

**THE SEQUENCE, EXPRESSION AND
IMMUNOLOGICAL CHARACTERISATION
OF THE HT-1 NEUROTOXIN FROM
THE AUSTRALIAN PARALYSIS TICK
*IXODES HOLOCYCLUS***

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DECLARATION

The experiments presented in this thesis were carried out by myself, except where indicated in the text. None of the material has been presented previously for the purpose of obtaining any other degree.

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ABSTRACT

The paralysis tick of Australia, *Ixodes holocyclus*, causes a severe toxicosis in domestic animals such as dogs and cats, livestock, and in some cases humans. It is characterised by a rapidly ascending flaccid paralysis. The causative agent of the toxicosis is a neurotoxin produced in the tick salivary glands. The current treatment for tick paralysis is in the form of a polyclonal dog antiserum. This antiserum treatment is expensive and effective only in the early stages of paralysis.

This thesis describes the isolation of the cDNA sequence for the neurotoxin HT-1 of *I. holocyclus*. In addition it describes the successful expression of the mature HT-1 toxin as a fusion protein in a bacterial host system and attempts at expression of this recombinant protein in the absence of a fusion partner in a baculovirus system. The antigenic and protective properties of the HT-1 fusion protein raised in mice and dogs is also discussed.

The complete cDNA sequence of the HT-1 neurotoxin was determined using the technique of RACE-PCR. The derived protein sequence of the mature toxin has a calculated molecular weight of 5.9 kDa and contains 8 cysteine residues, suggesting that it can form four disulphide bonds. The HT-1 protein sequence shares similarities with other arachnid neurotoxins. Examples of these similarities are its small size, number and arrangement of cysteine residues and in the length and composition of its signal sequence.

A recombinant form of the HT-1 toxin was produced as a fusion protein in an *E.coli* host system. The fusion protein was shown to be immunogenic by Western blot and ELISA analysis. The antibodies produced against the recombinant fusion protein show partial protection against native toxin in mouse assays. Dogs immunised with the recombinant fusion protein produced specific antibodies to HT-1 as demonstrated in ELISA assay. However, these antibodies were not protective when the dogs were challenged with numerous live ticks.

The results obtained have contributed a novel tick neurotoxin sequence which as a fusion protein produces antibodies that exhibit partial protection in mice against native toxin challenge. These results provide some optimism and basic understanding of what is required for the future development of a recombinant vaccine against paralysis caused by the tick *I. holocyclus*.

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ABBREVIATIONS

3'	three prime
5'	five prime
A	absorbance
Ab	antibody
AcNPV	<i>Autographa californica</i> NPV
Ag	antigen
AP	alkaline phosphatase
AVSL	Australian veterinary serum laboratories
Az	sodium azide
Bac	baculovirus
BCIP	5-bromo-4-chloro-3-indopyranoside
bp	base pair
BSA	bovine serum albumin
CAPS	3-[cylcohexylamino]-1-propane-sulfonic-acid
cDNA	complementary DNA
ChTx	charybdotoxin
CIAA	chloroform:isoamyl alcohol
CNBr	cyanogen bromide
Da	Dalton
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dNTP	deoxynucleotide triphosphate
ds	double stranded
DTT	dithiothreitol
ELISA	enzyme linked immuno-absorbent assay
EtBr	ethidium bromide
EtOH	ethanol
Fab	fragment antigen binding
Fwd	forward
HPLC	high pressure liquid chromatography
HT	holocyclus toxin
i.p.	intraperitoneal
IgG	immunoglobulin with γ isotype heavy chain
IMAC	immobilised metal affinity column
IPTG	isopropylthio- β -D-galactopyranoside
kDa	kiloDalton
KLH	keyhole limpet hemocyanin

kV	kilovolts
LB	Luria Bertani
LPS	lipopolysaccharide
mA	milliamps
Mab	monoclonal antibody
MBP	maltose binding protein
MCS	multiple cloning site
μF	micro-Farrad
mRNA	messenger RNA
MWCO	molecular weight cut off
NBT	nitro-blue tetrazolium
NMR	nuclear magnetic resonance
NPV	nucleopolyhedrovirus
OD	optical density
p-NPP	p-nitophenyl phosphate
PAGE	polyacylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
pmol	picomole
polyA	polyadenosine
PVDF	polyvinylidenedifluoride
RACE	rapid amplification of cDNA ends
RE	restriction endonuclease
Rev	reverse
RNA	ribonucleicacid
RNase	ribonuclease
s.c.	sub-cutaneous
ScFv	single chain fragment variable
SDS	sodium dodecyl sulphate
ss	single stranded
TBE	tris buffered ethylene diamine tetraacetate
TE	tris EDTA
Tris	tris-(hydroxymethyl)-aminomethane
UV	ultraviolet
V	volts
VH	heavy chain variable region
VL	light chain variable region
XGAL	5-bromo-4-chloro-3-indolyl-β-D-galactoside

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