

**An Investigation of
Porphyromonas gingivalis
Peptidylarginine Deiminase:
A Putative Virulence Factor in an
Animal Model of Inflammation**



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ABBREVIATIONS

ACPA	Anti-citrullinated protein antibodies
AKA	Anti-keratin antibodies
anti-CCP	Anti-citrullinated cyclic peptide antibodies
APF	Anti-perinuclear factor
Arg-Xaa	Arginine carboxy terminal peptide bond
ATP	Adenosine triphosphatase
AU	Absorbance unit
BAEE	Benzoyl-arginine ethyl ester
BHI	Brain-heart infusion
BSA	Bovine serum albumin
Ca ²⁺	Calcium ion
CaCl ₂	Calcium chloride
CO ₂	Carbon dioxide
C-terminal	Carboxy terminal
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid

EGTA	ethylene glycol tetraacetic acid
<i>et al.</i>	<i>et alia</i>
FeCl ₃	Iron (III) Chloride
FMN	Flavin mononucleotide
<i>g</i>	Gravitational force
GCF	Gingival crevicular fluid
H ₂	Hydrogen
HCl	Acid hydrochloric
IgA	Immunoglobulin A
IgG	Immunoglobulin G
kDa	kilo Dalton
Kgp	Lysine gingipain
Lys-Xaa	Lysine carboxy terminal peptide bond
M	Molar
mg cell protein ⁻¹ .min ⁻¹	Milligram per cell protein per minutes
mM	milliMolar
mPAD	Rabbit muscle/ mammalian PAD

N ₂	Nitrogen
nm	nanometre
nmoles	nanomoles
nmoles citrulline.unit ⁻¹ .min ⁻¹	nanomoles citrulline per unit per minute
N-terminal	Amino terminal
°C	Degree Celcius
OD ₅₆₀	Optical Density at 560nm
PAD	Peptidylarginine deiminase
PD	Periodontal disease
<i>Pg</i>	<i>Porphyromonas gingivalis</i>
<i>Pg</i> PAD	<i>Porphyromonas gingivalis</i> peptidylarginine deiminase
PMN	Polymorphonuclear
RA	Rheumatoid arthritis
RF	Rheumatoid factor
Rgp	Arginine gingipain
R-group	Amino acid functional group
<i>Sp.</i>	Species

TNF- α	Tumor necrosis factor alpha
TPCK	Tosyl phenylalanyl chloromethyl ketone
Tris-HCl	Tri sulphate – hydrochloric acid
v/v	Volume per volume
w/v	Weight per volume
μ L	microlitre
μ m	micrometre
met-arg-phe	Methionine-arginine-phenylalanine
H ₂ O ₂	Hydrogen peroxide

ABSTRACT

Porphyromonas gingivalis, an oral periodontopathogen linked to chronic periodontitis expresses peptidylarginine deiminase (PAD), an enzyme that converts peptide-bound arginine to citrulline. A relationship between human PADs and chronic inflammatory diseases has been proposed. Citrullinated α -enolase is a candidate auto-antigen in rheumatoid arthritis. Vimentin and fibrin are also likely target proteins in disease development. This study partially characterised the enzyme and the ability of *P. gingivalis* cells to citrullinate peptides and these rheumatoid arthritis relevant proteins. In addition, the influence of gingipains, key *P. gingivalis* virulence factors, on PgPAD activity was investigated. A limited histological survey was performed on selected tissues to investigate the effect of *P. gingivalis* in an animal model of adjuvant arthritis.

A colourimetric assay to quantify citrulline was developed and used to determine the effect of environmental pH and temperature on enzyme activity. Enzyme localization was investigated by comparing reaction rates of whole cells to cell sonicates. Enzyme specificity was determined by incubation of cells with a range of arginine analogues and arginine-containing peptides. The rates of citrullination of enolase, vimentin and fibrin by *P. gingivalis* cells were calculated. The influence of the gingipains on citrullination was measured by comparing the rate of citrullination of albumin in the presence and absence of the proteolytic inhibitors tosyl phenylalanyl chloromethyl ketone and leupeptin. Tissue sections from three regions of the animal heads were stained for polymorphonuclear cells and osteoclasts. In addition sponge samples were surveyed for polymorphonuclear cells and citrullinated proteins detected using immunohistochemical technique.

PgPAD activity was heat stable, predominantly cell-surface expressed and exhibited optimal activity between pH 7.5 and 8. The enzyme was highly specific for arginine and citrullinated arginine residues in all positions in the peptides tested. *PgPAD* was able to citrullinate all rheumatoid arthritis relevant proteins, at rates slower than peptides. Inhibition of the gingipains failed to influence the rate of citrullination of albumin. In the adjuvant arthritis animal study, pre-treatment with *P. gingivalis* produced increased inflammatory cellular infiltrate at the site of exposure but no similar affect in the head tissue. There was a significant increase numbers of polymorphonuclear cells in the bone marrow from the head region and in the implanted sponge infiltrate from rats with prior exposure to *P. gingivalis*. Although citrullinated proteins were detected in sponge sections from both adjuvant arthritis-induced rat groups, no difference between them was observed. A similar result was seen with osteoclasts, as both groups exhibited increased numbers over the control group.

This study has shown that *P. gingivalis* peptidylarginine deiminase has potential to influence the inflammatory process by citrullinating arginine containing peptides and rheumatoid arthritis relevant proteins. An examination of rats exposed to the bacterium in an animal model of rheumatoid arthritis did not appear to exacerbate inflammation in selected tissues.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Syatirah Najmi Abdullah and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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