Biochemical and Structural Characterization of *Streptococcus pyogenes C5a Peptidase*

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Abstract

Streptococcus pyogenes, also known as Group A Streptococci, is a common causative agent of bacterial infections of the human upper respiratory tract, skin, and soft tissue. Non-suppurative sequelae of *S. pyogenes* infections include rheumatic fever, rheumatic heart disease, and acute glomerulonephritis. Recently there has been a resurgence of rheumatic fever and rheumatic heart disease as well as an increase in aggressive streptococcal disease such as toxic shock syndrome and necrotizing fasciitis. *S. pyogenes* produce a formidable array of virulence factors, one of which is C5a peptidase. The human complement factor C5a is a potent chemoattractant, macrophage activator, and an anaphylatoxin. The C5a peptidase of both Group A Streptococci and Group B Streptococci cleave C5a within its polymorphonuclear neutrophil binding site rendering it inactive. Mouse infection models have demonstrated a functional C5a peptidase assists colonization by retarding the infiltration of phagocytes to the foci of infection. C5a peptidase is a multidomain cell surface subtilisin-like serine protease (subtilase) with an Asp, His, and Ser catalytic triad. Comparative sequence analysis shows C5a peptidase has considerable sequence homology to *Lactococcus lactis* PrtP, both of which are highly specific endopeptidases. Whereas the subtilisins in general show broad substrate specificity profiles, the cell envelope proteinases of lactic acid bacteria demonstrate remarkable substrate specificity. The greater specificity of the cell envelope proteinases is held attributable to changes in variable regions within the structurally conserved regions and the presence of the A-domain, both of which have been demonstrated to modify specificity in PrtP proteinases. The aims of this project were to study the structural and biochemical properties of the C5a peptidase of *Streptococcus pyogenes*. C5a peptidase and variants were cloned, expressed, purified, and assayed for activity in under-agarose lymphocyte migration assays and *in vitro* digestion assays. Absence of activity was found in a C5a peptidase variant in which the A-domain was absent. Purified recombinant C5a peptidase and derivatives were screened for crystallization conditions. Crystallization conditions were found for recombinant C5a peptidase. To combat both the increasing incidence of *S. pyogenes* associated diseases, and increasing antibiotic resistance, new chemotherapeutic agents are required. This study was designed to elucidate the structural and biochemical basis of the substrate specificity of C5a peptidase, which will assist the design of potent inhibitors of this powerful virulence factor.

Abbreviations

AGN	acute glomerulonephritis
bp	base pair
BSA	bovine serum albumin
C5P	C5a peptidase
cDNA	complementary DNA
CEP	lactic acid bacteria cell envelope proteinase
CO_2	carbon dioxide
DMSO	dimethyl sulphoxide
dNTPs	deoxynucleotide triphosphates
DTT	dithiothreitol
E. coli	*Escherichia coli*
EDTA	ethylene diamine tetraacetic acid
ELISA	enzyme linked immunosorbent assay
FPLC	fast protein liquid chromatography
GAS	group A streptococci
GBS	group B streptococci
HEPES	N-[2-hydodroxyethyl]piperazine-N'-[2-ethane sulfonic acid]
HPLC	high pressure liquid chromatography
IEFPLC	ion exchange FPLC
kb	kilobase
LAB	lactic acid bacteria
MAC	membrane attack complex
MEM	Eagle's minimal essential medium
MS	mass spectrometry
Mw	molecular weight
oligo	oligonucleotide
PAGE	polyacrylamide gel electrophoresis
pAMPSF	4-aminophenylmethane sulfonyl fluoride hydrochloride
PBS	phosphate buffered saline
PCR	polymerase chain reaction
pI	isoelectric point

PMSF	phenyl methyl-sulfonyl fluoride
PMN	polymorphonuclear neutrophil
PNK	polynucleotide kinase
RF	rheumatic fever
RPHPLC	reverse phase HPLC
SDS	sodium dodecylsulphate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
SEFPLC	size exclusion FPLC
SpeB	streptococcal cysteine proteinase
S. pyogenes	*Streptococcus pyogenes*
STSS	streptococcal toxic shock syndrome
TAE	tris acetate EDTA
Tris	tris (hydroxymethyl)-aminomethane
UV	ultra violet light
VR	variable region within structurally conserved catalytic domain of C5P
ZnAc	zinc acetate
ZnCl	zinc chloride

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