# CHARACTERIZATION OF VOLTAGE GATE ION CHANNELS, IN *GALLERIA MELLONELLA* USING PALUIT1

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*Key words: Ionic channels, Insecto-toxin δ-PaluIT1, Galleria mellonella*

**Introduction.** Spider venoms are powerful mixtures that may contain, small organic molecules like amino acids, nucleic acids, enzymes, polyamines, antimicrobials and neurotoxic peptides, so as proteins (1). Spider neurotoxic peptides can bind and recognize voltage gate ion channels such as: sodium $Na_V$, calcium $Ca_V$ and potassium $K_V$. Several spider neurotoxins have been reported to bind to site 4 of $Na_V$, among them δ-palutoxins (*P. luctuosus*), Curtatoxins (*Hololena curta*), μ-agatoxins (*Agelenopsis aperta*), and β/δ-agatoxins (4), they are structurally related since they are compose of 36–37 amino acid residues and cross-linked by four disulfide bridges forming an inhibitor cystine knot (ICK) motif (4). Among δ-palutoxins, δ-palutoxin IT1 (PaluIT1) has been described as an insecticidal toxin as potent as Lqh (*Leiurus quiquestratus*) scorpion toxin with 9.5 mg/gr $DL_{50}$ in *Galleria mellonella* (3). Using FITC-PaluIT1 in histochemical assays using the Central Nervous System (CNS: brain, subesophageal, thoracic and abdominal glanglia) has been identified as most abundant receptor tissue (3). PaluIT1 can be used as tool to study structural-function of voltage gated ion channels in Lepidoptera, to develop specific antibodies or labeled ligands for Western blot assays and develop specific insecticides or novel drugs such analgesics.

**Objective.** A rabbit antibody was produced, using PaluIT1 to identify voltage gated ion channel receptors in *Galleria mellonela* CNS.

**Methods.** An homogenate of *Galleria mellonela* glanglia nerve cord was prepared with a protein concentration of 2.9 mg/ml (Bradford assay). SDS-PAGE native and denaturing conditions as well as Western blot assays.

**Results.** In SDS-PAGE and Western blot experiments in native conditions a band around 250 kDa was detected. In denaturing conditions four bands were reveled, around ~68, 70, 75 kDa and another of 35 kDa (Fig 1).

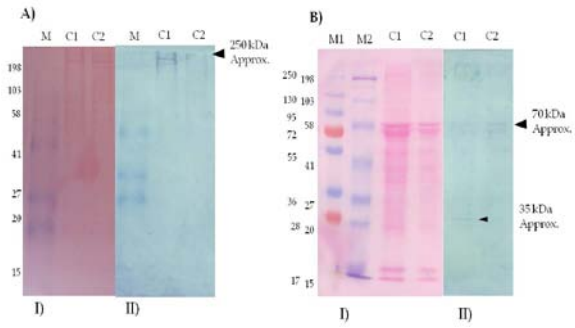


**Fig.1** 8% SDS-PAGE and Western Blot A) Native conditions I, M-molecular marker, C1-C2 supernatant homogenate stained with Ponceau red. II Peroxidase reveled membrane. B) Denaturing conditions 8%. M, I, II, same as A.

**Conclusions.** The 250 kDa band may correspond to a complete $Na_v$ in its native conformation. 70 kDa may corresponds to a specific site receptor according to (2), 35 kDa, could be the beta subunit of sodium channel. These results will being confirmed by proteomic experiments.

**Acknowledgements**. This work was supported by CONACyT 419235 grant, CEIB program degree and the Academic group of entomology and fitopatology.

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