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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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 diffirent 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_2O	-	Deionized distilled water
DMSO	-	Dimethyl sulfoxide
DNA	-	Deoxyribonucleic acid
dNTP	-	2'-deoxynucleoside-5'triphosphate
dsRNA	-	Double stranded ribonucleic acid
E.coli	-	*Escherichia coli*
EDTA	-	Ethylenediaminetetra-acetic acid
et al	-	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
M_r	-	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	-	*Spodoptera frugiperda*
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

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