



USING PaluIT1 TOXIN IN THE BIOCHEMICAL CHARACTERIZATION OF ION CHANNELS OF TWO LEPIDOPTERAN SPECIES

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Key words: ganglionic cord, PaluIT, Anti-PaluIT.

Introduction: The poisons produced by animals, are complex mixtures of peptides, which have been developed to capture prey and / or as a defense (1). The PalutoxinasIT, are a group of four toxins. (2). It has been reported PaluIT1 insecticidal activity against Lepidoptera, presenting a way of insect-specific action. It has been assessed in species as *Diatraea magnifactella*, *Galleria melanogaster* with an LD50 of 9.36 µg/g of insect and 9.5 µg/g for *Spodoptera liturgua* and 6.22 µg/g for *S. frugiperda* (2,3). Interaction has been identified in the cordon of PaluIT1 CNS nodal *S. frugiperda*, has also reported that the toxin PaluIT1 could have multiple receptors in the CNS, including a molecular weight higher than 200 kDa, possibly associated dependent sodium channel voltage and one with a weight of about 70 kDa (2). Objective. Elucidating the receiver *Spodoptera frugiperda* and *Diatraea magnifactella* where interacts the toxin with PaluIT1, by using antibodies PaluIT1.

Methods. Anti-PaluIT1: immunization was performed in a New Zealand rabbit race with increasing concentrations of toxin PaluIT1 for a period of 9 weeks. Was conducted immunoglobulin precipitation with caprylic acid. Obtaining insect CNS: ganglionic cord was extracted from *Spodoptera frugiperda* and from *Diatraea magnifactella* sixth instar larvae, a homogenate was performed and protein was measured by the Bradford method. Western-Blot: There were from SDS-PAGE 8%. Incubate with PaluIT1 toxin overnight followed by Anti-PaluIT1 and finally Goat Anti-Rabbit IgG (H + L)-HRP Conjugate Bio-Rad

Results. After 9 weeks of immunization and the precipitation of the immunoglobulin titers anti-PaluIT1 were obtained with a value of 1161. These were used in Western blot experiments, Figure 1 shows the result obtained, in this figure is seen in *Spodoptera frugiperda* as more than one receiver reports (3), the band with greater response has a molecular weight of about 96, 25 and 20 kDa and *Diatraea magnifactella* shows two

possible receivers with a weight of about 72 kDa and another at about 140 kDa.

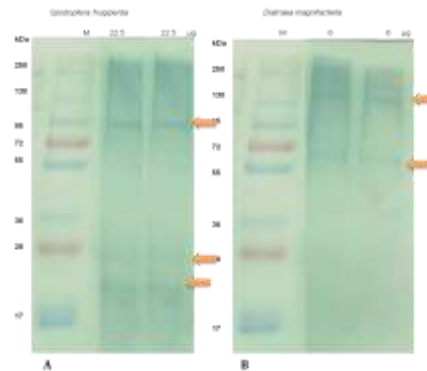


Fig.1. Results obtained by Western-blot with the two species of Lepidoptera. Panel A shows *Spodoptera frugiperda*, and in panel B *Diatraea magnifactella*.

Conclusions. This could be due to fragmentation of the sodium channel as possible PaluIT1 receiver as reports (2). It is suggested that the toxin is capable of recognizing sequences of the recipient, and it is not necessary to submit its three dimensional structure.

Acknowledgements. National Council for Science and Technology (CONACYT), by the scholarship to undertake this thesis Master with number 419248 and Basic Science project CONACYT **CB-2008-01** No. **106949**. Dr. Alejandro Alagon of IBT-UNAM for its help in the development of antibodies.

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