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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

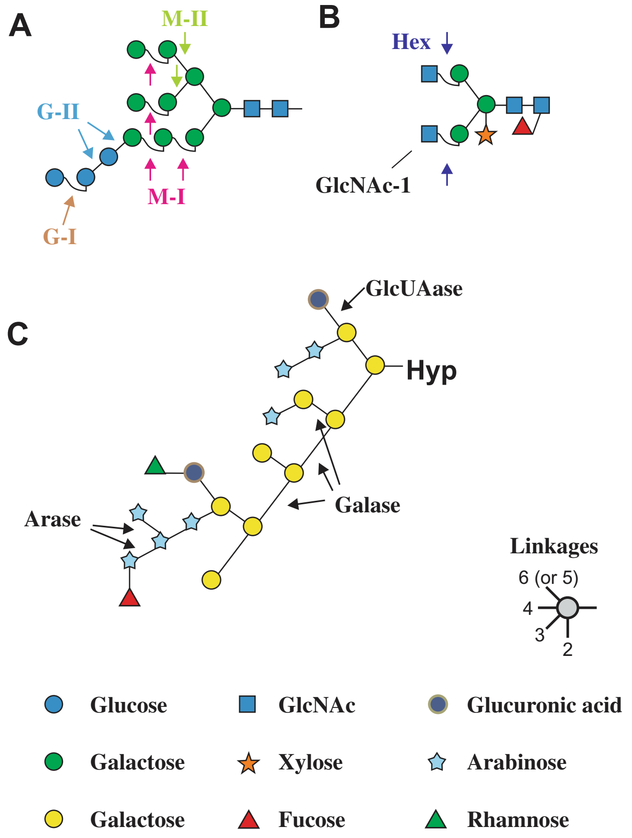

Fig. 1. Structures of selected substrates of plant glycosidases. (A) N-glycan precursor with glucose residues, G-I and G-II stand for α-glucosidases I and II; M-I and M-II for α-mannosidase I and II, respectively. (B) complex-type N-glycan with terminal GlcNAc residues. GlcNAc-1 is the residue attached 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 gradient density centrifugation using antibodies raised against the purified protein and noted the absence of a potential transmembrane domain on the enzyme (Kaushal et al., 1993). However, no peptide sequence was released from this work. The first identification of a plant gene encoding a glucosidase II was realized with a *Solanum tuberosum* gene that had initially been described as complementing a yeast maltase mutant. The antisense silencing of this gene, *MAL1*, led to a 90% decrease in the α-glucosidase II activity measured in potato microsomes (Taylor et al., 2000). The second characterized gene encoding the catalytic subunit of glucosidase II was identified through the study of an *A. thaliana* temperature-sensitive mutant, *rsw3*, affected in its cellulose synthesis and having a swollen roots phenotype (Burn et al., 2002). By map-based strategy, the mutated At5g63840 glucosidase II gene was shown to have a single base substitution leading to Ser 599 replacement by Phe in the protein. Sequence homology study with already identified animal genes showed that the catalytic α-subunit of the glucosidase II heterodimer was actually mutated in this plant and that the homologues to the β-subunit responsible for the retention in the endoplasmic reticulum in animals also existed in plants. An additional indication that At5g63840 actually encodes a glucosidase II came from the effective complementation of a *S. pombe* mutant for the α-subunit (Soussillane et al., 2008) and by the very high glucosylation rate of endo H obtained N-glycans of rsw3 shown by mass spectrometry (Soussillane et al., 2008). As no homozygous T-DNA insertion lines for At5g63840 were obtained so far, it is very likely that the complete lack of glucosidase II activity is lethal.

1.3. *α-Mannosidase I (EC 3.2.1.113)*

A processing mannosidase was first described in microsomal preparations from mung bean hypocotyls (Forsee, 1985). The enzyme was also purified from mung bean seedlings, and was confirmed to be a specific α1,2-mannosidase with enzymatic properties similar to animal mannosidase I (Szumilo et al., 1986b). The gene of a putative mannosidase I from *Glycine max* was identified by sequence homology with animal and fungal enzymes and expressed as a GFP-fusion protein for subcellular localization (Nebenfuhr et al., 1999). The same gene was used in further studies about the targeting mechanisms of plant N-glycan processing enzymes along the secretory pathway (Saint-Jore-Dupas et al., 2006). Nevertheless, the mannosidase I activity of the enzyme encoded by this gene was actually never proven, and this gap still remains unfilled. Inactivating this gene may help to obtain plants lines devoid of typical plant N-glycan epitopes, which pose a serious problem for biotechnological applications (Jin et al., 2008).

1.4. *Golgi α-Mannosidase II (EC 3.2.1.114)*

The Golgi α-mannosidase II participates in N-glycan processing in contrast to lysosomal or – in plants – vacuolar α-mannosidase (Kimura et al., 1999), which has a role in N-glycan degradation. Golgi mannosidase II was first purified to homogeneity from *Vigna radiata* (Kaushal et al., 1990b). The gene (At5g14950) of the *A. thaliana* mannosidase II was identified by its homologies with human and with *Drosophila melanogaster* protein sequence (Strasser et al., 2006). This At5g14950 encodes a type II transmembrane protein with an unusually long putative N-terminal cytoplasmic tail of 50 amino acids. The heterologous expression of *A. thaliana* mannosidase II in insect cells confirmed Man5Gn as the natural substrate of the enzyme and its absence of activity with Man5, highlighting the requirement for the presence of GlcNAc-1 (Fig. 1B). The N-glycan pattern of a mutant plant showed drastic changes as the complex-type major structures MMXF, GnMXF/MGnXF and GnGnXF were replaced by the hybrid-type glycans Man5XF and Man5GnXF (Strasser et al., 2006). No physiological consequence could be observed phenotypically under standard growth conditions. However, very recently it was reported that the lack of Golgi mannosidase II results in reduced salt tolerance of *A. thaliana* roots (Kang et al., 2008).

1.5. *β-N-Acetylglucosamidases (hexosaminidases) (EC, 3.2.1.52)*

Thus far, the trimming events are highly similar if not identical to those occurring in mammals (or in insects, fungi, etc.) cells. The biosynthetic routes diverge after the action of GlcNAc-transferase I (Gomord and Faye, 2004; Gorr and Altmann, 2007; Strasser et al., 1999). Plants contain substantial amounts of paucimannosidic N-glycans typically consisting of a pentasaccharide core with mannose residues at the non-reducing end decorated with xylose and fucose residues (Kurosaka et al., 1991; Wilson et al., 2001). These truncated oligosaccharides are not consistent with the specificities of the enzymes involved in their processing pathway, because Golgi α-mannosidase II, β1,2-xylosyltransferase and core α1,3-fucosyltransferase strictly require the presence of the terminal GlcNAc-1 residue added by β1,2-*N*-acetylglucosaminyltransferase I (Benćur et al., 2005; Leiter et al., 1999; Strasser et al., 1999; Strasser et al., 2006). These findings strongly indicate that paucimannosidic N-glycans are generated by the removal of the GlcNAc residue from the α1,3-linked mannosyl branch by either a very specific processing β-*N*-acetylglucosaminidase or a broad-spectrum enzyme involved in glycan degradation. Both types of β-hexosaminidases have been cloned and characterized from insects (Geisler et al., 2008; Léonard et al., 2006).

Paucimannosidic N-glycans have been described as major glycoforms on vacuolar proteins such as bean phaseolin (Sturm et al., 1987). Consequently, it was proposed that terminal GlcNAc residues are cleaved off in the vacuole or a pre-vacuolar compartment (Sturm et al., 1987; Vitale and Chrispeels, 1984). However, paucimannosidic N-glycans have also been found, albeit to a lesser extent, on extracellular glycoproteins (Fitchette-Lainé et al., 1997; Sturm, 1991) and on cell wall bound proteins (Kotake et al., 2001). The corresponding β-*N*-acetylglucosaminidase activities have been described in a variety of plants, e.g. in protein bodies from seeds, vacuoles from leaves and in suspension-cultured cells (Boller and Kende, 1979; Li and Li, 1970; Oikawa et al., 2003).

More recently, three *A. thaliana* β-*N*-acetylglucosaminidases have been cloned based on their sequence homology to the *Drosophila fdl* gene product, and their enzymatic properties have been characterized (Gutternigg et al., 2007; Strasser et al., 2007). These three enzymes termed HEXO1 (At3g55260), HEXO2 (At1g05590) and HEXO3 (At1g65590) are the only *A. thaliana* proteins, which have been assigned to the glycoside hydrolase family 20 (GH20: β-hexosaminidases) in the CAZy database (http://www.cazy.org/) (Cantarel et al., 2008). While HEXO2 and HEXO3 are putative type II membrane proteins with a short N-terminal cytoplasmic tail and a single transmembrane domain, HEXO1 is predicted to lack a transmembrane domain. The enzymatic characterization of the recombinant proteins, expressed as the secreted forms either in insect cells (Strasser et al., 2007) or in *P. pastoris* (Gutternigg et al., 2007), revealed that all the three HEXO proteins can cleave β-GlcNAc residues from pNP-GlcNAc. In contrast, β-GalNAc was not hydrolyzed very efficiently. With N-glycan substrates, GlcNAc release was clearly detected for HEXO1 and HEXO3, while for HEXO2 either no obvious activity (Gutternigg et al., 2007) or comparatively low specific activity was found (Strasser et al., 2007). With the physiologically relevant substrate GnGnXF, both HEXO1 and HEXO3 appeared to be the relevant enzymes for the generation of paucimannosidic N-glycans (Strasser et al., 2007). In contrast to the *Drosophila* processing β-*N*-acetylglucosaminidase, which exclusively removes the GlcNAc-1 residue (Léonard et al., 2006), the three HEXOs did not display a strict branch specificity and represent more broad-spectrum β-hexosaminidases comparable to the well-characterized jack bean β-hexosaminidase (Li and Li, 1970).

Based on enzymatic properties and *in silico* predictions for subcellular localization, Gutternigg et al. concluded that HEXO1 and HEXO3 are vacuolar enzymes (Gutternigg et al., 2007). However, in subcellular localization studies with fluorescence tagged enzymes, HEXO1 was found in the vacuole, while HEXO3 was targeted mainly to the plasma membrane (Strasser et al., 2007). The different specificities and subcellular localization indicate that the three Arabidopsis HEXOs are involved in different cellular processes. HEXO1 is most likely involved in the generation of MMXF structures on vacuolar glycoproteins, and thus has mainly a catabolic function. HEXO2 seems to be involved in other physiological processes, and could play a role in plant defense reactions since it efficiently releases GlcNAc from chitotriose (Gutternigg et al., 2007; Strasser et al., 2007). HEXO3 could be responsible for the removal of terminal GlcNAc residues from plasma membrane, cell wall and secreted glycoproteins.

2. Enzymes degrading N-glycans - *in vivo* or *in vitro*

2.1. β-N-acetylglucosaminidases (hexosaminidases) (EC, 3.2.1.52)

Jack bean (*Canavalia ensiformis*) hexosaminidase has been a useful reagent for decades (Li and Li, 1970). To the author's knowledge, the enzyme has, surprisingly, never been cloned. However, by mass spectrometric peptide sequencing this hexosaminidase was found to be highly homologous to the *A. thaliana* hexosaminidase HEXO1 (and also HEXO3) that was described above (J. Stadlmann, F. Altmann, and R. Strasser, unpublished data) (Strasser et al., 2007).

2.2. α-Fucosidases (EC 3.2.1.51 and EC 3.2.1.63)

Plants, e.g. sweet almonds, exhibit two kinds of fucosidase activities. Fucosidase I removes α1,3- and α1,4-linked fucose on GlcNAc or on galactose, whereas fucosidase II acts on α1,2-fucose residues linked to galactose within xyloglucan fragments (Kobata, 1982; Ogata-Arakawa et al., 1977). The purification of plant fucosidases has been realized in the earlier days of glycobiology (Imber et al., 1982; Scudder et al., 1990; Yoshima et al., 1979), but the identification of their genes was a process paved with confusions. Indeed, following the purification of an α1,2-fucosidase from *Pisum sativum* acting on xyloglucan oligosaccharides (Augur et al., 1993), the same authors published two years later the supposedly relevant cDNA sequence (Augur et al., 1995). Eight years later, another team showed that the corresponding protein was lacking fucosidase activity (Tarrago et al., 2003). More recently, de La Torre et al. published the sequence of two *A. thaliana* proteins, AtFuc1 (Q8GW72, At2g28100) and AtFXG1 (Q9FXE5, At1g67830), which they described as α1,2-fucosidases (de La Torre et al., 2002). Two years ago, we showed that AtFuc1 actually exclusively acted on α1,3- and α1,4-linkages (Zeleny et al., 2006). AtFXG1 on the other hand would rather be assigned as a lipase than a glycohydrolase by the electronic annotation applied to SwissProt entries (R. Fischl, M. Pabst, S. Lhernould, F. Altmann and R. Léonard, unpublished results). In 2008, we reported on the sequence and enzymatic characterization of an α1,2-fucosidase from *A. thaliana* acting on xyloglucan oligo- and polysaccharides (Léonard et al., 2008), which belongs to the CAZy glycoside hydrolase family 95.

At present, none of these plant fucosidases have been proven to be involved *in vivo* in the modification of N- or O-linked glycans. Nevertheless, fucosidase I can be used to remove *in vitro* the fucosyl residues in Lewis epitopes on glycoproteins or glycolipids. In the same way, fucosidase II, whose natural substrate is xyloglucan, is applied to the α1,2-linked fucose of blood group H oligosaccharides on human O-glycoproteins and glycolipids (Ishimizu et al., 2007).

2.3. α-Galactosidase (EC 3.2.1.22)

. . . or how an enzyme involved in hemicellulose degradation in plants ends up converting type B erythrocytes to type O cells thus rendering them useful for transfusion therapy (Smith et al., 2003; US patent 6630,339). *In planta*, the enzyme catalyzes the hydrolysis of the α1,6 link of galactose residues among oligomeric and polymeric galactomannans. Initially detected in and purified from coffee beans (Harpaz and Flowers, 1974), the enzyme also saw its gene identified for the first time in this plant (Zhu and Goldstein, 1994). α-galactosidase was also purified from the dicotyledons of *Phaseolus vulgaris* (Zhu et al., 1995) before the identification of its gene (Davis et al., 1997). The same work was also realized for *Oryza sativa* (Kim et al., 2002) and *Helianthus annuus* (Kim et al., 2003). Besides studies on the physiological roles of this enzyme notably in coffee bean (Marraccini et al., 2005), the practical usefulness of this enzyme for the conversion of erythrocytes with type B oligosaccharides to the universal donor group O was investigated with recombinant α-galactosidase (Zhu and Goldstein, 1994; Zhu et al., 1995). Finally, the use of *Glycine max* and *Phaseolus vulgaris* in this conversion was the subject of US patent (Smith et al., 2003; US patent 6630,339).

2.4. β-Galactosidases (EC 3.2.1.23)

β-Galactosidase from jack bean and almonds have been studied in the seventies (Arakawa et al., 1974; Li et al., 1975). In the recent years, genes of the β-galactosidase families of rice (15 members) and *A. thaliana* (16 close homologues and one further member of Cazy GH family 35) were identified. (Ahn et al., 2007; Iglesias et al., 2006; Tanthanuch et al., 2008).

Recombinantly expressed *A. thaliana* BGAL4 and two rice β-gals were active against β1,3-, β1,4- and β1,6-linked galactosides (Ahn et al., 2007). Thus, it appears likely that these enzymes relate to the "old" β-galactosidases, but this has not yet been established. Similarly, it can only be assumed that a biological role of these enzymes lies in pectin and hemicellulose metabolism, e.g. in xyloglucan degradation (Iglesias et al., 2006; Lee et al., 2007).

2.5. Endo-β-mannosidase (EC 3.2.1.152)

A structural analysis of Japanese pear RNases revealed the presence of chitobiose as the major N-linked glycan (Ishimizu et al., 1999), which implicated the existence of an enzyme that is able to hydrolyze the Manβ1-4GlcNAc linkage in the trimannosyl core structure. The activity of endo-β-mannosidase is clearly different from that of α-endomannosidase found in vertebrates, which cleaves the linkage between the glucose-substituted mannose and the rest of the oligomannosidic N-glycan; an activity that is absent in plants (Dairaku and Spiro, 1997). The corresponding enzymatic activity (EC 3.2.1.152) was purified from Lily flowers, and its gene was cloned and expressed in *E. coli* (Ishimizu et al., 2004). An enzyme exhibiting a similar activity was purified from a dicot plant, *Brassica oleracea*. This cabbage enzyme is made of four polypeptides encoded by a single gene with strong homologies to that of lily (Ishimizu et al., 2006). The enzyme showed no activity toward a xylosylated substrate.

2.6. Peptide:N-glycanase (PNGase) (EC 3.5.1.52)

Although often referred to as N-glycosidase, PNGases actually act as an amidase giving rise to a 1-amino-oligosaccharide (Takahashi and Nishibe, 1978). The enzyme from almonds, termed N-glycosidase A or almond glyco-amidase or PNGase A, transits through the secretory pathway and is therefore glycosylated (Altmann et al., 1998; Risley and Van Etten, 1985). PNGase A, like the bacterial enzyme PNGase F, with which it has no sequence similarity, has a sophisticated substrate specificity but unlike PNGase F it is not stopped by the presence of a core-α1,3-linked fucose (Altmann et al., 1995; Plummer and Tarentino, 1981; Tretter et al., 1991).

The nucleotide sequence of PNGase A was determined about 10 years ago and deposited in a patent application (Hiroyuki I., Masanori M., Ikunoshin K. Patent number US5710016, January 1998). The primary translation product (P81898) is cleaved into two non-covalently linked subunits (Altmann et al., 1998). Although homologues are found in *A. thaliana* (Q9LKB2, At3g14920; Q9FFG6, At5g05480), no functional study has so far been made.

Another peptide: N-glycanase was cloned from *A. thaliana* based on sequence homology to a murine PNGase (Diepold et al., 2007). This AtPNG1 (Q9FGY9, At5g49570) is a cytosolic, proteasome-associated enzyme involved in the ERAD-pathway of unfolded protein degradation also found in mammalian cells (Spiro, 2004). The two types of PNGases have no significant sequence homology.

3. Glycosidases acting on arabinogalactan-proteins (AGPs)

AGPs possess complex, highly branched carbohydrate moieties consisting of galactose, arabinose, glucuronic acid, rhamnose and fucose attached in various ways (Fig. 1C) (Tan et al., 2004). In addition, their involvement in many physiological mechanisms and the role played by their polysaccharide part (Motose et al., 2004; Shi et al., 2003) necessitates a permanent adaptation of the O-glycan profile of AGPs (Gibeaut and Carpita, 1991). For this reason, degradation and modification of these proteoglycans require the concerted action of several glycosidases among which an α-L-arabinofuranosidase, a β1,3-/β1,6-galactosidase and two β-glucuronidases have been molecularly characterized.

3.1. α-Arabinofuranosidase (EC 3.2.1.37)

Several plant α-L-arabinofuranosidases have been characterized and showed activity toward polysaccharides such as arabinan (Tanaka et al., 2001), pectins (Tateishi et al., 2005), or arabinoxylans (Lee et al., 2003). These enzymes are either monofunctional α-L-arabinofuranosidases (Tanaka et al., 2001) or bifunctional α-L-arabinofuranosidases/β-xylosidase such as the enzyme Ara-I from barley, which is able to hydrolyze both α-L-arabinosyl and β-xylosyl residues from arabinoxylans (Lee et al., 2003). Among bifunctional enzymes, an α-L-arabinofuranosidase/β-xylosidase from immature seeds of *Raphanus sativus* acting on AGPs has been recently purified, and its gene was identified (Kotake et al., 2006). When expressed in *A. thaliana*, the corresponding gene RsAraf1 confers a higher α-L-arabinofuranosidase activity toward synthetic *p*NP substrate and radish leaves and roots AGPs. In addition, transgenic plants contain slightly less arabinose in their cell wall (Kotake et al., 2006). A deeper study of enzymes with the same properties should be enabled by the existence of close homologues in *A. thaliana* (At5g64570 and At5g09730).

The hitherto identified plant α-L-arabinofuranosidases all belong to glycoside hydrolase family 3 in the Cazy database (Cantarel et al., 2008), whereas in other phyla arabinofuranosidases are found in families 3, 43, 51, 54 and 62.

3.2. β-Galactosidase (EC 3.2.1.23)

In addition to their role in the removal of β-galactose from xyloglucan and pectic galactans (Ishimaru et al., 2008), plants β-galactosidases are involved either in the modification or in the degradation of β1,3- and/or β1,6-galactans from AGPs (Kotake et al., 2005). Following a protein purification from radish seeds, Kotake et al. could obtain a cDNA encoding a putative β-galactosidase belonging to family 35 of glycosyl hydrolase. When expressed in *P. pastoris*, the enzyme RsBGAL1 (Q6L619) showed activity toward β1,3- and β1,6-galactan oligosaccharides. Despite its close homology to other glycosidases classified as lactases, RsBGAL1 was not active against β1,4-galacto-oligosaccharides. The recombinant enzyme did not show a strong activity toward native AGP due to the protection of the galactan by arabinose and glucuronic acid residues. On the contrary, when these AGPs were simultaneously treated with arabinofuranosidase and glucuronidase, the action of the recombinant galactosidase was strongly enhanced and up to 90% of the sugar content of the AGPs could be liberated.

3.3. β-Glucuronidase (EC 3.2.1.31)

In contrast to the bacterial β-glucuronidase (GUS) commonly used as a reporter for gene expression studies in plants, which is active at neutral pH, endogenous plant GUS has been described as an active enzyme under acidic conditions (Jefferson et al., 1987). This activity is associated with physiological processes such as cell elongation, and inactivation by the inhibitors is postponing the elongation of stem, roots and root hairs in tobacco seedlings (Sudan et al., 2006). Recently, the partial purification of a GUS from *A. thaliana* stems and the identification of the corresponding gene were realized (AtGUS2, At5g07830) (Eudes et al., 2008). The knock-out mutant for this gene exhibited a molar ratio of GlcA of AGPs 1.7 times higher than wild type, whereas AtGUS2 overexpressing plants possessed AGPs depleted from any detectable GlcA. Despite the presence of one close homologue of *AtGUS2* in *A. thaliana*, no GUS activity was measurable on the AtGUS2 knock-out mutant. The inactivation of GUS2 gene had no clear physiological impact on the elongation of plant organs. On the contrary, the GUS overexpressing plants had longer roots and stems, which illustrated the role of this enzyme in cell elongation.

4. Conclusion

The completion of the maturation and degradation map for N-glycans in plants seems to be within reach now. A few pieces of the puzzle remain to be inserted, e.g. the confirmation of the assignment of genes encoding mannosidases I and vacuolar mannosidases II. Also, there is evidence for the presence of endo-*N*-acetylglucosaminidase in plants (Berger et al., 1995; Ramessar et al., 2008; Vuylsteker et al., 2000).

Unknown glycosidases may lurk in the still not very well investigated field of arabinogalactan proteins. Here, it has to be admitted that for this review we did not try to cover glycosidases of the metabolic pathways or those acting on low mass plant glycosides.

The recent advances in the molecular biology of plant glycosidases can mostly be seen as a fruit of the concentration of efforts on model plants, especially on *A. thaliana*. We expect that the results obtained with *Arabidopsis* mutant lines will help to understand the enzymeś role in various other plants.

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