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Molecules of Interest

Plant glycosidases acting on protein-linked oligosaccharides

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ABSTRACT

Glycosidases have been used as invaluable tools in glycobiology research for decades, and their role in glycoprotein maturation has been amply studied. The molecular biological coverage of this large group of enzymes has only recently reached an appreciable level. In this review, we present an overview of plant glycosidases, whose DNA/protein sequence has been identified and for which recombinant enzymes have been characterized. The physiological role in the maturation of glycoproteins is discussed as well as the biotechnological prospects arising from knowing the enzymes responsible for the removal of terminal *N*-acetylglucosamine residues. The current knowledge on plant fucosidases and of the first bits of information on glycosidases acting on arabinogalactan proteins is presented.

In the early times of glycobiology, exoglycosidases from a wide variety of plants were investigated. The biosynthetic role of α-glucosidases and α-mannosidases was studied (Lerouge et al., 1998; Sturm et al., 1987), whereas α- and β-galactosidases, fucosidases, β-*N*-acetylglucosaminidases and, again, α-mannosidases found applications as valuable tools for the analysis of glycoproteins (Royle et al., 2006; Tomiya et al., 1987). This research was performed with classical biochemical methods, i.e., with purified natural enzymes. The molecular biological gold rush at the end of the last millennium hardly seized plant glycosidases. During the recent years, a few glycosidase genes were identified following protein purification and sequencing. More researchers took advantage of the ever increasing amount of DNA sequence data, which allowed spotting of potential plant orthologs of mammalian glycosidases. The decision to take the effort of expressing and characterizing the respective proteins was often prompted by a renewed interest for plant protein glycosylation stimulated by the perspective of producing recombinant therapeutics in plants (Strasser et al., 2007).

The glycosidases presented here are classified into three large groups despite the notorious fact that mother Nature appears recalcitrant to such efforts. The first group contains enzymes involved in the maturation of Asn-linked oligosaccharides (N-glycans). The second somewhat motley group comprises glycosidases that may fulfill catabolic functions *in vivo* as well as those used for analytical or preparative *in vitro* purposes. The third and last chapter is devoted to glycosidases that are assumed to act on arabinogalactan proteins (AGPs).

The amount of literature on plant glycosidases is overwhelming, and to a large part it dates back considerably. The criterion for selecting glycosidases and related manuscripts was the unambiguous identification of the respective DNA sequence (Table 1) and the proof of enzymatic activity with recombinant protein or mutant/silencing analysis.

1. Glycosidases involved in N-glycan maturation

In plants as in other eukaryotes, N-glycan is transferred *en block* co-translationally to the nascent protein in the form of $Glc_3Man_9GlcNAc_2$ (Fig. 1A). ER-resident enzymes, two α-glucosidases and one α-mannosidase lead to the oligomannosidic structures Man9 to Man5 (Gorr and Altmann, 2007). After the transfer of an *N*-acetylglucosamine (GlcNAc) residue, mannosidase II removes up to two additional mannoses to form glycoproteins carrying MGn, MGnF, MGnX or MGnXF (Fig. 1) depending on the presence of xylosyl- and α1,3-fucosyltransferase (Bondili et al., 2006; Gomord and Faye, 2004; Gorr and Altmann, 2007; Ko et al., 2008; Leiter et al., 1999; Strasser et al., 2000); these structures give birth either to more complex entities with elongated antennae or – by virtue of hexosaminidases - to paucimannosidic N-glycans.

1.1. α-Glucosidase I (EC 3.2.1.106)

After the transfer from dolichol to protein, the outermost α1,2-linked glucose of the N-glycan is hydrolyzed by glucosidase I (Grinna and Robbins, 1979; Hubbard and Robbins, 1979). The next two α1,3-linked glucoses are removed by glucosidase II (Kilker et al., 1981; Michael and Kornfeld, 1980). Both glucosidases reside in

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Table 1
Glycosidases and their genes.

Enzymatic activity	Protein (gene)	Organism	Publications leading to the gene identification
α-Glucosidase I	Q8GWC8_ARATH (At1g67490)	*A. thaliana*	Boisson et al., 2001; Gillmor et al., 2002; Furumizu and Komeda, 2008
α-Glucosidase II	Q9LEC9_SOLTU (*mal2*)	*Solanum tuberosum*	Taylor et al., 2000
	Q9FN05_ARATH (At5g63840)	*A. thaliana*	Burn et al., 2002
			Soussillane et al., 2008
α-Mannosidase II	Q9LFR0_ARATH (At5g14950)	*A. thaliana*	Strasser et al., 2006
Hexosaminidase	A7WM73_ARATH/Hexo1 (At3g55260)	*A. thaliana*	Strasser et al., 2007
	Q8L7S6_ARATH/Hexo3 (At1g65590)		Gutternigg et al., 2007
α1,3/4-Fucosidase	AtFUC1 (At2g28100)	*A. thaliana*	Zeleny et al., 2006
α1,2-Fucosidase	FUCO2_ARATH (At4g34260)	*A. thaliana*	Léonard et al., 2008
α-Galactosidase	AGAL_COFAR	*Coffea arabica*	Zhu and Goldstein, 1994
	Q41100_PHAVU	*Phaseolus. vulgaris*	Davis et al., 1997
	AGAL_ORYSJ	*Oryza sativa*	Kim et al., 2002
	Q84VQ7_HELAN	*Helianthus annuus*	Kim et al., 2003
Endo-β-mannosidase	EBM_LILLO	*Lilium longiflorum*	Ishimizu et al., 2004
	Q14U56_BRAOL	*Brassica oleracea*	Ishimizu et al., 2006
PNGase cytosolic	PNG1_ARATH (At5g49570)	*A. thaliana*	Diepold et al., 2007
PNGase secretory route	PNAA_PRUDU	*Prunus. dulcis*	Iroyuki et al., 1998 (patent)
α-Arabinofuranosidase	Q3V5Q1_RAPSA (*RsAraf1*)	*Raphanus sativus*	Kotake et al., 2006
β-Galactosidase	Q6L619_RAPSA (*RsBGal1*)	*R. sativus*	Kotake et al., 2005
β-Glucuronidase	HPSE1_ARATH (At5g07830, *gus2*)	*A. thaliana*	Eudes et al., 2008

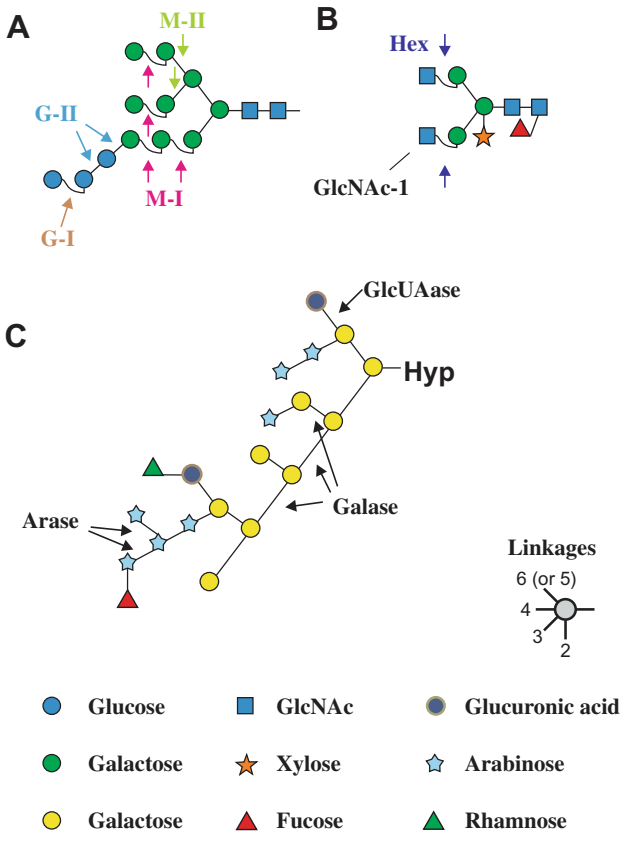

Fig. 1. Structures of selected substrates of plant glycosidases. (A) N-glycan precursor with glucose residues, G-I and G-II stand for α-glucosidases I and II; M-I and M-II for α-mannosidase I and II, respectively. (B) complex-type N-glycan with terminal GlcNAc residues. GlcNAc-1 is the residue attached to the α1,3-arm by GlcNAc-transferase I and Hex indicates the respective *N*-acetylglucosaminidase. (C) Example of a type II arabinogalactan linked to hydroxyproline as shown in (Tan et al., 2004) with potential target sites for α-arabinofuranosidase, β-glucuronidase and β-galactosidase.

the ER. This sequence of events was established for animal cells, but it has become evident that essentially the same steps occur in plants (Gomord and Faye, 2004).

The first purification of a plant glucosidase was realized in the eighties from mung bean seedlings (Szumilo et al., 1986a). The authors succeeded in separating the two forms of glucosidase activities and in purifying the glucosidase I 200-fold. This allowed them to confirm the inhibition of this plant enzyme by castanospermine and deoxynojirimycin. It was only in 1998 that a 3600-fold purification to apparent homogeneity of this glucosidase I was published (Zeng and Elbein, 1998). The enzyme did not seem to be affected by the number of mannose residues present on the N-glycan as Glc_3Man_5 was digested at the same rate as Glc_3Man_9. The sequence of four peptides obtained by trypsin digestion of the purified enzyme showed a significant homology with mammalian glucosidase I.

The first determination of a gene encoding a plant glucosidase I was allowed by the dramatic consequence of its inactivation on protein storage bodies of *A. thaliana* seeds. The T-DNA tagging led to the cloning of the glucosidase I gene (At1g67490). The glucosidase I mutant seeds could only accumulate low levels of storage proteins, had no typical protein bodies and showed a cell enlargement (Boisson et al., 2001). In the case of the T-DNA insertion within the glucosidase I gene, the mutation was lethal and the morphogenesis of the embryo appeared to be blocked at the heart stage of the development. Moreover, another study showed that glucosidase I mutant embryos carrying two null alleles due to a point mutation (*knf-14*) are deficient in cellulose and contain only 13% of the wt amount of crystalline cellulose (Gillmor et al., 2002). The authors concluded that the lack of N-glycan trimming was not directly affecting cellulose synthases but suggested that other proteins involved in cellulose synthesis, for instance KORRIGAN, require a proper N-glycan maturation. The finding of plants having a point mutated Glucosidase I and presenting a less severe phenotype, which is viable and fertile (Furumizu and Komeda, 2008), showed that these enzymes also play a role in the development of the plant in post embryonic stages and affect the stomatal density as well as the elongation and cell differentiation.

1.2. α-Glucosidase II (EC 3.2.1.84)

The first purification to homogeneity of a plant glucosidase II was realized in 1990 from a triton extract of mung bean seedlings (Kaushal et al., 1990a). The specificity of the enzyme for α1,3-glucosidic linkage was confirmed by inhibition studies with the α1,3-linked glucose disaccharide nigerose and the absence of inhibition by α1,2-, α1,4- and α1,6-linked glucose disaccharides. The same team demonstrated the ER localization of the enzyme by sucrose