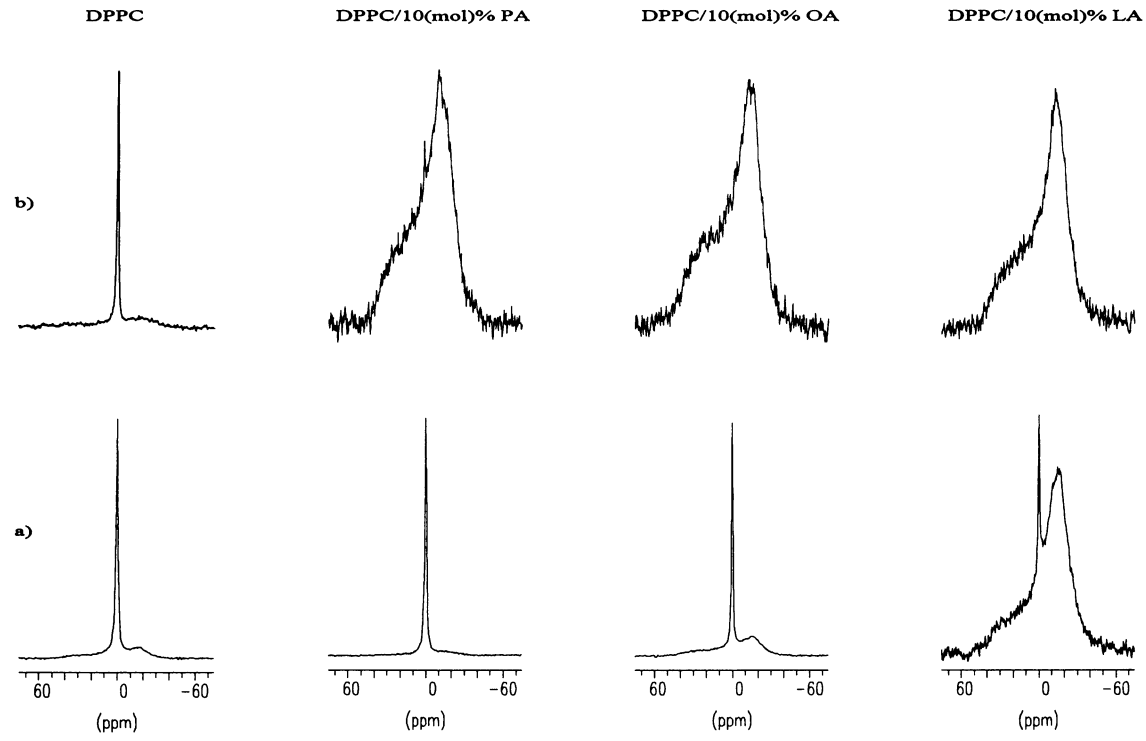

FIGURE 1 ^{31}P NMR spectra of samples containing 10(mol)% of palmitic, oleic, or linoleic acid compared to pure DPPC, in the presence of melittin (R_i = 40), at 26°C. (*a*) pH = 7.5; (*b*) pH = 9.5. The sample composition is indicated at the top of each column.

of the isotropic signal over the total area of the ^{31}P NMR spectrum (isotropic signal + powder pattern). The integration of the isotropic signal was achieved by fitting a polynomial at the base of the isotropic peak to delimit its surface. The reproducibility of the percentage micellization was about 5% for different samples of identical composition. The T_1 relaxation time is slightly different for both observed species (1.1 s for the edge of the powder pattern and 1.4 s for the isotropic component). This means that the fractional area of the isotropic component does not vary in direct proportion to the fraction of micellization, introducing a small systematic error in the determination of percentage micellization. However, the absolute error in percentage micellization is at the very most 4%. Therefore, the relative area of the isotropic peak provides a semiquantitative and straightforward estimate of the sample composition. Fig. 3 shows a larger proportion of micellization for the DPPC/PA mixture, for all $R_i > 20$, with respect to pure DPPC. Second, the presence of oleic and linoleic acid leads to a reduction in the percentage micellization compared to pure DPPC, the inhibition being more pronounced for the DPPC/LA mixture. For a constant R_i = 40, the percentage micellization is 82%, 58%, 34%, and 5% for the DPPC/PA, DPPC, DPPC/OA, and DPPC/LA mixtures, respectively. For this given peptide concentration, going from an unsaturated fatty acid to a saturated fatty acid leads to a change from almost intact bilayers to almost complete disruption. Comparable effects were obtained for DPPC samples containing 20(mol)% fatty acids (data not shown).

In an earlier study (Monette and Lafleur, 1995), a dramatic increase in melittin-induced micellization on DPPC bilayers containing 10(mol)% positively charged DPTAP or DOTAP was reported. Fig. 4 illustrates the ^{31}P NMR spectra obtained at 26°C for mixtures of DPPC containing 10(mol)% DPTAP and DOTAP at various R_i. In the absence of melittin, the powder patterns obtained are typical of gel-phase bilayers. The addition of a small amount of melittin (R_i = 100) leads to an extensive disruption of the DPPC/DPTAP vesicles, as inferred by the spectrum dominated by a large isotropic signal (width at half-height of about 1 kHz). For the DPPC/DOTAP mixture, the addition of the same amount of melittin causes only 50% micellization, and the line width of the isotropic signal is significantly smaller (width at half-height of 550 Hz) than that obtained from the DPPC/DPTAP mixture. Further addition of the toxin leads to the increase in the proportion of micellization for the mixture containing DOTAP (Fig. 4 *b*). At R_i = 40, a complete disruption of the vesicles is observed for both mixtures. For both systems, the phosphorus NMR line width of the isotropic component depends on the R_i; the values are 500, 350, and 250 Hz for R_i = 60, 40, and 30, respectively. The samples were heated above T_m, at 55°C. As discussed previously (Monette and Lafleur, 1995), the powder patterns recorded under these conditions (data not shown) indicate the fusion of the comicellar complexes into large lipidic assemblies in the fluid phase and show that the positively charged lipids do not affect the nature of the thermally induced macrostructural changes. In addition, a

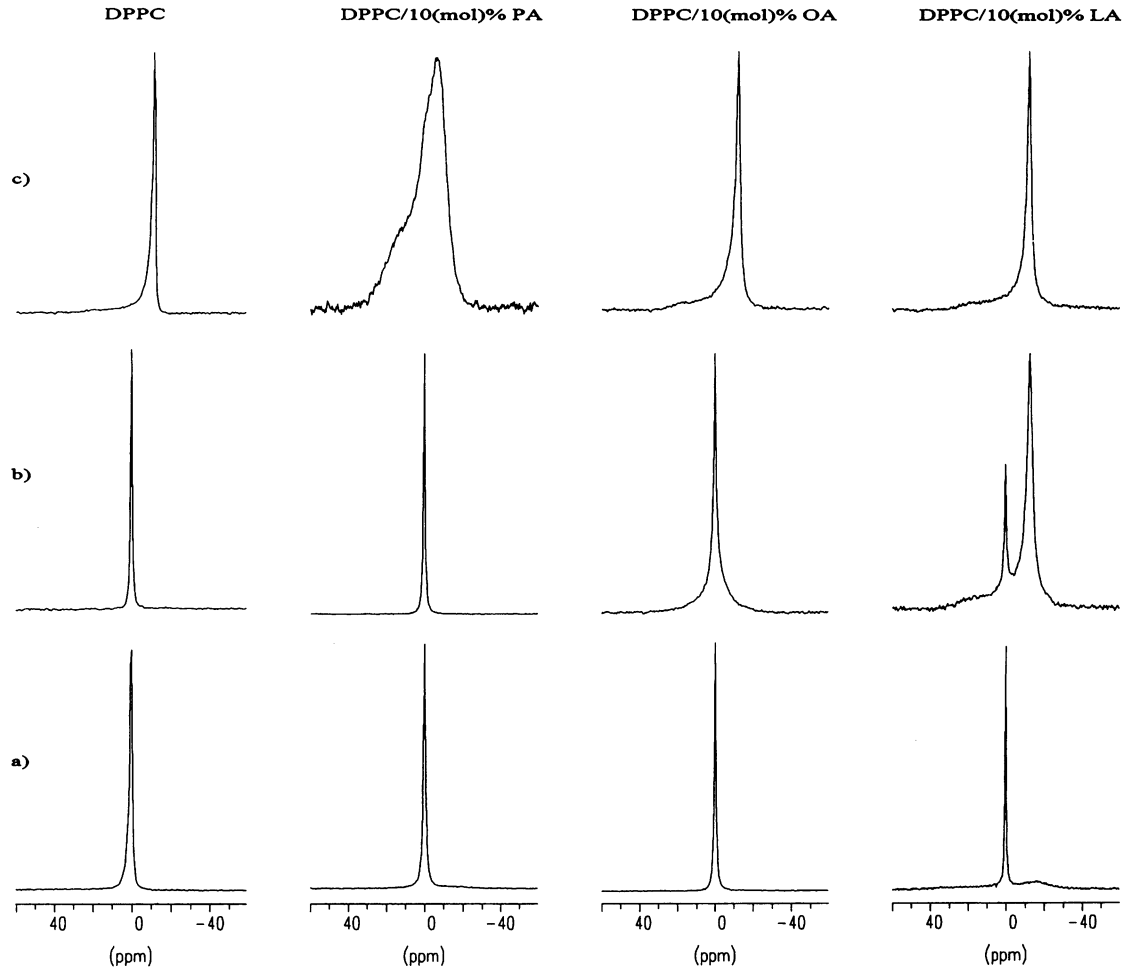

FIGURE 2 ^{31}P NMR spectra of samples containing 10(mol)% of palmitic, oleic, or linoleic acid compared to pure DPPC, in the presence of melittin (R_i = 20, except for the DPPC/PA mixture, R_i = 30) at pH = 7.5. (*a*) 26°C; (*b*) 35°C; (*c*) 45°C. The sample composition is indicated at the top of each column.

light-scattering control experiment was performed on a DPPC/DOTAP sample in the presence of melittin (R_i = 60).

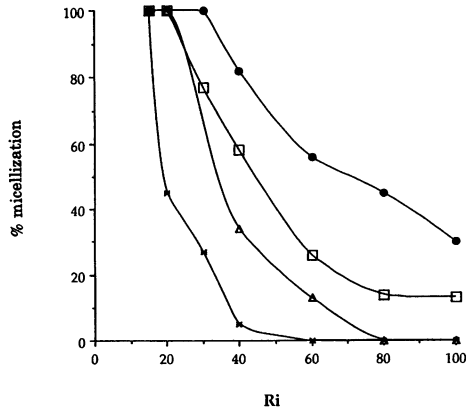

FIGURE 3 Percentage of micellization as a function of the lipid/peptide ratio (R_i) obtained at 26°C, pH = 7.5 for (□) pure DPPC, (●) DPPC/10(mol)% palmitic acid, (△) DPPC/10(mol)% oleic acid, and (*) DPPC/10(mol)% linoleic acid.

The toxin was added to the lipid mixture at room temperature, and the scattered light was measured before and after incubation above T_m. At 20°C, the scattered light intensity was drastically reduced after incubation at 60°C relative to that measured before incubation (data not shown). This result indicates a decrease in the size of the lipidic aggregates after interaction with melittin and supports our assignment of the isotropic component observed at low temperature to micelles.

Differential scanning calorimetry was used to correlate melittin-induced micellization with some physical properties of the DPPC bilayers containing different fatty acids. DSC thermograms for mixtures of DPPC-d_{62} with palmitic acid, oleic acid, and linoleic acid are shown in Fig. 5. The transition temperature of the pure DPPC-d_{62} is indicated by the dotted line (37.7°C according to Vist and Davis, 1990). In agreement with previous results (Mabrey and Sturtevant, 1977; Usher et al., 1978; Verma et al., 1980; Schullery et al., 1981; Ortiz and Gómez-Fernández, 1987), the presence of fatty acid in a DPPC matrix affects T_m and broadens the transition. Palmitic acid shifts the main transition toward a

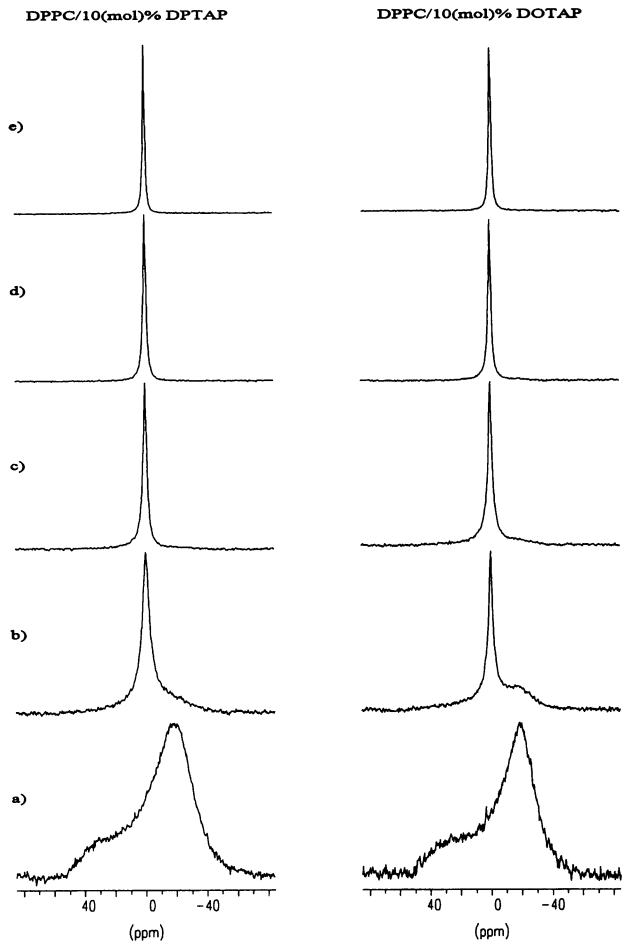

FIGURE 4 ^{31}P NMR spectra of DPPC samples containing 10(mol)% of DPTAP or DOTAP, at 26°C, pH = 7.5 (*a*) without melittin and (*b–e*) in the presence of melittin: (*b*) R_i = 100; (*c*) R_i = 60; (*d*) R_i = 40; (*e*) R_i = 30. The sample composition is indicated at the top of each column.

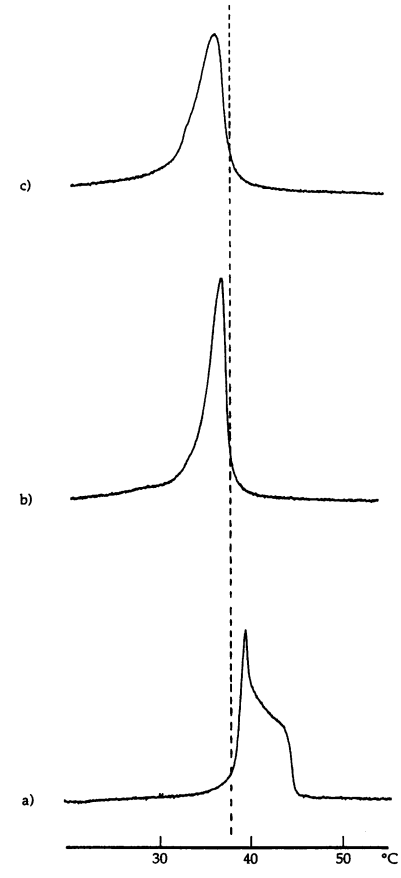

FIGURE 5 Differential scanning calorimetry traces for DPPC-d_{62} samples containing 10(mol)% of (*a*) palmitic acid, (*b*) oleic acid, and (*c*) linoleic acid. The scan rate was 10°C/h. The dotted line represents the transition temperature for pure DPPC-d_{62}: 37.7°C (Vist and Davis, 1990).

higher temperature (39.0°C). Conversely, the thermogram of the DPPC-d_{62}/oleic acid mixture indicates a small decrease in T_m (35.4°C) with respect to the pure lipid. The decrease is more pronounced in the case of DPPC-d_{62}/linoleic acid mixture (T_m = 34.7°C; Fig. 5 *c*). DSC calorimetry indicates that the presence of a small amount of saturated fatty acids in DPPC increases the T_m, whereas the presence of the same amount of unsaturated fatty acid leads to the reduction of in the T_m, in agreement with previous reports (Mabrey and Sturtevant, 1977; Usher et al., 1978; Verma et al., 1980; Schullery et al., 1981; Ortiz and Gómez-Fernández, 1987).

DISCUSSION

This study reports for the first time that melittin-induced micellization can be modulated by the degree of unsaturation of lipid acyl chains. Two sets of experimental results lead to this conclusion. First, the power of melittin to disrupt membranes increases in the following order: DPPC/10(mol)% LA < DPPC/10(mol)% OA < DPPC < DPPC/10(mol)% PA. The presence of unsaturated fatty acid inhibits melittin-induced micellization. Second, DPPC containing 10(mol)% DOTAP is less susceptible to micellization than is DPPC/10(mol)% DPTAP, leading to the same conclusion. This modulation, however, does not affect the nature of the polymorphism of lipid/melittin complexes. The fusion of the comicellar complexes is triggered by the gel-to-liquid crystalline phase transition of the lipid mixture, as in the well-established case of DPPC/melittin complexes (Dufourc et al., 1986).

We infer from this conclusion that unsaturation stabilizes the bilayer with regard to the micellization induced by melittin and/or destabilize the small lipid/peptide complexes. This influence on the disruptive properties of melittin is important, considering that a large reduction of the percentage micellization can be induced by a limited proportion of unsaturated acyl chains (10(mol)%). We have used limited amounts of fatty acids in the DPPC matrices, because this corresponds to their natural occurrence in certain membranes (Ray et al., 1969), as well as to avoid phase separation. It has previously been established that a DPPC/10(mol)%PA mixture forms a homogeneous system (Schullery et al., 1981; Koynova et al. 1988; Villalaín and Gómez-

Fernández, 1992). Similarly, such a limited amount of *cis*-unsaturated fatty acid (OA and LA) was shown to be miscible with saturated phosphatidylcholine (Ortiz and Gómez-Fernández, 1987).

To find a rationale for the modulation of melittin-induced micellization by acyl chain unsaturation, we have examined the effect of this unsaturation on the physical properties of the bilayer. For the first set of samples (DPPC/10(mol)% fatty acid), it is clear that the degree of unsaturation of the fatty acid modulates the properties of the membranes. The straightforward experimental result presented in this paper illustrating this is the shift of T_m, because we observe T_m(DPPC/10(mol)%LA) < T_m(DPPC/10(mol)%OA) < T_m(DPPC) < T_m(DPPC/10(mol)%PA). This progression is the same as that presented above for the propensity to form comicelles. These variations in T_m were expected and are well understood. Previous studies have shown that the introduction of PA into DPPC induces a shift of T_m toward high temperatures (Mabrey and Sturtevant, 1977; Usher et al., 1978; Schullery et al., 1981). This phenomenon was explained by the small size of the PA headgroup, leading to a tighter packing of the acyl chains and, as a consequence, enhanced interchain van der Waals interactions stabilizing the gel phase. Conversely, the introduction of unsaturation at the acyl chain level causes defects in the chain packing in the gel phase and leads to a shift of T_m toward low temperatures, as discussed previously (Verma et al., 1980; Usher et al., 1978; Ortiz and Gómez-Fernández, 1987). Therefore, it appears that the susceptibility of the membrane to undergo a micellization in the presence of melittin is associated with the cohesion of the hydrophobic core. It appears that the presence of unsaturation in the hydrophobic core leads to a more pronounced modulation of the micellization power of melittin than that observed for acyl chain length. When melittin-induced micellization was examined for DPPC, DSPC, and 1,2-diarachidoyl-sn-glycero-3-phosphocholine (DAPC) bilayers using ^{31}P NMR, it was found that the structural conversion from vesicle to comicelle occurs at the same melittin content (Faucon et al., 1995).

The stability of the comicelles as a function of the temperature provides insight into the rationalization of our results. The lipid/melittin comicelles are more stable in the gel phase than in the liquid crystalline phase. It has been suggested that melittin can be accommodated at the interface of a fluid bilayer because the acyl chains are flexible and can support the lateral expansion of the bilayer in response to the increase of interfacial area created by melittin (Dempsey and Watts, 1987). The gel phase, with its stiff chains, is not as accommodating and triggers the relocation of the peptide, leading to the formation of comicelles. We propose that the level of unsaturation of the hydrophobic core of the membrane modulates its ability to accommodate melittin in the gel-phase bilayer and, as a consequence, the percentage micellization. When the lipid chain packing is already perturbed by the presence of unsaturation, it may be easier for the membrane to adapt to the presence of melittin at the interface. Conversely, the presence of palmitic acid increases the cohesion of the hydrophobic core and, in this case, the location of melittin at the interface may be very unfavorable from a free energy point of view because of important interchain van der Waals interactions. In this case, the distribution of the peptide in the bilayer can favor enhanced micellization. A parallel inference has been proposed for pyrene solubility in phosphatidylcholine bilayer containing fatty acids (Usher et al., 1978). Fluorescence measurements have shown that gel-phase phosphatidylcholines are considerably affected by a low amount of fatty acids. The authors have shown that the presence of palmitic acid, in addition to inducing a shift of T_m toward high temperature, enhances the formation of pyrene probe clusters. Conversely, unsaturated acids (linoleic, oleic, and arachidonic acids in that study) lower T_m and decrease the ability of pyrene to form clusters. The fatty acids modulate the cohesion of the hydrophobic core of the bilayer and, as a consequence, the ability to accommodate bulky pyrene groups in a gel-phase membrane. The conclusions of that investigation are consistent with the relation we have established between hydrophobic core cohesion and melittin-induced micellization, as discussed above.

The change in macroaggregation occurring during micellization leads to at least two structural modifications. First, the radius of curvature of the lipid structure changes because the membrane undergoes a transition from a large vesicle to a flat discoidal micelle (if one supposes that the presence of 10(mol)% of other lipidic species in DPPC matrix does not affect the architecture of the comicelles). Second, the size of the aggregates decreases drastically, going from 0.5 to a few microns for multilamellar dispersions to about 200 Å for the comicelles (Faucon et al., 1995). Considering these two changes, the mechanical properties of the bilayer could also play an important role in the micellization process because the free energy associated with membrane curvature and local deformations of the bilayer such as surface undulations are likely affected by the structural transformation occurring during the micellization. The data in the literature on the micromechanical properties of bilayers and their variation as a function of the unsaturation degree are unfortunately too limited to discuss their potential contributions, but the modulation of these properties by unsaturation may be related to the role played by lipid chain in melittin-induced micellization. Finally, it should be mentioned that the inhibition of micellization by acyl chain unsaturation could be due to a lower partitioning coefficient of melittin in these bilayers, because the data are expressed in terms of lipid/melittin ratio during the incubation, without specifying the proportion of bound peptide. This is unlikely, however, because it was reported recently that the partition coefficient of melittin between the aqueous phase and the bilayer is basically the same for phospholipids with different levels of chain unsaturation (Subbarao and MacDonald, 1994).

A similar influence of lipid chain unsaturation on melittin-induced micellization is observed for the mixture containing the positively charged lipid. When the acyl chains of the positively charged lipid are saturated, the disruption of the membranes is more extensive than for DOTAP, as observed for R_i of 100 and 60 (Fig. 4). This inhibition is illustrated by a powder pattern significantly more intense with DOTAP than with DPTAP. This wide signal coexists with an isotropic line. The width of the isotropic component provides some information about the size of the comicelles (Burnell et al., 1980; Cushley et al., 1987). As deduced from the line width, the peptide/lipid comicelles formed by the DPPC/DPTAP mixture at a R_i of 100 are larger than those obtained with the DPPC/DOTAP mixture. If the structure of these comicelles is similar to those obtained with pure DPPC in the presence of melittin, the lipid/melittin ratio in the discoidal particle is directly proportional to its radius (Monette and Lafleur, 1995). We can therefore infer from the line width that, at a R_i of 100, the lipid/melittin ratio in the comicelle formed in the presence of DPTAP is higher than that obtained with DOTAP. This conclusion is in good agreement with the fact that a more pronounced micellization occurs with the saturated lipid, whereas more lipids still form large lipidic assemblies in the presence of DOTAP. Subsequent addition of melittin leads to a narrowing of the isotropic line for both samples, suggesting that the comicelles are broken up into smaller ones, in agreement again with a lower lipid/melittin ratio in the particles. These results show that the level of unsaturation of the hydrophobic core can modulate not only the extent of melittin-induced micellization but also, under certain conditions, the size of the resulting comicelles.

It should be noted that the proportion of melittin-induced micellization obtained in the presence of one or the other positively charged lipids in a DPPC matrix is always larger than that observed for pure DPPC (Monette and Lafleur, 1995). The electrostatic interactions appear to dominate those related to the cohesion of the hydrophobic core. Similarly, we showed (Monette and Lafleur, 1995) that the inhibition of the melittin-induced micellization was similar for PA, OA, and LA at high pH, when the fatty acid was unprotonated; in this case again, the electrostatic interactions appear to be the prime contribution. Therefore, the cohesion of the apolar core has an unquestionable role in melittin-induced micellization, but it is of second order relative to electrostatic interactions.

To conclude, it is interesting to highlight the fact that the influence of three components of biological membranes on melittin-induced micellization have been investigated: cholesterol (Monette et al., 1993; Pott and Dufourc, 1995), negatively charged lipids (Dempsey et al., 1989; Monette and Lafleur, 1995), and unsaturation (this paper). These three components have been shown to inhibit, via different mechanisms, the disruptive power of melittin. These conclusions provide interesting leads to understanding the greater resistance of the human erythrocyte membranes to melittin-induced micellization relative to model DPPC vesicles (Dufourc et al., 1989).

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