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Invertase Inhibitors from Sweet Potato (*Ipomoea batatas*): Purification and Biochemical Characterization

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Two proteinaceous invertase inhibitors, designated ITI-L and ITI-R, were purified to electrophoretic homogeneity. ITI-L was purified from acetone powder of sweet potato leaves through sequential steps entailing buffer extraction, acid treatment, DEAE-Sephacel ion-exchange chromatography, and Sephacryl S-100 gel filtration. ITI-R was purified from sweet potato tuberous roots by sequentially applying buffer extraction, Con A-Sepharose affinity chromatography, DEAE-Sephacel ion-exchange chromatography, Sephacryl S-200, and Superose 12 gel filtration. The optimal pHs for interaction between ITI-L and ITI-R and acid invertase from sweet potato leaves were 5.5 and 5.0, respectively. The molecular masses of ITI-L and ITI-R were 10 and 22 kDa, respectively, as estimated by both gel filtration and SDS-PAGE. Both inhibitors were thermostable (90% of the activity remained after incubation at 100 °C for 20 min), and Western blotting showed them to be immunologically related.

KEYWORDS: Sweet potato invertase inhibitor; purification; properties

INTRODUCTION

Invertases (β-D-fructofuranosidase; EC 3.2.1.26), which catalyze the hydrolysis of sucrose to glucose and fructose are widely distributed in higher plants, where they often occur in multiple forms having differing pH optima, isoelectric points, and subcellular localizations (*1*). However, although there have been many reports on the detection and characterization of invertases in various plants, little is known about the regulation of these enzymes. It is known, however, that large changes in invertase activity are associated with the growth and development of plants (*2*, *3*), as well as with environmental stimuli such as wounding and pathogenic infection (*4*, *5*); that low temperature (*6*), low oxygen (*7*), gravity (*8*), and drought (*9*−*11*) all enhance invertase levels, and that some invertases appear to be regulated by the hexose pool in plant tissues, as they are inhibited by the reaction-products, glucose and fructose (*12*, *13*). In addition, up-regulation of invertase genes by hormones such as gibberellins (*14*) and cytokinins (*15*) has also been observed in several plant species.

The presence of proteins that inhibit invertases in some plant tissues suggests another possible mechanism for the regulation of invertases. The first evidence for an endogenous proteinaceous invertase inhibitor was obtained from potato through analysis of invertase kinetics (*16*). Since then, proteinaceous invertase inhibitors with molecular masses ranging from 17 to 22.9 kDa have been found in red beet, sugar beets, sweet potatoes (*17*, *18*), maize endosperm (*19*), yams (*20*), and tomato fruit (*21*). In addition, we recently isolated two invertase inhibitors from sweet potato leaves (ITI-L) and tuberous roots (ITI-R). Both are low molecular mass proteins (10 and 22 kDa, respectively), but ITI-L, in particular, is much smaller than previously described plant invertase inhibitors. In this report, we describe the purification and characterization of ITI-L and ITI-R.

MATERIALS AND METHODS

Materials and Reagents. Sweet potato (*Ipomoea batatas* (L.) Lam. cv Tainong No. 57) leaves and tuberous roots were harvested immediately before use from a local farm. Millipore purified deionized water was used throughout these experiments. Unless otherwise specified, all chemicals used were purchased from Merck or Sigma and were of reagent grade.

Purification of Invertase Inhibitors. *1. Purification of ITI-L.* Fresh sweet potato leaves (100 g) were homogenized with 3 volumes of cold acetone for preparation of acetone powder. The acetone powder was later homogenized with 5 volumes of 50 mM sodium-phosphate buffer (pH 7.0 (Buffer A)) containing 1 mM EDTA and then centrifuged at 8500*g* for 30 min to remove insoluble substances. The supernatant (containing invertase and invertase inhibitor) was collected, and the invertase was inactivated by adjusting the pH to 1.5 with 1 M HCl. After incubation in a water bath at 37 °C for 40 min with occasional stirring, the precipitate formed was removed by centrifugation (8500*g*, 30 min), and the pH of the supernatant was adjusted to 4.0 with 1 M NaOH. After again centrifuging at 8500*g* for 30 min, the supernatant was readjusted to pH 7.0 and dialyzed overnight against Buffer A. The dialyzed sample was then applied to a DEAE-Sephacel column (2.6 × 15 cm) pre-equilibrated with Buffer A. After sample absorption, proteins

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Table 1. Purification of Invertase Inhibitors from Sweet Potato Leaves and Tuberous roots[a]

step	total activity (mU)[b]	total protein (mg)	specific activity (mU/mg)	purification (fold)	yield (%)
		sweet potato leaves			
crude extract	6990	159	44	1	100
acid treatment	1860	4.53	410	9.4	27
DEAE-Sephacel	790	1.07	738	16.8	11
sephacryl S-100	270	0.07	3857	87.7	3.8
		sweet potato tuberous roots			
crude extract	22 000	650	34	1	100
80% saturation $nh_4)_2so_4$	20 000	502	40	1.2	91
ConA-Sepharose (nonrestained)	17 000	405	42	1.2	77
acid treatment	9000	28	321	9.4	41
Sephacryl S-200	7100	10	710	20.9	32
DEAE-Sephacel	5200	2.5	2080	61.2	24
FPLC Superose 12	4800	1.4	3430	101	22

[a] Data were obtained from 100 g of sweet potato leaves and 400 g of tuberous roots. [b] One mU is 10^{-3} units and one unit of inhibitor is the amount of inhibitor that inhibits 50% of 1 unit invertase activity at pH 5.0 and 37 °C.

were eluted with 400 mL of a linear gradient of 0−0.3 M NaCl in Buffer A; the protein profile of the column eluate was monitored at 280 nm. Inhibitor activity in eluate was detected using an invertase inhibition assay. The main fractions containing inhibitor activity (fractions 60−74) were combined and dialyzed as above. The dialyzed sample was then loaded onto a Sephacryl S-100 column (1.6 × 90 cm) pre-equilibrated with Buffer A, after and the main fractions containing inhibitor activity were combined, dialyzed as above, and stored at −20°C until used.

2. Purification of ITI-R. Sweet potato tuberous roots (400 g) were peeled, sliced, and homogenized with 300 mL of Buffer A containing 1 mM EDTA in a Waring blender at 4 °C, after which the homogenate was centrifuged at 8500*g* for 30 min to remove the insoluble substances. Solid $(NH_4)_2SO_4$ was then added to the supernatant to 80% saturation, and the resultant precipitate was collected by centrifugation (8500*g*, 30 min), suspended in 35 mL of 50 mM sodium-phosphate buffer (pH 6.0 (Buffer B)), and dialyzed overnight against the same buffer. The dialyzed sample was applied to a Con A-Sepharose affinity column (1.6 × 10 cm) pre-equilibrated with Buffer B. In this case, the invertase was trapped by the column while the inhibitor activity was present in the eluate, which was treated with 1 M HCl and then purified by DEAE-Sephacel ion-exchange chromatography and Sephacryl S-200 gel filtration, as described above. Finally, the inhibitor was further purified on a Superose 12 column (1.0 × 30 cm) using an FPLC system (Pharmacia) at a flow rate of 0.5 mL/min.

Purification of Acid Invertase. Acid invertase was purified from sweet potato leaves using the procedure described by Charng et al. (*22*) and Wu et al. (*23*) with some modifications. Acetone powder of sweet potato leaves was homogenized with 5 volumes of Buffer A containing 1 mM EDTA, after which the homogenate was centrifuged at 8500*g* for 30 min to remove insoluble substances. The supernatant containing acid invertase activity was then further purified by Con A-Sepharose affinity chromatography and DEAE-Sephacel ion-exchange chromatography.

Measurement of Invertase and Inhibitor Activities. Invertase activity was determined by measuring reducing sugars formed from sucrose hydrolysis using the Somogyi method as described previously (*23*). The reaction was carried out at 37 °C in 0.1 M sodium acetate buffer (pH 5.0). The amount of reducing sugars produced was measured colorimetrically at 520 nm. Inhibitor activity was measured by preincubating the acid invertase (6 units) for 30 min with varying amounts of inhibitor in 0.12 mL of 0.1 M acetate buffer (pH 5.0) at 37 °C, after which 0.24 mL of 0.4 M sucrose (final concentration of 0.267 M) was added, and the remaining invertase activity was measured as described above. Invertase incubation mixture without added inhibitor served as a control. A unit of inhibitor was defined as the amount that inhibited the activity of one unit of invertase by 50% at pH 5.0 and 37 °C.

Protein concentrations were determined with the dye binding method (*24*), using bovine serum albumin as the standard.

Polyacrylamide Gel Electrophoresis. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on slab gels using the method of Laemmli (*25*) with a 4% stacking gel and a 10−20% gradient or 12.5% separating gel. One modification was that tricine was used instead of tris as the buffer component of the electrophoresis system for separation of low molecular weight peptides. The same system was used for native basic gels, except SDS was omitted and a 10% polyacrylamide gel was used. After electrophoresis, the separated proteins were stained with silver or Coomassie Brilliant Blue R-250.

Antiserum Preparation and Western Blotting. Polyclonal antibodies against the inhibitor were raised in rabbits inoculated with 200 μg of ITI-L, after which immunoglobulins were partially purified from the serum fraction by precipitation with solid $(NH_4)_2SO_4$ (50% saturation). In immunoassays, invertase inhibitor (6 μg) was subjected to SDS-PAGE, after which the resolved proteins were transferred to a poly(vinylidene difluoride) (PVDF) membrane (Immobilon-P transfer membrane purchased from Millipore). The protein bands were visualized by first incubating the membrane with horseradish peroxidase-conjugated secondary antibodies (Sigma Immunochemicals) and then with 3-3′ diaminobenzidine containing H_2O_2 for color development.

Determination of Optimal pH. Invertase from sweet potato leaves (6 units) was preincubated for 15 min at 37 °C with varying amounts of invertase inhibitor (3 and 6 μg of ITI-L or 3 and 5 μg of ITI-R) in an incubation mixture at pH 3 to 8, after which 0.24 mL of 0.4 M sucrose (final concentration, 0.267 M) was added, and the remaining invertase activity was measured as described above.

RESULTS

Purification of Invertase Inhibitors. The protocols for purification of ITI-L and ITI-R and their results are summarized in **Table 1**. Purification of invertase inhibitor from sweet potato leaves (ITI-L) and tuberous roots (ITI-R) was assessed during fractionation at each step by monitoring the inhibition of acid invertase purified from sweet potato leaves. Proteins extracted from acetone powder of sweet potato leaves were subjected to acid treatment (pH 1.5), which removed most proteins, including the invertase. The amount of inhibitory activity recovered at this step was rather low, however, perhaps because some inhibitor protein was denatured by the acid treatment. After this step, the inhibitor was further purified by DEAE-Sephacel anion exchange column chromatography, during which one major protein peak containing inhibitor activity was eluted using a linear NaCl gradient (0−0.3 M) (**Figure 1**). Final purification of the inhibitor was performed by gel filtration on Sephacryl S-100, and one protein peak containing inhibitor activity and two protein peaks without or with negligible inhibitor activity

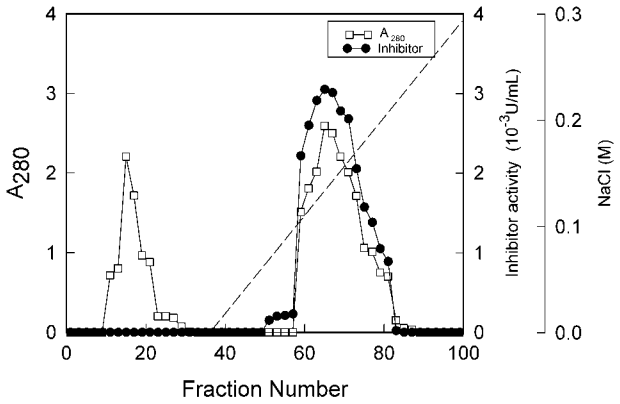

Figure 1. DEAE-Sephacel column chromatography of ITI-L obtained with acid treatment. The acid treated sample was applied to an anionic DEAE-Sephacel column (2.6 × 15 cm) previously equilibrated with Buffer A. Proteins were eluted with a 0–0.3 M NaCl gradient (in Buffer A) in a total volume of 400 mL; 3-mL fractions were collected. Protein profile (□) and inhibitor activity (●) were separately monitored as described in Materials and Methods.

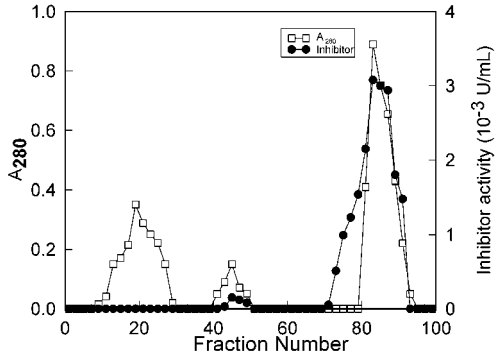

Figure 2. Sephacryl S-100 gel filtration of ITI-L obtained from DEAE-Sephacel column chromatography. The ITI-L obtained from the DEAE-Sephacel column was applied to a Sephacryl S-100 column (1.6 × 90 cm) previously equilibrated with Buffer A, and 3 mL fractions were collected. Protein profile (□) and inhibitory activity (●) were separately monitored.

were obtained (**Figure 2**). After gel filtration, the fractions containing the highest amounts of inhibitory activity (fractions 80−88) were pooled. The amount of inhibitor activity recovered at this step was also low, perhaps because some fractions containing lesser amounts of inhibitor activity were removed.

Proteins extracted from sweet potato tuberous roots were precipitated with an 80% saturated solution of $(NH_4)_2SO_4$ and then subjected to Con A-Sepharose affinity chromatography. The invertase inhibitor did not bind to the affinity adsorbent, though glycoproteins were adsorbed and emerged only after being eluted. Most of the invertase, for example, was removed by this procedure and was later eluted with phosphate buffer (pH 6.0) containing 0−0.3 M α-methyl-D-mannoside (**Figure 3**). The inhibitor was then further purified by acid treatment, gel filtration chromatography on Sephacryl S-200, and ion-exchange chromatography on a DEAE-Sephacel column. Final purification of the inhibitor on a Superose 12 column was performed using an FPLC system.

By use of the protocols summarized above, ITI-L was purified 87.7-fold with a yield of 3.8%, while ITI-R was purified 101-fold with a yield of 22% (**Table 1**). The SDS-PAGE electrophoretic patterns of ITI-L at various purification steps and ITI-R at various concentrations are shown in **Figure 4**, parts **A** and **B**, respectively. At the final step, the purified ITI-L was detected as a single protein band with a molecular mass of 10 kDa. Likewise, at all concentrations tested, ITI-R was detected as a single protein band, in this case with a molecular mass of 22 kDa.

Characteristics of Invertase Inhibitors. *Molecular masses*. The molecular masses of ITI-L and ITI-R estimated by SDS-PAGE were 10 kDa and 22 kDa, respectively (**Figure 4**). Both of these values were close to those determined by gel filtration on a Sephadex G-100 column (*27*) (data not shown), indicating that both inhibitors are monomers.

Effect of pH. The optimal pHs for invertase inhibition by ITI-L and ITI-R were 5.5 and 5.0, respectively (**Figure 5**), which were close to the optimal pHs previously determined for invertase inhibitors from potato and tomato fruit (*21*).

Thermostability. Both ITI-L and ITI-R were thermostable and retained 90% of their activity after being heated to 100 °C at pH 5.0 for 20 min.

Specificity of ITI-L Inhibition. As shown in **Figure 6**, ITI-L inhibited not only invertase from sweet potato leaves but also invertases from sweet potato tuberous roots and shoots of etiolated rice seedlings. The maximum inhibition of invertase from sweet potato leaves was 79%, whereas invertases from sweet potato tuberous roots and rice seedlings were inhibited by 41 and 47%, respectively. On the other hand, the inhibitor did not inhibit yeast invertase.

Specificity of Anti-ITI-L Antiserum. The specificity of anti-ITI-L antiserum was determined by Western blot analysis of the purified ITI-L and ITI-R plus analysis of ITI-R at various other stages of purification. The antiserum specifically reacted with the purified ITI-L and ITI-R (**Figure 7**), as well as with ITI-R protein in the crude extract and in the Con A-unbound fractions. It did not react with any other proteins in the crude extract or in the Con A bound fractions (**Figure 8**). This strongly suggests that ITI-L and ITI-R are immunologically related.

N-Terminal Sequence of ITI-L. The N-terminal amino acid sequence of the purified ITI-L was found to be GNPTVTNY, and a comprehensive database search revealed no homologous protein sequence. We recently isolated a full-length, ITI-L-encoding cDNA clone from sweet potato leaves. The N-terminus of the putative amino acid sequence from the cDNA was identical to the first 8 N-terminal amino acids of the isolated ITI-L protein, except Leu was substituted for Val at the 5 position, which might have been a microsequencer error.

DISCUSSION

The protocols for purification of ITI-L and ITI-R from sweet potato leaves and tuberous roots described here enabled us to purify both inhibitors to electrophoretic homogeneity. Because of its lability at low pH, treatment at pH 1.5 effectively removed invertase activity in both protocols. Bracho and Whitaker (*28*) observed that, at low pH, all invertase activity was lost from crude potato extracts, whereas ∼90% of the invertase inhibitor activity was retained. In the present study, however, only 30−50% of either inhibitor was recovered after low pH treatment, indicating that both ITI-L and ITI-R are less stable at low pH than potato invertase inhibitor.

The molecular masses of acid invertase inhibitors previously isolated from other plants reportedly range from 17 to 22.9 kDa (*17*, *18*, *21*, *28*). ITI-L and ITI-R were found to exist as single polypeptide chains with molecular masses of 10 kDa and 22 kDa, respectively; thus, the molecular mass of ITI-L is much lower than that of other plant invertase inhibitors, while that of ITI-R is similar to the 19.5 kDa previously reported for sweet potato (root) invertase inhibitor (*18*).

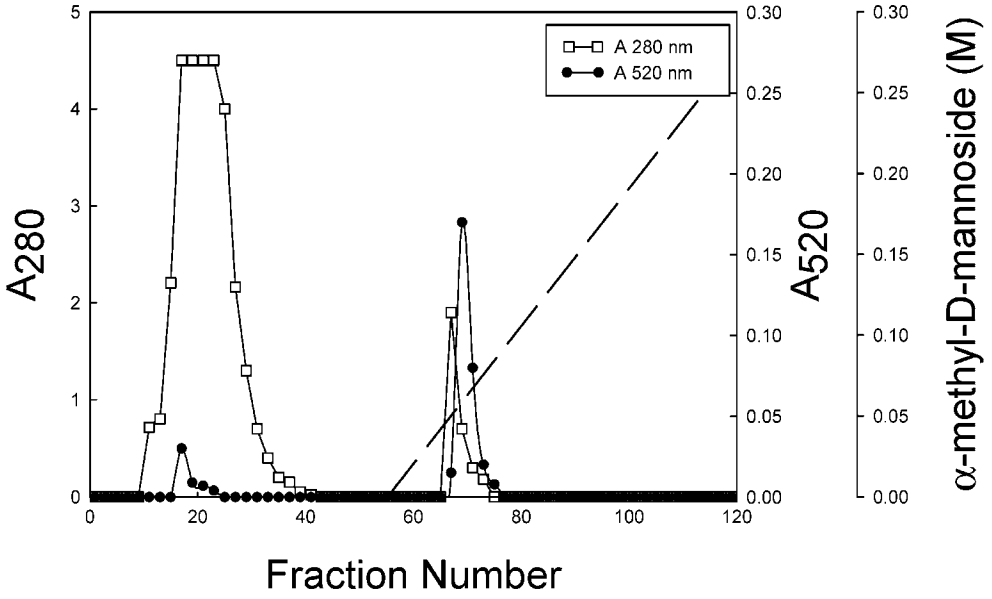

Figure 3. Con A-Sepharose column chromatography of invertase obtained from ammonium sulfate precipitation. Proteins precipitated with an 80% saturated $(NH_4)_2SO_4$ solution were applied to a Con A-Sepharose column (1.6 × 10 cm) previously equilibrated with Buffer B. Proteins bound to the column were eluted with Buffer B containing 0–0.3 M α-methyl-D-mannoside, and 3-mL fractions were collected. Protein profile (□) and invertase activity (A_{520} nm) (●) were separately monitored.

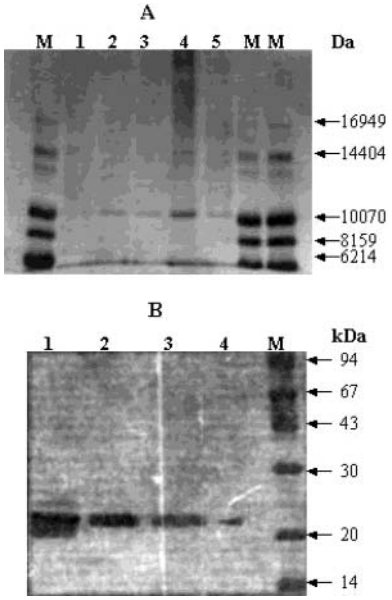

Figure 4. SDS-PAGE of invertase inhibitors. (A) SDS-PAGE of ITI-L at various purification steps was carried out using a 10–20% gradient gel: lane M, very low molecular weight protein markers (peptide marker kit, horse myoglobin peptides, Pharmacia); lane 1, Sephacryl S-100 column purified inhibitor; lanes 2 and 3, DEAE-Sephacel column purified inhibitor; lane 4, acid treated inhibitor; lane 5, crude extract of inhibitor. Proteins were visualized by silver staining. (B) SDS-PAGE of FPLC Superose 12 column purified ITI-R was carried out using a 12.5% gel: lane M, low molecular weight protein markers (Pharmacia); lanes 1, 2, 3, and 4, Superose 12 column purified ITI-R at concentrations of 12, 6, 4, and 2 μg, respectively. Arrowheads indicate the molecular masses of protein markers. Proteins were visualized by Coomassie blue staining.

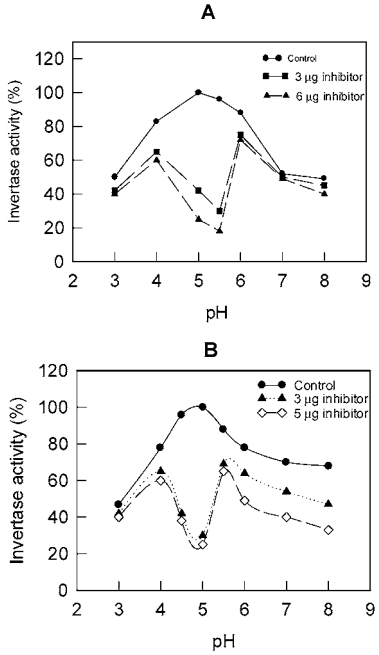

Figure 5. Effect of pH on the inhibition of invertase by ITI-L and ITI-R. Invertase from sweet potato leaves (6 units) was preincubated for 15 min at 37 °C with ITI-L (3 and 6 μg, (A)) or ITI-R (3 and 5 μg, (B)) in an incubation mixture at pH 3–8, after which the remaining invertase activity was measured. Buffers used in the incubation mixture were 0.1 M citric acid – 0.2 M phosphate buffer (pH 3–4), 0.1 M acetate buffer (pH 4–6) or 0.05 M phosphate buffer (pH 6–8).

Notably, ITI-L inhibited not only invertase from sweet potato leaves but also those from sweet potato tuberous roots and rice seedlings. The degree to which invertase inhibitors from a particular plant will inhibit invertases from other plants varies widely, ranging from virtually no inhibition to total inhibition (*17*, *18*, *21*). Our immunodetection analysis indicated that ITI-L and ITI-R are immunologically related and have at least some similar amino acid sequence. It seems likely that structural similarities among plant invertase inhibitors mean that their reactivities are not limited to their natural invertases.

Both ITI-L and ITI-R inhibit invertase by reversibly binding to it and require a short preincubation period for maximal inhibition (data not shown). This is identical to the behavior of the sweet potato invertase inhibitor described by Matsushita and

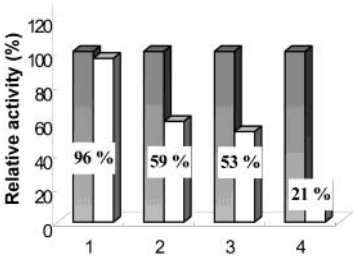

Figure 6. Inhibition of yeast and plant invertases by ITI-L. Samples of invertase isolated from (1) yeast (0.018 μg), (2) sweet potato tuberous roots (0.6 μg), (3) shoots of etiolated rice seedlings (0.6 μg), and (4) sweet potato leaves (0.6 μg) were reacted in the absence (solid bar) or presence (empty bar) of 3 μg of ITI-L.

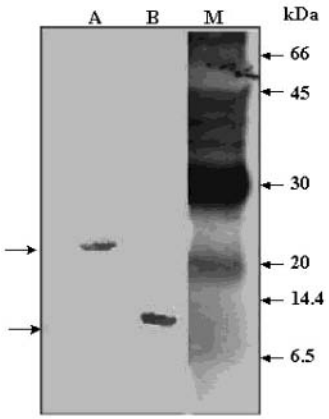

Figure 7. Western blot analysis of ITI-L and ITI-R. Purified ITI-L and ITI-R were subjected to SDS–PAGE on a 16.5% gel, after which the resolved proteins were transferred to a PVDF membrane and immunoblotted using antibodies against the purified ITI-L. Lane M contains low molecular weight protein markers (albumin, 66 kDa; ovalbumin, 45 kDa; carbonic anhydrase, 30 kDa; trypsin inhibitor, 20.1 kDa; α-lactoalbumin, 14.2 kDa and aprotinin, 6.5 kDa; purchased from Invitrogen). Lanes A and B contain ITI-L (10 kDa) and ITI-R (22 kDa), respectively.

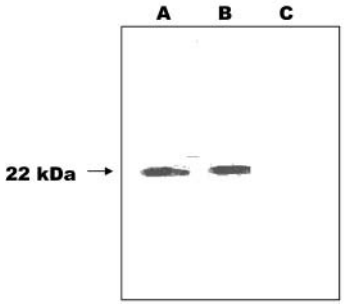

Figure 8. Western blot analysis of ITI-R after various purification steps. Proteins in crude extract of sweet potato tuberous roots (A) and in the Con A-Sepharose column unbound (B) and bound (C) fractions were resolved by SDS-PAGE on a 15% gel. The gel was then transferred to PVDF membrane and immunoblotted using antibodies against the ITI-L protein.

Uritani (*18*), as well as to that of the maize invertase inhibitor (*19*). By contrast, potato invertase inhibitor requires a longer preincubation time and forms a nondissociable complex with invertase (*29*).

The occurrence of proteins that inhibit invertase activities has been reported as another possible mechanism for the activity regulation of invertase in some plant species. Recently, Ordonez et al. (*30*) reported that the invertase inhibitor from *Cyphomandra betacea* Sendt fruits and several other plants (sugar beet, potato, and tomato) share lectin-like structural properties, including some common epitopes and some amino acid sequences. In our preliminary studies, we found that a recombinant invertase inhibitor from sweet potato leaves also showed lectin-like properties. It is well known that some lectins are components of the defense mechanisms higher plants use against fungi and bacteria, which suggests a probable function for proteinaceous inhibitors. The true physiological functions of ITI-L and ITI-R remain to be determined, however.

LITERATURE CITED

(1) Tymowska-Lalanne, Z.; Kries, M. The plant invertases: physiology, biochemistry and molecular biology. *Adv. Bot. Res.* **1998**, 28, 71−117.

(2) Sturm, A.; Tang, G. Q. The sucrose-cleaving enzymes of plants are crucial for development, growth, and carbon partitioning. *Trends Plant Sci.* **1999**, 4, 401−407.

(3) Hajirezaei, M. R.; Takahata, Y.; Trethewey, R. N.; Willmitzer, L.; Sonnewald, U. Impact of elevated cytosolic and apoplastic invertase activity on carbon metabolism during potato tuber development. *J Exp. Botany* **2000**, 51, 439−445.

(4) Rosenkranz, H.; Vogel, R.; Greiner, S.; Rausch, T. In wounded sugar beet (*Beta vulgaris* L.) tap-root, hexose accumulation correlates with the induction of a vacuolar invertase isoform. *J. Exp. Botany* **2001**, 52, 2381−2385.

(5) Sturm, A.; Chrispeels, M. J. cDNA cloning of carrot extracellular β-fructosidase and its expression in response to wounding and bacterial infection. *Plant Cell* **1990**, 2, 1107−1109.

(6) Balk, P. A.; de Boer, A. D. Rapid stalk elongation in tulip (*Tulipa gesneriana* L. cv. Apeldoorn) and the combined action of cold-induced invertase and the water-channel protein γTIP. *Planta* **1999**, 209, 346−354.

(7) Zeng, Y.; Wu, Y.; Avigne, W. T.; Koch, K. E. Rapid repression of maize invertases by low oxygen. Invertase/sucrose synthase balance, sugar signaling potential, and seedling survival. *Plant Physiol.* **1999**, 121, 599−608.

(8) Long, J. C.; Zhao, W.; Rashotte, A. M.; Muday, G. K.; Huber, S. C. Gravity-stimulated changes in auxin and invertase gene expression in maize pulvinal cells. *Plant Physiol.* **2002**, 128, 591−602.

(9) Kopka, J.; Provart, N. J.; Müller-Röber, B. Potato guard cells respond to drying soil by a complex change in the expression of genes related to carbon metabolism and turgor regulation. *Plant J.* **1997**, 11, 871−882.

(10) Pelleschi, S.; Guy, S.; Kim, J. Y.; Pointe, C.; Mahe, A.; Barthes, L.; Leonardi, A.; Prioul, J. L. Ivr2, a candidate gene for a QTL of vacuolar invertase activity in maize leaves. Gene-specific expression under water stress. *Plant Mol. Biol.* **1999**, 39, 373−380.

(11) Kim, J. Y.; Mahe, A.; Brangeon, J.; Prioul, J. L. A maize vacuolar invertase, *IVR2*, is induced by water stress. Organ/tissue specificity and diurnal modulation of expression. *Plant Physiol.* **2000**, 124, 71−84.

(12) Roitsch, T.; Bittner, M.; Godt, D. E. Induction of apoplastic invertase of *Chenopodium rubrum* by D-glucose and a glucose analogue and tissue-specific expression suggest a role in sink-source regulation. *Plant Physiol.* **1995**, 108, 285−294.

(13) Xu, J.; Avigne, W. T.; McCarty, D. R.; Koch, K. E. A similar dichotomy of sugar modulation and developmental expression affects both paths of sucrose metabolism: evidence from a maize invertase gene family. *Plant Cell* **1996**, 8, 1209−1220.

(14) Morris, D. A.; Arthur, E. D. Effects of gibberellic acid on patterns of carbohydrate distribution and acid invertase activity in *Phaseolus vulgaris*. *Physiol. Plant.* **1985**, 65, 257−262.

(15) Ehness, R.; Roitsch, T. Coordinated induction of mRNAs for extracellular invertase and a glucose transporter in *Chenopodium rubrum* by cytokinins. *Plant J.* **1997**, 11, 539−548.

(16) Schwimmer, S.; Makower, U.; Rorem, E. S. Invertase and invertase inhibitor in potato. *Plant Physiol.* **1961**, 36, 313−316.
(17) Pressey, R. Invertase inhibitors from red beet, sugar beet, and sweet potato roots. *Plant Physiol.* **1968**, 43, 1430−1434.
(18) Matsushita, K.; Uritani, I. Isolation and characterization of acid invertase inhibitor from sweet potato. *J. Biochem.* **1976**, 79, 633−639.
(19) Jaynes, T. A.; Nelson, O. E. An invertase inactivator in maize endosperm and factors affecting inactivation. *Plant Physiol.* **1971**, 47, 629−634.
(20) Nok, A. I.; Igwe, U. A.; Pai, S. J. Purification and characterization of invertase inhibitor from *Dioscorea Rotundata* tuber. *J. Enzyme Inhib.* **1992**, 6, 175−180.
(21) Pressey, R. Invertase inhibitor in tomato fruit. *Phytochemistry* **1994**, 36, 543−546.
(22) Charng, Y. Y.; Juang, R. H.; Su, J. C.; Sung, H. Y. Partial purification and characterization of invertase isozymes from rice grains (*Oryza sativa*). *Biochem. Mol. Biol. Int.* **1994**, 33, 607−615.
(23) Wu, L. J.; Huang, W. C.; Sung, H. Y. Partial purification and characterization of soluble acid invertase from sweet potato suspension cells. *Food Sci. Agric. Chem.* **2000**, 2, 49−54.
(24) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, 72, 248−254.
(25) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, 227, 680−685.
(26) Matsudaira, P. Sequence from picomole quantities of proteins electroblotted onto poly(vinylidene difluoride) membranes. *J. Biol. Chem.* **1987**, 262, 10035−10038.
(27) Whitaker, J. R. Determination of molecular weights of proteins by gel filtration on Sephadex. *Anal. Chem.* **1963**, *35*, 1950−1953.
(28) Bracho, G. E.; Whitaker, J. R. Purification and partial characterization of potato (*Solanum tuberosum*) invertase and its endogenous proteinaceous inhibitor. *Plant Physiol.* **1990**, 92, 386-394.
(29) Ewing, E. E.; McAdoo, M. H. An examination of methods used to assay potato tuber invertase and its naturally occurring inhibitor. *Plant Physiol.* **1971**, 48, 366−370.
(30) Ordonez, R. M.; Isla, M. I.; Vattuone, M. A.; Sampietro, A. R. Invertase proteinaceous inhibitor of *Cyphomandra betacea* Sendt fruits. *J. Enzyme Inhib.* **2000**, 15, 583−596.

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