

The characterization of inner core protein VP6 of African Horsesickness Virus

By

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The light shines in the darkness, and the darkness has never put it out.

John 1:5

**dedicated to my husband David
and my parents Jimmy and Jean**

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SUMMARY

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For the degree PhD

VP6 is one of the minor structural core proteins of African horsesickness virus. The minor core proteins VP1, VP4 and VP6 are presumed to constitute the dsRNA dependent RNA polymerase transcription complex of the virus. In the *Orbivirus* prototype bluetongue virus (BTV), VP6 has a helicase activity. The aim of this investigation was to characterize the primary structure and nucleic acid binding function of the inner core protein VP6 of African horsesickness virus (AHSV).

To characterize the primary structure of AHSV VP6, VP6 genes of serotypes 3 and 6 were cloned and sequenced. Both genes encode a 369 amino acid polypeptide.

A comparison to the VP6 proteins of other *Orbiviruses* indicated that in all cases the proteins are rich in basic residues and in glycine. The proteins are highly conserved within serogroups but the conservation between serogroups is low. VP6 of AHSV-3 and AHSV-6 have 93.5% identity and 96% similarity in amino acid residues. AHSV-6 VP6 has 27% identical and 46% similar amino acid residues to BTV-10 VP6. Phylogenetic analysis of four orbivirus VP6 genes indicated that AHSV and BTV are most closely related to each other. Motifs characteristic of known helicases were identified by sequence analysis. Glycine rich protein motifs and a N-glycosylation signal were present. No nucleic acid binding motifs identified in other proteins were found in AHSV VP6.

To characterize the VP6 protein of AHSV VP6, the genes were expressed using both a baculovirus and a bacterial expression system. Proteins were found to be soluble and the VP6 expressed in insect cells was found to be N-glycosylated.

The nucleic acid binding function of AHSV VP6 was investigated. Bacterially expressed VP6 was demonstrated to bind nucleic acids by electrophoretic mobility shift assays. Baculovirus expressed VP6 bound double and single-stranded RNA and DNA in nucleic acid overlay protein blot assays. Competition assays indicated that VP6 may have a preference for binding to RNA rather than DNA. Glycosylation was found to play no direct role in nucleic acid binding but the binding is strongly dependent on the NaCl concentration.

A series of truncated VP6 peptides were produced to investigate the importance of localized regions in nucleic acid binding. Two partially overlapping peptides were found to bind dsRNA at pH 7.0, while other peptides with the same overlap did not. Binding appeared to be influenced by charge as reflected by the isoelectric points (pI) of the peptides and experiments indicating the effect of pH on the binding activity. However, only peptides containing amino acid residues 190 to 289 showed binding activity. This region corresponded to the region on BTV VP6 that contains two binding domains. It is proposed that the dsRNA binding domain in AHSV VP6 is a sequence of positively charged amino acids constituting a domain that determines the nucleic acid binding characteristics of the peptide. The mechanism of binding of baculovirus expressed VP6 in a nucleic acid overlay protein blot is proposed to be charge related.

DECLARATION

I declare that the thesis which I hereby submit for the degree Philosophiae Doctor at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature:

Date:

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LIST OF ABBREVIATIONS

AHS	African horsesickness	FCS	foetal calf serum
AHSV	African horsesickness virus	g	gravitational acceleration
AHSV-6	African horsesickness virus serotype 6	HCV	hepatitis C virus
amp	ampicillin	hr/s	hour / hours
AMV	Avian myeloblastosis virus	h.p.i.	hours post infection
ATCC	American type culture collection	HPRI	human placental ribonuclease inhibitor
ATP	adenosine-5'-triphosphate	i.e.	it est (that is)
bp	base pairs	IPTG	isopropyl-β-D-thiogalactopyranoside
BRDV	Broadhaven virus	KAc	potassium acetate
BSA	bovine serum albumin	kb	kilobasepairs
BTV	bluetongue virus	kDa	kilodalton
°C	degrees Celsius	LacZ	β-galactosidase gene
cDNA	complementary DNA	LB	Luria-Bertani
CER	chicken embryo reticulocyte	M	molar
Ci	Curie	μg	microgram
CLP	core-like particle	μl	microlitre
cpm	counts per minute	mM	millimolar
Da	Daltons	mA	milliampere
dATP	2'-deoxyadenosine-5'-triphosphate	mCi	millicurie
dCTP	2'-deoxycytidine-5'-triphosphate	MCS	multiple cloning site
dGTP	2'-deoxyguanosine-5'-triphosphate	mg	milligram
dTTP	2'-deoxythymidine-5'-triphosphate	MHV	mouse hepatitis virus
DEPC	diethylpyrocarbonate	min	minutes
DMSO	dimethyl sulphoxide	ml	millilitre
DNA	deoxyribonucleic acid	mmol	millimol
ds	double-stranded	MMOH	methylmercuric hydroxide
DTT	1,4-dithiothreitol	m.o.i.	multiplicity of infection
EDTA	ethylenediaminetetra-acetic acid	M_r	molecular weight
e.g.	exempli gratia (for example)	mRNA	messenger ribonucleic acid
EHDV	epizootic haemorrhagic disease virus	NaAc	sodium acetate
ELISA	enzyme-linked immunosorbent assay	NaOH	sodium hydroxide
EMSA	electrophoretic mobility shift assays	nm	nanometre
et al.	et alia (and others)	NS	nonstructural
etc.	et cetera (and so forth)	OD	optical density
EtBr	ethidium bromide (3,8-diamino-6ethyl-5-phenylphenathridium bromide)	ORF	open reading frame
		OVI	Onderstepoort Veterinary Institute

PAGE	polyacrylamide gel electrophoresis	ss	single-stranded
PBS	protein buffered saline	SV40	simian virus 40
PCR	polymerase chain reaction	TCA	trichloroacetic acid
PEG	polyethylene glycol	TdT	terminal deoxynucleotidyl transferase
pfu	plaque forming units	TEMED	N,N,N',N',- tetramethylethylenediamine
p.i.	post infection	tet	tetracycline hydrochloride
pmol	picomol	Tris	Tris-hydroxymethyl-aminomethane
PNK	polynucleotide kinase	U	units
PSB	protein solvent buffer	UHQ	ultra high quality water
RC	replicase complex	UV	ultraviolet
RF	replicative form	V	volts
RNA	ribonucleic acid	VIB	virus inclusion bodies
rpm	revolutions per minute	VLP	virus-like particle
RT	room temperature	VP	virus protein
SDS	sodium dodecyl sulphate	w/v	weight per volume
sec	seconds	X-gal	5-Bromo-4-chloro-3-indolyl- β -D- galactopyranoside
Sf9	<i>Spodoptera frugiperda</i> (fall armyworm) cells		
SF	super families		

LIST OF BUFFERS

PBS:

137mM NaCl, 2.7 mM KCl, 4.3mM Na₂HPO₄.7H₂O, 14mMKH₂PO₄, pH 7.3

PSB (2x):

0.125M Tris-HCl pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol

SBB:

50mM NaCl; 1mM EDTA; 10mM Tris-HCl, pH 7; 0.02% Ficoll; 0.02% polyvinylpyrrolidone; 0.02% BSA

STE buffer:

0.15M NaCl, 0.01M Tris-HCl pH 7.6, 0.001M EDTA

STE-Tx buffer:

0.15M NaCl, 0.01M Tris-HCl pH7.6, 0.001M EDTA, 0.5% Triton-X100

TAE buffer:

0.04M Tris-acetate, 0.002M EDTA, pH 8.5

TE buffer:

0.01M Tris-HCl pH 7.6, 0.001M EDTA

TGS buffer:

0.025M Tris-HCL pH 8.3, 0.192M glycine, 0.1% SDS