myelin basic protein III. Interactions of the protein with lipid micelles by ^{1}H and ^{31}P NMR. *Aust. J. Chem.* 32:2631–2636.
10. Keniry, M. A., and R. Smith. 1979. Circular dichroic analysis of the secondary structure of myelin basic protein and derived peptides bound to detergents and to lipid vesicles. *Biochim. Biophys. Acta.* 578:381–391.
11. Uyemura, K., C. Tobari, S. Hirano, and Y. Tsukada. 1972. Comparative studies on the myelin proteins of bovine peripheral nerve and spinal cord. *J. Neurochem.* 19:2607–2614.
12. Chapman, B. E., G. E. James, and W. J. Moore. 1981. Conformations of P2 peripheral nerve myelin from nuclear magnetic resonance spectroscopy. *J. Neurochem.* 36:2032–2036.

INTERACTION OF BASIC PROTEINS WITH CHARGED PHOSPHOLIPIDS FOLLOWED BY FLUORESCENCE, DSC, AND RAMAN SPECTROSCOPY

E. BERNARD, J. F. FAUCON, AND J. DUFOURCQ,
Centre de Recherche Paul Pascal, Centre Nationale De Recherche Scientifique, 33405 Talence France

L. DUCHESNEAU AND M. PEZOLET, *Département de Chimie, Université Laval, Québec GIK 7P4, Canada*

Melittin (Mel) and cardiotoxins (CTX) are basic proteins which act on membranes as direct lytic factors. Mel binds to both negative and zwitterionic phospholipids, but it exhibits higher affinity toward the former (1). CTX interacts only with charged lipids (2). Studies on the perturbations of the bilayers induced by such toxins have been restricted to the effects of melittin on phosphatidylcholines (3–5). Here we report on the perturbations of Mel and CTX on bilayers of pure negative lipids or of binary mixtures as followed by fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH), calorimetry (DSC), and Raman spectroscopy.

RESULTS

Melittin-Phospholipid Interactions

According to their behavior in the presence of Mel, acidic phospholipids can be divided in two groups. Group one is constituted by mono- and divalent lipids in C_{14} ($DMPS^-$, $DMPG^-$, $DMPA^{--}$), divalent lipids in C_{16} ($DPPA^{--}$) and natural lipids such as PS^-. In this case, Mel leads to a progressive disappearance of the transition without any shift in temperature. For the second group of lipids, which includes monovalent lipids in C_{16} ($DPPG^-$, $DPPS^-$, $DPPA^-$) and also a monovalent C_{14} lipid ($DMPA^-$), two well-defined transitions are detected. One occurs at the same temperature as that of the pure lipid; the second one is 10–15°C lower. By increasing the amount of Mel, the high temperature transition progressively disappears while the lower one increases in amplitude (see Fig. 1).

Cardiotoxin-Phospholipid Interactions

For all the lipids used, CTX induces the same effects as those of Mel on group one lipids. The Raman spectra in the C–C stretching region (Fig. 2) for CTX-DMPA complexes show that even in the gel phase CTX induces formation of *gauche* conformers. At $R_i = 7.5$, the population of *gauche* rotamers is almost as high as that of pure DMPA in the liquid-crystalline state at 60°C. The C–H stretching region parameters indicate that before the occurrence of a significant disordering ($R_i > 30$), CTX induces a change in the packing of the aliphatic chains.

Interaction of Mel and CTX with Binary Mixtures of Lipids

In the presence of toxins the transition temperature as detected by fluorescence polarization for 1:1 mixtures PC-PA and PC-PS is always shifted toward that of the PC component. For example, the transition temperature of a DMPC-DMPA mixture is shifted downward by 13°C whereas in the case of DMPC-natural PA, an upward shift of 6°C is observed, the final transition being very close to that of pure DMPC (23°C) in both cases.

CONCLUSIONS

The presence of two well-defined transitions for complexes of group two lipids and Mel demonstrates that the latter induces phase separation between pure lipid regions and a peptide-lipid complex which melts at 10–15°C below the pure lipid. This phase separation could also occur for Mel and group one lipids as well as for CTX and all the lipids investigated in this study if the complexes formed do not give rise to a cooperative transition. We thus believe that Mel does not disrupt the bilayer structure formed by the group two lipids, which display stronger intermolecular interactions than group one lipids because the aliphatic chains are longer and/or the polar headgroups bear only one negative charge. The perturbations induced by Mel on group one lipids, which form less stable bilayers, and by CTX on all the lipids investigated are such that a cooperative transition can no longer occur.

The results obtained with mixtures indicate that Mel and CTX are able to induce phase separation between PC-rich regions that still give a transition and domains

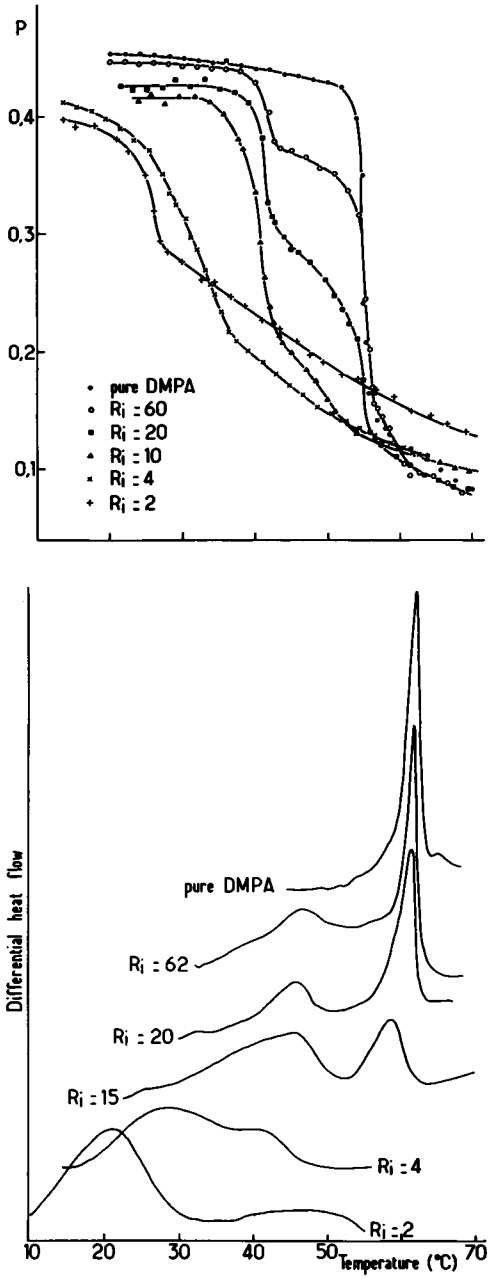

FIGURE 1 Effect of melittin on DMPA bilayers at pH 3.5 (20 mM acetate buffer) and at various lipid protein molar ratios, R_i. Top panel: fluorescence polarization of DPH ([DMPA] $\simeq$20 μM, [DPH] $\leq$ 0.2 μM). Lower panel: thermograms obtained by DSC and normalized to either 1 mg of DMPA (pure lipid, R_i = 62) or 2 mg (R_i = 20, 15, 4, 2).

formed by complexes of the toxins and the acidic lipids. Therefore, the specificity of the toxins for negatively charged lipids remains even in mixtures. This could have important implications in the mechanism of the lysis of natural membranes.

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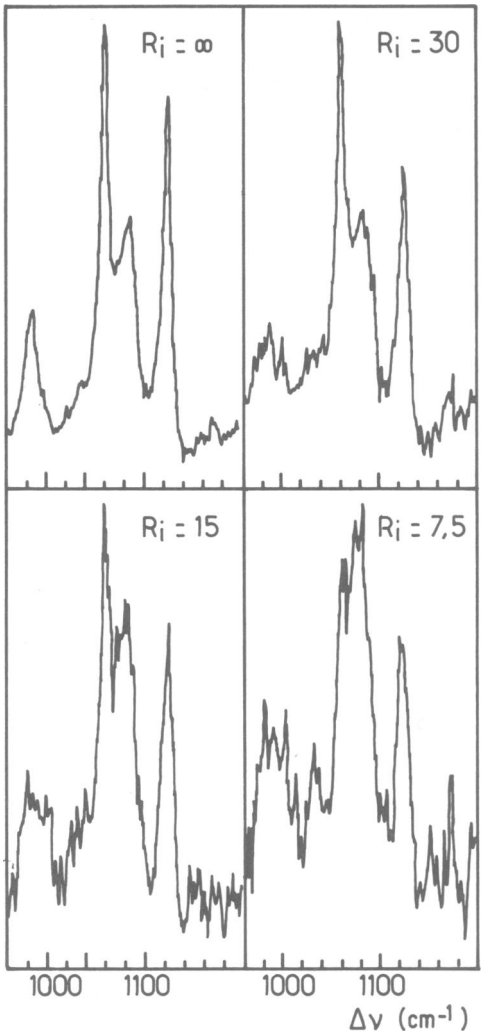

FIGURE 2 Effect of CTX on the C–C region of the Raman spectrum of DMPA bilayers at 37°C and pH 7.5 (100 mM phosphate buffer). Each spectrum is an average of 20 scans at 2 s/cm^{-1}.

REFERENCES

1. Dufourcq, J., and J. F. Faucon. 1977. Binding of melittin, an amphipathic peptide, to phospholipid vesicles. *Biochim. Biophys. Acta.* 467:1–11.
2. Dufourcq, J., and J. F. Faucon. 1978. Specific binding of a cardiotoxin from *Naja mossambica mossambica* to charged phospholipids detected by intrinsic fluorescence. *Biochemistry.* 17:1170–1176.
3. Mollay, C. 1976. Effect of melittin and melittin fragments on the thermotropic phase transition of dipalmitoyllecithin and on the amount of lipid-bound water. *Fed. Eur. Biochem. Soc. Lett.* 64:65–68.
4. Verma, S. P., and D. F. H. Wallach. 1976. Effect of melittin on thermotropic lipid state transitions in phosphatidylcholines. *Biochim. Biophys. Acta.* 426:616–623.
5. Lavialle, F., I. W. Lewin, and C. Mollay. 1980. Interaction of melittin with dimyristoylphosphatidylcholine liposomes. Evidence for boundary lipids by Raman spectroscopy. *Biochim. Biophys. Acta.* 600:62–71.