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Investigation of the expression profile of streptokinase

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Investigation of the expression profile of streptokinase

* A thesis submitted in (partial) fulfilment of the requirements for the award of the degree

Master of Science (Research)

from

UNIVERSITY OF WOLLONGONG

by

Priya Shyam

Department of Biological Sciences

2006

DECLARATION

This thesis is submitted under the regulations of the University of Wollongong in partial fulfilment of the degree of Master of Science (Research). It does not include any material published by another person except where due reference is made in the text. The experimental work described in this thesis is original work and has not been submitted for a degree in any University.

Priya Shyam

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ABSTRACT

Streptococcus pyogenes (group A streptococcus; GAS) is a Gram-positive bacterium that causes invasive and non invasive infections in human skin and mucosal tissue. Since the mid-1980s, a conspicuous increase in the incidence and severity of invasive infections caused by GAS has been observed worldwide. *S. pyogenes* infections are endemic amongst many Aboriginal communities of Northern Australia. With rates of infection in these communities being amongst the highest in the world, there is an urgent need for identifying the key virulence factors involved in the initiation and progression of invasive GAS diseases. Streptokinase, a secreted group A streptococcal protein, activates host plasminogen converting it to the broad spectrum protease plasmin. Several plasmin(ogen) binding receptors present on the GAS cell surface facilitate the acquisition of cell surface plasmin activity. The gene encoding encoding streptokinase (*ska*) is present in all GAS isolates. In this study, allelic variants of *ska* have been characterised in various GAS isolates from the Northern Territory of Australia. The region encoding the β domain of *ska* in these isolates was sequenced and phylogenetically analysed to identify the specific allelic variant genotype for each isolate. Culture supernatants of these strains were examined for the presence of streptokinase and the cysteine protease, SpeB, via immunoblotting using rabbit polyclonal antisera directed against these proteins. Two GAS strains (ALAB49 and 5448) and their isogenic *ska* and *speB* mutants were used to demonstrate the specificity and applicability of this technique for detecting the presence of streptokinase and SpeB in the culture supernatants. Using this methodology, streptokinase expression in culture supernatants of NT GAS isolates was investigated. Streptokinase was found to be expressed in mid-log, late-log and early stationary growth phases for all NT isolates examined in this study. It was also found that different *ska* alleles expressed streptokinase proteins of variable molecular weights. The streptokinase

expressed by strains possessing alleles of *ska* 1 cluster (NS53, NS730 and NS931) and strains possessing alleles of *ska* 2a sub-cluster (NS696, 5448) express a 48 kDa streptokinase protein and strains possessing alleles of the *ska* 2b sub-cluster (NS13, ALAB49, NS88.2 and NS455) express a 50 kDa streptokinase protein. Culture supernatants from all GAS strains in early stationary growth phase were found to contain pro-SpeB (inactive precursor). Mature SpeB was only detected in the early stationary phase culture supernatants of strain NS53. Examples of the three known allelic *ska* variants were cloned from strains NS931, 5448 and ALAB49. DNA sequence analysis of these clones showed a high percentage of identity with previously published *ska* sequences. The recombinant plasmid pSka1 (containing the *ska* insert from NS931) was expressed and purified. The yield of the purified protein obtained was 1.16 mg/ml. Future work will determine the functional differences between the recombinant proteins expressed by these clones and it is hoped that this will help gain a better understanding of the role of the allelic variation of streptokinase in GAS disease virulence.

TABLE OF CONTENTS

DECLARATION.....	ii
ABSTRACT.....	iv
TABLE OF CONTENTS.....	v
LIST OF FIGURES.....	ix
LIST OF TABLES.....	xi
ABBREVIATIONS	xii
Chapter 1 Streptokinase as a virulence factor in Group A Streptococcal invasive diseases: an overview	1
1.1 Introduction.....	1
1.1.1 Diseases caused by group A streptococcus.....	4
1.1.1.1 <i>Non-Invasive Diseases</i>	4
1.1.1.2 <i>Invasive Diseases</i>	4
1.1.1.3 <i>Disease sequelae</i>	4
1.2 Classification of group A streptococcus.....	5
1.2.1 Lancefield Classification	5
1.2.2 M-Serological Typing.....	5
1.2.3 Emm-Sequence Typing.....	6
1.2.4 Vir-Typing	6
1.3 Plasminogen activation system	6
1.4 Streptokinase	10
1.4.1 Structure of streptokinase	11
1.4.2 Mechanism of action.....	11
1.4.3 Variability of streptokinase.....	17
1.4.3.1 <i>β domain of streptokinase</i>	18
1.4.3.2 <i>Evolution of ska</i>	20
1.5 GAS and the host plasminogen activation system	20
1.5.1 Direct pathway of plasminogen binding	21
1.5.1.1 <i>GAS cell surface receptors</i>	21
1.5.2 Indirect pathway of plasminogen binding.....	26
1.6 Group A streptococcal cysteine protease (SpeB)	26
1.7 Research Objectives.....	31
Chapter 2 Materials and methods	33
2.1 Bacterial strains, plasmids and media	33
2.2 Preparation of GAS culture supernatants.....	33
2.3 Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis	35
2.4 Western blot analysis.....	36
2.5 Bicinchoninic acid assay for protein concentration determination.....	37
2.6 DNA manipulation	37

2.6.1 Cloning of <i>ska</i>	37
2.6.2 Chromosomal DNA extraction	38
2.6.3 Polymerase Chain Reaction (PCR)	39
2.6.4 Restriction Enzyme Digestion	39
2.6.5 DNA Purification	41
2.6.6 Ligation of Recombinant Plasmids	41
2.6.7 Preparation of Electrocompetent <i>E. coli</i>	42
2.6.8 Transformation of <i>E. coli</i>	42
2.6.9 Plasmid DNA Extraction	43
2.6.10 Agarose Gel Electrophoresis	44
2.6.11 DNA Sequence Analysis	44
2.7 Recombinant protein expression and purification.....	45
2.7.1 Pilot expression	45
2.7.2 Preparation of whole cell lysates and testing the solubility of the target protein	46
2.7.3 Large scale expression	46
2.7.4 Native purification of 6xHis tagged fusion proteins using Nickel-NTA Affinity chromatography.	47
RESULTS	
Chapter 3 Analysis of streptokinase expression in <i>ska</i> alleles of GAS.....	49
3.1 GAS streptokinase genotypes.....	49
3.2 Protein detection in GAS culture supernatants	50
3.2.1 Growth of wild type and isogenic mutants of 5448 and ALAB49	50
3.2.2 Immunodetection of streptokinase	53
3.2.3 Immunodetection of SpeB	56
3.3 Protein detection in Northern Territory GAS isolates	57
3.3.1 Growth of NT GAS isolates	57
3.3.2 Immunodetection of streptokinase	56
3.3.3 Immunodetection of SpeB	58
Chapter 4 Cloning and expression of <i>ska</i> 1, <i>ska</i> 2a, and <i>ska</i> 2b.....	62
4.1 PCR amplification of <i>ska</i> 1, <i>ska</i> 2a, and <i>ska</i> 2b	64
4.2 Design of constructs for expression of <i>ska</i> 1, <i>ska</i> 2a and <i>ska</i> 2b.....	64
4.3 DNA sequence analysis	66
4.3.1 Sequence analysis of pSka1	67
4.3.2 Sequence analysis of pSka2a	73
4.3.3 Sequence analysis of pSka2b	71
4.4 Allelic variation in pSka1, pSka2a and pSka2b	78
Chapter 5 Expression, purification and <i>in vitro</i> activity of recombinant streptokinase.....	84
5.1 Pilot expression of recombinant streptokinase.....	84
5.2 Native purification of recombinant streptokinase	84
5.3 <i>In vitro</i> production of recombinant streptokinase	87
5.3.1 Optimisation of <i>in vitro</i> production of recombinant streptokinase	87
Chapter 6 References.....	103

Chapter 7 Appendices.....	112
Appendix 1.....	112
Appendix 2.....	114
Appendix 3.....	116
Appendix 4.....	117

LIST OF FIGURES

Figure 1.1 Diagrammatic representation of the closed and open conformations of plasminogen.....	8
Figure 1.2 The role of plasmin in the plasminogen activation system	9
Figure 1.3 Two proposed mechanisms of plasminogen activation.....	16
Figure 1.4 Model of streptokinase binding to plasminogen.	17
Figure 1.5 Domain structure of streptokinase protein.	18
Figure 1.6 Phylogenetic tree for the β -domain-encoding region of <i>ska</i> of 90 GAS isolates.....	19
Figure 1.7 Representation of plasminogen activation by GAS secreted streptokinase. ..	25
Figure 1.8 Proposed interactions of plasmin(ogen) with GAS plasminogen binding proteins.....	22
Figure 2.1 Amplification of <i>ska</i>	39
Figure 3.1 Phylogenetic tree for the β -domain-encoding region of <i>ska</i>	52
Figure 3.2 Growth of GAS strains in THY broth at 37°C.	52
Figure 3.3 Protein detection in GAS culture supernatants.....	57
Figure 3.4 Growth of NT GAS isolates monitored for 10 h in THY broth at 37°C.....	59
Figure 3.5 Protein detection in TCA precipitated culture supernatants of NT GAS isolates.....	63
Figure 4.1 PCR amplification of the <i>ska</i> alleles.	65
Figure 4.2 Screening of transformants for the presence of recombinant plasmids containing insert DNA.....	66
Figure 4.3 DNA sequencing analysis.....	72
Figure 4.4 ClustalW multiple alignment of the deduced amino acid sequences encoded by the <i>ska</i> gene from NS931(pSka1).	72
Figure 4.5 DNA sequence data from plasmid pSka2a.....	76
Figure 4.6 Results of the ClustalW multiple alignment of the deduced amino acid sequence encoded by the <i>ska</i> gene from strain 5448 (pSka2a).....	78
Figure 4.7 DNA sequence and deduced amino acid sequence (shown below the DNA sequence) of <i>ska</i> from ALAB49.....	81
Figure 4.8 ClustalW pairwise sequence alignment of the insert in plasmid pSka2b with the published full length sequence of <i>ska</i> in ALAB49	82
Figure 4.9 ClustalW multiple sequence alignment of the inserts in the recombinant plasmids pSka1, pSka2a and pSka2b.....	83
Figure 5.1 Pilot expression of recombinant 6xHis tagged fusion proteins.....	85
Figure 5.2 Purification of recombinant streptokinase expressed by pSka1	86
Figure 5.3 Schematic representation of the cloning strategy used to incorporate the Factor Xa Protease recognition sequence in the clone pSka1.....	88
Figure 5.4 Incorporation of the Xa protease recognition site in pSka1.....	89
Figure 5.5 DNA sequence data from pSka1xa.....	92
Figure 5.6 Pilot expression of recombinant 6xHis tagged fusion proteins.....	93
Figure 5.7 Purification of recombinant streptokinase expressed by pSka1xa.....	95
Figure 7.1 Standard curve of PageRuler Protein Ladder Log ₁₀ molecular weight (kDa) is plotted against migration distance (cm) in SDS-PAGE.....	115
Figure 7.2 Standard curve of 1 kb DNA hyperladder used for determining molecular weight of the recombinant <i>ska</i>	116
Figure 7.3 Map of expression vector pQE30 used to express recombinant polyhistidine tagged fusion proteins.....	116

Figure 7.4 ClustalW alignment of full length published sequences of *ska* alleles of 15 GAS strains..... 123

LIST OF TABLES

Table 1	Group A streptococcal adhesins and their binding host cell receptors/proteins.....	3
Table 2	Characteristics of the bacterial strains used in this study.....	34
Table 3	Characteristics of the various plasmids used in this study.....	34
Table 4	PCR specifications for amplification of <i>ska</i> 1, <i>ska</i> 2a and <i>ska</i> 2b...	40
Table 5	Molecular weight and migration distance of PageRuler Protein Ladder in SDS-PAGE	115
Table 6	Molecular weight and migration distance of 1 kb DNA hyperladder in agarose gel electrophoresis.....	115

ABBREVIATIONS

μF	Microfaraday
μg	Microgram
μl	microlitre
μm	micrometre
A_{562}	absorbance at 562 nm
A_{600}	absorbance at 600 nm
Ap	ampicillin
APSGN	acute post-streptococcal glomerulonephritis
ARF	acute rheumatic fever
bps	base-pairs
cm	centimetre
DAB	3,3'-diaminobenzidine
dH ₂ O	glass distilled H ₂ O
DNA	deoxyribonucleic acid
ECM	extracellular matrix
EDTA	ethylenediaminetetraacetic acid
<i>g</i>	9.8 ms ⁻²
g	gram
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GAS	group A streptococcus
h	hour
IPTG	isopropyl- β -D-thiogalactopyranoside
kb	kilobase-pairs
kDa	kilodalton
Km	kanamycin
kV	kilovolt
L	litre
LB	Luria-Bertani
M	molar
mg	milligram
MilliQ	H ₂ O purified with the milliQ system
min	minute
ml	millilitre

mM	millimolar
mV	millivolt
NF	Necrotising fasciitis
ng	nanogram
NTP	NH ₂ terminal peptide
°C	degrees Celsius
ORF	open reading frame
PAI-1	plasminogen activator inhibitor 1
PAI-2	plasminogen activator inhibitor 2
PAM	plasminogen-binding group A streptococcal M-like protein
PBS	phosphate buffered saline
PBST	phosphate buffered saline containing 0.05% Tween-20
PCR	polymerase chain reaction
pmol	picomole
rpm	revolutions per minute
SDS	sodium lauryl sulfate
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
sec	second
SEN	group A streptococcal surface enolase
STSS	streptococcal toxic shock syndrome
TAE	Tris-Acetate-EDTA
tPA	tissue-type plasminogen activator
Tris-base	Tris(hydroxymethyl)aminomethane
Tris-HCl	Tris(hydroxymethyl)aminomethane hydrochloride
Tween-20	polyoxyethylenesorbitan monolaurate
uPA	urokinase-type plasminogen activator
v/v	volume/volume
w/v	weight/volume
Ω	ohm