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Cell Wall Invertase in Tobacco Crown Gall Cells[1]

Enzyme Properties and Regulation by Auxin

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ABSTRACT

The cell wall invertase from an *Agrobacterium tumefaciens*-transformed *Nicotiana tabacum* cell line (SR1-C58) was purified. The heterogeneously glycosylated enzyme has the following properties: M_r 63,000, pH optimum at 4.7, $K_{m\ sucrose}$ 0.6 millimolar (at pH 4.7), pI 9.5. Enzyme activity is inhibited by micromolar concentrations of $HgCl_2$ but is insensitive to H_2O_2, *N*-ethylmaleimide and dithiothreitol. Upon transfer of transformed cells from the stationary phase to fresh medium, a cycloheximide- and tunicamycin-sensitive *de novo* formation of cell wall invertase is demonstrated in the absence or presence of sucrose. While in an auxin mutant (lacking gene 1; SR1-3845) 1 micromolar 1-naphthaleneacetic acid led to a further increased activity, the wild-type transformed cell line (SR1-C58) responded with a decreased activity compared to the control. An analysis of cell wall invertase in and around tumors initiated with *Agrobacterium tumefaciens* (strain C58) on *Nicotiana tabacum* stem and *Kalanchoë daigremontiana* leaves revealed gradients of activity. The results indicate that the auxin-stimulated cell wall invertase is essential for the establishment of the tumor sink.

Recently, a number of higher plant cell wall invertases (EC 3.2.1.26) have been purified and characterized (3, 5, 6, 9, 13, 15, 17, 22–24, 37). The enzyme, which has an acidic pH optimum and is positively charged (pI 9–10) at the pH of the cell wall, hydrolyzes sucrose in the apoplast in rapidly growing tissues and has been proposed to be involved in establishing metabolic sinks (25, 33, and literature cited therein). The cell wall invertase from suspension-cultured cells of *Daucus carota* has been shown to be a heterogeneously glycosylated protein, containing one high-mannose and two complex glycans (17). Polyclonal antibodies against the glycosylated protein did not cross-react with the chemically deglycosylated invertase but reacted with a number of other cell wall proteins (18), whereas polyclonal antibodies against the deglycosylated invertase were rather specific (17). The secretion of cell wall invertase follows the classic pathway, even when tunicamycin inhibits glycosylation (4). However, the nonglycosylated form is very unstable and does not accumulate in the cell wall (4).

Although it is well established that the cell wall invertase is primarily expressed in fast growing tissues, little is known about the factors involved in gene regulation. In view of the established effects of auxin on expression of several cell wall proteins (32), we started from the hypothesis that synthesis and/or secretion of cell wall invertase may be regulated by this hormone. Earlier reports suggested that an exogenous application of auxin and gibberellic acid stimulates invertase activity, but the specificity of the effect for the cell wall form was not demonstrated (25). As a model system, we used *Agrobacterium tumefaciens*-transformed, suspension-cultured tobacco cells for two reasons: (a) transformed cells are strong metabolic sinks independent of exogenous hormones due to a genetically defined auxin biosynthetic pathway encoded by two genes in the T-DNA (14, 26); (b) transformed cell lines with mutations in the T-DNA auxin gene 1 and 2 are available which show a decreased auxin content and a dramatically changed cytokinin/auxin ratio (12, 31, 35). Here we report the purification of the tobacco cell wall invertase and the effect of auxin on its *de novo* formation in different tobacco cell lines. For comparison, cell wall invertase gradients across tumors induced on stem of *Nicotiana tabacum* and leaves of *Kalanchoë daigremontiana* are presented.

MATERIALS AND METHODS

Plant Material

The following *Agrobacterium tumefaciens*-transformed *Nicotiana tabacum* (cv 'Petit Havana') cell lines were a gift from Prof. H. Van Onckelen (University of Antwerp, Belgium): SR1-C58 (wild type) and SR1-3845 (−gene 1 mutant). A nontransformed cell line (SR1) served as a control (35). Cell suspension cultures were grown in the dark on Linsmaier-Skoog medium (19) and transferred every 3 weeks to fresh medium. The nontransformed cell line received 0.3 mg/L benzylaminopurine and 2 mg/L 1-NAA.[2] For transformation of intact plants, *N. tabacum* stems (6-week-old plants) or *Kalanchoë daigremontiana* leaves (8-week-old plants) were inoculated with *A. tumefaciens* (strain C58). Plants were cultivated under natural light in the greenhouse and tumors with surrounding tissues were harvested after 4 weeks.

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[2] Abbreviations: 1-NAA, 1-naphthaleneacetic acid; Endo H, endoglucosidase H; 1-OMG, 1-*O*-methyl-D-glucose; NEM, *N*-ethylmaleimide; TFMS, trifluoromethanesulfonic acid.

Cell Wall Isolation

Cells were harvested after the indicated time intervals and extracted with mortar and pestle in buffer A: 100 mM Mops, 250 mM sorbitol, 10 mM $MgCl_2$, 10 mM KCl, 2 mM DTT, adjusted with KOH to pH 6.0. The homogenate was sonicated for 1.5 min (Branson B12, output control 3.5) and then centrifuged at 3000*g* for 15 min. The pellet was washed in buffer A. After a 10-min treatment with buffer A containing 1% Triton X-100 and a further washing step, the partially purified cell wall fraction was used for extraction of invertase. Alternatively, intact cells were incubated in 1 M NaCl for 6 h at 4°C with gentle shaking, and the supernatant was used directly for $(NH_4)_2SO_4$ precipitation.

For quantitative comparisons of total cell wall invertase activities, the residual activity not released by NaCl treatment plus two bursts of sonification was determined and added for the calculation.

Enzyme Purification

Invertase was extracted from the partially purified cell wall fraction by incubation with 1 M NaCl in buffer A for at least 3 h with gentle stirring (here again, an initial sonification step was included). After $(NH_4)_2SO_4$ precipitation, the 45 to 90% fraction was collected and desalted on a Sephadex G 25 column. After binding to a Con A column and elution with 1-OMG, the active fractions were pooled and further purified by two alternative procedures. Procedure A: after chromatography on a Sulfopropyl-Sephadex column with a pH-gradient (20 mM triethanolamino hydrochloride [pH 8] 30 mM L-arginine [pH 12.4], the active fractions were pooled and loaded on the same column type but eluted with a NaCl-gradient (100–300 mM, in 100 mM Na-acetate [pH 5.6]). All fractions from both gradients were analyzed by SDS-PAGE according to Laemmli (16), and the cell wall invertase was identified by copurification of the 63 kD band with enzyme activity. Procedure B: the active fraction from the Con A step was subjected to electrophoresis on a native cathodic, polyacrylamide gradient gel according to Gogarten (7). Invertase activity was localized in the gel by introducing the gel slices directly into the assay buffer (see below). Again, the 63 kD peptide copurified with invertase activity. After SDS-PAGE gels were silver stained according to Heukeshoven (11). The identification was further supported by a cross-reaction of polyclonal antibodies raised against the *Beta vulgaris* vacuolar invertase (a gift from Dr. R. Milling, Rothamsted Experimental Station, U.K.) with the 63 kD polypeptide as well as its enzymatic and chemical deglycosylation products (see below) as shown by Western blotting.

Isoelectric Focusing

To determine the isoelectric point, the desalted fraction (after $[NH_4]_2SO_4$ precipitation) was subjected to granular preparative isoelectric focusing (2% ampholine pH 9–11, Sephadex G 75 superfine) according to Righetti (30).

Invertase Assay

The assay medium contained 20 mM sucrose in 20 mM triethanolamine hydrochloride/6.6 mM citric acid/KOH (pH 4.7) buffer. After incubation at 37°C for the indicated time intervals, aliquots were assayed for liberated D-glucose with hexokinase/glucose-6-phosphate dehydrogenase according to Bergmeyer *et al.* (1). One nkatal of invertase activity is defined as the amount of enzyme hydrolyzing 1 nmol of sucrose per second at 37°C.

Enzymatic and Chemical Deglycosylation

Deglycosylation with endoglucosidase H (Endo H) followed the procedure of Trimble *et al.* (34): 10 milliunits Endo H (10 μL) were added to purified cell wall invertase dissolved in 50 μL 50 mM Na-citrate (pH 5.5), 2 mM PMSF, 0.02% SDS, 0.1 M β-mercaptoethanol. After incubation for 20 h at 37°C, the complete mixture was analyzed by SDS-PAGE according to Laemmli (16). Chemical deglycosylation was performed with TFMS according to Edge *et al.* (2), while the final neutralization step followed the procedure of Harrison (10). Before SDS-PAGE analysis, the sample was dialyzed against water.

Protein Determination

Protein was determined according to Peterson (28) with bovine serum albumin as standard.

Treatment of Data

All experiments were repeated two or three times. For all quantitative comparisons, the standard error of the mean never exceeded 5%. The invertase activities of the different cell lines are expressed on a g fresh weight basis as their respective dry weights differed ≤10% (SR1-C58: 6.0 ± 0.4%; SR1-3845: 5.7 ± 0.4%; SR1: 6.8 ± 0.5%).

RESULTS

Purification and Characterization of the Cell Wall Invertase in Transformed *Nicotiana tabacum* Cells

The purification procedure for the cell wall invertase (Table I) resulted in the tentative identification of a polypeptide with the apparent molecular mass of 63 kD as judged by SDS-PAGE analysis (Fig. 1). The identification is based on copurification of the 63 kD band with invertase activity after chromatography on Con A Sepharose, sulfopropyl-Sephadex (pH-gradient and NaCl-gradient) and native cathodic gradient gel electrophoresis. Furthermore, the enzymatically (apparent molecular mass 61 kD) or chemically (apparent molecular mass 59 kD) deglycosylated protein cross-reacted with polyclonal antibodies raised against the vacuolar invertase from *Beta vulgaris* (M Weil, unpublished data). Although the final invertase preparation contained other peptides with molecular mass of 68, 60, 56, and 40 kD, these peptides did not copurify with invertase enzyme activity.

The cell wall invertase eluted from sulfopropyl-Sephadex at about pH 9.5 (Fig. 2A) and 175 mM NaCl (Fig. 2B, corresponding to 275 mM Na^+ since a 100 mM Na acetate buffer was used) for pH-gradient and NaCl-gradient chromatography and from Con A Sepharose at 1% 1-OMG (Fig. 2C), respectively. The enzyme showed a pH optimum of 4.7 and

Table I. *Purification of Cell Wall Invertase (EC 3.2.1.26) from dark-grown N. tabacum Cells Transformed with A. tumefaciens (SR1-C58, Wild Type)*

	Total Protein	Total Activity	Specific Activity	Yield	Purification Factor
	mg	*nkat*	*nkat/ mg protein*	%	
NaCl-solubilized fraction	60	320	5.3	100	1.0
$(NH_4)_2SO_4$ precipitation (45–90%)	27	227	8.5	71	1.6
SP-column, pH gradient	2.13	142	66.8	44	12.5
SP-column, NaCl gradient	0.37	78	212	25	39.7
Con A column	0.06	19	289	6	54.1

had a pI of 9.5 as judged from pH gradient chromatography on sulfopropyl-Sephadex and confirmed by isoelectric focusing (M Weil, unpublished data). The K_m for sucrose at pH 4.7 was 600 μM. Enzyme activity was sensitive to micromolar concentrations of $HgCl_2$ (65% inhibition at 1 μM). However, neither H_2O_2, DTT, nor NEM (10-min preincubation at pH 7.5) had any effect.

Regulation of Cell Wall Invertase in Transformed *N. tabacum* Cells and the Effect of Auxin

Three tobacco cell lines, namely a nontransformed line (SR1) and two transformed lines (wild-type: SR1-C58; −gene 1 mutant: SR1-3845), were compared with respect to their cell wall invertase activities (Fig. 3). For all three cell lines, a rapid initial increase during the first day after transfer was followed by a decline during a culture period of 21 d. How-

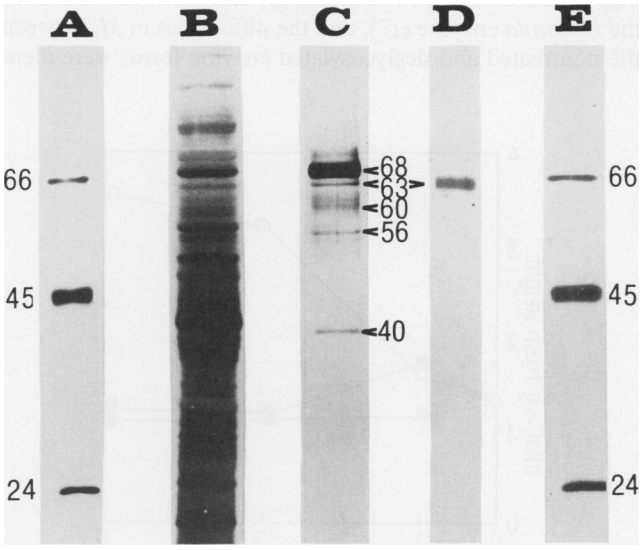

Figure 1. SDS-PAGE analysis of the partially purified *N. tabacum* cell wall invertase preparation (C) in comparison to the initial $(NH_4)_2SO_4$ cell wall protein fraction (B). (D) presents the electroeluted putative acid invertase, for which an apparent M_r of 63,000 was calculated. (A) and (E) are molecular weight standards (bovine serum albumin, 66,000; ovalbumin, 45,000; trypsinogen, 24,000).

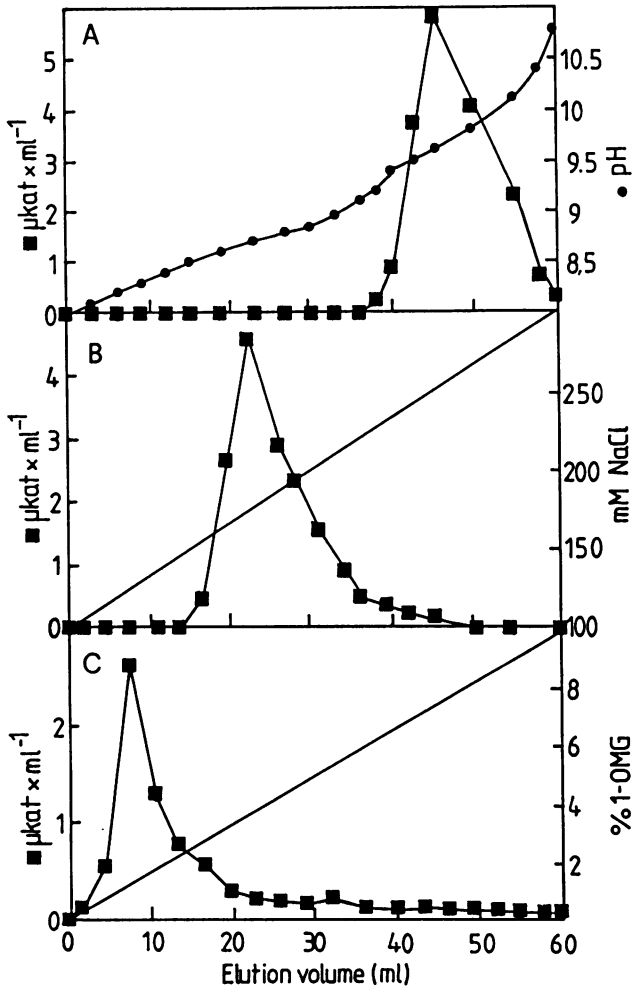

Figure 2. Representative elution profiles of *N. tabacum* cell wall acid invertase from pH-gradient (A) and NaCl-gradient chromatography (B) on sulfopropyl-Sephadex, and chromatography on Con A Sepharose (C).

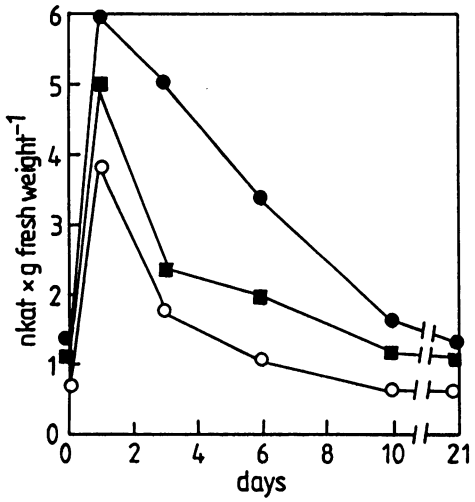

Figure 3. Time-course of cell wall invertase activity in dark-grown suspension cultures of three different *N. tabacum* cell lines grown on Linsmaier-Skoog medium containing 3% (w/v) sucrose: (○), untransformed line, SR1; (●), transformed line, SR1-C58; (■), transformed auxin mutant (−gene1), SR1-3845.

ever, a difference in the time-course becomes apparent when the relative decrease of activity between the first and the sixth day is compared. In the wild type, cell wall invertase decreased only by 32%, but the decline was 60 and 72% for the −gene 1 mutant and the nontransformed line, respectively. In two independent experiments, the relative order of maximum invertase activity followed the pattern wild type > −gene 1 mutant > nontransformed.

To characterize the initial increase of cell wall invertase activity after transfer of cells to fresh medium, we compared the effects of two inhibitors, the translation inhibitor, cycloheximide (10 μM), and the *N*-glycosylation inhibitor, tunicamycin (10 μM; it inhibits the transfer of *N*-acetyl-glucosamine-1-phosphate to dolichol-phosphate). In the presence of tunicamycin (no preincubation), cell wall invertase activity initially increased and then decreased slightly below its initial activity. Cycloheximide prevented any increase in activity after transfer of cells to fresh medium (Fig. 4). These results are in agreement with a *de novo* formation of cell wall invertase.

The initial increase of cell wall invertase activity after transfer to fresh medium was not due to the presence of sucrose and was not prevented by replacing sucrose with D-glucose (Table II). The highest invertase activity 24 h after transfer was found in cells incubated in medium without N- and C-sources.

To test the hypothesis that the cell wall invertase activities in the three tobacco cell lines are affected by their different endogenous auxin status, we compared the effect of exogenous auxin on the three cell lines (Table III). While 1-NAA was stimulatory in the −gene 1 mutant and the nontransformed cell line, it decreased cell wall invertase activity in the wild type. It is noteworthy that the nontransformed cell line responded to a higher exogenous 1-NAA concentration than the −gene 1 mutant. The cell wall invertase activities in the nontransformed cell line at 10 μM 1-NAA were not changed by the presence of 1.3 μM benzoyladenine (data not shown).

Gradients of Cell Wall Invertase around Crown Gall Tumors Induced on *N. tabacum* Stems and *Kalanchoe daigremontiana* Leaves

The cell wall invertase activities in cell cultures grown heterotrophically in suspension are in agreement with activities around tumors induced on *N. tabacum* stems or *K. daigremontiana* leaves (Table IV). Gradients of activity were observed. While the spatially well-defined tumors had the highest activities, the surrounding nontransformed tissue also showed elevated enzyme activity.

DISCUSSION

Characterization of the Cell Wall Invertase

Recently, the cell wall invertase has been purified and characterized from a number of dicot and monocot plants. The *Nicotiana tabacum* enzyme (Table I; Figs. 1 and 2, A–C) resembles other cell wall invertases in many respects: (a) its M_r is identical to the *Daucus carota* enzyme (17) and is similar to the enzymes from *Urtica dioica* (M_r 58,000; 3), *Hordeum vulgare* (M_r 60,000; 13) and *Chenopodium rubrum* (M_r 56,000; 7); (b) its pH optimum at 4.7 is comparable to all other cell wall invertases; (c) its K_m value of 600 μM is rather low but similar to the enzymes from *Chenopodium rubrum* (7) and *Beta vulgaris* (22, 23); and (d) its pI (9.5) is comparable to the *Urtica dioica* enzyme (3).

Plant cell wall invertases are glycoproteins. With an antiserum against the deglycosylated enzyme, a heterogeneous glycosylation has been demonstrated by differential deglycosylation with Endo H and TFMS (17). The presence of one high mannose and two complex glycans was established for the *D. carota* enzyme (17), and the differences in M_r between the nontreated and deglycosylated enzyme forms were iden-

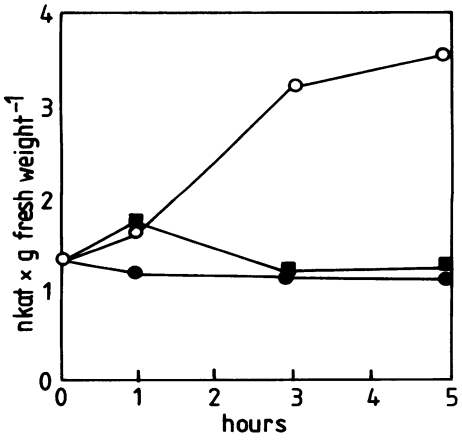

Figure 4. Effect of cycloheximide and tunicamycin on cell wall invertase activity in *N. tabacum* cells transformed with *A. tumefaciens* (SR1-C58). Cycloheximide (●) or tunicamycin (■) were added at 0 h; (○), control. Cells were transferred on day 21. Hours indicate time after transfer to fresh medium.

Table II. *Effect of Medium Composition on Cell Wall Invertase Activity in N. tabacum Cells Transformed with A. tumefaciens (SR1-C58)*

Cells were transferred on the 21st day. Activity was measured at the indicated times after transfer to fresh medium.

Medium composition	Invertase Activity				
	0 h	2 h	5 h	15 h	24 h
	nkat × g fresh wt^{-1} (% of 0 h)				
1. Linsmaier-Skoog (L.S.) medium, 3% sucrose	1.83 (100)	3.35 (183)	3.60 (197)	4.00 (218)	4.67 (255)
2. L.S. medium, no sucrose	1.83 (100)	3.27 (174)	3.57 (195)	4.45 (243)	4.97 (272)
3. Modified L.S. medium: KNO_3 replaced by KCl; no NH_4NO_3; 3% sucrose	1.83 (100)	3.50 (191)	3.65 (199)	4.05 (221)	4.13 (226)
4. Like 3; no sucrose	1.83 (100)	3.30 (180)	3.53 (193)	4.67 (255)	5.38 (294)
5. Like 2; 3% D-glucose	1.83 (100)	3.32 (181)	3.43 (187)	4.15 (227)	4.85 (265)

tical to those found for the tobacco enzyme (M Weil, unpublished results). The effect of the glycosylation inhibitor tunicamycin on the *de novo* formation of cell wall invertase (Fig. 4) most likely reflects a decreased stability of the nonglycosylated enzyme after secretion and not the result of an inhibited secretion as demonstrated recently in an elegant study with suspension-cultured *D. carota* cells (4).

The essentiality of SH-group(s) is supported by the strong inhibition by micromolar concentrations of $HgCl_2$ as was also found for the *H. vulgare* enzyme (13). A strong inhibition of the cell wall invertases from *U. dioica* (3) and *Triticum aestivum* (15) was demonstrated for micromolar concentrations of *p*-chloromercuribenzoyl sulfonate, while millimolar concentrations of DTT (3) were needed to activate the enzyme. The absence of any inhibitory effect of NEM, as previously reported for the *T. aestivum* enzyme (15), suggests that the sensitive SH-group(s) may be either exposed only at the enzyme's pH optimum (here NEM is not reactive) or during the catalytic cycle. The binding of cell wall invertase to a organo-mercurial derivative of Bio-gel (13) in the absence of substrate argues against the second interpretation, but different SH-groups may be involved.

The characteristics of the enzyme obtained either from intact cells or from partially purified cell wall fractions were identical, indicating that no other (cytoplasmic, vacuolar) invertase isoform bound to the cell wall during extraction. The culture medium did not contain any detectable acid invertase activity (even after concentration by $(NH_4)_2SO_4$-precipitation). This shows that the secreted enzyme bound completely to the cell wall by ionic forces and, possibly, steric hindrance of diffusion (8). The latter possibility is supported by the effect of sonication on the release of cell wall invertase in the presence of 1 M NaCl. Usually, 60 to 70% of total cell wall invertase activity could be dissociated from the cell wall by NaCl treatment alone, and sonication released another 10 to 15%. The decrease in cell wall invertase activity by cycloheximide after 3 h (14%; Fig. 4) was due to lowered activity of the extractable portion (1 M NaCl and sonication), while the nonextractable portion remained unchanged. Nagahashi and Seibles (27) provided a possible explanation with their observation of a gradient for structural density of the primary

Table III. *Effect of 1-Naphthaleneacetic Acid on Cell Wall Invertase Activity in Different N. tabacum Cell Lines*

Cells were transferred on d 21. Activity was measured at the indicated times after transfer to fresh medium.

Cell Line	1-NAA	Invertase Activity		
		0 h	3.5 h	15 h
	μM	*nkat/g fresh wt (% of 0 h)*		
Nontransformed (SR1)	0	0.44 (100)	0.44 (100)	2.20 (500)
	1	0.44 (100)	0.57 (130)	2.25 (511)
	10	0.44 (100)	0.92 (209)	2.65 (602)
Wild type (SR1-C58)	0	1.60 (100)	3.79 (237)	5.55 (347)
	1	1.60 (100)	3.93 (246)	4.64 (290)
	10	1.60 (100)	3.84 (240)	4.02 (251)
-gene 1 mutant (SR1-3845)	0	1.12 (100)	3.09 (276)	3.34 (298)
	1	1.12 (100)	3.64 (325)	4.10 (366)
	10	1.12 (100)	3.07 (274)	3.58 (319)

Table IV. *Gradients of Cell Wall Invertase Activity around Crown Gall Tumors Induced on N. tabacum Stems and K. daigremontiana Leaves with A. tumefaciens Strain C58*

Tissue	Invertase Activity
	nkat × g fresh wt^{-1}
N. tabacum cv 'Petit Havana'	
Tumor (total area 0.93 cm^2)	1.75
Surrounding tissue (0–5 mm)	0.98
Stem above tumor (5–15 mm)	0.45
Stem below tumor (5–15 mm)	0.20
K. daigremontiana	
Tumor (total area 1.5 cm^2)	1.08
Surrounding tissue (0–5 mm)	0.77
Surrounding tissue (5–10 mm)	0.45

cell wall from the middle lamella toward the plasma membrane in plant cell walls.

In summary, our data show that: (a) qualitatively the cell wall invertase from *N. tabacum* cells is similar to the invertase enzymes from a number of nontransformed plants; (b) the secreted invertase activity bound completely to the cell wall; and (c) there is no indication for an additional specific isoform related to the transformed state of the cells. Therefore, it was possible to study the effect of auxin and other factors on cell wall invertase by comparing total activities from partially purified cell wall fractions.

Regulation of Cell Wall Invertase by Auxin

The results on the *de novo* formation of cell wall invertase after transfer of cells to fresh medium (Table II) exclude substrate and nitrogen sources as being involved in the induction process. The fact that, upon transfer to fresh medium, 75% of the sucrose in the medium is hydrolyzed within the first 6 d (*i.e.* during the early log phase of growth; S Weigand, M Weil, unpublished data) confirms that, at least in suspension-cultured tobacco cells, the cell wall invertase activity is not related to sucrose availability. It is noteworthy that omitting the nitrogen source and the carbon source results in an even more pronounced increase in invertase activity after transfer.

Several experiments support the hypothesis that auxin may be involved as one factor regulating synthesis and/or secretion of the cell wall invertase (Fig. 3; Tables III and IV): (a) enzyme activity correlated with previously determined auxin levels in the different cell lines used (31), the highest cell wall invertase activity being found in the wild type-transformed cells known to contain the highest endogenous auxin level; (b) the response of enzyme activity toward exogenous auxin reflects the endogenous auxin status, the wild type-transformed cell line being even inhibited at micromolar concentrations of 1-NAA, while both the −gene 1 mutant and the nontransformed cell lines were stimulated in their activity; and (c) the cell wall invertase gradients around tumors initiated *in vivo* on the intact plant parallel auxin gradients (36), which are probably the result of auxin leakage from tumors to surrounding cells.

The effect of exogenous auxin on the release of a number of cell wall glycoproteins has been demonstrated (32), and, in the wild type-transformed tobacco cell line used in this study, 1-NAA (10 μM) was previously shown to specifically inhibit the release of an anionic peroxidase isoenzyme (pI 3.7, M_r 37,000) into the medium (T Rausch, unpublished data). Morris (25) has performed a number of studies on the stimulatory effect of auxin on acid invertase activity, but the localization of the enzyme was not determined.

The gradients of cell wall invertase around tumors induced on tobacco stems or *Kalanchoë* leaves support a crucial role of the enzyme in establishing the metabolic sink. Marx *et al.* (20, 21) have shown that the levels of D-glucose, D-fructose, and sucrose in *Kalanchoë* leaf tumors are elevated 4-fold, 40-fold, and 8-fold, respectively, compared to the surrounding mesophyll cells. Recently, Marx (personal communication) demonstrated the efficiency of the tumor sink on *Kalanchoë* leaves by feeding $^{14}CO_2$ to noninfected leaves of the same plant; the tumor competed efficiently with the apex and young leaves, and the label accumulated mainly in D-glucose, D-fructose, and sucrose. In the tobacco cell lines used in this study, D-glucose concentrations were 0.9 μmol/g fresh weight, 4.6 μmol/g fresh weight, and 7.7 μmol/g fresh weight for the nontransformed, the wild type-transformed, and −gene 1 mutant, respectively (M Weil, unpublished data). This comparison indicates that the cell suspension cultures may serve as relevant model systems for hormonally regulated sink tissues.

We conclude that: (a) the cell wall invertase in transformed cells is an essential factor in sink establishment; (b) enzyme induction is not dependent on carbon or nitrogen sources; and (c) auxin regulates secretion and, possibly, synthesis of the enzyme. The increase in cell wall invertase activity after transfer to fresh medium in nontransformed cells indicates that factors other than auxin may also be involved in regulation of gene expression of this enzyme. However, amino acid-conjugated 1-NAA formed during the previous culture (29) may be hydrolyzed after transfer to fresh medium; this could obscure the hormone dependence of cell wall invertase induction in the nontransformed cells.

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