

An investigation into nonstructural proteins NS3 and NS3A of African Horsesickness virus

By

Tracy Leonora Meiring

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Enough of it.

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SUMMARY

An investigation into nonstructural proteins NS3 and NS3A of African horsesickness virus

By

Tracy Leonora Meiring

Supervisor: Dr. V van Staden
Department of Genetics
University of Pretoria

Co-supervisor: Prof. H. Huismans
Department of Genetics
University of Pretoria

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The aims of this investigation were to compare the nonstructural proteins, NS3 and NS3A, of African horsesickness virus (AHSV) and to further characterise the cytotoxic properties of these proteins.

NS3 and NS3A are encoded from two in-phase overlapping reading frames on the smallest double-stranded (ds) RNA genome segment, segment 10 (S10) of AHSV. The proteins differ only with respect to the presence of an additional 10 or 11 amino acids, depending on the serotype, at the N-terminal of NS3. All known orbiviruses have been shown to encode two closely related proteins from S10. Sequence analysis of the N-terminal region of the NS3 proteins of the different serotypes of AHSV and various orbiviruses revealed that this region is not highly conserved.

Both AHSV NS3 and NS3A are membrane-associated and cytotoxic to insect cells causing membrane permeabilisation and eventual cell death when expressed individually (Van Staden *et al.*, 1995; Van Staden *et al.*, 1998; Van Niekerk *et al.*, 2001a). AHSV infection of Vero cells results in the synthesis of both proteins in equimolar amounts (Van Staden, 1993). The effect on the cytotoxic properties of these proteins when expressed together in insect cells was therefore investigated here.

Whether co-expressed or expressed individually NS3 and NS3A caused a dramatic decrease in the viability of insect cells. The NS3 protein is therefore representative of the NS3 and NS3A proteins together in terms of its cytotoxic effect.

The effect of the exogenous addition of NS3 on the membrane permeability of Vero cells was also investigated. The NS3 protein was found to cause a rapid increase in the membrane permeability of Vero cells. The cytotoxic properties of NS3 appear therefore not to be limited to their endogenous effects on insect cells.

The AHSV-3 NS3 and NS3A proteins were expressed as histidine tagged recombinants in the baculovirus expression system, to allow for the purification of large quantities of protein for functional and comparative studies. The resulting NS3 histidine fusion product, however, displayed a decrease in solubility, probably as a result of incorrect folding due to the presence of the histidine tag extension at the N-terminus of the protein.

To produce antibodies that detect NS3, and not NS3A, in AHSV infected cells, the N-terminal region unique to AHSV-3 NS3 was displayed on the surface of the AHSV core protein, VP7. The chimeric protein VP7-NS3 displayed the same structural characteristics as the wildtype VP7 protein, aggregating into highly insoluble crystals. Antiserum was prepared against purified VP7-NS3 and analysed in terms of its ability to recognise denatured and non-denatured AHSV-3 NS3. Although the antiserum was shown to contain antibodies directed against VP7 epitopes no immune reaction with NS3 was observed. The use of alternate sites on the surface region of VP7 for the display of such a small peptide needs to be investigated.

Although no functional differences between NS3 and NS3A were identified in this investigation, the finding that NS3 causes membrane permeability or damage to Vero cells represents the first indication that this AHSV protein causes extracellular membrane damage in mammalian cells. Many viral membrane damaging proteins or viroporins are thought to contribute significantly to the severity of virus-induced pathogenesis. The mechanism of membrane damage and the contribution of the membrane damaging properties of NS3 to AHSV-induced pathogenesis needs to be investigated.

OPSOMMING

'n Studie van nie-strukturele proteïene NS3 en NS3A van Perdesiekte virus

Deur

Tracy Leonora Meiring

Promotor: Dr. V. van Staden
Departement Genetika
Universiteit van Pretoria

Mede-promotor: Prof. H. Huismans
Departement Genetika
Universiteit van Pretoria

vir die graad MSc

Die doel van hierdie studie was om die nie-strukturele proteïene, NS3 en NS3A van perdesiekte virus (PSV), te vergelyk en om die sitotoksiese eienskappe van hierdie proteïene verder te karakteriseer.

NS3 en NS3A word gekodeer vanaf twee oorvleuelende in-fase oop leesrame op die kleinste dubbeldraad (dd) RNA genoomsegment, S10 van PSV. Hierdie twee proteïene verskil slegs van mekaar ten opsigte van 'n addisionele 10 of 11 aminosure, afhangend van die serotipe, aan die N-terminus van die NS3 proteïen. Alle bekende orbivirusse kodeer vir twee byna identiese proteïene vanaf S10. Aminosuur volgorde analise van die N-terminale gebiede van die NS3 proteïene van die verskillende PSV serotipes en verskeide orbivirusse, het bewys dat hierdie gebied nie hoogs gekonserveerd is nie.

Beide NS3 en NS3A is sitotoksies vir *Sf9* selle wanneer hulle alleen uitgedruk is in die baculovirus ekspressie sisteem (Van Staden *et al.*, 1995; Van Staden *et al.*, 1998; Van Niekerk *et al.*, 2001a). PSV infeksie van Vero selle lei tot die ekspressie van beide NS3 en NS3A in omtrent dieselfde hoeveelhede (Van Staden, 1993). Om die sitotoksiese effek van ko-ekspressie van NS3 en NS3A in insekselle te bestudeer, is

insekselle geïnfekteer met rekombinante baculovirusse wat NS3 en NS3A uitdruk. Dit is bewys dat ko-ekspressie van NS3 en NS3A nie die sitotoksiese effek van hierdie proteïene verander nie.

Die effek van eksogeniese NS3 op die selmembraan permeabiliteit van Vero selle is ook bestudeer. NS3 veroorsaak 'n vinnige toename in die permeabiliteit van Vero selmembrane. Die sitotoksiese eienskappe van NS3 is daarom nie beperk tot hulle endogeniese effek op insekselle nie.

Om groot hoeveelhede van die PSV-3 NS3 en NS3A proteïene te bekom, is die S10 geen en 'n verkorte vorm van die S10 geen uitgedruk as histidien fusieproteïene in die baculovirus ekspressie sisteem. Die NS3 fusieproteïen het 'n sterk afname in solubiliteit getoon in vergelyking met die wildetipe NS3 proteïen en is vir hierdie rede nie gebruik vir die suiwing van NS3 nie.

Om tussen NS3 en NS3A te onderskei in PSV geïnfekteerde selle is antiserum teen die N-terminale gebied van NS3 berei deur gebruik te maak van die PSV strukturele proteïen, VP7. Die eerste 12 aminosure van NS3 is op die oppervlakte van VP7 vertoon. Dit is bewys dat die VP7-NS3 chimera dieselfde strukturele eienskappe as die wildetipe VP7 proteïen vertoon. Antiserum teen die VP7-NS3 proteïen is in hase berei, maar dit is aangetoon dat geen immuun reaksie met NS3 voorkom nie. Alternatiewe posisies op die oppervlakte van VP7 moet ondersoek word.

Die bevinding dat NS3 membraan permeabilisering in Vero selle veroorsaak is die eerste aanduiding dat die PSV proteïen eksogeniese membraan beskadiging veroorsaak. Baie sitotoksiese proteïene of viroporins dra by tot virus geïnduseerde patogenese. Die bydra van NS3 tot PSV geïnduseerde patogenese moet bepaal word.

ABBREVIATIONS

| | |
|-----------------|-------------------------------------|
| aa | amino acids |
| AHS | African horsesickness |
| AHSV | African horsesickness virus |
| Amp | ampicillin |
| Amps | amperes |
| ATCC | American type culture collection |
| bp | base pairs |
| BRD | Broadhaven virus |
| BTV | bluetongue virus |
| °C | degrees Celcius |
| cDNA | complementary deoxyribonucleic acid |
| CHV | Chuzan virus |
| Ci | Curie |
| CLP | core-like particle |
| cm ³ | centimeter cubed |
| CPE | cytopathic effect |
| cys | cysteine |
| Da | Dalton |
| DEPC | diethylpyrocarbonate |
| DNA | deoxyribonucleic acid |
| dNTP | deoxyribonucleotide triphosphate |
| ds | double stranded |
| EC | endothelial cells |
| EDTA | ethylenediaminetetra-acetic acid |
| EHDV | Epizootic hemorrhagic disease virus |
| EM | electron microscope |
| ER | endoplasmic reticulum |
| <i>et al.</i> | and others |
| EtBr | ethidium bromide |
| fcs | foetal calf serum |
| Fig | figure |
| FMDV | Foot-and-mouth disease virus |
| g | gravitational force |
| G | gauge |
| gent | gentamycin |
| GTP | guanosine triphosphate |
| h | hour/s |
| HBsAgs | HBV surface antigens |
| HBV | hepatitis B virus |
| HD | hydrophobic domain |
| His | histidine |
| HIV | Human Immunodeficiency virus |
| h.p.i | hours post infection |
| Hyg B | hygromycin B |
| i.e. | that is |
| IM | intramuscular |
| IP3 | inositol triphosphate |
| IPTG | isopropyl-β-D-thiogalactopyranoside |
| IRES | internal ribosome entry site |
| ISA50 | incomplete seppic adjuvant |
| k | kilo |
| kan | kanamycin |

| | |
|-----------------|--|
| L1,L2,L3 | large segments 1, 2 or 3 |
| LB | Luria broth |
| LLP1 | lentivirus lytic peptide 1 |
| M | molar |
| M4,M5,M6, M7 | medium segments 4,5,6 or 7 |
| MEM | minimal essential medium |
| min | minute/s |
| ml | millilitre |
| mM | millimolar |
| MMOH | methyl mercuric hydroxide |
| MOI | multiplicity of infection |
| mRNA | messenger ribonucleic acid |
| ng | nanograms |
| nm | nanometers |
| NS1, NS2,NS3 | nonstructural proteins 1, 2 or 3 (refers to <i>orbivirus</i>) |
| NSP4 | nonstructural protein 4 (refers to <i>rotavirus</i>) |
| NTA | nitro-tri-acetic acid |
| OIE | Office international des Epizooties |
| ORF | open reading frame |
| OVI | Onderstepoort Veterinary Institute |
| P | particulate |
| PAGE | polyacrylamide gel electrophoresis |
| pBS | bluescribe plasmid |
| PBS | phosphate buffered saline |
| PCR | polymerase chain reaction |
| pfu | plaque forming units |
| p.i. | post infection |
| pmol | picomolar |
| PSB | protein solvent buffer |
| PSV | perdesiekte virus |
| RNA | ribonucleic acid |
| rNTP | ribonucleic acid triphosphate |
| rpm | revolutions per minute |
| RRL | rabbit reticulolysate |
| RT | reverse transcriptase |
| S | supernatant |
| S1 – S10 | segments 1 to 10 (refers to <i>orbiviruses</i>) |
| ³⁵ S | radioactive Sulphur 35 |
| SDS | sodium dodecyl sulphate |
| sec | second/s |
| Sf9 | <i>Spodoptera frugiperda</i> insect cells |
| SIV | Simian immunodeficiency virus |
| SSP | single shelled particle |
| ss | single stranded |
| TE | Tris EDTA |
| tet | tetracyclin |
| TM | transmembrane |
| U | units |
| UHQ | ultra high quality water |
| µg | micrograms |
| µl | microlitres |
| µm | micrometers |
| UP | University of Pretoria |
| V | volts |

| | |
|---------|--|
| VIB | virus inclusion body |
| VLP | virus-like particle |
| VMP | viral membrane protein |
| VP1 – 7 | virus protein 1 to 7 |
| v/v | volume per volume |
| v/w | volume per weight |
| X-gal | 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside |
| 3-D | three dimensional |

LIST OF BUFFERS

Elution buffer:

20 mM Tris-HCl pH 8.5, 100 mM KCl, 100 mM imidazole, 10 mM 2-mercaptoethanol, 10% (v/v) glycerol

Lysis buffer without detergent:

50 mM Tris-HCl pH 7.5, 300 mM NaCl, 1 mM PMSF, 1 mM 2-mercaptoethanol

Na-K-P buffer:

0.075 M $\text{KH}_2\text{PO}_4/\text{NaH}_2\text{PO}_4$, pH7.4

PBS:

137 mM NaCl, 2.7 mM KCl, 4.3 mM $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 1.4 mM KH_2PO_4 ; pH7.3

Protein solvent buffer (PSB)(2x):

0.125 M Tris-HCl pH8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol

STE buffer:

0.01 M NaCl, 0.01 M Tris-HCl pH 7.6, 0.0001 M EDTA

TAE buffer:

0.04 M Tris, 0.002 M EDTA; pH8.5

TBS buffer:

500 mM NaCl, 25 mM Tris, pH7.6

TE buffer:

0.01 M Tris-HCl pH7.6, 0.001 M EDTA

TGS buffer:

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

Wash buffer:

20 mM Tris-HCl pH8.5, 500 mM KCl, 20 mM imidazole, 10 mM 2-mercaptoethanol, 10% (v/v) glycerol

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