The characterization of inner core protein VP6 of African Horsesickness Virus

By

Pamela Jean de Waal

A thesis submitted in partial fulfilment of the requirements for the degree Philosophiae Doctor in the Faculty of Natural and Agricultural Sciences

University of Pretoria

Pretoria **May 2005**

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

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to:

Professor Henk Huismans for his guidance and support throughout this study.

Professor Paulette Bloomer, Drs Mandy Bastos and Wayne Delport for their assistance with the phylogenetic analyses.

Members of the Genetics Department at the University of Pretoria for assistance and support, especially Sharon Cormack, Eben von Well and Ruan van Rensburg.

Members of the School of Biological Sciences, especially Professor Jacques Theron and Dr Ben Mans for their advice.

My husband, David, for his assistance with the manuscript, his patience, unfailing support and encouragement.

My parents, parents-in-law, brothers and friends for their interest and encouragement.

SUMMARY

The characterization of inner core protein VP6 of African Horsesickness Virus

by

Pamela Jean de Waal

Promoter: Prof. H. Huismans
Department of Genetics
University of Pretoria

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: ……..

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

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

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

LIST OF BUFFERS

PBS:

137mM NaCl, 2.7 mM KCl, 4.3mM $Na_2HPO_4.7H_2O$, 14mMKH_2PO_4, 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% polyvinylpyrollidone; 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