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PNGases: A diverse family of enzymes related by function rather than catalytic mechanism

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2010

PNGases:
A diverse family of enzymes related by function rather than catalytic mechanism

A thesis presented in partial fulfilment of the
requirements for the degree of

Doctor of Philosophy

in the

Institute of Molecular BioSciences
Massey University
Palmerston North, New Zealand

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2010

ABSTRACT

Peptide:*N*-glycanases (PNGases, EC 3.5.1.52) release *N*-linked glycan moieties from glycoproteins and glycopeptides. They catalyse the cleavage of the amide bond between the proximal *N*-acetylglucosamine and the asparagine side chain of the polypeptide, resulting in the conversion of the asparagine residue to aspartic acid and the concomitant release of the intact glycan and free ammonia. PNGases, especially PNGase F, are valuable tools for the removal of glycan moieties from glycoproteins for subsequent analyses of the released glycan and/or protein.

In the first part of this work, a classification for PNGases has been proposed, dividing these enzymes into three types based on their primary amino acid sequence, and also on their subcellular localisation, phylogenetic distribution (to date) and physiological function (if known). It appears that the three PNGase-types developed by convergent evolution. Gene expression studies for one putative type I (*Deinococcus radiodurans*) and two putative type II (*Aspergillus niger*, *Streptomyces avermitilis*) PNGases showed that these proteins were expressed in their native organisms. Recombinant expression of these proteins and the putative PNGase from *Sulfolobus solfataricus* yielded soluble protein for the *S. avermitilis* and *D. radiodurans* proteins and PNGase activity could be shown once for the latter enzyme.

In the second part of this work, site-specific mutants of PNGase F, the only characterised type I PNGase to date, were generated, expressed and characterised using enzyme kinetic methods. From the kinetic results obtained here, a catalytic mechanism can be proposed for PNGase F. In this mechanism a bound water molecule acts as the nucleophile after being activated by the abstraction of a proton by a conserved glutamate residue. The carbonyl carbon of the scissile bond is primed for the nucleophilic attack by another conserved residue, Arg248, probably by the donation of a proton.

A 1.57 Å crystal structure of the recombinant wildtype PNGase F that has three glycerol molecules non-covalently bound in the active site is also presented. This crystallographic analysis shows that the recombinant protein has a structure identical to that of the native protein, validating the basis of the kinetic studies, and showing why glycerol acts as an inhibitor of this enzyme.

ACKNOWLEDGEMENTS

Many people have greatly supported me during my time working on this project. It is impossible to mention everyone here, but I would like to thank especially the following people for their support and assistance:

My supervisor Gill Norris who has given me the opportunity to ‘start over’ during what was a difficult time for me. Thank you for your encouragement, advice and guidance in good times and in times of frustration and despair.

My *co*-supervisor Mark Patchett, who has always been approachable and never short of excellent advice if things were not going to plan.

All my colleagues and friends in the X-lab and the ‘Neighbour-lab’ past and present, especially Alice Clark, Meekyung Ahn, Jan Richter, Judith Stepper, Simon Oakley, Matthew Bennett and Greg Sawyer. A big thank-you to Trevor Loo for his assistance with all sorts of equipment and general good advice.

Bryan Anderson and Geoff Jameson for their assistance with X-ray crystallography.

Everyone in the Institute of Molecular BioSciences, who has supported me during my time here.

Finally, all this would not have been possible without the unconditional love, support and constant encouragement of my family in particular my Mum, my Grandmas, my sister Rauna and my husband Viatcheslav. You were always able to raise my spirits even in the most difficult times. Thank you so much! Большое спасибо! Vielen, vielen Dank! Diese Arbeit ist Euch gewidmet!

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ABBREVIATIONS

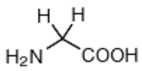
AMFR	Autocrine Motility Factor Receptor
Ani	*Aspergillus niger*
Amp	Ampicillin
BLAST	Basic Local Alignment Search Tool
bp	base pair(s)
BTP	Bis-Tris propane
BVES	Baculovirus expression system
°C	degree Celsius
CBM	Carbohydrate-binding module
Cm	Chloramphenicol
CNBr	Cyanogen bromide
CV	Column Volume(s)
Da	Dalton
DEPC	Diethyl pyrocarbonate
dn	denatured
DNA	Deoxyribonucleic acid
Dra	*Deinococcus radiodurans*
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetate
et al.	*et alteri* (and others)
ER	Endoplasmic Reticulum
ERAD	ER Associated Degradation
EtOH	Ethanol
FPLC	Fast Protein Liquid Chromatography
Fuc	Fucose
g	gram
g	g-force
Gal	Galactose
GalNAc	*N*-Acetylgalactosamine
GlcNAc	*N*-Acetylglucosamine

GOL	Glycerol
GST	Glutathione S-Transferase
h	hour(s)
h...	human...
H	α-Helix
His$_6$	hexahistidine-tag
HPLC	High Performance Liquid Chromatography
Hyl	Hydroxylysine
Hyp	Hydroxyproline
IMAC	Immobilised Metal Affinity Chromatography
IPTG	Isopropyl-β-D-thiogalacto-pyranoside
Kan	Kanamycin
kDa	kilodalton
L	Litre
LB	Luria-Bertani
m...	mouse...
M	Molar, Mega...
MALDI-TOF-MS	Matrix Assisted Laser Desorption/Ionization-Time Of Flight-Mass Spectrometry
MBP	Maltose Binding Protein
mg	milligram
min	minute
mL	millilitre
mM	millimolar
Man	Mannose
Mw	Molecular weight
n	native
NCBI	National Centre for Biotechnology Information
OD$_{600}$	Optical Density at Wavelength of 600 nanometres
OmpA	Outer Membrane Protein A
ORF	Open Reading Frame
Pa	Pascal
PAGE	Polyacrylamide-Gel-Electrophoresis

PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PEG	Polyethylene glycol
pfu	plaque forming unit
PNGase	Peptide:*N*-glycanase
pI	Isoelectric Point
PUB	PNGase/Ubiquitin-associated or UBX-containing Protein Domain
rmsd	Root mean square deviation
RNase B	Ribonuclease B
rpm	Revolutions per Minute
RT	Room Temperature
***S**av*	*Streptomyces avermitilis*
SDS	Sodium-dodecylsulfate
SEC	Size Exclusion Chromatography
Sso	*Sulfolobus solfataricus*
Tc	Tetracycline
UBA	Ubiquitin-Associated Domain
UBL	Ubiquitin-Like Domain
UBX	Ubiquitin Regulatory X Domain
UV	Ultraviolet
v/v	volume per volume
w/v	weight per volume
XPCB	Xeroderma pigmentosum protein C-Binding Domain
y...	yeast...

AMINO ACIDS

Small



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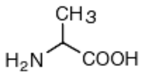
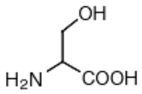
MW: 57.05

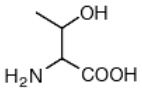


Alanine (Ala, A)
MW: 71.09

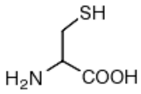
Nucleophilic



Serine (Ser, S)
MW: 87.08, $pK_a \sim 16$

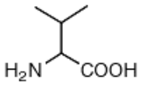


Threonine (Thr, T)
MW: 101.11, $pK_a \sim 16$

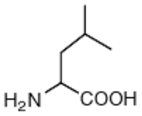


Cysteine (Cys, C)
MW: 103.15, $pK_a = 8.35$

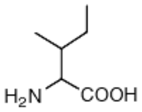
Hydrophobic



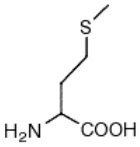
Valine (Val, V)
MW: 99.14



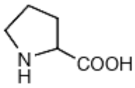
Leucine (Leu, L)
MW: 113.16



Isoleucine (Ile, I)
MW: 113.16

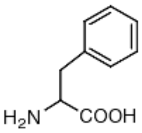


Methionine (Met, M)
MW: 131.19

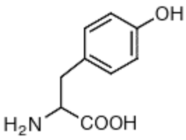


Proline (Pro, P)
MW: 97.12

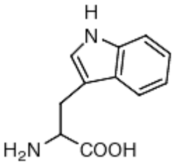
Aromatic



Phenylalanine (Phe, F)
MW: 147.18

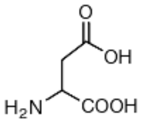


Tyrosine (Tyr, Y)
MW: 163.18

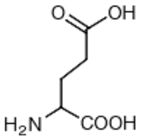


Tryptophan (Trp, W)
MW: 186.21

Acidic

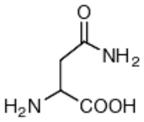


Aspartic Acid (Asp, D)
MW: 115.09, $pK_a = 3.9$

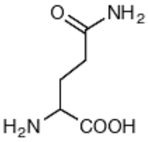


Glutamic Acid (Glu, E)
MW: 129.12, $pK_a = 4.07$

Amide

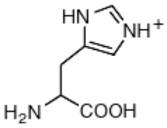


Asparagine (Asn, N)
MW: 114.11

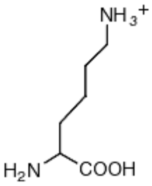


Glutamine (Gln, Q)
MW: 128.14

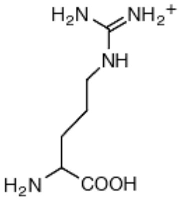
Basic



Histidine (His, H)
MW: 137.14, $pK_a = 6.04$



Lysine (Lys, K)
MW: 128.17, $pK_a = 10.79$



Arginine (Arg, R)
MW: 156.19, $pK_a = 12.48$

NUCLEIC ACID ABBREVIATIONS

A	Adenine
T	Thymine
C	Cytosine
G	Guanine
U	Uridine
R	G or A
Y	T or C
K	G or T
M	A or C
S	G or C
W	A or T
B	G or T or C
D	G or A or T
H	A or C or T
V	G or C or A
N	Any

STANDARD GENETIC CODE

	T			**C**			**A**			**G**		
T	TTT	F	Phe	TCT	S	Ser	TAT	Y	Tyr	TGT	C	Cys
	TTC	F	Phe	TCC	S	Ser	TAC	Y	Tyr	TGC	C	Cys
	TTA	L	Leu	TCA	S	Ser	**TAA**	*	**Stop**	**TGA**	*	**Stop**
	TTG	L	Leu	TCG	S	Ser	**TAG**	*	**Stop**	TGG	W	Trp
C	CTT	L	Leu	CCT	P	Pro	CAT	H	His	CGT	R	Arg
	CTC	L	Leu	CCC	P	Pro	CAC	H	His	CGC	R	Arg
	CTA	L	Leu	CCA	P	Pro	CAA	Q	Gln	CGA	R	Arg
	CTG	L	Leu	CCG	P	Pro	CAG	Q	Gln	CGG	R	Arg
A	ATT	I	Ile	ACT	T	Thr	AAT	N	Asn	AGT	S	Ser
	ATC	I	Ile	ACC	T	Thr	AAC	N	Asn	AGC	S	Ser
	ATA	I	Ile	ACA	T	Thr	AAA	K	Lys	AGA	R	Arg
	ATG	**M**	**Met**	ACG	T	Thr	AAG	K	Lys	AGG	R	Arg
G	GTT	V	Val	GCT	A	Ala	GAT	D	Asp	GGT	G	Gly
	GTC	V	Val	GCC	A	Ala	GAC	D	Asp	GGC	G	Gly
	GTA	V	Val	GCA	A	Ala	GAA	E	Glu	GGA	G	Gly
	GTG	V	Val	GCG	A	Ala	GAG	E	Glu	GGG	G	Gly