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Construction of a new peptide insertion site in the top domain of major core protein VP7 of African horsesickness virus

BY

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SUMMARY

Construction of a new peptide insertion site in the top domain of major core protein VP7 of African horsesickness virus.

BY

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For the degree MSc(Agric)

VP7 is the major core protein of AHSV. It is a 38kDa protein composed of 349 amino acids. VP7 is a highly insoluble protein and when expressed in large quantities in AHSV-infected cells (Burroughs *et al.*, 1994) or by recombinant baculovirus in insect cells (Chuma *et al.*, 1992; Maree *et al.*, 1998a; Maree *et al.*, 1998b), it aggregates into large crystal structures that are visible under the light microscope. These crystals, with a large surface area for the display of multiple epitopes, show potential for use as a vaccine delivery system. Two requirements for this system would be the ability of VP7 to accommodate epitope sequences without affecting its structural characteristics and, secondly, to present the epitope sequences in an effective manner to the immune system to generate a protective immune response. These two conditions were investigated in this study.

Two sites for the insertion of epitope sequences had been created by Maree (2000). In this study, a third site was created between amino acids 144 and 145 of AHSV-9 VP7. Restriction enzyme sites *Sma*1, *Eco*R1 and *Xho*1 were introduced by PCR amplification. The effect of the insertion was investigated in terms of its influence on VP7 structure, its association into trimers and further aggregation into VP7 crystalline structures. In the second stage of the investigation, a twenty-five amino acid sequence of a VP2 neutralising epitope (Venter *et al.*, 2000; Bentley *et al.*, 2000; Martínez-Torrecuadrada *et al.*, 2001) was chosen to insert into the created site at position 144. This was achieved by PCR-amplification of the VP2 epitope to introduce *Eco*R1 and *Xho*1 sites for directional cloning into site 144. The appropriate presentation of the insert was investigated in terms of its effect on VP7 structure, its effect on trimer formation and further aggregation of trimers into

crystalline particulate structures. Furthermore, the presentation of the insert was investigated in terms of its ability to generate antibodies with the potential to provide a protective immune response.

The insertion of restriction enzyme sites and resulting six amino acids at position 144, as well as the further insertion of the twenty-five amino acid VP2 epitope, did not affect the ability of VP7 to form trimers. The subsequent association of trimers into particulate structures was affected by an increase in the proportion of smaller structures, in comparison to that observed for the wild-type VP7. This was concluded to be a consequence of an increase in solubility of the proteins, caused by weakened hydrophobic interactions between trimers. This resulted in a reduced stability of larger particulate structures. The efficient presentation of the VP2 epitope could not be determined. A poor immune response was generated against the VP7mt144-VP2 trimer in comparison to other proteins. The result was an inability of the antiserum to recognise native AHSV-9 VP7 and VP2 protein by western blot analysis. More informative conclusions regarding the presentation of the VP2 epitope on the surface of the VP7 epitope display vehicle may be drawn from a future virus neutralisation assay.

ABBREVIATIONS

AHS	African horsesickness
AHSV	African horsesickness virus
AHSV-9	African horsesickness virus serotype 9
AmAc	Ammonium Acetate
amp	ampicillin
A. P.	ammonium persulphate
BHK	Baby hamster kidney
bp	base pairs
BTV	Bluetongue virus
°C	Degrees Celsius
cDNA	complementary DNA
CLP	Core-like particle
DMSO	dimethylsulphoxide
DNA	Deoxyribonucleic acid
dH_2O	distilled water
ddH_2O	deionized distilled water
dNTP	deoxyribonucleoside-triphosphate
ds	double-stranded
EDTA	ethylenediaminetetra-acetic acid
EEV	Equine encephalosis virus
e.g.	*exempli gratia* (for example)
EHDV	Epizootic hemorrhagic disease virus
et al.	*et alia* (and others)
FCA	Freund's complete adjuvant
FCS	Fetal calf serum
FIA	Freund's incomplete adjuvant
Fig.	Figure
g	gram
HBcAg	Hepatitis B core antigen
HBV	Hepatitis B virus
hr	hour

i.e.	*Id Est* (that is to say)
IPTG	isopropyl-β-D-thiogalactopyranoside
KAc	Potassium Acetate
kDa	kilodalton
kb	kilobase pairs
kV	kilovolts
l	litre
LB	Luria Bertani
log	logarithmic
M	molar
MCS	multiple cloning site
mg	milligram
min	minutes
ml	millilitre
mM	millimolar
MOI	Multiplicity of Infection
M_r	molecular weight
mRNA	messenger ribonucleic acid
NaAc	sodium acetate
ng	nanogram
NS	non-structural
OD	optical density
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
pmol	picomol
polh	polyhedrin
PSB	protein solvent buffer
RNA	ribonucleic acid
RNase	ribonuclease
rpm	revolutions per minute
SDS	sodium dodecyl sulphate
S.E.M.	scanning electron microscopy

Sf	*Spodoptera frugiperda*
ss	single-stranded
TCA	trichloroacetic acid
TEMED	N,N,N',N'-tetramethylethylenediamine
tet	tetracyline hydrochloride
Tris	Tris-hydroxymethyl-aminomethane
TSB	Transformation suspension buffer
TSBG	Transformation suspension buffer with glucose
µg	microgram
µl	microlitre
U	units
UV	ultraviolet
v	volume
V	volts
VIB	viral inclusion body
VLP	virus-like particles
VP	viral protein
w	weight
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

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