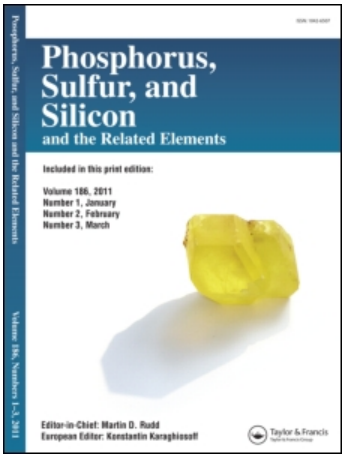

Phosphorus, Sulfur, and Silicon and the Related Elements

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

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To cite this Article Dufourcq, J. , Dufourc, E. J. , Maillet, J. -C. , Cornut, I. , Thiaudiere, E. and Bonmatin, J. M.(1993) 'Peptide-Induced Changes in Structure, Dynamic and Barrier Properties of Liposomes and Membranes', Phosphorus, Sulfur, and Silicon and the Related Elements, 77: 1, 113 — 116
To link to this Article: DOI: 10.1080/10426509308045632
URL: http://dx.doi.org/10.1080/10426509308045632

Phosphorus, Sulfur, and Silicon, 1993, Vol. 77, pp. 113–116
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PEPTIDE-INDUCED CHANGES IN STRUCTURE, DYNAMIC AND BARRIER PROPERTIES OF LIPOSOMES AND MEMBRANES

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Abstract: Peptides which have the plasma membrane as a target may induce colloid osmotic lysis of cells. This is reflected by changes in phospholipid structure and dynamics as documented by spectroscopic methods and by modifications of their supramolecular properties such as phase stability, vesicularization, fusion and micellization. From similar changes induced both on model systems and on natural membranes, mechanisms are proposed for biological membrane destabilization.

INTRODUCTION

Proteins and peptides may interact with phospholipids. As a result, changes may be observed in the structure and dynamics of both components. This is well documented for cytotoxins which are proposed to act through changes in the structure and properties of the phospholipid framework. Changes can be conveniently monitored on the acyl chains by ^{2}H-NMR and on the phosphate head group by ^{31}P-NMR.

These interactions may essentially be electrostatic, between oppositely charged partners, or hydrophobic resulting in the burying of peptide apolar residues within the membrane core. Different typical situations will be presented to illustrate each case and rationalize the perturbations according to the respective positions of peptides and lipids within the membrane.

PEPTIDE-INDUCED CHANGES AT THE MOLECULAR LEVEL

Lipid-to-peptide interactions dominated by electrostatic forces

Polylysines (PLL) are well known polycations able to bind at the charged lipid interface where they generally induce a phase separation in the membrane plane by aggregation in domains of PLL-coated phopholipids. These domains of different curvature have a higher gel-to-fluid phase transition temperature[1,2].

By monitoring fluid phase dimyristoylphophatidic acid (DMPA) by ^{2}H-NMR one observes the classical orientational order parameter gradient of the CD_2 groups with a plateau up to the ninth position followed by a sharp decrease towards the center of the bilayer (Figure 1A). The addition of long (MW=200 000) PLL increases the order parameter all along the chain up to a maximal effect when the 1:1 Lysine residue-to-PO_4 group molar ratio is reached. Then charge neutralization at the interface by the polypeptide results in a better packing of the lipid chains down to the bilayer core. PLL

with lower molecular weight (4000) is unable to induce phase separation[2] but increases the lipid order. Hovewer, this effect does not propagate to the center of the bilayer as seen on Figure 1A. By Raman spectroscopy of the same systems one can obtain informations on lipid order and peptide structure. When bound to these lipids, long

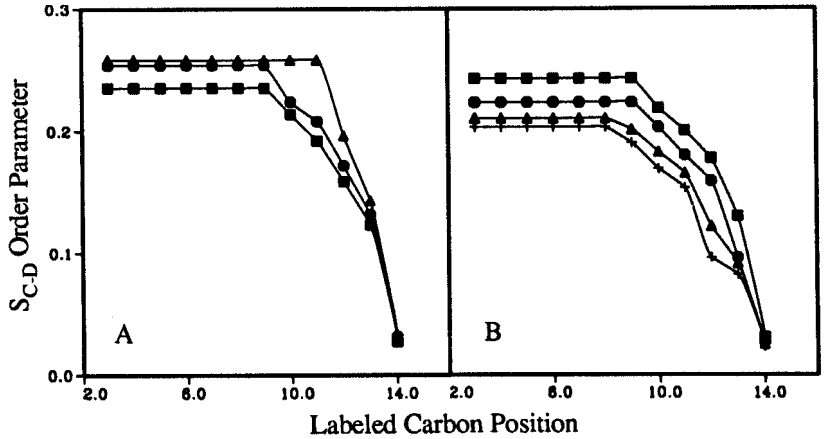

FIGURE 1 Orientational order parameter *vs* DMPA labeled carbon position (*sn*-2 acyl chain). A: 55°C, pH= 6.5, (■) pure lipid, (▲) with long PLL, (●) with short PLL. B: 47 °C, pH=4.2, (■) pure lipid, with melittin (●) 0.5 mole %, (▲) 16 mole %, (+) 32 mole %.

are structured in β-sheets whatever the temperature. At the opposite, for the short PLL one observes a concomitant drop of the lipid order and a peptide unfolding at the transition temperature, T_C. The same behaviour is also observed with dipalmitoylphosphatidic acid. The lipid order-disorder transition therefore triggers the conformational change of the peptide at the interface[3]. The differences seen in Figure 1A according to peptide length therefore result from different peptide structures at the interface, the more rigid β-sheet imposing more restricted lipid motions.

Lipid-to-peptide interactions dominated by hydrophobic forces

Melittin, also a strongly basic peptide, has an apolar segment and structures itself as an amphipathic α-helix at lipid interfaces[4]. As seen on Figure 1B it has the reversed effect on the DMPA acyl chain order when compared to PLL: the more melittin bound, the greater the decrease. This also occurs all along the lipid chain and whatever the temperature.

Melittin also interacts with zwitterionic lipids and as shown on Figure 2B induces a very similar effect on dipalmitoylphosphatidylcholine (DPPC) at high temperatures. δ-Toxin, another cytotoxic amphipathic peptide which has no net charge, gives an almost identical decrease in the order parameter of the aliphatic chains, Figure 2B. Embedding the apolar part of these peptides in the fluid membrane therefore promotes a CD_2 order parameter decrease.

When the temperature is decreased towards T_C, the effects induced by melittin

decrease while those due to δ-Toxin are totally reversed (Figure 2A): near T_C, it strongly increases the lipid order all along the chain as observed for PLL. The perturbations induced by the toxins therefore severely change both in strength and direction, according to temperature.

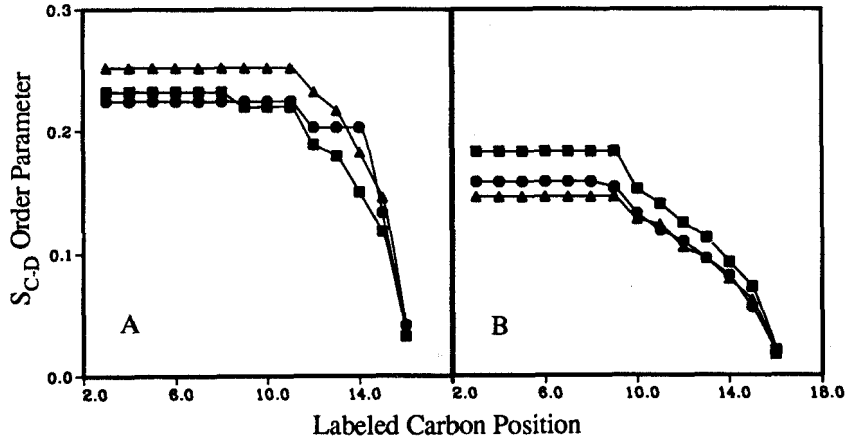

FIGURE 2 Orientational order parameter profile *vs* DPPC labeled carbon position (*sn*-2 chain). A: (■) pure lipid, 41°C, (●) with 5 mole % melittin, 41 °C, (▲) with 9 mole % δ-Toxin, 45°C. B: 65 °C (■) pure lipid, (●) with 5 mole % melittin, (▲) with with 9 mole % δ-Toxin.

On going to the gel phase the differences between the two toxins become even more striking. δ-Toxin has no effect on the lipid chains but ^{31}P-NMR clearly indicates that the toxin changes the structure of the polar head by remaining in contact with the bilayer an probably stays at interface without entering into the hydrophobic core[5]. At the opposite melittin induces even more drastic changes since at $T<T_C$ both ^{2}H and ^{31}P-NMR spectra show an isotropic line (small isotropically tumbling structures) superimposed onto the powder pattern of lamellar phase lipids.

PEPTIDE-INDUCED CHANGES AT THE SUPRAMOLECULAR LEVEL

As soon as the lipid-peptide mixtures, which have been incubated at $T>T_C$, are brought at $T<T_C$, melittin transforms the lamellar structures into new discoidal particles well characterized by gel filtration, light scattering and NMR. They correspond to *ca.* one-to-two thousand lipid molecules packed as a bilayer fragment surrounded by a "tire" of amphipathic peptides[6,7]. Increasing the acyl chain length above 22 carbon atoms lowers the ability of melittin to fragment the bilayers. ^{31}P-NMR conveniently allows to monitor the stability of these fast tumbling structures[8], it also documents a reversible disc-to-large vesicle transition triggered by the acyl chain order-disorder transition[7].

When cholesterol is present in the bilayer, it decreases the ability of melittin to induce discoidal particles. Conversely when melittin interacts with phosphatidyl-

ethanolamine, well known for undergoing a lamellar-to-hexagonal phase transition on increasing the temperature, this transition can be totally abolished as sensitively detected by NMR through changes in the ^{31}P chemical shift anisotropy.

Finally, when melittin is assayed on cell membranes such as erythrocyte ghosts, the ^{31}P-NMR spectra are changed very similarly to what has been described here above for gel phase lipids[8]. The amount of isotropically tumbling lipids increases in parallel with the amount of toxin added. This also parallels with the amount of lipids which are not pelleted with the ghosts by centrifugation and the amount of hemolysis on whole cells[9]. Then it can be proposed that the fragmentation-fusion events characterized on liposomes can also occur when the amphipathic peptides act on cells.

CONCLUSION

As opposed to the very weak effects generally observed with intrinsic membrane proteins[10] we have shown drastic changes in the lipid order and dynamics both when electrostatic and hydrophobic forces stabilize basic and/or amphipathic polypeptides in the membrane. Such changes can be rationalized either by an improved packing of the lipids due to charge neutralization at the interface, or conversely by the insertion of part of the peptide at the contact of the lipid chains which decreases the order of lipid chains, *i.e.* the hydrophobic thickness, which may in turn result in a decreased bilayer stability. Such changes are also modulated by the physical state of the lipids, which leads to the proposal that very delicate changes in the position of the polypeptides with respect to the bilayer can lead to quite different perturbations. In a way similar to what has been developed for the lipids by Israelashwilii, one may define an equivalent geometry for peptides like δ-Toxin, melittin or analogues. These "cone shaped molecules" or inverted ones may act differently on membranes according to the position of the amphipathic rod with respect to the lipid interface.

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