

Jaburetox-2EC, a Recombinant Urease-Derived Insecticidal Peptide: Site-Directed Mutagenesis Studies.

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Ureases (E.C. 3.5.1.5) are metalloenzymes widespread in plants, fungi and bacteria that participate in nitrogen bioavailability and defense mechanisms in the source organism. Our group described the insecticidal properties of canatoxin, an isoform of *Canavalia ensiformis* (jack bean) urease, against different insect species. Its toxicity relies on an internal 10 kDa peptide (pepcanatox), released by hydrolysis of the protein by cathepsins in the digestive system of susceptible insects. Based on N-terminal sequence of pepcanatox, a 270-bp fragment corresponding to pepcanatox (*jaburetox-2Ec*) was amplified from JBURE-II cDNA and cloned into pET 101 vector to obtain heterologous expression in *Escherichia coli*. The recombinant Jaburetox-2Ec, carrying V5 and His tag, was fed to the cotton stainer bug, *Dysdercus peruvianus*, the kissing bug *Rhodnius prolixus*, the german cockroach *Blattella germanica* and the armyfall worm *Spodoptera frugiperda* and 100% mortality was observed in all the tested models after ingestion of few micrograms of the peptide. Modeling of Jaburetox-2Ec revealed a prominent β -hairpin motif consistent with an insecticidal activity based on either neurotoxicity or cell permeation. A site-directed mutagenesis approach was chosen to identify motifs involved in entomotoxic activity. Combinations of the aminoacids Lys, Glu, Asp in the hairpin were exchanged for Ala using the Quikchange site-directed mutagenesis kit (Stratagene). The mutagenized jaburetox, containing the His tag will be confirmed by sequencing and tested for entomotoxic activity in insect models (CAPES, CNPq, FAPERGS, FINEP).