J. Plant Biol. 38(3) : 251~258 (1995)

Purification and Characterization of Soluble Acid Invertase from the Hypocotyls of Mung Bean (*Phaseolus radiatus* L.)

Lee, Dong-Hee and Young-Sang Kim*
Department of Biology, Pusan National University, Pusan 609-735, Korea

The soluble acid invertase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26) was isolated and characterized from the hypocotyls of mung bean (*Phaseolus radiatus* L.). The enzyme was purified to apparent homogeneity by consecutive step using diethylaminoethyl (DEAE)-cellulose anion exchange, Concanavalin (Con) A affinity and Sephacryl S-300 chromatography. The overall purification was about 148-fold with a yield of about 15%. The finally purified enzyme exhibited a specific activity of about 139 μmol of glucose produced mg^{-1} protein min^{-1} at pH 5.0 and appeared to be a single protein by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and nondenaturing PAGE. The enzyme had the native molecular weight of 70 kD and subunit molecular weight of 70 kD as estimated by Sephadex G-200 chromatography and SDS-PAGE, respectively, suggesting that the enzyme was composed of a monomeric protein. On the other hand, the enzyme appeared to be a glycoprotein containing N-linked high mannose oligosaccharide chain on the basis of its ability to bind to the immobilized Con A. The enzyme had a Km for sucrose of 1.8 mM at pH 5.0 and maximum activity around pH 5.0. The enzyme showed highest enzyme activity with sucrose as substrate, but the activity was slightly measured with raffinose and cellobiose. No activity was measured with maltose and lactose. These results indicate the soluble acid invertase is a β-fructofuranosidase.

Keywords: soluble acid invertase, purification, mung bean, hypocotyls

Invertases (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26) hydrolyze sucrose, one of the predominant form of carbohydrate translocation, into its constituent monosaccharides, glucose and fructose in higher plants (ap-Rees, 1984; Sturm and Chrispeels, 1990). It has been suggested that the transportation of invertase synthesized on the rough endoplasmic reticulum are regulated by golgi complex (Lauriere *et al.*, 1988). Invertases are present in multiple form in a given tissues. Invertases are divided into two groups on the basis of the pH required for maximum activity, nomenclaturing acid (pH 4.0-5.0) and alkaline (pH 7.0-8.0) invertase (Chin and Weston, 1973; Matsushita and Uritani, 1974).

Acid invertase participates in enhancing sink strength of developing plant organs (Ho, 1984). The enzyme can be subdivided into 2 groups according to its different subcellular location, defined as extra- (insoluble) and intracellular (soluble) invertase (Leigh *et al.*, 1979; Giaquinta *et al.*, 1983). Extracellular acid invertase bound ionically to cell wall gives rise to maintain a steep sucrose concentration gradient between source and sink organs in phloem unloading (Fahrendorf and Beck, 1990; Sturm and Chrispeels, 1990). Soluble acid invertase is thought to be predominantly found in rapidly growing tissues, at sites of emerging secondary roots and in developing tap roots and leaves (Eshrich, 1980). When plant cell ceases growth, the cell exhibits the decrease in the enzyme activity, thus finally results in the disappearance of the activity. Intracellular acid invertase is known to be vacuolar (Leigh *et al.*, 1979; Giaquinta *et al.*, 1983), but it is proposed that the enzyme is sometimes present in the cytosol (Fahrendorf and Beck, 1990; Karuppiah *et al.*, 1990).

*Corresponding author: Fax +82-51-581-2962
© 1995 by Botanical Society of Korea, Seoul

High intracellular invertase activity is usually present in young seedlings and mature fruits (Ricardo and ap-Rees, 1970; Krishnan and Pueppke, 1990).

The purification of acid invertase has been studied to elucidate the biochemical characterizations of the enzyme from several plants of potato and banana (Anderson and Ewing, 1978; Sum *et al*., 1980; Iwatsubo *et al*., 1992; Obenland *et al*., 1993; Walker and Pollock, 1993). The purification of invertases, however, is difficult because of high lability of their biochemical characteristics. Thus, few soluble acid invertase has been successfully purified. Therefore, we performed the isolation and extensive purification of soluble acid invertase from mung bean (*Phaseolus radiatus*) to understand the biochemical and molecular regulation of sucrose metabolism. In this report we describe the purification and biochemical characterization of soluble acid invertase from the hypocotyls of mung bean.

MATERIALS AND METHODS

Plant materials and reagents

The seeds of mung bean (*Phaseolus radiatus* L.) were soaked in running tap water for 2 h and planted on the pot, and grown in growth chamber at 28± 1℃ with 70% humidity under dark condition for 10 days. The elongating hypocotyls of 10 day-old seedlings were used for experimental materials.

Chemicals and assay enzymes were obtained from Sigma and Boehringer Chemical Co. Reagents for electrophoresis and chromatography such as DEAE-cellulose, Con A-Sepharose 4B, Sephacryl S-300 and Sephadex G-200 chromatography were purchased from Sigma.

The buffer solutions used were as follows: A, 50 mM HEPES, pH 7.0, 1 mM Mg-acetate, 1 mM Na-EDTA, 1 mM DTT and 1 mM PMSF; B, 50 mM HEPES, pH 7.0, 1 mM Na-EDTA and 1 mM PMSF; C, 50 mM HEPES, pH 7.0, and 1 mM PMSF.

Assays for invertase activities and protein

Invertase activity was determined by measuring glucose content formed from sucrolysis. For the assay of acid invertase, 1.6 mL aliquot of reaction mixtures containing 50 mM phosphate-citrate buffer, pH 5.0, 1 mM Mg-acetate, 100 mM sucrose and a suitable amount of enzyme solutions was incubated at 25℃ for 10 min, and then boiled for 3-5 min to cease reaction. The amount of glucose formed was measured by the modified glucose oxidase-peroxidase method (Bergmeyer and Bernt, 1974). A 0.8 mL of the glucose oxidase-peroxidase mixture (pH 7.0, 0.8 unit of each enzyme) contained 800 μg *o*-dianisidine dihydrochloride was added to reaction mixture, and then incubated at room temperature for at least 30 min until color development. After a 0.8 mL of 5 M HCl was added to reaction mixture, the amount of glucose was measured at 540 nm with spectrophotometer. The unit is defined as the formation of 1 μmol glucose from sucrose per min per 1 mL of enzyme solution at 25℃ at pH 5.0 for acid invertase.

The amount of protein was determined according to the modified Bradford (1976) method using BSA (bovine serum albumin) as the standard protein.

Crude invertase extraction

Crude invertase extraction was performed by the modified Chen and Black (1992) method. Approximately 500 g of elongating hypocotyls rinsed three times with distilled water was homogenized with Waring blender in buffer A with ratio of 1 g of elongating hypocotyls: 1 mL of buffer A. The homogenate was filtered through four layers of cheesecloth, then centrifuged at 12,000 *g* for 15 min. The supernatant was designated as the crude extract. All separations for enzyme purification were performed at 4℃.

Separation of acid invertase by ammonium sulfate precipitation

Crude extract, which was prepared from about 500 g of mung bean hypocotyls, was precipitated from 50 to 70% saturation with enzyme grade $(NH_4)_2SO_4$ powder. The precipitates were collected after centrifugation, and dissolved in buffer B, then dialyzed overnight against the same buffer. At each purification step, protein purity was examined by SDS-PAGE.

DEAE-cellulose chromatography

The dialyzed enzyme solutions were applied to DEAE-cellulose column (3×15 cm) preequilibrated with buffer B. The column was washed with the same buffer to remove unaimed proteins, then eluted to a 1 L linear gradient, 0 to 0.3 M NaCl in buffer B at a flow rate of 0.8 mL/min. 8 mL fractions, which occurred activity peak at pH 5.0, were precipitated to 70% saturation with ammonium sulfate, and then dissolved in buffer B. The precipitates were dialyzed against the same buffer or desalted with a Sephadex G-25 column.

Con A-Sepharose 4B chromatography

About 10 mL of concentrated enzyme solutions obtained from DEAE-cellulose chromatography were applied to the Con A-Sepharose 4B column (1.5×10 cm) previously equilibrated with buffer B. The column was washed with the same buffer until the A_{280} was decreased to minimum level, and then eluted with 0.2 M methyl-α-D-mannopyranoside in buffer B at a flow rate of 0.5 mL/min. 5 mL fractions containing enzyme activity peak were pooled and concentrated by ammonium sulfate precipitation as above. The precipitates dissolved in buffer C were dialyzed against the same buffer.

Sephacryl S-300 chromatography

About 5 mL of the enzyme solutions obtained from Con A step were carefully loaded to the top of the Sephacryl S-300 column (1.5×60 cm) preequilibrated with buffer C. The column was run at a flow rate of 0.25 mL/min. 1 mL fractions, which showed activity peak at pH 5.0, were pooled and concentrated by Amicon ultracentrifugation (Amicon Diaflo ultrafiltration membranes, 10 XM50, 43 mm).

Native molecular weight estimation

The native molecular weight of the enzyme was determined by using Sephadex G-200 chromatography. Purified enzyme solution, together with molecular standard markers were subjected. After collection at a flow rate of 0.1 mL/min, 1 mL fractions measured the A_{280} and acid invertase activity. The void volume of the column was measured by using blue dextran. The standard markers used were as follows: ferritin, 450 kD; catalase, 240 kD; aldolase, 158 kD; BSA, 68 kD; albumin from hen egg, 45 kD; chymotrypsinogen A, 25 kD; cytochrome C, 12.5 kD.

Electrophoresis

SDS-PAGE was performed by the modified method of Ausubel *et al.* (1987). Proteins were separated on a 10% resolving gel at 200 V. After electrophoresis, the gels were stained with silver or Coomassie brilliant blue. The standard proteins used for SDS-PAGE were myosin (200 kD), galactosidase (116.3 kD), phosphorylase (97.4 kD), BSA (66.3 kD), glutamic dehydrogenase (55.4 kD), lactate dehydrogenase (36.5 kD), carbonic anhydrase (31 kD), trypsin inhibitor (21.5 kD), lysozyme (14.4 kD), apotinin (6 kD), and insulin B chain (3.5 kD).

Nondenaturing PAGE were performed in a similar way to SDS-PAGE except that SDS and β-mercaptoethanol were not added to the gels.

RESULTS

Purification of soluble acid invertase

Purification of soluble acid invertase from the hypocotyls of mung bean is summarized in Table 1. Attempts to fractionate the crude extract with ammonium sulfate were successful, as the activity measured at pH 5.0 mainly exhibited from 50 to 70% ammonium sulfate saturation. The similar separations have been achieved in the case of soybean (Chen and Black, 1992). The apparent electrophoretic homogeneity of consecutive purification step using DEAE-cellulose anion exchange, Con A affinity and Sephacryl S-300 chromatography for soluble acid invertase from mung bean is shown in Fig. 1. The overall purification of soluble acid invertase was about 148-fold with a yield of about 15% from the starting material (Table 1). The finally purified enzyme had a specific activity of approximately 139 μmol of glucose produced mg^{-1} protein min^{-1} at pH 5.0 (Table 1), and showed a single protein in SDS-PAGE (Fig. 1).

Fig. 2 shows the elution profile of soluble acid

Table 1. Purification protocol of acid invertase in the hypocotyls of *Phaseolus radiatus*[a]

Steps	Total protein (mg)	Total activity (U)	Specific activity (U mg^{-1} protein)	Purification (fold)	Yield (%)
Crude extract	647.46	605.69	0.94	1	100
50-70% ammonium sulfate precipitate	118.27	172.83	1.46	1.55	28.5
DEAE-cellulose chromatography	17.84	121.76	6.83	7.27	20.1
Con A chromatography	1.07	104.02	97.21	103.41	17.2
Sephacryl S-300 chromatography	0.63	87.55	138.97	147.84	14.5

a Unit (U) is defined as the formation of μmol of glucose from sucrose per min per mL of enzyme solution at 25℃ at pH 5.0.

[a]From about 500 g of elongating hypocotyls.

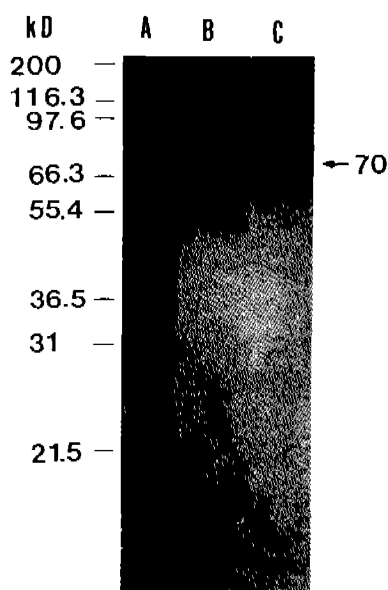

Fig. 1. SDS-PAGE of purification of soluble acid invertase (indicated by the arrow) in the hypocotyls of *Phaseolus radiatus*. The gel was stained by silver. Lane A, DEAE-cellulose chromatography step; Lane B, Con A affinity chromatography step; Lane C, Sephacryl S-300 chromatography step. The standard proteins used for SDS-PAGE were myosin (200 kD), galactosidase (116.3 kD), phosphorylase (97.4 kD), BSA (66.3 kD), glutamic dehydrogenase (55.4 kD), lactate dehydrogenase (36.5 kD), carbonic anhydrase (31 kD), trypsin inhibitor (21.5 kD), lysozyme (14.4 kD), apotinin (6 kD), and insulin B chain (3.5 kD).

invertase after chromatography on DEAE-cellulose column. The soluble acid invertase was eluted with NaCl gradient ranging from 0.05 to 0.1 M because the enzyme was weakly bound to DEAE-matrix. The chromatography was an effective step for removing impurities, since the most impurities were not bound and washed through the column with the buffer.

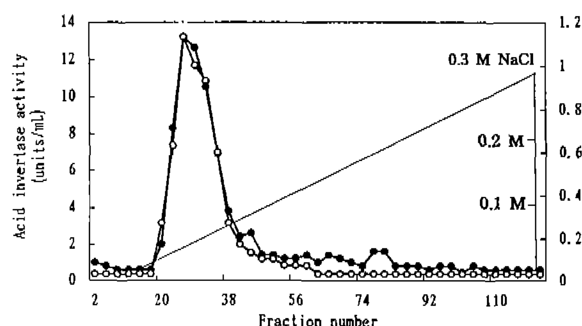

Fig. 2. DEAE-cellulose anion exchange chromatography of acid invertase in the hypocotyls of *Phaseolus radiatus*, with elution with a linear gradient from 0 to 0.3 M NaCl. Fractions (8 mL) were assayed for acid invertase activity (●) and protein content (○) by A_{280}.

The pooled fractions containing soluble acid invertase activity were subjected to Con A affinity chromatography (Fig. 3). Soluble acid invertase retained to Con A-Sepharose 4B was eluted as a single activity peak with 0.2 M methyl-α-D-mannopyranoside. Con A affinity chromatography, which alone induced about 103-fold purification, was a major step in the purification of the enzyme. Unbound proteins were reloaded onto Con A-Sepharose 4B column to ascertain column overloading, no further retaining occurred. Also, the mobility of the purified enzyme digested with endoglycosidase H was faster than that of the intact enzyme during SDS-PAGE (data not shown). From these result it is suggested that the soluble acid invertase is probably a glycoprotein and characteristically has N-linked high mannose oligosaccharide chains.

There were some contaminating proteins in the pooled Con A-Sepharose 4B fractions (Fig. 1). To remove the impurities the fractions were subjected

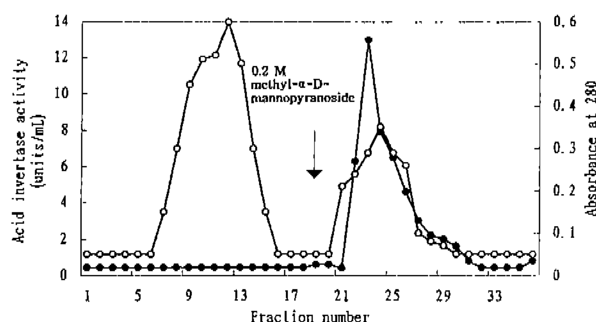

Fig. 3. Con A Sepharose affinity chromatography of acid invertase in the hypocotyls of *Phaseolus radiatus*, with elution with 0.2 M methyl-α-D-mannopyranoside. Fractions (5 mL) were assayed for acid invertase activity (●) and protein content (○) by A_{280}.

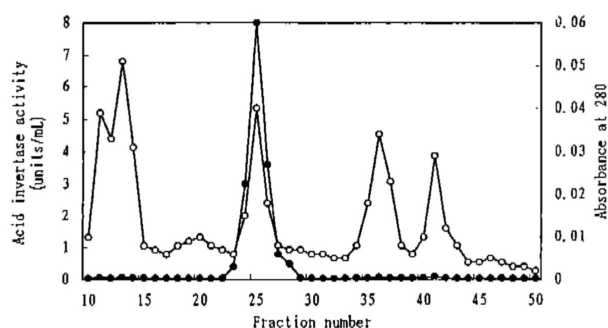

Fig. 4. Sephacryl S-300 chromatography of acid invertase in the hypocotyls of *Phaseolus radiatus*. Fractions (1 mL) were assayed for acid invertase activity (●) and protein content (○) by A_{280}.

to gel filtration on Sephacryl S-300 column (Fig. 4). The soluble acid invertase, which was finally separated from other proteins. represented a single protein as shown by SDS-PAGE (Fig. 1) and non-denaturing PAGE (data not shown).

Molecular weight determination

The native molecular weight determination by gel filtration on a Sephadex G-200 column with protein standards showed that purified soluble acid invertase had a molecular weight of about 70 kD (Fig. 5). SDS-PAGE of purified invertase gave a single band which estimated molecular weight of about 70 kD on the basis of their mobility relative to those of standard proteins (Fig. 1). These results indicate that the soluble acid invertase is composed of a single polypeptide chains, thus seems to be a monomer of 70 kD polypeptides.

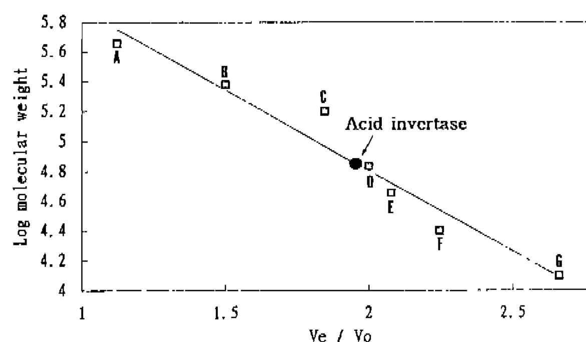

Fig. 5. Determination of molecular weight of native acid invertase by gel filtration in the hypocotyls of *Phaseolus radiatus*. Standard proteins (□) and acid invertase (●) eluted from Sephadex G-200 column. Fractions (1 mL) were assayed for acid invertase. A, ferritin (450 kD); B, catalase (240 kD); C, aldolase (158 kD); D, albumin from bovine serum (68 kD); E, albumin from hen egg (45 kD); F, chymotrypsinogen A (25 kD); G, cytochrome C (12.5 kD).

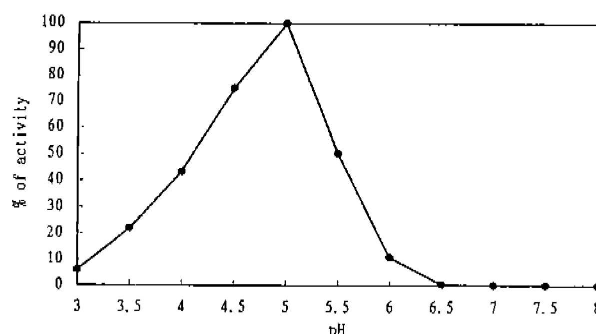

Fig. 6. The pH profile of acid invertase activities of *Phaseolus radiatus*. The buffers used included acetate (pH 3.0-5.0), sodium citrate (pH 5.5-6.5), and HEPES (pH 7.0-8.0). The reactions were carried out at 25℃ for 10 min.

pH and substrate specificity

The soluble acid invertase exhibited a pH optimum of about 5.0, as shown in some reports using soybean (Fig. 6). The purified enzyme activity versus sucrose concentration fit Michaelis-Menten kinetics and the Km values for sucrose obtained from Lineweaver-Burk double reciprocal plots was about 1.8 mM (Fig. 7). The enzyme was most active with sucrose whereas 40 mM raffinose and cellobiose as substrates caused the hexose release of 14 and 7%, respectively, compared to sucrose. No hydrolytic activity was detected with maltose and lactose (Table 2). Consequently, the soluble acid invertase seems to be

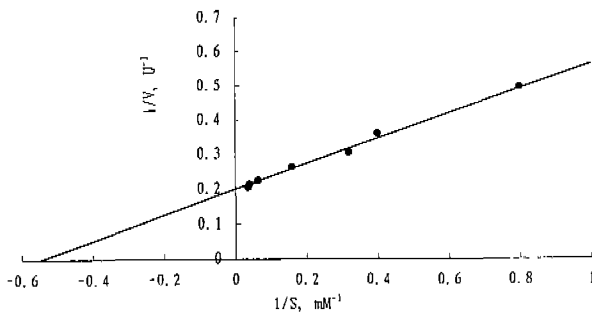

Fig. 7. Kinetics of partial purified acid invertase activities of *Phaseolus radiatus* versus sucrose concentration (3 to 30 mM).

Table 2. Substrate specificity of the purified soluble acid invertase from the hypocotyls of *Phaseolus radiatus*

Substrate	Invertase activity (% with sucrose)
Sucrose	100
Raffinose	14
Cellobiose	7
Maltose	0
Lactose	0

a β-fructofuranosidase on the basis of substrate specificity.

DISCUSSION

For the soluble acid invertase from mung bean hypocotyls consecutive purification protocol using DEAE-cellulose anion exchange, Con A affinity and Sephacryl S-300 chromatography were found to be effective, produced a homogeneous protein that was about 148-fold purified with 15% yield of the activity from crude extract (Table 1). In the purification step, Con A affinity chromatography showed a good increase in the specific activity (Table 1). Thus it is suggested that endogenous acid invertase inhibitor may be mainly dissociated with Con A affinity chromatography, as shown in some report on the potato invertase (Bracho and Whitaker, 1990). The finally purified soluble acid invertase exhibited a specific activity of about 139 μmol of glucose produced mg^{-1} protein min^{-1} at pH 5.0, which was higher than that of the coleoptiles of wheat (Krishnan *et al.*, 1985), the elongating stem of barley (Karuppiah *et al.*, 1989), and the hypocotyls of soybean (Chen and Black, 1992). Bracho and Whitaker (1990) suggested that the enzyme could represent the different value of specific activity from different sources, as well as even the same plants. After Sephacryl S-300 chromatography, the invertase showed a single band in SDS-PAGE (Fig. 1).

The native molecular weight of the purified soluble acid invertase was estimated to be about 70 kD by gel filtration using Sephadex G-200 column (Fig. 5). SDS-PAGE of the purified enzyme gave a single band with an estimated molecular weight of about 70 kD, indicating that the native enzyme was composed of a single polypeptide chain. Therefore, the soluble acid invertase from mung bean hypocotyls seems to be a monomer. The enzyme showed the diverse configuration of dimer in potato and soybean (Bracho and Whitaker, 1990; Chen and Black, 1992), tetramer in wheat (Krishnan *et al.*, 1985), and heptamer in *Ricinus communis* (Prado *et al.*, 1985). This fact agrees with previous reports for the purified acid invertase from washed discs of storage roots of red beet being a monomeric protein with the molecular weight of 65 kD (Milling *et al.*, 1993). The molecular weight of native soluble acid invertase from the hypocotyls of mung bean is similar to that from the leaves of *Ricinus communis*, 77.9 kD (Prado *et al.*, 1985) and the leaves of barley seedlings, 63 kD (Lee *et al.*, 1992). On the other hand, the subunit molecular weight of the enzyme obtained by SDS-PAGE is consistent with that from date palms, 70 kD (Al-Bakir and Whitaker, 1978) and bamboos, 70 kD (Cheng *et al.*, 1990). However, plant invertase exhibits surprising variability in their native molecular weight ranging from 48.5 kD for radish seedlings (Faye *et al.*, 1981) to 450 kD for *Lilium pollens* (Singh and Knox, 1984). The variability may be attributed to glycosylated nature of the enzyme or different degrees of polymerization of the enzyme subunits, although accurate reasons for the variability of the molecular weight are not clear yet.

The soluble acid invertase from the hypocotyls of mung bean may be a glycoprotein on the basis of its ability to bind to the immobilized Con A, as shown in several plants (Anderson and Ewing, 1978; Fahrendorf and Beck, 1990). Also this enzyme may contain N-linked high mannose oligosaccharide chains according to SDS-PAGE using the purified enzyme digested with endoglycosidase H. These results are in agreement with those from *Ricinus com-*

munis (Lauriere *et al.*, 1988). However, we could not describe whether the enzyme contained O-linked carbohydrate chains or not. Avigad (1982) proposed that the glycosylation of the enzyme would be necessary for its transportation.

The soluble acid invertase from mung bean had maximum activity around pH 5.0 (Fig. 6), as shown in the other group known as acid invertase, whose activity are maximum around this pH (Chen and Black, 1992). The response versus sucrose concentration of the enzyme fit Michaelis-Menten kinetics and the Km value for sucrose obtained from Lineweaver-Burk double reciprocal plots was about 1.8 mM (Fig. 7). This Km value was similarly low with that of acid invertase from *Lilium* pollens, 0.65 mM (Singh and Knox, 1984), and barley leaves, 1.0 and 0.7 mM (Obenland *et al.*, 1993). The enzyme was most active with sucrose as substrate, but the activity was slightly measured with raffinose and cellobiose. On the other hand, no activity was measured with maltose and lactose (Table 2). These results indicate that the enzyme is a β-fructofuranosidase. Because the physiological importance of these enzymes to metabolite the storage sucrose, further studies are needed on the purification of cell wall-bound and alkaline forms in the same tissues as well as their gene structure and their regulation mechanisms by environmental stimuli using antibody obtained from the purified soluble acid invertase.

ACKNOWLEDGEMENTS

This research was supported by a Genetic Engineering Research Grant (1994) from the Ministry of Education, Korea.

LITERATURE CITED

Avigad, G. 1982. Sucrose and other disaccharides. *In* Encyclopedia of Plant Physiology. F.A. Loewus and W. Tanner (eds.), Vol. 13A, Springer, Berlin, pp. 217-347.

ap-Rees, T. 1984. Sucrose metabolism. *In* Storage Carbohydrates in Vascular Plants. D.H. Lewis (ed.), Cambridge Univ. Press, London, pp. 53-73.

Anderson, R.S. and E.E. Ewing. 1978. Partial purification of potato tuber invertase and its proteinaceous inhibitor. *Phytochemistry* **17**: 1007-1018.

Al-bakir, A.Y. and J.R. Whitaker. 1978. Purification and properties of invertase from dates (*Phoenix dactylifera* L., var. Zahdi). *J. Food Biochem.* **2**: 132-160.

Ausubel, F.M., R. Bernt, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith and K. Struhl. 1987. Analysis of proteins. *In* Current Protocols in Molecular Biology. Green/Wiley-Interscience, New York, pp. 10.0.1-10.2.9.

Bergmeyer, H.U. and E. Bernt. 1974. D-Glucose. *In* Methods of Enzymatic Analysis. H. U. Bergmeyer (ed.), Vol. 2, Academic Press, New York, pp. 1205-1212.

Bracho, G.E. and J.R. Whitaker. 1990. Purification and partial characterization of potato (*Solanum tuberosum*) invertase and its endogenous proteinaceous inhibitor. *Plant physiol.* **92**: 386-394.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**: 248-254.

Chen, J.Q. and C.C. Black. 1992. Biochemical and immunological properties of alkaline invertase isolated from sprouting soybean hypocotyls. *Arch. Biochem. Biophys.* **296**: 1-9.

Cheng, J.F., N. Mitsuya, R.H. Juang and H.Y. Sung. 1990. Purification and characterization of invertase isozymes from bamboo shoots. *Biochem. international* **21**: 497-506.

Chin, C.K. and G.D. Weston. 1973. Distribution in excised *Lycopersicum esculentum* roots of the principal enzymes involved in sucrose metabolism. *Phytochemistry* **12**: 1229-1236.

Eshrich, T. 1980. Free space invertase, its possible role and in phloem unloading. *Ber. Deutsch. Bot. Ges.* **93**: 363-378.

Fahrendorf, T. and E. Beck. 1990. Cytosolic and cell-wall-bound acid invertases from leaves of *Urtica dioica* L.: a comparison. *Planta* **180**: 237-244.

Faye, L., C. Berjonneau and P. Rollin. 1981. Studies on β-fructosidase from radish seedlings. I. Purification and partial characterization. *Plant Sci. Lett.* **22**: 77-87.

Giaquinta, R.T., W. Rin, N.L. Sadler and V.R. Franceschi. 1983. Pathway of phloem unloading of sucrose in corn roots. *Plant Physiol.* **72**: 362-367.

Ho, L.C. 1984. Partitioning of assimilates in fruiting tomato plants. *Plant Growth Regula.* **2**: 277-285.

Iwatsubo, T., H. Nakagawa, N. Ogura, T. Hirabayashi and T. Sato. 1992. Acid invertase of melon fruits: Immunochemical detection of acid invertases. *Plant Cell Physiol.* **33**: 1127-1133.

Karuppiah, N., B. Vadlamudi and P.B. Kaufman. 1989. Purification and characterization of soluble (cytosolic) and bound (cell wall) isoforms of invertases in barley (*Hordeum vulgare*) elongating stem tissue. *Plant Physiol.* **91**: 993-998.

Krishnan, H.B., J.T. Blanchette and T.W. Okita. 1985. Wheat invertases. *Plant Physiol.* **78**: 241-245.

Krishnan, H.B. and S.G. Pueppke. 1990. Cherry fruit invertase: Partial purification, characterization and activity during fruit development. *J. Plant Physiol.* **135**: 662-666.

Lauriere, C., M. Lauriere, A. Sturm, L. Faye and M.J. Chrispeels. 1988. Characterization of β-fructosidase, an ex-

tracellular glycoprotein of carrot cells. *Biochimie* **70**: 1483-1491.

Lee, M.A., J.S. Maeng, S.G. Kim and Y.M. Kwon. 1992. Changes of sucrose content and invertase activity in leaves of barley seedlings under low temperature. *Korean J. Bot.* **35**: 91-95.

Leigh, R.A., T. ap-Rees, W.A. Fuller and J. Banfield. 1979. The location of acid invertase activity and sucrose in the vacuoles of storage root of red beet (*Beta vulgaris*). *Biochem. J.* **178**: 539-547.

Matsushita, K. and I. Uritani. 1974. Changes in invertase activity of sweet potato in response to wounding and purification and properties of its invertases. *Plant Physiol.* **54**: 60-65.

Milling, R.J., J.L. Hall and R.A. Leigh. 1993. Purification of an acid invertase from washed discs of storage roots of red beet (*Beta vulgaris* L.). J. Exp. Bot. **44**: 1679-1686.

Obenland, D.M., U. Simmen, T. Boller and A. Wiemken. 1993. Purification and characterization of three soluble invertases from barley (*Hordeum vulgare* L.) leaves. *Plant Physiol.* **101**: 1331-1339.

Prado, F.E., M.A. Vattuone, O.L. Fleischmacher and A.R. Sampietro. 1985. Purification and characterization of *Ricinus communis* invertase. *J. Biol. Chem.* **260**: 4952-4957.

Ricardo, C.P.P. and T. ap-Rees. 1970. Invertase activity during the development of carrot roots. *Phytochemistry* **9**: 239-247.

Singh, M.B. and R.B. Knox. 1984. Invertases of *Lilium* pollen. *Plant Physiol.* **74**: 510-515.

Sturm, A. and M.J. Chrispeels. 1990. cDNA cloning of carrot extracellular β-fructosidase and its expression in response to wounding and bacterial infection. *Plant Cell* **2**: 1107-1119.

Sum, W.F., P.J. Rogers, I.O. Jenkins and R.D. Guthrie. 1980. Isolation of invertase from banana fruit (*Musa cavendishii*). *Phytochemistry* **19**: 399-401.

Walker, R.P. and C.J. Pollock. 1993. The purification and characterization of soluble acid invertase from coleoptile of wheat (*Triticum aestivum* L. cv. Avalon). *J. Exp. Bot.* **44**: 1029-1037.

(Received July 18, 1995)

녹두의 하배축에서 분리한 Soluble Acid Invertase의 정제와 특성

李 動 熙 · 金 永 相*

釜山大學校 自然科學大學 生物學科

적 요

녹두(*Phaseolus radiatus* L.)의 하배축에서 분리한 soluble acid invertase를 DEAE-cellulose 음이온교환, Con A 친화성, 그리고 Sephacryl S-300 크로마토그라피의 순서로 정제하였다. 본 효소는 정제과정을 통하여 약 15%의 회수율과 약 148배의 정제배율로 정제되었다. 정제된 효소는 pH 5.0에서 약 139 μmol of glucose produced mg^{-1} protein min^{-1}의 specific activity를 가졌으며, SDS-PAGE와 nondenaturing PAGE로 조사한 결과 단일 밴드를 가졌다. 본 효소의 분자량은 70 kD이었고, 한 개의 소단위로 구성된 monomer인 것으로 추측된다. 한편 본 효소가 Con A 칼럼에 흡착되는 것으로 보아 이 단백질은 당단백질이며 많은 mannose를 함유한 N-linked oligosaccharide 사슬을 가지는 것으로 보인다. 서당에 대한 Km은 1.8 mM이고 최적활성 pH는 5.0이었다. sucrose, raffinose, cellobiose, maltose 그리고 lactose 등의 여러 가지 기질에 대한 특이성을 조사해 본 결과, 본 효소는 sucrose에 대하여 최적활성을 나타내었다. 따라서 본 효소는 β-fructofuranosidase인 것으로 추정된다.

주요어 : soluble acid invertase, 정제, 녹두, 하배축

*교신저자: Fax (051) 581-2962