ARTÍCULO DE INVESTIGACIÓN

Characterization of a Mutant *Bacillus thuringiensis* δ-endotoxin With Enhanced Stability and Toxicity

Caracterización de una delta endotoxina mutante de *Bacillus thuringiensis* con estabilidad y toxicidad aumentadas

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Summary

The centrally located α-helix 5 of *Bacillus thuringiensis* δ-endotoxins is critical for insect toxicity through ion-channel formation. We analyzed the role of the highly conserved residue Histidine 168 (H168) using molecular biology, electrophysiology and biophysical techniques. Toxin H168R was ~3-fold more toxic than the wild type (wt) protein whereas H168Q was 3 times less toxic against Manduca sexta. Spectroscopic analysis revealed that the H168Q and H168R mutations did not produce gross structural alterations, and that H168R (T_m= 59 °C) was more stable than H168Q (T_m= 57.5 °C) or than the wt (T_m= 56 °C) toxins. These three toxins had similar binding affinities for larval midgut vesicles (K_{conn}) suggesting that the differences in toxicity did not result from changes in initial receptor binding. Dissociation binding assays and voltage clamping analysis suggest that the reduced toxicity of the H168Q toxin may result from reduced insertion and/or ion channel formation. In contrast, the H168R toxin had a greater inhibition of the short circuit current than the wt toxin and an increased rate of irreversible binding (k_{obs}), consistent with its lower LC_{50} value. Molecular modeling analysis suggested that both the H168Q and H168R toxins could form additional hydrogen bonds that could account for their greater thermal stability. In addition to this, it is likely that H168R has an extra positive charge exposed to the surface which could increase its rate of insertion into susceptible membranes.

Key words: α-helix 5, Circular dichroism, molecular modeling, site-directed mutagenesis, thermal stability, *Bacillus thuringiensis*

Resumen

La α-Hélice 5 del domino I de las δ-endotoxinas de *Bacillus thuringiensis*, es crítica para la toxicidad de las toxinas contra insectos al participar en la formación de canales iónicos. La participación en la función tóxica del residuo Histidina 168 (H168) –el cual es altamente conservado– fue estudiada mediante técnicas de biología molecular, electrofisiología y biofísica. La toxina mutante H168R fue ~ 3 veces más tóxica que la toxina silvestre (ts) en Manduca sexta, mientras que H168Q fue 3 veces menos tóxica. Los análisis espectroscópicos indicaron que las mutaciones no producen alteraciones estructurales significativas y que la toxina H168R (T_m= 59 °C) es más estable que las toxinas H168Q (T_m= 57.5 °C) y wt (T_m= 56 °C). Las tres toxinas exhibieron uniones de afinidad similares (K_{conn}) en vesículas de intestino de larvas de insecto, indicando

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