The Molecular Biology of Venom Genes from Death Adder and King Brown Snake

Kah-Leong Sung

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CERTIFICATE OF AUTHORSHIP/ORIGINALITY

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Abstract

A complex mixture of post-synaptic and pre-synaptic neurotoxins have been identified in both King Brown snake and Death Adder venom. However, since the experiments were conducted using pooled venom samples from different snakes, it could be argued that the large number of homologous toxins previously reported were due to individual (intra-species) variation. Furthermore, previous studies on King Brown snake and Death Adder toxins were mainly at a protein level and there have been few studies at a genotypic level. Thus, a series of experiments were conducted to ascertain whether individual King Brown snakes and Death Adders express an array of toxins, or whether the results were due to the use of pooled venom samples.

In order to isolate all venom genes and their homologues from the genome of both snakes, individual snakes from both species were sacrificed and cDNA libraries were prepared from their excised venom glands. The venom gland cDNA libraries were then screened exhaustively for the presence of any post-synaptic and pre-synaptic neurotoxin genes i.e. the Phospholipase A_2 gene and its homologues, as well as the alpha-neurotoxin genes (both long and short chain α-neurotoxins genes). While the results from King Brown snake cDNA library supported the hypothesis that individual snakes express a number of homologues of PLA_2 enzymes, some of which might be toxins, the actual number of venom genes and the variation between each homologue were lower than previously reported values. Multiple copies of PLA_2 genes were isolated supporting the contention that there was gene duplication of these venom genes. Regions of hyper-variability were also observed within the PLA_2 genes, further supporting the idea that these neurotoxin genes evolved at an accelerated rate. However, with respect to Death Adder cDNA library, the results indicated that there was only a single copy of the PLA_2 γενε, ανδ συππορτεδ τηε νυλλ ηψποτηεσισ τηατ ϖαριατιον μαψβε δυε εντιρελψ το ποολεδ ϖενομ σαμπλεσ.

Υνφορτυνατελψ, νο ρεσυλτσ ωερε οβταινεδ φορ α-neurotoxin genes despite numerous attempts at isolating the long and short chain α-neurotoxin genes. Thus no conclusions could be drawn regarding the evolution and complexity of post-synaptic neurotoxins.