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PARTIAL PURIFICATION OF POTATO TUBER INVERTASE AND ITS PROTEINACEOUS INHIBITOR*

ROGER S. ANDERSON and ELMER E. EWING
Department of Vegetable Crops, Cornell University, Ithaca, NY 14853, U.S.A.

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Key Word Index—*Solanum tuberosum*; Solanaceae; potato tubers; enzymes; invertase; proteinaceous inhibitor; Concanavalin A-Sepharose chromatography; enzyme–inhibitor complex dissociation.

Abstract—Improved purification of potato tuber invertase was achieved by utilizing a form of affinity chromatography between the enzyme and Concanavalin A (Con A) bound to Sepharose. Twenty-fold increases in specific activity were routinely obtained with this step and the enzyme was purified 190-fold over that found in the crude homogenate. The Con A-Sepharose chromatography step gave a greater purification than any other step in the invertase isolation procedure. There was up to 170% recovery of the activity loaded onto the column. α-Methyl-D-mannoside, sucrose, D-glucose and D-fructose eluted the enzyme from the Con A-Sepharose column with similar recoveries, although the volume of eluent required varied with the sugar. This unusually high recovery of invertase activity was obtained with some batches of tubers but not with others. There was evidence to suggest that the high recovery, or activation, may be due to the release of an inhibitor from the enzyme in the presence of Con A-Sepharose. Adsorption of invertase to Con A-Sepharose could be eliminated by incubation of the enzyme with α-mannosidase and β-glucosidase, indicating that potato tuber invertase is a glycoprotein. Proteinaceous inhibitor purification was improved by treatment of the tuber extract at low pH.

INTRODUCTION

Potato tubers contain substantial invertase (β-fructofuranosidase, EC 3.2.1.26) activity when stored at 5° [1]. At 10°, the invertase activity is much lower because of the presence of a proteinaceous inhibitor. This inhibitor binds to the enzyme protein to form an inactive enzyme–inhibitor complex [1–3] which is normally undissociable [4]. Pressey found that this inhibitor could be destroyed, and functional enzyme could be released from the inactive enzyme–inhibitor complex, by rapidly stirring the protein solution in a Waring [2] or VirTis [3] blender. Bubbling nitrogen through the solution accomplishes the same results but more slowly [4]. It appears that the inhibitor protein is much more labile to surface denaturation than is the enzyme. The present paper describes a method for recovering functional inhibitor from the complex.

Pressey [2, 3, 5] developed purification methods for potato tuber invertase. Sasaki *et al.* [6] found that 5 forms of invertase activity from potato tubers could be separated by chromatography on DEAE cellulose. However, we found that even a combination of the procedures developed by Pressey and Sasaki *et al.* resulted in a rather impure preparation; and we attempted to purify the enzyme further.

Yeast [7], *Neurospora* [8] and *Phytophthora megasperma* var. *sojea* [9] invertases are glycoproteins and, although it has not been shown if the enzyme from potato tubers is also a glycoprotein, it seemed probable. Based on its supposed glycoprotein nature, an attempt was made to purify the enzyme by chromatography on a column containing a carbohydrate-binding lectin immobilized on a solid support. A number of other glycoproteins have been purified using this technique [10]. The lectin, Concanavalin A (Con A), was chosen because of its widespread use and commercial availability. This paper describes a form of affinity chromatography between invertase and Con A to agarose. Elution from columns could be accomplished with several different sugars. Considerable purification of potato tuber invertase was achieved by this method.

RESULTS

Con A-Sepharose chromatography of invertase

Elution profiles of proteins and invertase activity obtained from the Con A-Sepharose column are shown in Fig. 1. Twenty-fold increases in specific activity were achieved with this step, and invertase activity recovered from the column was about 1.7 times greater than the activity loaded onto the column. Passing the enzyme through a column of CNBr-activated Sepharose 4B with its active groups blocked with glycine resulted in neither retention of the enzyme activity nor an increase in activity. Table 1 shows details of the partial purification. The fact that more activity was eluted from the column than was loaded onto it is an interesting aspect of this purification step. All four sugars used for elution resulted in similar recoveries.

Experiments were designed to explain this high recovery. All the protein loaded on the column could be accounted for in the eluted fractions. The increased invertase activity was not caused by high concentrations of NaCl or sugar or the salts $MgCl_2$, $CaCl_2$ or $MnCl_2$ in contact with the enzyme prior to dialysis. An aliquot of

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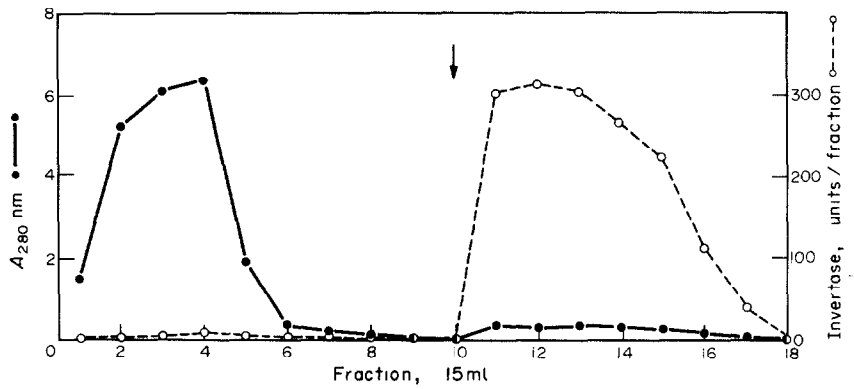

Fig. 1. Purification of potato tuber invertase by chromatography on Con A-Sepharose. Invertase from the DEAE cellulose column (840 units with a sp. act. of 2.3 units/mg protein) was made 0.1 M with NaOAc, pH 4.75, M with NaCl and 1 mM with $MgCl_2$, $CaCl_2$ and $MnCl_2$ and loaded on a 0.9 × 12.0 cm Con A-Sepharose column equilibrated with the same buffer as the enzyme. Protein not adsorbed by the column was eluted with the equilibration buffer until the A_{280} of the eluent was 0. This buffer eluted 318 mg of the 362 mg of protein loaded on the column. The second elution buffer containing 0.1 M methyl mannoside in the equilibration buffer was started at the arrow Invertase activity (1545 units with a sp. act. of 81.5 units/mg protein) was eluted with this buffer. The sp. act. of the invertase prepn was increased 35-fold. Recovery of enzyme activity from the Con A-Sepharose column was 184% of that loaded on it.

CNBr-activated Sepharose 4B with its active groups blocked with glycine or free Con A (0.001–0.1%) did not increase the activity of Con A-Sepharose purified invertase. However, an aliquot of Con A-Sepharose caused increased invertase activity in enzyme preparations that had been passed through the DEAE column but not in preparations that had also been passed through the Con A-Sepharose column.

The possibility was examined that an invertase inhibitor, such as the one described by Pressey [2, 3, 5], was released from the enzyme on passing through the Con A-Sepharose column. No inhibitor activity was observed in the enzyme preparation collected from the DEAE column. Fractions eluted from the Con A-Sepharose column by the equilibration buffer did contain an inhibitor. Pressey's inhibitor was not retained by this column and was also eluted by the equilibration buffer. To see whether enzyme–inhibitor complex could be dissociated by passing over the Con A-Sepharose column, purified inhibitor was incubated for 1 hr at 37° [4] with an excess of Con A-Sepharose purified enzyme. The mixture, containing enzyme–inhibitor complex and excess enzyme, was loaded on the column. Elution with the equilibration buffer removed inhibitor activity as well as a small amount of invertase activity from the column (Table 2). This may represent invertase that cannot be affected by the inhibitor. The difference between the enzyme activity eluted from the column by methyl mannoside and the activity loaded on the column was almost identical to the inhibitor activity eluted by the equilibration buffer. The results in Table 2 show that 15% of the inhibitor activity originally present in the enzyme–inhibitor mixture could be removed from the enzyme. When a different batch of inhibitor was prepared, the equilibration buffer removed only 6% of the inhibitor applied to the column. No further inhibitor was released into the equilibration buffer when this

Table 1. Summary of invertase partial purification including the Con A-Sepharose chromatography step, from potato tubers

Fraction	Volume (ml)	Total protein (mg)	Specific activity (units/mg)	Total activity (units)	Fold-increase in specific activity
Tuber homogenate	2600	34108	0 37	12688	
Foaming	2500	25613	0 6	15240	1 6
Ethanol	160	1176	1 5	1766	4 0
Sephadex G-100	178	384	3 0	1154	8 1
DEAE-cellulose	142	186	3 5	652	9 5
Con A-Sepharose	60	16	69 9	1108	188 9

Values are from the average of 11 invertase purifications made from April through October 1976, during which period the recoveries of invertase activity from the Con A-Sepharose columns were in excess of 100% of the activity loaded on the column. One unit of invertase activity equals 1 μmole of reducing sugar formed per hour.

Table 2. Dissociation of invertase enzyme-inhibitor complex on passage over a Con A-Sepharose column

Preparation	Activity units Enzyme	Inhibitor
Original enzyme	71.5	—
Original inhibitor	—	56.0*
Enzyme–inhibitor mixture loaded on column	15.5	0
Equilibration buffer eluate	1.8	8.6†
Methyl mannoside buffer eluate	23.9	0

* Obtained by subtraction.

† Based on reduction in activity of an aliquot of invertase after incubation with the eluate.

Inhibitor was mixed with an excess of invertase and the mixture was incubated to allow the proteins to bind [4]. The mixture was loaded on a Con A-Sepharose column and the column was eluted with the equilibration buffer, followed by the methyl mannoside buffer. Invertase enzyme and inhibitor activities were assayed in each eluate Note that the increased enzyme activity of the methyl mannoside buffer eluate (8.4 units more than in the enzyme–inhibitor mixture loaded on the column) is nearly equal to the inhibitor activity (8 6 units) eluted from the column.

enzyme–inhibitor complex was passed through the column a second time.

Recovery of invertase activity in excess of 100% of that loaded on the Con A-Sepharose column was achieved with preparations from tubers harvested in September 1975 and used from April 1976, when these experiments began, until October 1976. Tubers from the 1976 harvest gave preparations with these high recoveries when used after January 1977. A decline in recovery of enzyme activity from the Con A-Sepharose column was observed with increasing time of storage at 4° of the enzyme collected from the DEAE cellulose column.

To examine the glycoprotein nature of potato tuber invertase, the enzyme (100 units) was eluted from Con A-Sepharose and incubated for 24 hr at 30° in 0.1 M sodium acetate, pH 4.75, with 2 units of α-mannosidase (EC 3.2.1.24, Sigma, Type III, from Jack Beans) and/or 2 units of β-glucosidase (EC 3.2.1.21, Sigma, from almonds). The invertase preparations treated with glycosidases were passed over Con A-Sepharose columns of 0.5 ml bed volumes. Elution of these columns with the equilibration buffer released 1.4% of the non-glucosidase treated enzyme, 2.0% of the β-glucosidase-treated enzyme, 8.7% of the α-mannosidase-treated invertase and 37.0% of the enzyme incubated with both glycosidases. Invertase activity was unaffected by the glycosidase treatments (data not shown).

The 4 sugars used in the equilibration buffer to elute invertase activity from the Con A-Sepharose column did so with differing efficiencies at 0.1 M. Most efficient was methyl mannoside, which eluted invertase activity with the least volume of buffer. Peak activity eluted with 3 bed volumes. Fructose required 3.5 while glucose and sucrose each required about 4 bed volumes. At 0.5 M all four sugars eluted invertase activity with the same efficiency as 0.1 M methyl mannoside.

Polyacrylamide gels of the enzyme preparation before and after passage through the Con A-Sepharose column are depicted in Fig. 2. Invertase activity was located in the band indicated by the arrow. The proteins eluted by all 4 sugars gave the same gel patterns.

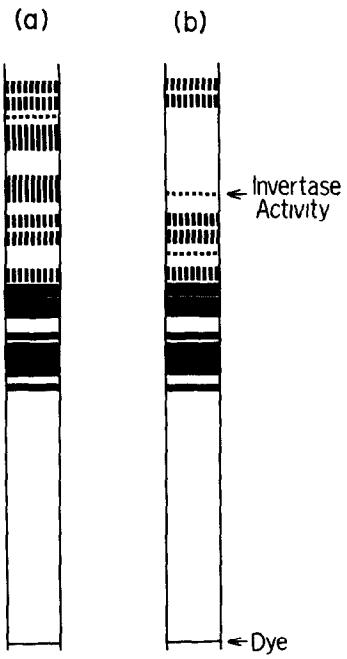

Fig. 2. Polyacrylamide gels (8%) of (a) the proteins in the invertase preparation loaded on the Con A-Sepharose column and (b) the proteins adsorbed by a column and eluted with 0.1 M sucrose, D-glucose, D-fructose or methyl mannoside. Details are given in the Experimental.

Low pH *dissociation of the enzyme–inhibitor complex*

Acidification of potato invertase to pH 1 for 1 hr at 37° resulted in almost complete inactivation (Table 3, line b). When the inhibitor alone was subjected to the same low pH conditions and then allowed to bind enzyme, there was still a large amount of inhibitor activity remaining (c). The recovery of inhibitor from the low pH treatment was 80% of the inhibitor activity when compared to the activity of inhibitor not subjected to low pH (d). The experiment described in line e showed that low pH treatment of the enzyme–inhibitor complex dissociated the complex, inactivated the enzyme and released active inhibitor which was again able to bind enzyme. Recovery of inhibitor activity from the complex was 96% of that observed with inhibitor not treated at low pH (d). In both cases (c and e), the inhibitor protein showed remarkable stability to acidic conditions. The amount of inhibitor activity recovered after low pH treatment of the inhibitor alone (c) was less than that recovered from the complex (e). Perhaps the inhibitor, which was slightly inactivated by the low pH, was protected partially by the complex or nonspecifically by the protein impurities associated with the enzyme preparation.

The addition of a second aliquot of enzyme to the previously formed complex resulted in no further inhibition; the treatment in line f is essentially equal to the sum of a plus d. Thus, no inhibition of the second aliquot of enzyme could occur unless the complex was first treated with acid. Comparison of lines e and f indicates that functional inhibitor was released from the low pH treated complex.

Table 3. Effect of low pH on invertase, inhibitor and complex

	Reaction components and protocol* (E = enzyme, I = inhibitor, EI = enzyme–inhibitor complex)		Units of invertase activity	Reduction in invertase activity
	Initial components	Added after 1 hr	Mean ± s.e.	(%)
a	E		0.811 ± 0.011	
b	E + acid†	base†	0.052 ± 0.009	93.6
c	I + acid	base + E	0.190 ± 0.009	76.7
d	E + I		0.032 ± 0.007	96.1
e	EI + acid	base + E	0.058 ± 0.009	92.5‡
f	E + I	E	0.845 ± 0.014	0.0‡

* All reactions were made 0.25 M with sucrose after 2 hr.

† Sufficient acid (HCl) was added to give pH 1, and sufficient base (NaOH) to neutralize the acid.

‡ Inhibition of the second aliquot of enzyme that was equal to the first.

Summary of experiments showing invertase enzyme inactivation, inhibitor stability, enzyme–inhibitor complex dissociation and release of functional inhibitor from the complex by treatment at pH 1 for 1 hr at 37°.

A pH of 2 or less was required for maximum enzyme inactivation. When 0.390 unit of invertase activity was incubated at pH 2, 1 or 0.5 for 1 hr at 37°, 0.008 unit of activity was observed in all cases when assayed at pH 4.75. Invertase (0.438 unit) inactivation was more than 99% complete after low pH treatment at 37° for 1 hr, whereas activity was reduced only 5% on ice (data not shown).

Inclusion of the low pH treatment in Pressey's [3] procedure for inhibitor isolation increased both the specific activity and the total activity of the inhibitor preparation. An increase in specific activity from 1.2 to 4.3 inhibitor units/mg protein and an increase in yield from 9.4 to 35.7 inhibitor units were obtained when the low pH treatment was used.

DISCUSSION

Invertase enzymes obtained from other sources [7–9] are known to be glycoproteins. Evidence from this paper shows that potato tuber invertase is also a glycoprotein. It is retained on Con A-Sepharose columns and is eluted from them with methyl mannoside. Retention of potato tuber invertase on these columns could be reduced after incubating the enzyme with certain glycosidases. The carbohydrate moiety contains residues that can be hydrolyzed from the protein with either α-mannosidase or a combination of this enzyme and β-glucosidase. External yeast invertase was shown to contain D-glucosamine and mannose residues in its carbohydrate portion [11]. The effect of α-mannosidase was therefore not surprising, but the synergistic effect of the β-glycosidase needs further investigation. After this work was conducted Ziegler and Albersheim [9] reported the use of a Con A-Sepharose column to provide part of their evidence that *Phytophthora megasperma* Drechs. var *sojea* invertase was a glycoprotein. Their enzyme was retained by the column and it was eluted with methyl mannoside.

Con A can adsorb a large number of mono- and polysaccharides including sucrose, D-glucose and D-fructose [12, 13] as well as methyl mannoside. Thus it is not surprising that all these sugars elute proteins and invertase activity from the Con A-Sepharose column, although the efficiency of elution differs. Ranking the sugars for efficiency in eluting enzyme from the column based upon the volume of 0.1 M sugar solution required, gives the same ranking as that reported [13] for their ability to inhibit the binding between dextran and Con A. Since sucrose was not the most efficient eluent of invertase activity from this column, in the strict sense this is not an example of true affinity chromatography. In light of the above, the use of Con A-Sepharose chromatography would not be expected to result in a completely pure preparation. This does not rule out the possibility that sucrose may alter the enzyme's conformation when it binds to the invertase molecule and cause release of invertase from the Con A-sepharose column. The conformational change could alter the binding or the accessibility of the sugar groups on the enzyme for the column.

The Con A-Sepharose chromatography technique gave far greater purification of potato tuber invertase than any other step in the isolation procedure (Table 1). Incorporation of this step yielded an enzyme preparation with about 190-fold increase in specific activity over that in the homogenate. Without the Con A-Sepharose chromatography, there was only a 10-fold purification.

Chromatography of potato tuber invertase on Con A-Sepharose gave a recovery of enzyme activity from the column which was in excess of that applied to it. The results reported here suggest that the unusually high recovery of invertase activity eluted from the column may have been due to the release of an inhibitor, such as the proteinaceous one described by Pressey [2, 3, 5]. Perhaps the adsorption of the enzyme or the enzyme–inhibitor complex to the Con A-Sepharose altered the protein enough to cause the release of the inhibitor.

However, this high recovery of invertase activity from Con A-Sepharose columns was not achieved in every case. Two factors seemed to decrease recovery of activity from this column. One was an effect of storage of the enzyme preparation eluted from the DEAE column before Con A-Sepharose chromatography, and the second was the time of year in which tubers were used for an enzyme preparation. The decline in recovery from the Con A-Sepharose column with increasing time of storage at 4° of the enzyme collected from the DEAE column may have been due to a decrease in the enzyme protein's stability. This decreased stability also may have caused the decreased activity and decreased temperature optimum that have been observed for invertase preparations with storage at this temperature. The loss in the ability to achieve greater than 100% recovery of invertase activity eluted from the Con A-Sepharose column with aging of the DEAE fractions could also have been due to a loss of enzyme–inhibitor complex from these fractions with aging.

The seasonal variability observed in invertase preparations was perhaps due to changes in the endogenous levels of invertase and its proteinaceous inhibitor [1] or to the form of the enzyme [6]. The changing levels of invertase and its inhibitor during storage could also explain the wide range of recoveries that we have observed in the purification of both proteins throughout the year.

The low pH technique described in this paper for dissociating the invertase enzyme–inhibitor complex is similar to one described by Kunitz [14] to dissociate the pancreatic trypsin–soybean inhibitor complex and to recover active inhibitor. Pressey [15] used a pH 1.5 treatment step in the purification of sugar beet and sweet potato invertase inhibitors and suggested that low pH may increase inhibitor activity by dissociating the inactive enzyme–inhibitor complex and removing the enzyme activity.

Although the inhibitor from potato was precipitated at pH 1, and in fact is insoluble at pH 4 [3], Pressey [15] found that the sweet potato and sugar beet inhibitors were not preciptated at pH 1.5. While all three inhibitors are stable to low pH it would appear that they differ in solubility.

EXPERIMENTAL

Invertase was isolated from 'Kennebec' or 'Katahdin' tubers stored at 10° a few weeks after harvest and then transferred to 5° storage until extraction. Tubers treated with the sprout inhibitor maleic hydrazide were used for enzyme prepns made during the longer storage times. No differences in recoveries of invertase activity eluted from the Con A-Sepharose columns were observed between treated and non-treated tubers. The enzyme was partially purified according to the method of ref. [3]. The tubers were homogenized in an Acme Juicerator, 1 ml M Na_2SO_3 and 1 g PVP were added immediately to each 100 ml of juice. The juice was centrifuged at 20000 *g* for 20 min and

the supernatant fluid collected. The supernatant fluid was made 0.2 M with NaOAc, pH 5, and stirred in a Waring blender set at top speed for 5 min in order to inactivate the endogenous inhibitor [2]. The protein was fractionated at −11° with the addition of EtOH to the enzyme prepn. The protein precipitating between 20 and 45% EtOH was collected by centrifugation at 8500 *g* for 10 min and was resuspended in 0.2 M NaCl. The resuspended protein was loaded on a 2.6 × 100.0 cm Sephadex G-100 column equilibrated with 0.2 M NaCl. The column was eluted with this same soln. Fractions containing invertase activity were pooled, concd on an Amicon XM-50 membrane and dialyzed against 0.05 M NaOAc, pH 6.2. The dialyzed enzyme prepn was loaded on a 2.6 × 100.0 cm DEAE cellulose column [6] that had been equilibrated with the dialysis buffer. The invertase activity was eluted with a linear gradient starting with the equilibration buffer and ending with 0.1 M NaOAc, pH 3.9. Although 2 or more peaks eluted, depending upon the storage history of the tubers [6], the major fraction of activity consistently eluted immediately after the void vol. This first fraction was collected for the Con A-Sepharose chromatography step. All procedures following homogenization were carried out at 2° unless otherwise specified. Invertase inhibitor was isolated from tubers stored at 10° until used. The inhibitor was partially purified according to a modified method of ref. [3]. Tubers having inhibitor activity in the crude homogenate were homogenized in an Acme Juicerator, 1 ml M Na_2SO_3 and 1 g PVP were added immediately to each 100 ml of juice. The juice was centrifuged at 20000 *g* for 20 min and the supernatant fluid was collected. The supernatant fluid from the tuber homogenate was incubated at pH 1 for 1 hr at 37° to destroy invertase activity, dissociate the invertase enzyme-inhibitor complex and recover functional inhibitor activity. The prepn was adjusted to pH 4 and the resulting ppt. was collected by centrifugation at 8500 *g* for 10 min. The ppt. was resuspended in 0.2 M NaCl, the pH was adjusted to 6 and the suspension was stirred for 1 hr. The suspension was centrifuged at 8500 *g* for 10 min and the supernatant fluid collected. The supernatant fluid was adjusted to pH 5.5 and solid $(NH_4)_2SO_4$ was added to give 25% satn. The supernatant fluid was collected by centrifugation at 8500 *g* for 10 min, adjusted to pH 4 and solid $(NH_4)_2SO_4$ was added to give 35% satn. The resulting ppt. was collected by centrifugation at 8500 *g* for 10 min, resuspended in 0.05 M NaOAc, pH 6.2, and dialyzed against this same buffer *ca* 18 hr. The dialyzed prepn was loaded on a 2.6 × 100 cm DEAE cellulose column equilibrated with the buffer. The column was washed with 0.1 M NaOAc, pH 3.9, and inhibitor activity was eluted from the column with 0.2 M NaCl. All steps following homogenization were done at 2° except for the low pH treatment. The resulting inhibitor had a sp. act. of 16 units/mg protein. Con A-Sepharose was prepared by binding Con A (Sigma, Grade IV) covalently to CNBr-activated Sepharose 4B (Pharmacia), 8 mg/ml sedimented gel, according to the instructions accompanying the Sepharose. The binding of Con A to the Sepharose was followed by monitoring the decrease with time in A_{280} of the supernatant fluid. Con A-Sepharose was equilibrated with a pH 4.75 buffer that contained 0.1 M NaOAc, M NaCl and 1 mM $MgCl_2$, $CaCl_2$ and $MnCl_2$. Preliminary expts were done on columns in Pasteur pipets with 0.5 ml bed vols, and later expts in 0.9 × 12.0 cm columns. The invertase fraction collected from the DEAE column was made to the same pH and salt concn as the equilibration buffer before loading on the Con A-Sepharose column. Proteins not adsorbed by the Con A-Sepharose were eluted with the equilibration buffer and elution was continued until the A_{280} of the eluent was 0. Up to 7500 units of invertase activity (sp. act. 5 units/mg protein) have been loaded on the 0.9 × 12.0 cm column without loss of activity into the equilibration buffer. Invertase activity could be removed from the Con A-Sepharose column by elution with 0.1 M sucrose, D-glucose, D-fructose or α-methyl-D-mannopyranoside (methyl mannoside) in the equilibration buffer, although the latter was the most efficient eluent and was therefore routinely used. Fractions containing enzyme activity were dialyzed against 0.1 M NaOAc, pH 4.75, to remove the NaCl, $MgCl_2$, $CaCl_2$, $MnCl_2$ and sugar before assaying. The above procedures were conducted at 2°. The Con A-Sepharose columns appear to be reusable indefinitely. Invertase activity was assayed according to ref. [4]. An appropriate quantity of enzyme was incubated in 1 ml reaction mixture containing 0.25 M sucrose and 0.1 M NaOAc, pH 4.75, at 37° for 1 hr. One unit of invertase activity was defined as the activity which yields 1 μmol of reducing sugar per hr under the above assay conditions. Inhibitor activity was measured by incubating an appropriate quantity of the inhibitor with an aliquot of enzyme for 1 hr at 37° in 0.1 M NaOAc, pH 4.75. The reaction mixture was then made 0.25 M with sucrose and the remaining free, unbound enzyme assayed as described above. The quantities of enzyme and inhibitor used in the assay were such that the enzyme was in slight excess and that the binding reaction would be completed within 1 hr. Percent inhibition was defined as [1-(enzyme activity with inhibitor/enzyme activity)] × 100 and is directly correlated with the quantity of inhibitor when assayed under the above conditions. Inasmuch as this inhibitor binds very tightly to the enzyme, 1 unit of inhibitor activity was defined as that which inhibits 1 unit of enzyme activity [4]. Protein was measured by the Lowry method as adapted in ref. [16] with BSA as the standard, or it was monitored by absorbance at 280 nm. Some Lowry protein measurements were conducted on protein pptd in the presence of 6% TCA and 125 μg/ml deoxycholate to remove any interfering compounds from the protein [17]. This was done to remove, in particular, any phenolic compounds from the preps since potatoes can contain rather high levels of these materials. No difference was found in the measured Lowry protein in the enzyme prepn either before or after passing over the Con A-Sepharose column. Thus, the large purification of invertase obtained with the Con A-Sepharose chromatography technique was not simply due to the removal of phenolic compounds. Invertase preps both before and after loading on the Con A-Sepharose column were run on 8% polyacrylamide gels using 150 μg protein. Gels were run according to ref. [18], except that 7 cm running gels and stacking gels polymerized with ammonium persulfate were used and the proteins were loaded on the gels in 40% glycerine in Tris-HCl at pH 6.9. Proteins were stained with Coomassie blue G. Invertase activity was located in gels by assaying 2 mm slices for 8 hr using the assay conditions described above.

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