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Kinetics of Interaction between Insecticidal Cry1A Toxins from *Bacillus thuringiensis* and Artificial Lipid Membrane Vesicles (Liposomes).

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The insecticidal mechanism of Cry toxins, especially the interaction between Cry toxins and larval midgut apical cell membranes (brush border membrane, BBM), has not been fully elucidated. We employed liposomes as a simplified model to study the interaction of Cry toxins with membranes and evaluate their destructive efficacy. Cry1Ab disrupted phosphatidylcholine/phosphatidylserine (PC/PS) liposomes and PC/PS liposomes containing cholesterol and oligosaccharylceramide from *Plutella xylostella* midgut more extensively. When PC liposomes containing Triton X-100 soluble proteins from BBM vesicles of *Bombyx mori* were exposed to Cry1Ab, the pore formation activity increased 17-fold compared to that in PC liposomes, suggesting that Cry1Ab achieved higher penetration into various liposomes than the two other toxins that were tested (Cry1Aa and Cry1Ac).

Bacillus thuringiensis Cry toxins bind to specific receptors on larval midgut apical cell membranes (brush border membranes, BBM) and form pores that lead to insect death. Cry toxins have a narrow toxicity spectrum against insect larvae and are used as biopesticides to control insect pests worldwide. However, the appearance of resistance in insects (1, 2) like the diamondback moth, *Plutella xylostella*, represents a major threat to the use of Cry toxins. Cry1Ac-susceptible *Plutella xylostella* (PXS) larvae are killed by Cry1Aa, Cry1Ab and Cry1Ac at relatively low toxin concentrations (i.e., LC50 = 0.3 µg/g of diet for Cry1Aa and Cry1Ac, and 0.1 µg/g of diet for Cry1Ab) while Cry1Ac-resistant *P. xylostella* (PXR) larvae are only killed by Cry1Aa and Cry1Ab. The insecticidal mechanism of these toxins has not been fully elucidated yet due to the complexity of the BBM structure. Therefore, we used liposomes to estimate the interaction between Cry toxins, lipids and BBM components.

We analyzed the binding of Cry1Aa, Cry1Ab and Cry1Ac to CHAPS-soluble brush border membrane vesicle (BBMV) proteins from PXR and PXS using surface plasmon resonance (SPR) (Fig. 1) (3). Cry1Aa and Cry1Ac bound to BBMV proteins from both PXR and PXS, but Cry1Ab did not. There are two possible explanations for these results. Firstly, the receptor for Cry1Ab may differ from that of Cry1Aa and/or Cry1Ac and Cry1Ab could bind to other membrane components besides BBM proteins. Secondly, the binding of Cry1Ac to midgut proteins may not result in insecticidal activity. We have found that PXR and PXS BBMs differ substantially in the sugar moiety of oligosaccharyl-ceramide (OSC, a glycosphingolipid) (4). We hypothesized therefore that the microenvironment of PXR BBM is responsible for resistance to Cry1Ac.

To test the above hypothesis, we used different types of calcein-loaded liposomes made of phosphatidylcholine (PC), phosphatidylserine (PS), cholesterol (CHL), OSC from PXS or PXR, and Triton X-100-soluble BBM proteins. They were essentially prepared as described by Doron and Shai (5).

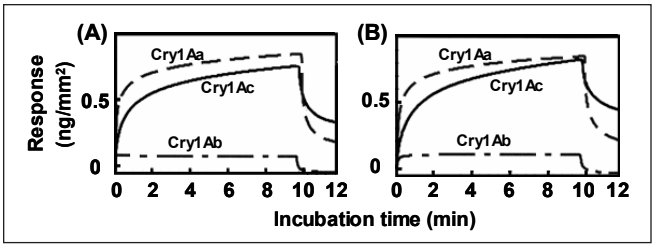

FIG. 1. SPR analysis of binding between Cry1A toxins and CHAPS-soluble BBM proteins using IAsys. Cry1A toxins were reacted with the BBM proteins from PXS (A) and from PXR (B). Response represents Cry1A toxins bound to gold thin film of IAsys.

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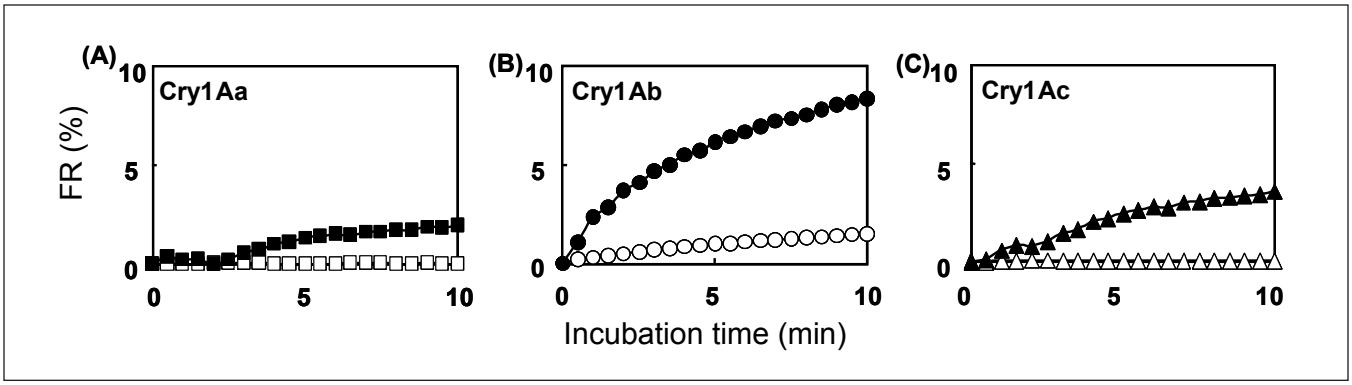

FIG. 2. Release of calcein from PC- and PC/Proteoliposomes upon disruption by Cry1A toxins. PC/Proteoliposomes contained TritonX-100-soluble BBM proteins from *Bombyx mori*. PC-Liposomes were used as control. White and black symbols show amount of fluorescence released from PC- and PC/Proteoliposomes, respectively.

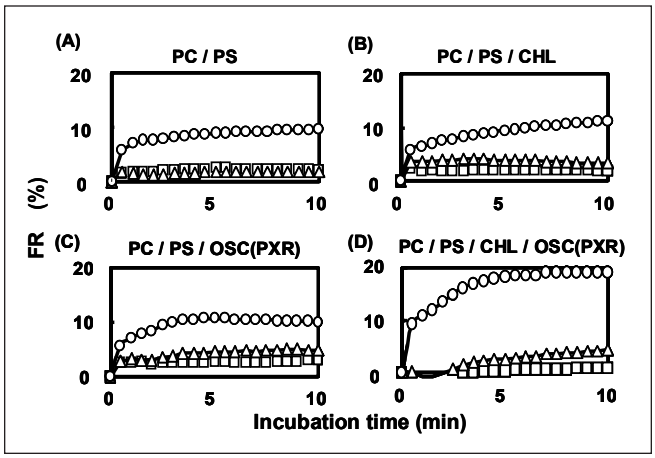

FIG. 3. Release of calcein from various liposomes upon disruption by Cry1A toxins. Liposomes were composed of PC/PS (A), PC/PS/CHL (B), PC/PS/OSC(PXR) (C), and PC/PS/CHL/OSC(PXR) (D), respectively. Squares represent Cry1Aa; circles, Cry1Ab; and triangles, Cry1Ac.

The ability of Cry1A toxins to form pores in PC proteoliposomes was measured by a calcein release assay (Fig. 2) (6). The fluorescence intensity (FI) of calcein excited at 490 nm was measured at 520 nm. The amount of calcein released (FR) was expressed as the percentage of the maximum FI measured by the addition of Triton X-100 .

When PC proteoliposomes were exposed to Cry1Ab, the pore formation activity of the toxin, determined as the initial FR rate, increased 17-fold compared to that observed in PC liposomes, suggesting that Cry1Ab is more active in the presence of a complex of lipids and BBMV proteins.

To find out whether the microenvironment of BBMs is involved in the mechanism of resistance, we prepared six types of liposomes to assess Cry1A toxin-mediated pore formation. Fluorescence measurements on PC/PS/CHL/OSC(PXS) liposomes showed the same kinetics as that from PC/PS/CHL/OSC(PXS) liposomes. Therefore, only the PXR results (6) are shown in Fig. 3.

Simultaneous enrichment of liposomes with CHL and OSC resulted in augmented pore formation by Cry1Ab, whereas Cry1Ac hardly formed pores in all types of liposomes. We, therefore, speculate that CHL and OSC change the structure of liposome membrane. However, this does not support our previous hypothesis that OSC from PXR reduces the interaction between the plasma membrane and Cry1Ac. More precise fractionation of OSC will be necessary to evaluate further the role of PXR oligosaccharyl-ceramide.

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