LOCALIZATION OF THE *BACILLUS THURINGIENSIS* TOXIN RECEPTOR IN THE GYPSY MOTH BY IMMUNO ELECTRON MICROSCOPY

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ABSTRACT

Bacillus thuringiensis (Bt) biopesticides have proved to be highly valuable for controlling a variety of agricultural and forest insect pests. Bts are characterized by the production of insecticidal crystal (Cry) proteins, which interact with receptors on the midgut epithelial cells. After binding to their receptors, the Bt toxins insert into the membrane, form pores, and destroy the gut cells. Two Bt receptors have been identified in the gypsy moth, *Lymantria dispar*, that bind with high affinity the lepidopteran-specific Cry1A Bt toxins: a 120 kDa glycosylphosphatidyl-inositol (GPI)-anchored aminopeptidase N (APN); and a 270 kDa glyconjugate (BTR-270). Analysis of toxin binding to APN and BTR-270 by probing western blots with Cry1A toxins and by surface plasmon resonance showed that gypsy moth APN binds Cry1Ac toxin but does not interact with other Bt toxins (i.e., Cry1Aa or Cry2A toxins), which are highly toxic to the gypsy moth. In contrast, binding studies showed that BTR-270 interacts with all of these toxins, and the affinities of the toxins to BTR-270 correlate with their respective toxicities.

Immunolocalization of Bt toxin binding sites in lepidopteran insects has been previously reported. These reports have demonstrated that the Bt Cry toxins accumulate at the apical microvilli of sensitive insects. Little or no accumulation was reported in the goblet cells, cytoplasm, nucleus, or other locations. Although BTR-270 has been identified as a high-affinity receptor for Bt toxins in the gypsy moth, the localization of this Bt receptor in the insect midgut tissue has not been determined.

In this study, we used an immunogold electron microscopic procedure to study the distribution of BTR-270 in the gut tissue of third-instar and fifth-instar gypsy moth larvae. The distribution of the immunogold label for BTR-270 was essentially similar to that reported for the localization of Bt toxins in midgut tissue of lepidopteran larvae. The label was concentrated on the microvilli on the brush border membrane of midgut epithelial cells. Little or no labeling was observed in goblet cells or in the cytoplasm, confirming that BTR-270 is an intrinsic and specific component of the gypsy moth brush border membrane microvilli.