The molecular characterization of equine encephalosis virus non-structural protein NS3

by

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The Road Not Taken

By Robert Frost
(1874-1963)

Two roads diverged in a yellow wood,
And sorry I could not travel both
And be one traveler, long I stood
And looked down one as far as I could
To where it bent in the undergrowth;

Then took the other, as just as fair,
And having perhaps the better claim,
Because it was grassy and wanted wear;
Though as for that the passing there
Had worn them really about the same,

And both that morning equally lay
In leaves no step had trodden black.
Oh, I kept the first for another day!
Yet knowing how way leads on to way,
I doubted if I should ever come back.

I shall be telling this with a sigh
Somewhere ages and ages hence:
Two roads diverged in a wood, and I--
I took the one less travelled by,
And that has made all the difference.

Dedicated to my parents and to Theunis, who made all of this possible through their love, encouragement and patience

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SUMMARY

The molecular characterization of equine encephalosis virus non-structural protein NS3

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Equine encephalosis virus (EEV) is a member of the *Orbivirus* genus within the family *Reoviridae*. The 10 dsRNA genome segments of these viruses encode 7 structural proteins and 3 non-structural proteins. It has been shown that the smallest of the non-structural proteins, NS3, is an integral membrane protein that plays a role in virulence and pathogenicity. The NS3 protein has two hydrophobic regions that can form transmembrane helices to allow the protein to incorporate itself into the cellular membrane. In the case of AHSV, NS3 is a highly variable protein with 36% variation within the serogroup. The variation of BTV NS3 is much less and in the case of EEV, NS3 has never been characterized.

The aim of this study was to investigate the EEV NS3 protein by looking at the structural characteristics of the protein; the variation found between different EEV NS3 proteins and its expression, using a suitable expression system.

The NS3 gene of EEV Bryanston (EEV-1) was cloned and sequenced and the deduced amino acid sequence determined. In addition, the nucleotide sequences of the NS3 gene of 7 different serotypes and 7 field isolates, as well as their deduced amino acid sequences, were also determined. These sequences represent the first sequence data for S10/NS3 of EEV.

A number of conserved structural features were identified in EEV S10/NS3, namely two putative initiation codons, two hydrophobic regions with the potential to form transmembrane helices, a proline-rich region at the N-terminal, a conserved region containing a conserved myristylation motif followed by a stretch of positively charged amino acids that together constitute a putative bipartite membrane targeting signal, glycosylation sites, conserved cysteine residues and a variable region.

These results suggested that EEV NS3 was an integral membrane protein, similar to BTV and AHSV NS3.

The level of variation observed in S10 and NS3 of EEV was determined using phylogenetic analysis, and the level of variation observed in the EEV NS3 protein was determined to be 16.7%, a value that is higher than that of BTV NS3, but lower than that of AHSV NS3. Based on S10/NS3 sequences, the EEV serogroup formed a lineage independent to that of the other orbiviruses and this lineage segregated into two clusters that corresponded to the northern and southern regions of South Africa. This geographic distribution of the EEV serotypes might be related to the distribution of the *Culicoides* vector subspecies in South Africa.

The NS3 gene of EEV Bryanston (EEV-1) was also studied by means of expression in an *in vitro* translation system using denatured EEV dsRNA, as well as by means of the baculovirus expression system. These expression studies indicated that approximately equal levels of EEV NS3 and NS3A were expressed during *in vitro* translation studies, whereas low levels of EEV NS3 expression was observed in the baculovirus expression system.

ABBREVIATIONS

A	adenine or adenosine
aa	amino acids
AcNPV/AcMNPV	*Autographa californica* nuclear polyhedrosis virus
AHS	African horsesickness
AHSV	African horsesickness virus
AMV	avian myeloblastosis virus
ATP	adenosine-5'- triphosphate
bp	basepairs
BHK	baby hamster kidney
BLAST	Basic Local Alignment Search Tool
BRDV	Broadhaven virus
BTV	bluetongue virus
°C	degrees Celsius
C	cytosine or cytidine
cDNA	complementary deoxyribonucleic acid
CLP	core-like particle
cm	centimetre
Da	Dalton
dATP	2'-deoxyadenosine-5'-triphosphate
dCTP	2'-deoxycytidine-5'-triphosphate
dGTP	2'-deoxyguanosine-5'-triphosphate
ddH_2O	double-distilled water (ultra high quality water)
dH_2O	distilled water
dNTP	deoxynucleoside-triphosphate
dTTP	2'-deoxythymidine-5'-triphosphate
DEPC	diethylpyrocarbonate
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dsRNA	double-stranded RNA
DTT	1,4-dithiothreitol
EDTA	ethylenediaminetetra-acetic acid
EE	equine encephalosis
EEV	equine encephalosis virus
e.g.	*exempli gratia* (for example)
EGTA	ethylene glycol bis(β-aminoethyl ether) N,N,N',N'-tetra-acetic acid

EHDV	epizootic haemorrhagic disease virus
ELISA	enzyme-linked immunosorbent assay
EMBL	European Molecular Biology Laboratory
ER	endoplasmic reticulum
et al.	*et alia* (and others)
EtBr	ethidium bromide
FCS	foetal calf serum
Fig.	figure
g	gram; centrifugal force
G	guanine or guanosine
GTP	guanosine-5'-triphosphate
h	hour(s)
i.e.	*id est* (that is to say)
IPTG	isopropyl-β-D-thiogalactopyranoside
IUB	International Union of Biochemistry
IUPAC	International Union of Pure and Applied Chemistry
kDa, K	kilodalton
LB	Luria-Bertani
M_r	relative molecular weight
M	molar
MCS	multiple cloning site
mg	milligram
min	minute(s)
ml	millilitre
mM	millimolar
MMOH	methylmercuric hydroxide
MOI	multiplicity of infection
mRNA	messenger ribonucleic acid
NCBI	National Center for Biotechnology Information
nm	nanometer
NS	non-structural
OD_{550}	optical density at 550 nm
OD_{600}	optical density at 600 nm
ORF	open reading frame
OVI	Onderstepoort Veterinary Institute
PAGE	polyacrylamide gel electrophoresis
pBS	plasmid Bluescribe
PBS	phosphate buffered saline

PCR	polymerase chain reaction
PDB	protein databank
PEG	polyethylene glycol
pI	isoelectric point
p.i.	post infection
pmol	picomole
PSB	protein solvent buffer
RER	rough endoplasmic reticulum
RNA	ribonucleic acid
RNase	ribonuclease
rpm	revolutions per minute
rRNA	ribosomal ribonucleic acid
RT	reverse transcriptase; reverse transcription
RT-PCR	reverse transcriptase polymerase chain reaction
S10	genome segment 10
SA	South Africa
SDS	sodium dodecyl sulphate
sec	second
Sf	*Spodoptera frugiperda*
ss	single-stranded
SV40	Simian virus 40
T_m	melting (or midpoint temperature); thermal denaturation
T	thymine or thymidine
TEMED	N,N,N',N'-tetramethylethylenediamine
Tris	tris-hydroxymethyl-aminomethane
tRNA	transfer ribonucleic acid
µCi	microcurie
µg	microgram
µl	microlitre
U	unit; uracil or uridine
USA, US	United States of America
UV	ultraviolet
V	Volts
VIB	viral inclusion body
v/v	volume per volume
VP	virus protein
w/v	weight per volume
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

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