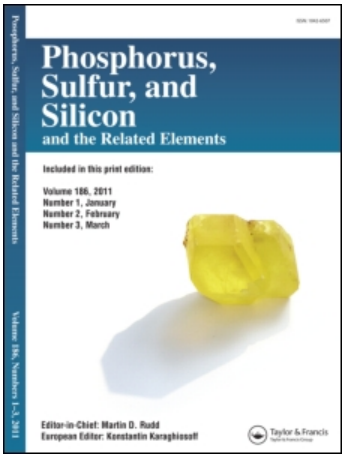

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

Mechanistic Study on Membrane Lysis by Bee Venom

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To cite this Article Nishiya, T. , Lam, R. T. -T. and Robibo, D.(1993) 'Mechanistic Study on Membrane Lysis by Bee Venom', Phosphorus, Sulfur, and Silicon and the Related Elements, 77: 1, 117 — 120
To link to this Article: DOI: 10.1080/10426509308045633
URL: http://dx.doi.org/10.1080/10426509308045633

Phosphorus, Sulfur, and Silicon, 1993, Vol. 77, pp. 117–120
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MECHANISTIC STUDY ON MEMBRANE LYSIS BY BEE VENOM

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Abstract Interaction of phospholipid bilayer with melittin and/or phospholipase A_2 (PLA) have been studied with circular dichroism (CD), fluorescence, differential scanning calorimetry (DSC), and pH-stat.

INTRODUCTION

Melittin, an amphipathic 26-amino acid peptide, and PLA are main constituents of the bee venom. Melittin induces a wide variety of structural perturbation of the lipid bilayer, depending on the type of lipid and lipid/melittin molar ratio (Ri), and the activity of PLA depends on the phase state of membrane matrix. In this report, the interactions of lipid bilayers composed of phosphatidylcholine or phosphatidylethanolamine with melittin and/or PLA are discussed.

INTERACTION OF PHOSPHATIDYLCHOLINE WITH MELITTIN AND/OR PLA

Interaction of melittin and/or PLA with CD active phospholipid, bis(4'-n-octanoxazobenzene-4-carboxyl)-L-*α*-phosphatidylcholine (CDPC)[1], were studied. In the presence of melittin at Ri of 5, multilamellar dispersion, composed of CDPC and DPPC with a molar ratio of 1, underwent morphological change to form small melittin-lipid particles. When PLA was added to these particles at 24°C, the CD band at 222 nm exhibited a remarkable enhancement depending on Ri, indicating the formation of melittin-PLA-lipid complex.[2] Fluorescein-labeled PLA (FLU-PLA) was used to study the effects of

the substrate binding on the internal rotation of FLU-PLA in the presence or absence of melittin. In this study, the change in fluorescence polarization (P) of FLU-PLA, excitation at 490 nm and emission at 520 nm, can be investigated without interfering effects of the perturbation from melittin. In the presence of melittin, the increase in P of FLU-PLA as a function of DMPC conc. is more pronounced than that in the absence of melittin, suggesting the formation of a ternary complex composed of melittin, FLU-PLA and DMPC (Fig. 1).

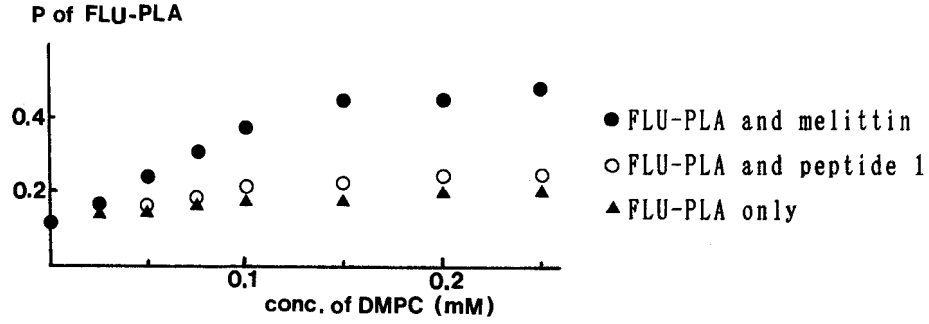

FIGURE 1 Effects of melittin and peptide 1 on P of FLU-PLA

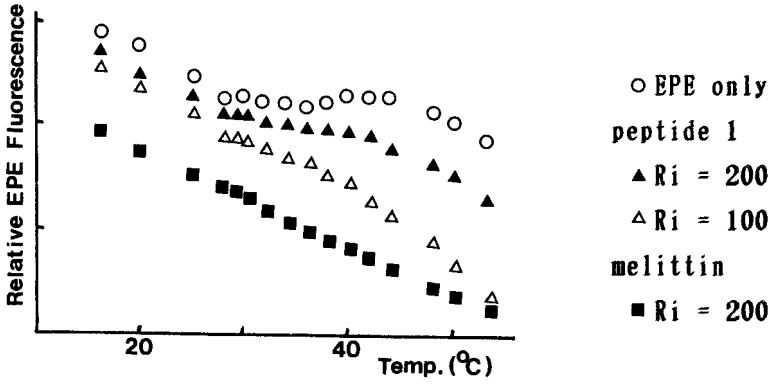

FIGURE 2 Effects of melittin and peptide 1 on EPE bilayer

INTERACTION OF PHOSPHATIDYLETHANOLAMINE WITH MELITTIN AND/OR PLA

The effects of melittin on the bilayer-to-hexagonal (H_{II}) phase transition of egg phosphatidylethanolamine (EPE), and the influence of the phase state of membrane matrix on hydrolysis of EPE by PLA have been studied. The phase transitions were measured using the fluorescent probe N-(7-nitro-2,1,3-benzoxadiazol-4-yl)-phosphatidylethanolamine (N-NBD-PE) and DSC. In the presence of melittin, the phase transition of EPE disappeared, indicating that melittin stabilizes the bilayer structure (Fig.2). The fluorescence intensity of the tryptophan residue of melittin is sensitive to the phase transition and the wavelength of emission maxima shifted from 352 to 337 nm upon addition of EPE. Kinetic parameters for PLA-catalyzed hydrolysis of EPE in bilayer and H_{II} phases showed that H_{II} phase of EPE is a poorer substrate for PLA.[3]

EFFECTS OF MELITTIN ANALOGUE ON MEMBRANE DYNAMICS

Melittin-induced morphological changes in the membranes may be attributed to a wedgelike penetration of a hydrophobic part of the protein molecule into the lipid bilayer structure. To test this hypothesis, the effects of melittin analogue, peptide 1 (Fig.3), on lipid bilayer was studied. As shown in Fig.3, residues 1-20 of peptide 1 have been chosen to form an amphiphilic helix with minimum homology to melittin while maintaining a hydrophobic-hydrophilic balance related to the two amphiphilic α helical segments of melittin (2-13) and (15-21). The most important difference between peptide 1 and melittin is that peptide 1 forms a longer amphiphilic segment than melittin without the break of helix, because the helix breaker proline at position 14 in melittin is replaced by serine in peptide 1.

Interestingly, peptide 1 has the different effects from melittin on the lipid bilayers. In the presence of peptide 1, the change in P of FLU-PLA as a function of DMPC conc. was much smaller than that in the presence of melittin (Fig. 1), indicating that peptide 1 does not form a ternary complex with DMPC and FLU-PLA. The bilayer-to- H_{II} phase transition of EPE could be observed in the presence of peptide 1 at Ri of 200 and 100, while the phase transition disappeared in the presence of melittin even at Ri of 200, suggesting that peptide 1 does not stabilize the EPE bilayer (Fig. 2).

PEPTIDE 1

H_2N-LEU-LEU-GLN-SER-LEU-LEU-SER-LEU-LEU-GLN-
SER-LEU-LEU-SER-LEU-LEU-LEU-GLN-TRP-LEU-
LYS-ARG-LYS-ARG-GLN-GLN-$CONH_2$

MELITTIN

H_2N-GLY-ILE-GLY-ALA-VAL-LEU-LYS-VAL-LEU-THR-
THR-GLY-LEU-PRO-ALA-LEU-ILE-SER-TRP-ILE-
LYS-ARG-LYS-ARG-GLN-GLN-$CONH_2$

FIGURE 3 Amino acid sequences of peptide 1 and melittin

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