2006

Investigation of the expression profile of streptokinase

Priya Shyam
University of Wollongong

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* 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.

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Priya Shyam

ACKNOWLEDGEMENTS

Firstly, I would like to thank my supervisor Mark Walker for giving me the opportunity to work on this project.

I would also like to thank my co-supervisor Jason McArthur for his direction and assistance throughout the year, sharing practical knowledge and reading drafts.

I would also like to thank the other members of the Walker lab: Amanda, Amie, Ania, Anna, Andrew, Carola, Christine, Fay, Jake, Jason, Martina and Tracey. Their support and assistance has been invaluable throughout the year.

I express my gratitude to Fiona McKay for providing me with the assay data.

My parents and brother were a great help in dispatching this thesis.

Last but not the least, I am thankful to my husband Mayank, and my in laws for all the moral support when the going was tough.

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 ß 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

LIST OF FIGURES

LIST OF TABLES

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_2O	glass distilled H_2O
DNA	deoxyribonucleic acid
ECM	extracellular matrix
EDTA	ethylenediaminetetraacetic acid
g	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_2O purified with the milliQ system
min	minute
ml	millilitre

mM	millimolar
mV	millivolt
NF	Necrotising fasciitis
ng	nanogram
NTP	NH_2 terminal peptide
ºC	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