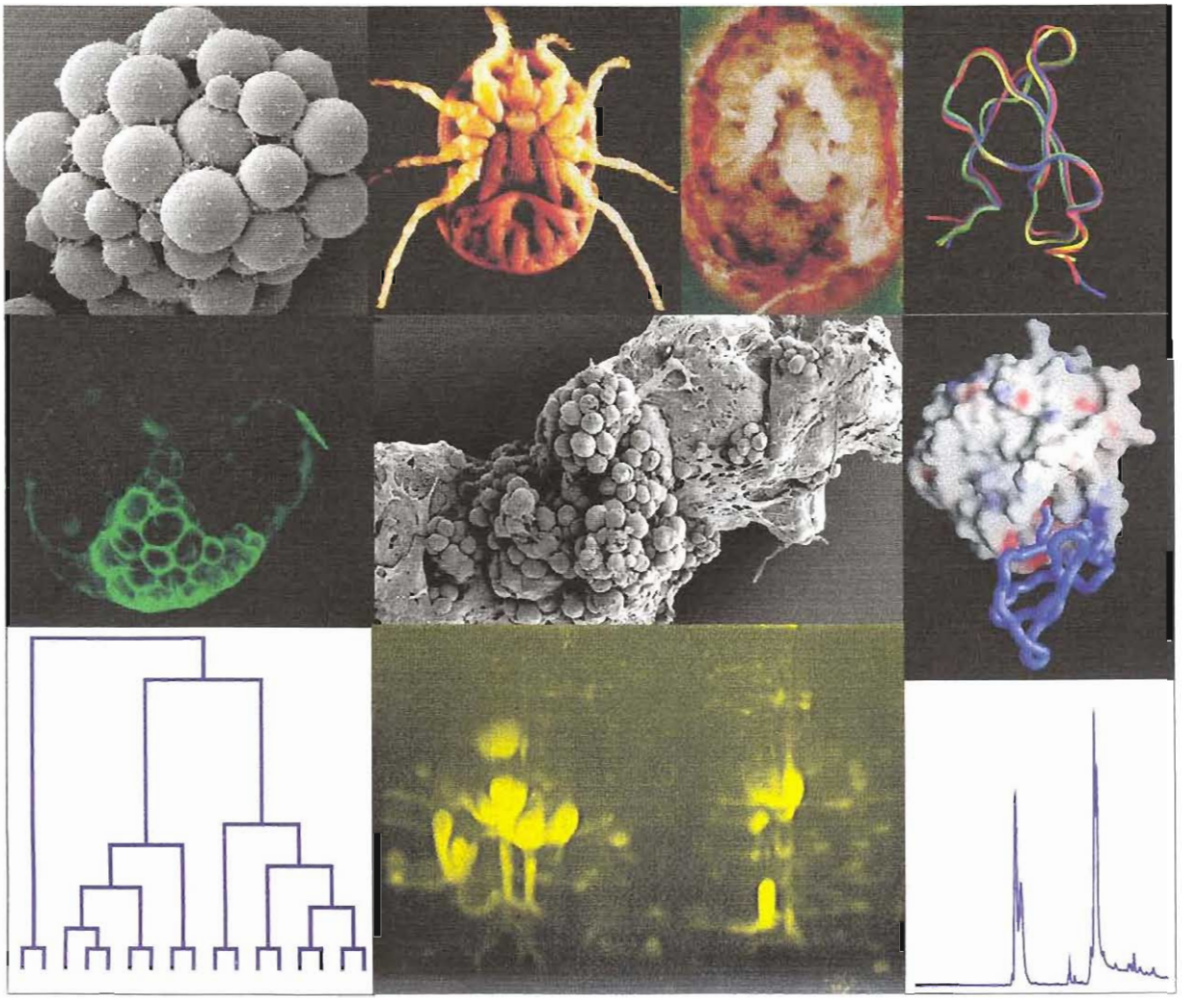
Functional perspectives on the evolution of argasid tick salivary gland protein superfamilies



Functional perspectives on the evolution of argasid tick salivary gland protein superfamilies

by

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Abbreviations

AA	arachidonic acid
ADP	adenosine diphosphate
ADS	antibody dilution solution
AEHPLC	anion exchange HPLC
ATP	adenosine triphosphate
BAPNA	N-α-benzoyl-L-arginine-4-nitranilide
BLAST	basic local alignment search tool
BMTI	*Boophilus microplus* trypsin inhibitor
BPTI	bovine/basic pancreatic trypsin inhibitor
BSA	bovine serum albumin
CEC	cation exchange chromatography
CI	collagen specific inhibitor
COX	cyclo oxygenase
CTI	C-terminal domain of the soft tick thrombin inhibitors
DAG	diacyl glycerol
DAB	3,3' diaminobenzidine
DMEM	Dulbecco's modified Eagle's medium
DMSO	dimethyl sulfoxide
DTT	dithiothreitol
DEPC	diethyl pyrocarbonate
EDTA	ethylene diamine tetra-acetic acid
EGTA	ethylene-bis(oxyethylene nitrilo) tetra-acetic acid
ELISA	enzyme linked immunosorbent assay
ER	endoplasmic reticulum
ESMS	electrospray mass spectrometry
FAP	focal adhesion point
FCS	fetal calf serum
FXaI	fXa inhibitors
GdCl	guanidinium chloride
GSP	gene specific primer
HIHPLC	hydrophobic interaction HPLC
HPLC	high performance liquid chromatography
IP_3	inositol triphosphate
IPTG	isopropyl β-D-thiogalactopyranoside
ISG	immature secretory granule

MALDI-TOF-MS	matrix assisted laser desorption ionization time of flight mass spectrometry
MOPS	3-(N-morpholino)propane sulphonic acid
MSA	methanesulfonic acid
NJ	neighbor joining
4-NPGB	p-nitrophenyl-p'-guanidinobenzoate
NTI	N-terminal domain of the soft tick thrombin inhibitors
OrnGD	Ornatin-glycine-aspartic acid
PAF	platelet activating factor
PAGE	poly-acrylamide gel electrophoresis
PAI	platelet aggregation inhibitors
PBS	phosphate buffered saline
PDB	protein databank
PG	prostaglandin
PGD_2	prostaglandin D2
PGI_2	prostglandin I2
PKC	protein kinase C
PLC	phospholipase C
RGD	arginine-glycine-aspartic acid
RMSD	root mean square deviation
RNAse	ribonucleic acid hydrolase
RPHPLC	reversed phase HPLC
SCR	structural conserved region
SCOP	structural classification of proteins
SD	standard deviation
SDS	sodium dodecyl sulphate
SEHPLC	size exclusion HPLC
SEM	scanning electron microscopy
SGE	salivary gland extract
SGS	salivary gland secretion
TAE	Tris-acetate-EDTA
TAI	tick adhesion inhibitor
TCH	thiocarbohydrazide
TEM	transmission electron microscopy
TEMED	N,N,N',N'-tetramethyl-ethylenediamine
TFPI	tissue factor pathway inhibitor

TGN	trans-Golg
TRAP	thrombin receptor activating peptide
Tris	tris(hydroxymethyl) aminomethane
Tricine	N-[Tris(hydroymethyl) methyl] glycine
TSGP	tick salivary gland proteins
TXA_2	thromboxane A2
4-VP	4-vinyl pyridine
vWf	von Willebrandt's factor
UPGMA	unweighted pair group method with arithmetic mean
UTR	untranslated region
X-gal	5-bromo-4-chloro-3-indolyl β-D-galacto-pyranoside

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Thesis title: Functional perspectives on the evolution of argasid tick salivary gland protein superfamilies

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Department: Biochemistry

Degree: Philosophia Doctor

Ticks evolved an obligate, hematophagous lifestyle approximately 120 million years ago while the vertebrate hemostatic system has existed for at least 400 million years. This implies that ticks adapted to an established and efficient hemostatic system. Adaptation to a new environment at a molecular level implies the gain of new protein functions. Mechanisms for the acquisition of new protein functions include gene duplication and subsequent gain/loss of protein function. This predicts the presence of multi-gene or protein families. The present study investigated the adaptation of ticks to a blood-feeding environment through the use of such multi-gene families present in the salivary gland proteins of the soft tick *Ornithodoros savignyi*.

In this study, a family of platelet aggregation inhibitors named savignygrins was characterized. These savignygrins for which gene duplication was indicated inhibit platelet aggregation induced by various agonists, disaggregate aggregated platelets and inhibit the binding of the monoclonal antibody P2 to integrin $\alpha_{IIb}\beta_3$ and $\alpha_{IIb}\beta_3$ to fibrinogen. This indicates that the savignygrins target the fibrinogen receptor, which was confirmed by sequence identity to disagregin, a fibrinogen receptor antagonist from the closely related tick specie *Ornithodoros moubata*. Savignygrin, however, differs from disagregin due to the presence of the integrin recognition motif RGD.

The thrombin inhibitor savignin was cloned and sequenced. Savignin consists of two BPTI-Kunitz domains. Homology modeling using the structure of ornithodorin, a thrombin inhibitor from *O. moubata*, shows similar mechanisms of inhibition. This

includes targeting of thrombin's active site with its N-terminal BPTI-domain and thrombin's fibrinogen recognition exosite with its C-terminal domain.

Protein fold prediction as well as phylogenetic analysis indicated that the savignygrins share the BPTI-fold with thrombin and fXa inhibitors previously described for the *Ornithodoros* genus. A model of protein evolution for the tick BPTI-inhibitors is proposed that indicates a sequential evolution of inhibition of the substrate recognition capability of thrombin (targeting of the fibrinogen binding exosite), its catalytic capability (targeting of the active site), the catalytic capability of fXa (similar to that of thrombin) and platelet aggregation. This model accounts for the different inhibitory mechanisms of the tick anti-coagulants relative to that of the canonical BPTI-family. The unique presentation mode of the RGD motif on the BPTI substrate-presenting loop of the platelet aggregation inhibitors is also explained.

Four highly abundant proteins (TSGPs) of the lipocalin family were characterized. It was proposed that these proteins function during salivary gland granule biogenesis. TSGP2 and TSGP4 were also identified as toxins that affect the cardiac system. In contrast to savignygrin and apyrase, which localizes to two specific salivary granule types, the TSGPs localize to all the different granule types identified in the salivary glands. Localization studies also indicate that instead of the previously described three granular cell types in soft tick salivary glands, there are five. Phylogenetic analysis of the tick lipocalins indicates a series of gene duplication events and subsequent gain/loss of protein function. The absence of the toxins in the salivary glands of *O. moubata* suggests that the toxins as well as the non-toxic TSGP3 might be recent gene duplications that occurred after the divergence of these two tick species.

OPSOMMING

Titel van tesis:	Functional perspectives on the evolution of argasid tick salivary gland protein superfamilies
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Bosluise het 'n verpligte bloedvoedende lewenstyl ongeveer 120 miljoen jaar gelede ontwikkel. Werweldiere se hemostatiese sisteem bestaan al ongeveer 400 miljoen jaar. Bosluise moes dus by 'n bestaande en hoogs ontwikkelde hemostatiese sisteem aanpas. Aanpassing op molekulêre vlak impliseer die ontwikkeling van nuwe proteïen funksie. Meganismes vir die ontwikkeling van nuwe proteïen funksie sluit geen duplikasie en die daaropvlogende wins/verlies van proteïen funksie in. Dit voorspel die bestaan van multi-geen of proteïen families. Die huidige studie ondersoek die aanpassing van bosluise by 'n bloedvoedende lewenstyl deur gebruik te maak van multi-geen families teenwoordig in die speekselkliere van die sagte bosluis *Ornithodoros savignyi*.

'n Familie van bloedplaatjie aggregasie inhibitore is gekarakteriseer. Geen duplikasie kon aangetoon word. Bloedplaatjie aggregasie deur verskeie agoniste sowel as binding van die monoklonale teenliggaam P2 aan die integrien $\alpha_{IIb}\beta_3$ en $\alpha_{IIb}\beta_3$ aan fibrinogeen is deur savignygrin voorkom. Dit identifiseer die fibrinogeen reseptor as teiken van savignygrin. Identiteit aan die fibrinogeen reseptor antagonis disagregien bevestig die hipotese. Savignygrin verskil egter van disagregin a.g.v. die teenwoordigheid van die integrien herkennings motief, RGD.

Die trombien inhibitor, savignin se geen volgorde is bepaal deur klonering. Savignin bestaan uit twee Kunitz-BPTI domeine. 'n Struktuur model gegrond op ornithodorin , 'n trombien inhibitor van *O. moubata,* voorspel soortgelyke inhibitor meganismes. Dit sluit die teikening van trombien se aktiewe setel deur die N-terminale BPTI-domein en die fibrinogeen herkennings setel deur die C-terminale BPTI-domein in.

Proteïen-vou voorspelling en filogenetiese analise dui aan dat savignygrin die BPTI-vou deel saam met die trombien en fXa inhibitore geidentifiseer in the genus *Ornithodoros*. 'n Model vir die opeenvolgende onwikkeling van die bosluis BPTI-inhibitor funksie word voorgestel. Dit sluit in die teiken van trombien se substraat herkennings setel, trombien se aktiewe sentrum, fXa se aktiewe setel en bloedplaatjie aggregasie. Die model verklaar die uiteenlopende inhibisie meganismes van the bosluis BPTI-inhibitore ten opsigte van die kanonikale BPTI-inhibitore. Die unieke presentering van die RGD motief op die BPTI-substraat herkennings lus word ook verklaar.

Vier van die mees volopste speekselklier proteïene (TSGPs) wat deel is van die lipokalien familie is gekarakteriseer. 'n Funksie in die biogenese van speekselklier granules is voorgestel. TSGP2 en TSGP4 is ook as toksiene aangedui wat die hart aantas. In teenstelling met savignyrin en apirase wat in slegs twee granule tipes gevind word, word die TSGPs in al die granule sel tipes gevind. In plaas van die oorspronklike drie granule seltipes van sagte bosluise, is vyf aangedui. Filogenetiese analise van die lipokaliene dui 'n reeks geen duplikasies aan met dienooreenkomstige wins/verlies van funksie. Die afwesigheid van die toksiene in die speekselklier ekstrakte van *O. moubata* dui ook aan dat die toksiene sowel as die nie-toksiese TSGP3 geen duplikasies mag wees wat plaasgevind het na die spesiasie van die twee bosluis spesies.