

**Biochemical and molecular characterization of
putative immunoprotective molecules of the soft
tick, *Ornithodoros savignyi* Audouin (1827)**

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Abbreviations

A

A5C	Actin 5C
AA	Arachidonic acid
ACN	Acetonitrile
AEBSF	4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride
AMP	Antimicrobial peptide
APS	Ammonium persulphate
ATCC	American type culture collection
α 2M	α 2-Macroglobulin

B

BAB	Biogenic amine-binding
BLAST	Basic Local Alignment Search Tool
BLASTP	Protein-protein BLAST

C

C	Control
CAPS	3'-cyclohexylamino-1-propanesulfonic acid
cB	Bacterial protein control
CDD	Conserved domain database
cHL	Total hemolymph plasma protein
CID-MS	Collision-induced dissociation mass spectrometry

D

Da	Dalton
DAE	<i>Dermacentor andersoni</i> embryonic cell line
dddH ₂ O	Double distilled deionized water
DEPC	Diethylpyrocarbonate
DGNBP	<i>Drosophila</i> Gram-negative binding protein
DTE	Dithioerythritol
DTT	1,4 Dithiothreitol

E

E-64	N-(trans-epoxysuccinyl)-L-leucine-4-guanidinobutylamide
EDTA	Ethylenediaminetetra-acetic acid
EST	Expressed sequence tag

F

FB	Fat body
----	----------

G

GNBP	Gram-negative binding protein
GST	Glutathione S-transferase

H

HBP	Histamine binding protein
HC	Hemocyte
HL	Hemolymph
HMM	High molecular mass protein
HPLC	High performance liquid chromatography
HSP	High score pairing



I

IAA	Iodoacetamide
IDE 12	<i>Ixodes scapularis</i> embryonic cell line 12
IMD	Immunodeficiency pathway
IPTG	Isopropyl β -D-1-thiogalactopyranoside
Ir-LBP	<i>Ixodes ricinus</i> lipocalin leukotriene B4 protein

L

L	Transmembrane
LB	Luria-Bertani broth
LPS	Lipopolysaccharide
LBP	LPS binding protein
LIR6	Lipocalin of <i>I. ricinus</i>
LMM	Low molecular mass marker
LTB ₄	Leukotriene B4
LTC ₄	Leukotriene C4

M

MALDI-MS	Matrix assisted laser desorption ionization mass spectrometry
MG	Midgut
MS	Mass spectrometry
MS/MS	Mass spectrometry/ Mass spectrometry

N

NaCl	Sodium chloride
NAG	N-acetylglucosamine
NAID	National institute of allergy and infectious diseases
NAM	N-acetylmuramic acid
NaN ₃	Sodium azide
N/D	Not determined
NEG	Negative control
NF- κ B	Nuclear factor kappa-light chain enhancer for B cells
NIH	National institute of health
NJ	Neighbour joining

O

OD	Optical density
OMCI	<i>Ornithodoros moubata</i> complement inhibitor

P

PAGE	Polyacrylamide gel electrophoresis
PAMPs	Pathogen associated molecular patterns
PBS	Phosphate buffered saline
PDB	Protein databank
PGBP	Peptidoglycan recognition protein
PGRP	Peptidoglycan recognition receptor
PGN	Peptidoglycan
PMM	Peptide mass marker
PO	Phenol oxidase
proPO	Prophenoloxidase
PRRs	Pathogen recognition receptors
PSI-BLAST	Position-Specific Iterated BLAST



PTU Phenyl thiocarbamide or Phenyl thiourea
PVDF Polyvinylidene fluoride

R

RMSD Root mean square deviation
ROS Reactive oxygen species
RP-HPLC or RPHPLC Reversed phase- High performance liquid chromatography
RT-PCR Reverse transcription polymerase chain reaction

S

S Secreted
SDS-PAGE Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SERPIN Serine proteinase inhibitor
SG Salivary gland
SGE Salivary gland extract
SHBP Serotonin and histamine binding protein

T

TAF Tick actin fragment
TAM Tick alpha-macroglobulin
TBB Tick bleeding buffer
TBLASTN Search translated nucleotide database using a translated nucleotide query
TEMED N,N,N',N'-Tetramethylethylenediamine
TFA Trifluoroacetic acid
TOF Time-of-flight mass spectrometer
TPL *Tachypleus* lectin
Tris-HCl Tris (hydroxymethyl)aminomethane Hydrochloride
TSGP Tick salivary gland protein
TXA₂ Thromboxane A2

U

UN Unchallenged ticks
U/mg Unit per milligram
UTR Untranslated region

X

X-GAL Bromo-chloro-indolyl-galactopyranoside

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Summary

Most studies on innate immunity in ticks have focused on the antimicrobial peptides from hemolymph, such as defensins and lysozyme, while less is known about bacterial recognition molecules, or antimicrobial mechanisms in other tissues. The current study attempted to identify novel antimicrobial mechanisms, with a focus on bacterial recognition by hemolymph proteins and antimicrobial activity in salivary gland extracts.

Using bacteria as affinity beads, two high molecular mass molecules (Protein X and Protein Y) have been identified in tick hemolymph. These proteins are thought to interact with the bacterial surface via ionic interactions. Tandem mass spectrometry analysis followed by *de novo* sequencing indicated that these proteins are novel as no homologs could be identified from sequence databases.

In an attempt to clone Protein X, using a degenerate primer obtained from a *de novo* sequence, an unrelated hemocyte protein was identified. This protein, named savicalin, was shown to belong to the lipocalin family based on bioinformatical analysis. Transcriptional profiling indicated that savicalin is found in hemocytes, midgut and ovaries, but not in the salivary glands. To date, this is the first tick lipocalin not derived from salivary glands. Interestingly, up-regulation of its mRNA transcript in response to bacterial challenge suggests that this protein could be involved in antimicrobial activity. Up-regulation after feeding also suggests a role in the post-feeding development of the tick.

Two different approaches were used to purify the Gram-positive antibacterial activity from salivary gland extracts. The first attempt entailed a two-step separation approach. Tricine SDS-PAGE of the active fraction showed 3 components (~20, ~10 and ~7 kDa). BLAST searches using the N-terminal sequences of the latter proteins identified the ~20 kDa protein as savignin, while the other two proteins could not be matched. The second strategy included an

ultrafiltration step (10 kDa cut-off) and MS-analysis of the active fraction in this case indicated the presence of various components with molecular masses ranging from 0.99 – 7.182 kDa, with 12 predominant components ranging from 0.99 - 4.448 kDa. Further tandem mass spectrometry analysis of the active fraction revealed the presence of three tick actin-derived fragments. This is of interest as actin fragments have been implicated in innate immunity of other invertebrates. In this study, synthetic peptides corresponding to one of the detected tick actin fragments as well as actin5C (detected in *Drosophila* hemolymph) were found not to inhibit the growth of *Bacillus subtilis* when tested up to a concentration of 100 µg/ml.

It is envisaged that future studies of immunoprotective molecules of the tick, *O. savignyi*, may contribute to the development of novel anti-infective agents and potential targets for anti-tick vaccine design.