**Plant invertases: structure, function and regulation of a diverse enzyme family**

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Invertase is a key metabolic enzyme which hydrolyzes the disaccharide sucrose (the major type of sugar transported through the phloem of higher plants) to glucose and fructose. In higher plants, invertase exists in several isoforms with different biochemical properties and subcellular locations. The specific functions of the different invertase isoforms are not clear, but they appear to regulate the entry of sucrose into the different utilization pathways. Invertases, alone or in combination with plant hormones, are involved in regulating developmental processes, carbohydrate partitioning, as well as biotic and abiotic interactions. The current knowledge about this isoenzyme family, with special reference to recent key findings, is reviewed here.

**Key words:** invertase, sugar metabolism, gene expression, regulation.

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**INTRODUCTION**

Plant invertases (b-fructofuranosidase, EC 3.2.1.26) comprise a family of enzymes which catalyze the hydrolysis of sucrose to glucose and fructose. Higher plants contain several invertase isoenzymes, which can be distinguished by their subcellular localization (cell wall, vacuole or cytosol), solubility (soluble or insoluble in low ionic strength buffer), optimum pH (acid or neutral/alkaline) and isoelectric point (pI) (Sturm & Chrispeels, 1990). Cell wall and vacuolar invertases are glycosylated forms with an acid pH optimum, while cytosolic invertase is most likely a non-glycosylated form with a neutral/alkaline optimum pH. Glycosylation of acid invertases is required for their transport across either the plasma membrane or the tonoplast, hence their localization in the cell wall or the vacuole (Tymowska-Lalanne & Kreis, 1998a). Cell wall invertases are ionically bound to the cell wall via positive charges due to their neutral/basic pI at low pH (Kim *et al*., 2000). However, Carlson & Chourey (1999) have demonstrated the presence of contaminating cell wall invertase in the soluble fraction of developing maize kernels, suggesting that these ionic bonds are rather weak. The main characteristics of the three types of plant invertases are summarized in Table 1.

**Table 1. Properties of plant invertases**

<table>
<thead>
<tr>
<th>Type of invertase</th>
<th>pH optimum</th>
<th>Solubility</th>
<th>Glycosylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell wall (acid)</td>
<td>4.5 - 5.0</td>
<td>Insoluble</td>
<td>+</td>
</tr>
<tr>
<td>Vacuolar (acid)</td>
<td>4.5 - 5.0</td>
<td>Soluble</td>
<td>+</td>
</tr>
<tr>
<td>Cytoplasmic (alkaline)</td>
<td>7.0 - 7.8</td>
<td>Soluble</td>
<td>–</td>
</tr>
</tbody>
</table>

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ENZYMATIC PROPERTIES OF PLANT INVERTASES

Vacuolar and cell wall invertases share some biochemical properties, e.g., they cleave sucrose most efficiently between pH 4.5 and 5.0 and attack the disaccharide from the fructose residue. Both types of invertases are β-fructofuranosidases and also hydrolyze other fructofuranosides such as raffinose and stachyose but with significantly reduced cleavage efficiency. Contrary to the acid invertases, neutral and alkaline invertases appear to be sucrose specific (Sturm, 1999).

Acid invertases have a $K_m$ for sucrose in the low mM range. Activity is inhibited by heavy metal ions such as $\text{Hg}^{2+}$ and $\text{Ag}^+$, suggesting the presence of a sulfhydryl group at the catalytic site. Acid invertases are also inhibited by their reaction products, with glucose acting as a non-competitive inhibitor and fructose as a competitive inhibitor. The majority of the mature polypeptides have molecular masses between 55 and 70 kD. In the case of cytosolic invertases, most native polypeptides are homotetramers composed of subunits with a molecular mass of 54 to 65 kD. The polypeptides preferentially hydrolyze sucrose with a $K_m$ of 10 mM. They are strongly inhibited by glucose and fructose, but not by heavy metal ions, suggesting marked differences between the catalytic sites of neutral/alkaline and acid invertases. It should be noted that cytosolic invertases are extremely labile and enzyme activity is rapidly lost after tissue homogenization, thus rendering their purification very difficult.

Analysis of some of the purified proteins on denaturing SDS gels under reducing conditions revealed the presence of proteolytic fragments (for review, Sturm, 1999). Under native conditions, these fragments appear to be tightly associated and, in a complex, possess enzymatic activity. Fragmentation does not appear to be an artifact of protein purification, but instead seems to be under developmental control. For example, Arai et al. (1991) have demonstrated that the full-length proteins predominate in very young hypocotyls of mung bean, whereas with increasing hypocotyl age the N- and C-terminal fragments were more abundant. Their functional importance however is not yet fully understood.

MOLECULAR PROPERTIES AND GENE STRUCTURE OF PLANT INVERTASES

Many genes and cDNAs coding cell wall (acid insoluble) and vacuolar (acid soluble) invertases have been cloned from various plant species (for review, Tymowska-Lalanne & Kreis, 1998a; Goetz & Roitsch, 2000). In comparison, only a few cDNAs or genes encoding neutral/alkaline cytosolic invertases have been reported in plants (Gallagher & Pollock, 1998; Sturm et al., 1999). According to Tymowska-Lalanne & Kreis (1998a), cell wall and vacuolar invertases are synthesized as pre-proproteins, with a long leader sequence which is cleaved off during transport and protein maturation (Fig. 1). This leader sequence most likely consists of a signal peptide, required for entry into the endoplasmic reticulum and, thus, into the secretory pathway (Blobel, 1980), and an N-terminal propeptide, thought to either play a role in protein folding and stability (Klionsky et al., 1988) and/or in the regulation of enzyme activity (Hasilik & Tanner, 1987). Contrary to the cell wall enzyme, the vacuolar proteins contain a short hydrophobic C-terminal extension, which might be involved in the vacuolar targeting of the protein (Unger et al., 1994).

![Cell wall invertase schematic](image1)

**FIG. 1.** Schematic representation of cell wall and vacuolar invertases. The peptide sequences NDPN and WEC_PDF represent the β-fructosidase motif and the catalytic site, respectively. Diagram adapted from Sturm (1999).
In general, plant invertase genes have a fairly similar structure and contain six to eight exons (Sturm, 1999). With the exception of a cloned gene encoding a cell wall-bound invertase in carrot (InvDC1; Ramloch-Lorenz et al., 1993), all other invertase genes contain one conserved exon (exon 2), which is only 9 bp long (Tymowska-Lalanne & Kreis, 1998a). This exon is the smallest functional exon known in the plant kingdom (Kim et al., 2000) and encodes three amino acids (DPN) which are part of the highly conserved β-fructosidase motif NDPN (Goetz & Roitsch, 2000). This motif together with the well-conserved cysteine catalytic site (WECV/PDF) might have an important function in enzyme

FIG. 2. Phylogenetic relationships of different, characterized and putative, plant invertases. The dendrogram shown was generated using the DRAWTREE program of the PHYLIP package (Felsenstein, 1989) by comparison of the deduced amino acid sequences of plant invertases. Light oval indicates vacuolar invertase isoforms, whereas dark oval indicates cell wall invertase isoforms. Arabidopsis invertase isoforms are boxed. Gene names and EMBL accession numbers: Arabidopsis thaliana: Atbfruct1, X74515 (Schwebel-Dugué et al., 1994); Atbfruct2, U11033 (Mercier & Gogarten, 1995); Atbfruct3, X99111 (Haouazine-Takvorian et al., 1997); Atbfruct4, Y11559 (Haouazine-Takvorian et al., 1997); carrot (Daucus carota): InvDC1, X09321 (Ramloch-Lorenz et al., 1993); InvDC2, X78424 (Lorenz et al., 1995); InvDC3, X78423 (Lorenz et al., 1995); tomato (Lycopersicon esculentum): InvLe23g, Z12027 (Elliott et al., 1993); tomato (Lycopersicon pimpinellifolium): InvLp6g, Z12028 (Elliott et al., 1993); maize (Zea mays): Zmivr1, U16123 (Xu et al., 1995); Tobacco (Nicotiana tabacum): Ntbfruc1, X81834 (Greiner et al., 1995); pea (Pisum sativum): Ps11, AY112702 (Zhang, 2002). Other genes (At2g36190, At3g13784) represent uncharacterized, putative Arabidopsis invertase isoforms, as determined by MIPS database (MAdDB) searches and sequence analysis. (It should be noted that the putative Arabidopsis cell wall invertase genes At1g55120 and At5g11920 were not included in the tree, because a research paper just published by De Coninck et al. (2005) has shown that At1g55120 and At5g11920 show in fact fructan exohydrolase activity, as demonstrated by heterologous expression in Pichia pastoris).
conformation or catalytic activity.

Detailed amino acid analysis has identified that cell wall-bound invertases have a proline residue in the cysteine catalytic site, while vacuolar invertases have a valine residue (Tymowska-Lalanne & Kreis, 1998a). Goetz & Roitsch (2000) have also demonstrated that the aspartate (D) from the NDPN box and the glutamate (E) and cysteine (C) from the WECV/DF box are essential for enzyme activity, by introducing specific amino acid substitutions by site-directed mutagenesis and heterologously expressing the mutated genes in an invertase deficient Saccharomyces cerevisiae strain.

The comparison of the deduced amino acid sequences for cell wall and vacuolar invertases has also demonstrated that the two forms belong to two different classes (Tymowska-Lalanne & Kreis, 1998a). A higher similarity is seen between members of the same form (cell wall or vacuolar) from different species compared with different forms from the same species (Tymowska-Lalanne & Kreis, 1998a). For example, the Arabidopsis thaliana cell wall invertases are more closely related to the carrot cell wall invertases than to the Arabidopsis vacuolar invertases (Fig. 2). This implies that perhaps invertase gene duplication and divergence occurred prior to the separation of these plant species during evolution (Tymowska-Lalanne & Kreis, 1998a). Interestingly, in addition to the two major classes being identified during sequence analysis of plant invertase genes, multiple isoenzymes or isoforms of invertase have been shown to exist in the same plant tissue (Weber et al., 1995; Godt & Roitsch, 1997; Sherson et al., 2003).

PHYSIOLOGICAL ROLE AND EXPRESSION OF PLANT INVERTASES

The physiological functions of invertase isoforms are complex and depend upon the kind of tissue and the subcellular location (Sturm & Tang, 1999; Tang et al., 1999; Roitsch & González, 2004) (Fig. 3). The acid soluble (vacuolar) invertases not only mobilize sucrose and/or control sugar composition in vacuoles but they also play a role in establishing sink (area of growth or storage) tissue in storage organs, such as in the mature tomato fruit (Klann et al., 1993). Vacuolar invertases may also play a role in maintaining cell turgor and cell expansion (Sebkova et al., 1995). Tissues undergoing rapid cell expansion usually have a high hexose and low sucrose concentration suggesting an important role for sucrose-metabolizing enzymes (Sebkova et al., 1995). More recently, Mitsuhashi et al. (2004) have detected high transcript levels of the vacuolar invertases A1fruct3

FIG. 3. Subcellular locations and proposed functions of plant invertases. Plant cells contain different invertase isoforms in the apoplast, cytoplasm and vacuole. Hexoses generated by the activities of the different enzymes have different functions (a few important examples are indicated in italics). Diagram adapted from Sturm (1999).
Plant invertase gene expression and enzyme activity are both known to be influenced by a variety of intracellular and extracellular factors. These factors modulate invertase activity either by activation or by repression, acting either at the level of gene expression and/or at the level of protein activity (Tymowska-Lalanne & Kreis, 1998a). The following section briefly reviews these factors.

**FACTORS AFFECTING INVERTASE EXPRESSION**

Plant invertase gene expression and enzyme activity are both known to be influenced by a variety of intracellular and extracellular factors. These factors modulate invertase activity either by activation or by repression, acting either at the level of gene expression and/or at the level of protein activity (Tymowska-Lalanne & Kreis, 1998a). The following section briefly reviews these factors.
Sugars

Sugars, known to act as signaling molecules regulating a variety of genes in different physiological pathways (Koch, 1996; Sheen et al., 1999), also modulate the activity of invertase, although the mechanisms of the signaling pathways remain unclear. Burch et al. (1992) have shown that the activity of vacuolar invertase from potato leaves and tubers is inhibited in a competitive manner by fructose and in a non-competitive manner by glucose. This confirms the observation by Isla et al. (1991) about the competitive inhibition of potato tuber invertase by fructose, while Lopez et al. (1988) have shown that acid invertase from Carica papaya fruits was inhibited by fructose but not by glucose. Cho et al. (2005) have recently demonstrated that the expression of the acid invertase OsCIN5 in excised rice leaves was significantly suppressed when leaves were treated with sucrose.

In contrast to the above reports about direct inhibition of invertase enzyme activity by sugars, several authors have demonstrated the stimulation of invertase enzyme levels by sugars. The increase in enzyme activity of cell wall invertase by glucose in C. rubrum was shown to run parallel to the increased level of mRNA of CIN1 (Roitsch et al., 1995). Expression of a cell wall invertase isoform has also been shown to be induced by glucose in tobacco (Krausgrill et al., 1996), Arabidopsis (Tymowska-Lalanne & Kreis, 1998b) and tomato (Sinha et al., 2002). However, other studies have shown that sugars do not modify invertase activity in various cell types or organs (Sturm & Chrispeels, 1990; Weil & Rausch, 1990). Although contradictory, these results might simply stem from the fact that isoenzymes, which were extracted from different organs, were either repressed or enhanced by sugar availability (Tymowska-Lalanne & Kreis, 1998a). Results obtained by Xu et al. (1996) indicate that this is probably the case. Their data showed that the expression of two vacuolar invertase genes from maize is sugar-regulated, involving different patterns of sugar induction and repression. One maize gene (Ivr1) encoding vacuolar invertase is repressed by the presence of sugars and up-regulated by their depletion, whereas a second gene (Ivr2) is up-regulated by increasing sugar supply. Similar induction of Ivr2 by glucose in maize leaves has also been reported in a more recent study by Trouverie et al. (2004).

Plant growth regulators

Plant growth regulators (PGRs) play an integral role in controlling growth, differentiation and development of plants. There is accumulating evidence that invertases are regulated by various PGRs which, in most cases, can be related to the increased carbohydrate demand of growth-stimulated tissues (Roitsch et al., 2003). For example, gibberellic acid (GA₃) has been reported to increase invertase activity in several plant organs (for review, Tymowska-Lalanne & Kreis, 1998a). Invertase mRNA from shoots of dwarf pea plants (Pisum sativum) was induced after GA₃ treatment, indicating that the expression of the shoot cell wall invertase gene could be regulated by GA₃ at transcriptional and/or translational levels (Wu et al., 1993). Similarly, Mitsuhashi et al. (2004) have shown an induction of both vacuolar and cell wall invertase genes in Arabidopsis seeds following treatment with GA₄, an active gibberellin.

It has been reported that the activity of cell wall invertase is also stimulated by auxin (Weil & Rausch, 1990). Morris & Arthur (1984) have observed that during cell expansion in bean stems, the highest level of invertase activity occurs simultaneously with the peak of indolyl-3-acetic acid concentration, while the exogenous application of indolyl-3-acetic acid promoted an increase in vacuolar invertase activity. Similarly, there is preliminary evidence for the significance of the induction of cell wall invertase by abscisic acid obtained with transgenic tobacco plants expressing a fusion between the promoter of the tomato cell wall invertase Lin6 and the β-glucuronidase reporter gene (Roitsch et al., 2003). Cytokinins have also been implicated in affecting invertase expression. Tissues with elevated activities of cell wall invertase, such as rapidly growing tissues, are known to contain elevated cytokinin concentrations (Godt & Roitsch, 1997), while studies using autotrophic cell cultures showed that the cell wall invertases CIN1 from Chenopodium rubrum (Ehness & Roitsch, 1997) and Lin6 from tomato (Godt & Roitsch, 1997) are highly up-regulated in response to physiological concentrations of different cytokinins. Abiotic stress

A variety of abiotic stress factors such as low temperature, oxygen deficiency, wounding, drought and salinity have been considered to affect the level of invertase activity (for reviews, Tymowska-Lalanne & Kreis, 1998a; Roitsch et al., 2003; Roitsch & Gon-
Záalez, 2004). Zhou et al. (1994) have detected vacuolar invertase transcripts in potato tubers stored at 1 °C, but not in those stored at 10 °C, while Zeng et al. (1999) showed that low oxygen stress lead to a decrease in expression of the vacuolar invertase isoforms Inv1 and Inv2 in maize root tips. Such a response reveals an important implication in acclimation to low oxygen stress by conserving sucrose and ATP and reducing the hexose-based sugar-signaling system (Zeng et al., 1999).

Wounding constitutes one of the most dramatic forms of environmental (abiotic) stress (Ehness et al., 1997). When plant tissues become damaged, (e.g. mechanical wounding), plant defense mechanisms are elicited, since plant responses to injury and wounding are very similar to those against pathogen attacks (Isaac, 1996). However, activation of defense reactions requires energy and thus induction of the sink metabolism (Ehness et al., 1997). Such increased demand in carbohydrate supply could potentially be met by the localized up-regulation of invertase in response to the stress-related stimulus (Roitsch et al., 2003). Evidence supporting this theory included the studies by Sturm & Chrispeels (1990) and Ehness et al. (1997), who showed that the mRNAs for the cell wall invertases InvDC1 of carrot and Cin1 of C. rubrum are induced in response to wounding.

Inhibitors

The low and variable invertase activity might be attributed, at least in part, to the presence of endogenous inhibitors (Greiner et al., 1998). Several invertase inhibitor proteins have been isolated and purified from a number of plant species (for review, Tymowska-Lalanne & Kreis, 1998a; Rausch & Greiner, 2004), and cDNA clones coding for cell wall and/or vacuolar invertase inhibitors have now been isolated and analyzed from tobacco (Greiner et al., 1998, 1999), Arabidopsis (Link et al., 2004), and maize (Bate et al., 2004). Interestingly, fairly low concentrations of sucrose prevent an inhibitor purified from a suspension culture of tobacco binding to the enzyme (Weil et al., 1994), thus raising questions about its postulated function. The deduced amino acid sequence of NtCIF shows homology to several sequences identified in the genomes of other plant species, including 15 genes in Arabidopsis (Sherson et al., 2003). A recombinant Nt-inh1-encoded protein was shown to inhibit the activities of cell wall invertases from tobacco and C. rubrum and vacuolar invertase from tomato in vitro, but no inhibitory activity was found towards two yeast invertases (Greiner et al., 1998), suggesting that an inhibitory effect on fungal pathogen invertases is rather unlikely (Greiner et al., 1998).

The physiological role of invertase inhibitors during plant development is not yet fully understood, however it has been hypothesized that the invertase inhibitor may operate as a regulatory switch for cell wall invertase, with the inhibitor always being bound to the cell wall invertase, but inducing the inhibitory conformational change only when sucrose concentration decreases below a certain threshold (Weil et al., 1994).

Pathogen infection

Of particular interest is the potential effect of pathogen infection on invertase activity. There is a growing body of evidence supporting the concept that infection by plant pathogens leads to increased levels of invertase activity, both acid and alkaline (for review, Hall & Williams, 2000). The majority of such reports refer to acid invertase activity (Storr & Hall, 1992; Clark & Hall, 1998; Chou et al., 2000), although increases in alkaline invertase have also been noted (Storr & Hall, 1992). An interesting point is that increases in invertase activity could be (partly) attributed to the potential presence of fungal invertase isoforms as well. Previous reports have identified invertase isoforms from biotrophic pathogenic fungi (Chou et al., 2000), which, if located peripherally in the fungal tissue or secreted into the surrounding medium, would further contribute to the invertase activity already present within the host (Mendgen & Hahn, 2002). In any case, it is not clear whether as a result of this increase in invertase activity in the infected tissues, the observed increased hexose levels act to support the defense responses or serve to provide a supply of hexoses for the growing pathogen (Fotopoulos et al., 2003).

Molecular investigations of expression have also provided evidence to support the observed increases in invertase activity following pathogen attack. Sturm & Chrispeels (1990) have reported that the infection of carrot roots and leaves with the bacterial pathogen Erwinia carotovora results in a rapid increase in the mRNA levels of cell wall invertase, with maximal expression observed one hour after the first contact with the pathogen. Similarly, Chou et al.
ed (Sherson et al., 2003), and detailed analysis of these mutants should provide important information on the function of individual invertase enzymes. The knowledge gained will help to understand one of the most fundamental processes in plants, which may allow the successful biotechnological manipulation of carbohydrate metabolism and partitioning in order to improve yield and quality in crop plants.

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