



**Construction and Structural Evaluation of Viral Protein 7 of African Horse
Sickness Virus as a Particulate, Multiple Peptide Vaccine Delivery System.**

BY

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dedikasi kajian ini saya ingin menyerahkan kepada gia, sumber inspirasi saya.

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Summary

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For the degree MSc

The highly hydrophobic viral protein (VP) 7 of African horse sickness virus (AHSV) folds into a trimeric structure that aggregates to form flat, hexagonal crystals (Chuma et al., 1992). These crystals are composed of flat sheets of hexameric rings, similar to the rings of trimers seen in the outer core surface layer. The crystals have been shown to be highly immunogenic when used as a subunit vaccine and are able to elicit a strong immune response against subsequent viral infections (Wade-Evans *et al.*, 1997). The aim of this study is to investigate the structural constraints of using these structures as a particulate, multiple peptide vaccine delivery system.

Three hydrophilic regions at amino acid position 144, 177*and 200 on the VP7 surface of this trimeric structure were targeted for insertion of peptides and a new vector was constructed in this study with a multiple cloning site at each one of the three top domain sites. The newly constructed three-site VP7 mutant gene was expressed in the Bac- To-Bac expression system and the recombinant proteins were investigated for its solubility and crystal formation by sucrose density gradient centrifugation. The structure and stability of the modified, trimeric VP7 was confirmed and further analyzed. Scanning electron microscopy showed the formation of large structures by the trimeric modified VP7 protein units. These structures differed from the hexagonal crystals formed by unmodified VP7, resulting in rough-looking, flat circular structures attached by protein cables. The high yield of protein expression and the ease, with which these particles can be purified, makes this vector ideal for vaccine use. These protein structures also seemed to remain stable after being stored under different conditions. Studies were also conducted on the stability of these structures after sonication, enabling a range of different size particles to be presented to the immune system.

The purpose for the creation of multiple cloning sites was for the vaccine to be able to accommodate and efficiently present multiple epitopes to the immune system. An investigation was launched into the effect of peptide insertion at one or more of the multiple cloning sites. The initial study included the

insertion of two small peptides from AHSV VP2 at amino acid sites 144 and 177 respectively. The size of the peptides that can be inserted is also very important in the use of virus-like particles as antigen carriers. In order to utilize the full potential of the VP7 particles as an antigen presentation system, it must be possible to accommodate large epitope-containing insertions. At the extreme, a stretch of 250 amino acids from AHSV VP2 was inserted into the 177 amino acid multiple cloning site of the three-site VP7. Structural evaluation of all these expressed proteins indicated that the structure of the VP7 subunit vaccine is stable and still retains the ability to form large aggregated structures from the trimeric units. Scanning electron microscope revealed that all these peptide-containing constructs retain approximately the same structural shape as the structures formed by the three-site VP7 mutant.



List of Abbreviations

AA	-	Amino acid
AHS	-	African Horse Sickness
AHSV	-	African Horse Sickness Virus
Amp	-	Ampicillin
ATP	-	Adenosine-5'triphosphate
BHK	-	Baby hamster kidney cells
Bp	-	Base pair
BTV	-	Bluetongue virus
BLV	-	Bovine Leukemia virus
°C	-	Degree celsius
CLP	-	Core-like particle
cm ³	-	Cubic centimeters
ddH ₂ O	-	Deionized distilled water
DMSO	-	Dimethyl sulfoxide
DNA	-	Deoxyribonucleic acid
dNTP	-	2'-deoxynucleoside-5'triphosphate
dsRNA	-	Double stranded ribonucleic acid
<i>E.coli</i>	-	<i>Escherichia coli</i>
EDTA	-	Ethylenediaminetetra-acetic acid
<i>et al</i>	-	et alia (and others)
Fig.	-	Figure
g	-	Gram
GP	-	Glyco protein
h	-	Hour
HbsAg	-	Hepatitis B virus surface antigen
IPTG	-	Isopropyl-β-D-thiogalactopyranoside
kDa	-	Kilodalton
KB	-	Kilobase
KV	-	Kilovolt
LB	-	Lauria-Bertani
Log	-	Logarithmic
M	-	Molar
MHC	-	Major histone compatability
min	-	Minutes
ml	-	Millilitre
mm	-	Millimeter
mM	-	Millimolar
M.O.I.	-	Multiplicity of infection
<i>M_r</i>	-	Molecular weight
MW	-	Molecular weight
μg	-	Microgram
μl	-	Microlitre
N	-	Normal
NaAc	-	Sodium acetate
ng	-	Nanogram
NS	-	Non-structural



PAGE	-	Polyacrylamide gel electrophoresis
PBS	-	Phosphate buffered saline
PCR	-	Polymerase chain reaction
pfu	-	Plaque forming units
pmol	-	Picomolar
PSB	-	Protein solvent buffer
RNA	-	Ribonucleic acid
rpm	-	Revolutions per minute
SDS	-	Sodium dodecyl sulphate
sec	-	Seconds
S. E. M.	-	Scanning electron microscopy
Sf	-	<i>Spodoptera frugiperda</i>
TEMED	-	N,N,N',N'-tetramethylethelenediamide
Tet	-	Tetracycline hydrochloride
Tris	-	Tris(hydroxymethyl)-aminomethane
TSB	-	Transformation suspension buffer
U	-	Units
UHQ	-	Ultra high quality
UV	-	Ultraviolet
V	-	Volt
VP	-	Viral protein
VLP	-	Virus-like particles
v/v	-	Volume per volume
w/v	-	Weight per volume
X-gal	-	5-bromo-4chloro-3indolyl- β -D-galactopyranoside

Table of Contents

Dedication	i
Acknowledgements	i
Summary	iii
List of abbreviations	v
Chapter 1: Literature Review	
1.1. Vaccines	1
1.2. Types of Vaccines	2
1.2.1. Inactivated Vaccines	2
1.2.2. Live Attenuated Vaccines	2
1.2.2.1. Biological Attenuation	3
1.2.2.2. Genetic Attenuation	4
1.2.3. Nucleic Acid Vaccines	5
1.2.3.1. DNA Vaccines	5
1.2.3.2. RNA Vaccines	6
1.2.4. Live Recombinant Vaccine Vehicles	7
1.2.5. Antibody-based Vaccines	8
1.2.5.1. Passive Vaccination Strategies	8
1.2.5.2. Anti-idiotypic Antibody Vaccines	9
1.2.6. Subunit Vaccines	10
1.2.6.1. Particulate and Fusion Vaccines	10
1.2.6.2. Synthetic Peptide Vaccines	12
1.3. African Horse Sickness Virus	14
1.3.1. African Horse Sickness Virus Viral Protein 7	15
1.3.2. African Horse Sickness Virus Viral Protein 7 Vaccine	17
1.4. Aims and Strategy of this Study	19
Chapter 2: The Creation of the Three Site VP7 Construct and the Characterisation of the Structural Features and Stability.	
2.1. Introduction	21
2.2. Materials and Methods	23
Materials	23
2.2.1. Polymerase Chain Reaction	23

2.2.2. Agarose Gel Electrophoresis	23
2.2.3. Purification of Amplified DNA Fragments	24
2.2.4. Restriction Enzyme Digestion	24
2.2.5. Dephosphorylation	25
2.2.6. Ligation	25
2.2.7. Preparation of Competent <i>E.coli</i> Cells	25
2.2.8. Transformation of Competent Cells	25
2.2.9. Plasmid DNA Isolation and Purification	26
2.2.10. Nucleotide Sequence Determination	26
2.2.11. Hydropathy Predictions	27
2.2.12. Cells and Media	27
2.2.13. Transposition	27
2.2.14. Isolation of Composite Bacmid DNA	28
2.2.15. Transfection	28
2.2.16. Large-Scale Recombinant Protein Expression and Isolation	28
2.2.17. SDS-Polyacrylamide Gel Electrophoresis	29
2.2.18. Coomassie Brilliant Blue Staining	29
2.2.19. Sucrose Gradient Studies	29
2.2.20. Density Analysis	30
2.2.21. Sonication	30
2.2.22. Trimerization Assay	30
2.2.23. Storage Stability Studies	30
2.2.24. Scanning Electron Microscopy (S. E. M.)	31
2.3. Results	31
2.3.1. Construction of Cloning Site 177	31
2.3.2. Nucleotide Sequence Determination and Hydropathy Predictions	34
2.3.3. Baculovirus Expression	38
2.3.4. Solubility and the Effect of Sonication on VP7 Aggregation	39
2.3.5. Trimerization Assays	40
2.3.6. Storage Stability Studies	41
2.3.7. Scanning Electron Microscopy (S.E.M.)	41
2.4. Discussion	50

Chapter 3: Structural Effects caused by Inserting Small Hydrophilic Peptides into Multiple Sites on African Horse Sickness Virus Viral Protein 7.

3.1. Introduction	53
3.2. Materials and Methods	54
Materials	54
3.2.1. Annealing of Oligonucleotide Primers	54
3.2.2. Cloning of Epitopes into the Modified VP7mt144/177/200 Construct and Expression of the Recombinant Proteins	54
3.3. Results	56
3.3.1. Epitope Insertion	56
3.3.2. Nucleotide Sequence Determination and Hydropathy Predictions	56
3.3.3. Baculovirus Expression	58
3.3.4. Purification of Recombinant VP7 Particles on Sucrose Gradients	60
3.3.6. Scanning Electron Microscopy (S.E.M.)	62
3.4. Discussion	67

Chapter 4: The Effect of Various Lengths of Viral Protein 2 of African Horse Sickness Virus on Modified Viral Protein 7.

4.1. Introduction	69
4.2. Materials and Methods	70
Materials	70
4.2.1. Polymerase Chain Reaction	70
4.2.2. Cloning of VP2 Regions into the Modified VP7mt144/177/200 Construct and Expression of the Recombinant Proteins	71
4.3. Results	71
4.3.1. Synthesis of the Size-Constructs	71
4.3.2. Nucleotide Sequence Determination and Hydropathy Predictions	76
4.3.3. Baculovirus Expression	80
4.3.4. Purification of VP7 Particles on Sucrose Gradients	82
4.3.6. Scanning Electron Microscopy (S.E.M.)	83
4.4. Discussion	89



Chapter 5: Concluding Remarks	91
References	93
Addendum	104
Appendices	107
Appendix A	107
Appendix B-1	109
Appendix B-2	111
Appendix B-3	113
Appendix C-1	116
Appendix C-2	120
Appendix C-3	123
Appendix C-4	129