P210k: THE PUTATIVE BINDING PROTEIN FOR Bacillus thuringiensis CrylA(c)δ-ENDOTOXIN IN THE MIDGUT BRUSH-BORDER MEMBRANE OF THE LEPIDOPTERAN Diatraea saccharalis

Roberto I Vázquez, Dmitri L Prieto, Ariel Martínez and Gustavo A de la Riva

Department of Environmental Biotechnology, Center for Genetic Engineering and Biotechnology, P.O. Box 6162, Havana 10600, Cuba.

Introduction

Bacillus thuringiensis is a gram-positive soil bacterium, able to produce insecticidal parasporal bodies during sporulation. These bodies -commonly of crystalline shape are composed of subunits called -endotoxins, or Cry proteins, encoded by *cry* genes, which form a large gene family. The Cry proteins are classified in classes and subclasses according to aminoacid sequence homology and target specificity (1).

The mode of action of *B. thuringiensis* insecticidal proteins involves several stages (2). Upon ingestion by the insect larvae, the Cry proteins are proteolytically processed in the midgut, and bind to high-affinity specific membrane receptors located on the brush-border epithelium. Then, cation-specific channels are formed causing an electrolytic imbalance that ultimately kills the larva. Several authors report the identification and purification of Cry protein receptors from various insect species, as well as cloning and sequencing of their genes.

Materials and Methods

Preliminary studies of the toxicity of CryI proteins against *D. sacharalis* were done employing recombinant CryIA(b) and CryIA(c) proteins expressed in *Escherichia coli* and 3-instar larvae at 10 per toxin concentration. Extracts from untransformed *E. coli* were used as negative control. The bioassay was standardised using *B. thuringiensis* var *kurstaki* HD-1δ -endotoxin.

To identify the toxin binding proteins in the midgut epithelial cell membranes of *D. saccharalis*, BBMV (3) proteins have been electrophoretically separated by SDS-PAGE and transferred to nitrocellulose membranes in a submarine transfer apparatus. The membrane was submitted to a ligand-blot analysis using activated natural CryIA(c) δ -endotoxin and immunopurified subclass-specific anti-CryIA polyclonal antibodies. Immunochemical recognition was detected by anti-rabbit peroxidase using ECL chemoluminiscent system. Preliminary competition assays were done.

Results and Discussion

Bioassays performed with recombinant CryIA(b) and CryIA(c) proteins and third instar *D. saccharalis* larvae, showed that the values of LD_{50} were 1,2 μg/ml diet for CryIA(b) and 7.8 μg/ml diet for CryIA(c). These results are correlated with the fact of strong self-pesticide activity, observed in transgenic sugarcane plants expressing recombinant CryIA(b) toxin.

The ligand-blot experiment allowed a well-defined band for a putative CryIA(c) binding protein with MW value of approximately 210 kDa. Preliminary competition experiments confirmed the possibility that this toxin binds to a putative receptor located on the brush-border membrane of the midgut epitelial cells. The protein with binding activity was called P210k.

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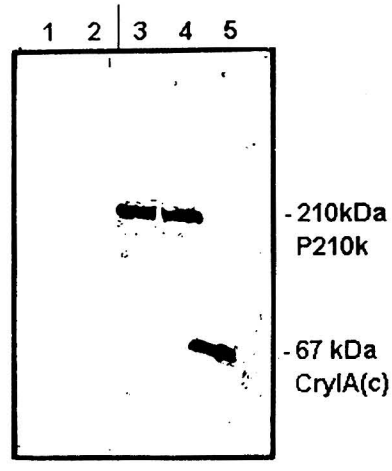

Figure 1. *Detection of P210 on the midgut brush-border membrane of* D. saccharalis *by Ligand Blotting. Lane 1: 10 μg total BBMV proteins not incubated with CrylA (c). Lane 2: 20 μg total BBMV proteins not incubated with CrylA (c). Lane 3: 10 μg total BBMV proteins incubated with CrylA (c). Lane 4: 20 μg total BBMV proteins incubated with CrylA (c). Lane 5: 10 μg trypsin-treated natural CrylA (c) toxin from* B thuringiensis *HD-73.*