THE DEVELOPMENT OF A PURIFICATION PROCEDURE FOR PEPTIDE:*N*-GLYCOSIDASE A FROM *PRUNUS AMYGDALUS*

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ABSTRACT

Peptide-N^4-(*N*-acetyl-β-glucosaminyl) asparagine amidases cleave the amide bond between N-linked glycans at N-acetylglucosamine and asparagine, liberating intact oligosaccharide chains from glycoproteins. Although PNGase A is commonly used by glycobiologists for removal of N-linked glycans from plant sources, much less is known about it than about PNGase F, an enzyme that is more commonly used for deglycosylating proteins.

New studies on PNGase A have been initiated, with the aim of carrying out complete biochemical and structural studies in order to determine the substrate specificity, isoelectric point, primary, secondary and tertiary structures. Comparisons will then be made with PNGase F, whose three-dimensional structure is known.

The first step in these studies is therefore to obtain some pure protein and amino acid sequence. Although purification protocols have been published previously, it was difficult to produce a homogeneous preparation following these methods and they have hence been modified. The methods used are described in *Chapter 2* and the results of four preparations, using almond meal and almond emulsin as starting materials, are reported in *Chapters 3-6*.

Although PNGase A had not been purified to homogeneity, an active band excised from a native gel and analysed by SDS-PAGE showed four major bands. Which band represents PNGase A remains to be determined.

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LIST OF ABBREVIATIONS

BCA	Bicinchoninic acid
BME or βME	β-mercaptoethanol
BSA	Bovine serum albumin
CM	Carboxymethyl
DEAE	Diethylaminoethyl
DIG	Digoxigenin
EDTA	Ethylenediamine tetra-acetic acid (di-sodium salt)
ENGase	Endo-*N*-acetyl-β-D-glucosaminidase or endoglycosidase
FPLC	Fast protein liquid chromatography
GLC	Gas-Liquid chromatography
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
NaAc	Sodium acetate buffer
NH_4Ac	Ammonium acetate buffer
$(NH_4)_2SO_4$	Ammonium sulphate
PAGE	Polyacrylamide gel electrophoresis
PMSF	Phenyl methyl sulfonyl fluoride
PNGase	Peptide-N^4-(*N*-acetyl-β-D-glucosaminyl) asparagine amidase A
PVDF	Polyvinylidene difluoride
Q- or QAE	Quaternary amino (hydroxypropyl diethyl aminoethyl)
RPC	Reverse phase chromatography
SDS	Sodium dodecyl sulphate
TFA	Trifluoroacetic acid
TRIS	Tris(hydroxymethyl)aminomethane

Three and one letter code for amino acids

Ala	A	Alanine
Arg	R	Arginine
Asn	N	Asparagine
Asp	D	Aspartic acid
Cys	C	Cysteine
Gln	Q	Glutamine
Glu	E	Glutamic acid
Gly	G	Glycine
His	H	Histidine
Ile	I	Isoleucine
Leu	L	Leucine
Lys	K	Lysine
Met	M	Methionine
Phe	F	Phenylalanine
Pro	P	Proline
Ser	S	Serine
Thr	T	Threonine
Tyr	Y	Tyrosine
Val	V	Valine
	H_s	Homoserine lactone

Sugar abbreviations

GlcNAc	N-acetylglucosamine	Fuc	fucose
GalNAc	N-acetylgalactosamine		

Linkages are described using conventional carbon ring numbers connected by a slash and anomericity is denoted by α or β. For example: fuc α1-3 linked to GlcNAc.

TABLE OF CONTENTS

LIST OF FIGURES

FIGURE PAGE

LIST OF TABLES