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Mechanism of Detoxification of Cry1Ac in *Bombyx mori*, Hybrid Shunrei x Shogetsu

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Toxicity of Cry1Ac to *Bombyx mori,* Shunrei x Shogetsu, was 3,200 times lower than that of Cry1Aa. However, non toxic Cry1Ac binds to midgut BBM proteins as well as that of highly toxic Cry1Aa. We found that Cry1Ac was trapped by some peritrophic membrane (PM) proteins via N-acetylgalactosamine of a sugar side chain. P252 was purified from *B. mori BBM* as Cry1Ac binding proteins. Furthermore, we also found Cry1Ac binding proteins, such as 105-, 100-, 96- and 75-kDa BBM proteins. We thought that a majority of these Cry1Ac binding proteins may act as pseudo-receptors to quench the toxicity against *B. mori* as well as PM proteins.

B. mori, hybrid Shunrei x Shogetsu, is susceptible to Cry1Aa and insensitive to Cry1Ac (1). It has been believed that the toxicity is correlated with the presence of a specific receptor in midgut epithelial cell membrane of insects. In ELISA and ligand blot analysis, however, many kinds of BBM proteins were shown to bind to Cry1Ac with almost equal intensity in both resistant and susceptible insects. These suggest that a majority of the bindings between Cry1Ac and BBM proteins of *B. mori* may be pseudo-binding. Therefore, we hypothesized that those pseudo-bindings to Cry1Ac should not lead to insect death. As Cry1A must pass through the PM before reaching the BBM, PM is an important step for the activation of Cry1A toxin as well as BBM. To elucidate the Cry1Ac non-susceptibility of *B. mori*, we searched Cry1Ac binding proteins which are involved in the pseudo-binding in PM and BBM of this insect. Here, we present evidences showing some PM and BBM proteins may act as a pseudo-receptor for Cry1Ac in *B. mori*.

First of all, we evaluated the permeability of Cry1Aa or Cry1Ac through the PM. An apparatus to estimate the passage through PM was constructed as described in (2). BSA (66-kDa), Carbonic anhydrase (29-kDa) and Cry1Aa passed through the PM at 0.37 μg/mm^2 PM/h. These passages seemed to be a diffusion process because their rates were similar, even though their molecular sizes were different. In contrast, Cry1Ac did not pass through the PM for the first 2 h. However, during the third hour, Cry1Ac passed through the PM at 0.34 μg/mm^2 PM/h, because of a destruction of the PM or a saturation of Cry1Ac binding site on the PM.

The Cry1Ac trapped during the first two hours correlated closely to an interaction of the toxin with some PM proteins. We performed ligand blot analysis of Cry1A toxins with PM proteins. As expected, Cry1Ac binding proteins were detected in detergent soluble fractions (Fig. 1).

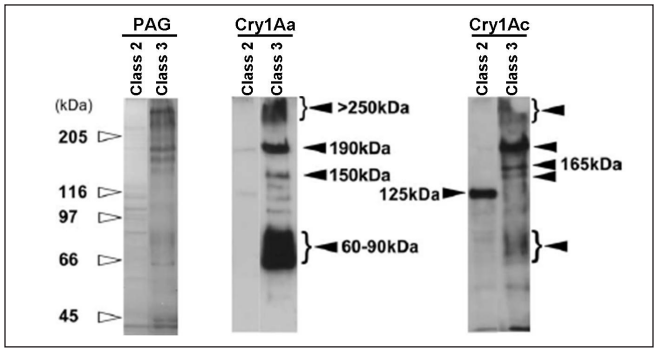

FIG. 1. Ligand blot analysis of PM proteins from *B. mori* treated with Cry1Aa and Cry1Ac. Class 2 and class 3 PM proteins were seperated by SDS-7.5% PAGE and strained with CBB (PAG). PM proteins were then transferred to a PVDF membrane and analysed by ligand blot using Cry1Aa and Cry1Ac. The migration of molecular weight markers is indicated by open arrow heads. Major PM proteins bound to Cry1Aa and/or Cry1Ac are indicated by filled arrow heads.

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In particular, 125- (P125), 100- (P100), 95- (P95) and 55- (P55) kDa proteins from class 2 which were obtained with 1% Triton X-100 and 165 (P165) kDa proteins of class 3 which was extracted with 2% SDS and 2% mercaptoethanol were shown to bind to Cry1Ac only but not Cry1Aa (2). Our results suggest that the prevention of Cry1Ac from passing through the PM occurs by trapping of PM proteins.

Cry1Ac recognizes N-acetylgalactosamine (GalNAc) occurring as sugar side-chain of proteins localized in BBM (3). Cry1Ac trapping by whole PM proteins was inhibited by 70% in the presence of GalNAc, but Cry1Aa trapping was not. In ligand blot, the bindings between Cry1Ac and P125, P95, P55 and P165 were almost completely inhibited by GalNAc. We estimated the GalNAc effect on the Cry1Ac passage through the PM and co-inoculation of Cry1Ac and GalNAc facilitate was shown to Cry1Ac passage through the PM with rate of 0.45 μg/mm^2 PM/h. GalNAc residue of PM protein seemed to be involved in Cry1Ac trapping.

We focused on the Cry1Ac binding proteins in class 2 PM and tried to purify them using DEAE ion-exchange chromatography. Whole proteins of this class were fractionated into 8 peaks and those were subjected to ligand blot analysis after SDS-PAGE. Four proteins, *i. e.*, P125, P100, P95 and P55, were detected as Cry1Ac binding protein and the bindings between those proteins and Cry1Ac were inhibited by GalNAc with respective degree. Further characterizations of those proteins are under way in our laboratory.

We also detected several kinds of Cry1Ac binding proteins in *B. mori* whole BBM protein, Triton X-100 soluble and insoluble BBM proteins (Fig. 2).

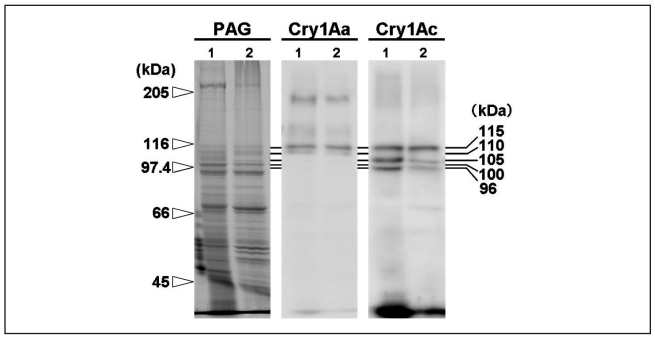

FIG. 2 Ligand blot analysis of BBMV proteins with Cry1Aa and Cry1Ac. Whole BBMV or Triton X-100-soluble proteins from BBMV were applied to SDS-PAGE and proteins were blotted onto PVDF membrane. Ligand blot was performed with Cry1Aa and Cry1Ac. Lane 1: whole BmBBMV proteins, Lane 2: Triton X-100-soluble BmBBMV proteins. The migration of molecular weight markers is indicated by open arrow heads.

Although Cry1Ac has no toxicity to *B. mori*, many kinds of Cry1Ac binding proteins, such as 252-, 115-, 105-, 100-, 96- and 75-kDa, were detected. We purified 96-kDa APN (APN96) and 252-kDa (P252) proteins (1, 4) as Cry1Ac binding proteins in Triton X-100 soluble fraction of *B. mori* BBM. We determined the K_D constant of APN96 and P252 for Cry1Ac binding using SPR analysis. The K_D value of APN96–Cry1Ac interaction was 1.83 μM (1) and the K_D value for Cry1Aa, Cry1Ab and Cry1Ac of P252 were 28.9, 178.5 and 20 nM, respectively (4).

We believe that a majority of these Cry1Ac binding proteins from both PM and BBM may act as pseudo-receptors to quench the toxicity of Cry1Ac. We propose here a model of mechanism for detoxification of Cry1Ac in *B. mori*. Activated Cry1Ac is initially trapped by PM proteins. In case, some Cry1Ac molecules pass through the PM and bind to BBM proteins, such as APN96, P252, 100-, 105- and 75-kDa proteins. The bindings with these proteins further reduce the toxicity of Cry1Ac. It was reported that both the cadherin like protein and APN have been shown to relate with Cry1Ab toxicity in *Manduca sexta* (3, 5, 6). We hypothesize, however, that there is no such involvement of these proteins in Cry1Ac binding in *B. mori*. As Cry1Ac must bind to PM and/or BBM proteins and stay there unchange for some time, Cry1Ac cannot form pores on BBM.

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