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

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The alkaline 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 by consecutive step using diethylaminoethyl (DEAE)-cellulose anion exchange, 1st Sephadex G-200, DEAE-Sephadex A50 and 2nd Sephadex G-200 chromatography. The overall purification was about 77-fold with a yield of about 6%. The finally purified enzyme exhibited a specific activity of about 48 μmol of glucose produced mg^{-1} protein min^{-1} at pH 7.0 and appeared to be a single protein by nondenaturing polyacrylamide gel electrophoresis (PAGE). The enzyme had the native molecular weight of 450 kD and subunits molecular weight of 63 kD and 38 kD as estimated by Sephadex G-200 chromatography and SDS-PAGE, respectively, suggesting that the enzyme is a heteromultimeric protein composed of two types of subunits. On the other hand, the enzyme appeared to be not a glycoprotein according to the results of Con A chromatography and glycoprotein staining. The enzyme had a Km for sucrose of 19.7 mM at pH 7.0 and maximum activity around pH 7.5. The enzyme was most active with sucrose as substrate, compared to raffinose, cellobiose, maltose and lactose. These results indicate the alkaline invertase is a β-fructofuranosidase.

Keywords : alkaline invertase, purification, mung bean, hypocotyls

Sucrose, the major carbohydrate produced by photosynthesis, is principal form of the carbon translocation and storage compound in higher plants. It is synthesized in the cytosol of photosynthetic cells, transiently stored in the vacuole, and translocated via phloem to nonphotosynthetic cells. Invertases (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26) irreversibly catalyze the hydrolysis of sucrose into glucose and fructose available for energy and carbon requirements of plant cells (ap-Rees, 1984; Sturm and Chrispeels, 1990). Lauriere *et al.* (1988) have proposed that the transportation of invertase, synthesized on the rough endoplamic reticulum (ER) of cytosol of source organs, are regulated by Golgi complex. Invertases occur multiple forms in plant cells based on the pH required for maximum activity and their subcellular locations. According to their pH optima, the enzymes are divided into acid (pH 4.0-5.0) and alkaline (pH 7.0-8.0) forms. Acid invertase can be subdivided into extra- and intracellular forms according to its subcellular location (Chin and Western, 1973; Matsushita and Uritani, 1974). Intracellular acid invertase may participate in facilitating sucrose uptake concomitant with its hydrolysis as well as in enhancing sink strength of developing plant organs (Ho, 1984; Stommel and Simon, 1990). Extracellular acid invertase bound to cell wall ionically results in maintaining a steep sucrose concentration gradient between source and sink organs in phloem unloading (Fahrendorf and Beck, 1990; Sturm and Chrispeels, 1990).

Alkaline invertase known to be a cytoplamic enzyme occurs in many plant tissues, particularly mature organs (Ricardo and ap-Rees, 1970; Avigad, 1982), and it also coexists with acid invertase in some tissues such as cultured carrot cells (Stommel and Simon, 1990), sugar beet cells (Masuda *et al.*,

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1988) and root tissues of sweet potato (Matsushita and Uritani, 1974). Ricardo (1974) has proposed that alkaline invertase is more important for breakdown of sucrose in mature tissues in which the activity of acid invertase is significantly low. To clarify the biochemical properties of alkaline invertase, the enzyme has been partially purified in some plants, for example, infected soybean nodules (Morell and Copeland, 1984), sugar beet cells (Masuda *et al.*, 1988), and cultured carrot cells (Stommel and Simon, 1990). The purification of invertase, however, is difficult because of high lability of its biochemical characteristics. Recently, alkaline invertase has been successfully purified in soybean hypocotyls (Chen and Black, 1992). Therefore, we performed the isolation and extensive purification of alkaline invertase from the hypocotyls of mung bean (*Phaseolus radiatus*) to understand the biochemical regulation of sucrose metabolism.

In the present report, we describe the purification and biochemical characterization of alkaline invertase from the hypocotyls of mung bean as well as a comparison of the biochemical properties of alkaline invertase and acid invertase extensively purified in the same plant tissues.

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 seedings were used for experimental materials.

Chemicals and assay enzymes were obtained from Sigma and Boehringer Chemicals Co. Reagents for electrophoresis and chromatography such as DEAE-cellulose, Sephadex G-200, DEAE-Sephadex A50 and Con A-Sepharose 4B chromatography were purchased from Phamacia and 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 Mg-acetate 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 the hydrolysis of sucrose. For the assay of alkaline invertase, 1.6 mL aliquot of reaction mixtures containing 50 mM HEPES buffer, pH 7.0, 1 mM Mg-acetate, 100 mM sucrose and a suitable amount of enzyme solutions was incubated for 60 min at 25℃, and boiled for 3-5 min to cease reaction. The amount of glucose formed was measured by the modified glucose oxidase-peroxidase method (Bermeyer and Bernt, 1974). A 0.8 mL of glucose oxidase-peroxidase mixture consisted of 50 mM HEPES, pH 7.0, 0.8 unit of glucose oxidase, 0.8 unit of peroxidase and 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. A unit is defined as the formation of 1 μmol glucose from sucrose per min per 1 mL of enzyme solution at 25℃ at pH 7.0 for alkaline invertase.

The amount of protein was determined according to the Bradford method (1976) 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 1 g of 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 alkaline invertase by ammonium sulfate precipitation

Crude extract, which was prepared from about 500 g of mung bean hypocotyls, was precipitated from 20 to 40% saturation with enzyme grade ammonium sulfate powder. The precipitates were collected after centrifugation, and dissolved in buffer B, then dialy-

zed overnight against the same buffer. At each purification step, protein purity was examined by SDS-PAGE.

DEAE-cellulose chromatography

About 15 mL of the dialyzed enzyme solution was 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 1.0 M NaCl in buffer B at a flow rate of 0.8 mL/min. 8 mL fractions, which showed activity peak at pH 7.0, were precipitated to 40% saturation with ammonium sulfate, and then dissolved in buffer C. The precipitates were dialyzed against the same buffer or desalted with a Sephadex G-25 column.

1st Sephadex G-200 chromatography

About 7 mL of enzyme solution obtained from DEAE-cellulose chromatography was loaded carefully onto top of Sephadex G-200 column (2.0×85 cm) previously equilibrated with buffer C. The column was run at a flow rate of 0.2 mL/min. 4 mL fractions, which showed activity peak at pH 7.0, were pooled and concentrated by Amicon ultracentrifugation (Amicon Diaflo ultrafiltration membranes, 10 XM50, 43 mm).

DEAE-Sephadex A50 chromatography

About 5 mL of concentrated enzyme solution obtained from 1st Sephadex G-200 chromatography was subjected to the DEAE-Sephadex A50 column (3×15 cm) preequilibrated preequilibrated with buffer C. The column was washed with the same buffer to remove impurities, and then eluted to a 400 mL linear gradient, 0 to 1.0 M NaCl in buffer C at a flow rate of 0.5 mL/min. 5 mL fractions, which occurred activity peak at pH 7.0, were precipitated to 40% saturation with ammonium sulfate, and then dissolved in buffer C. The precipitates were desalted with a Sephadex G-25 column and concentrated by Amicon ultracentrifugation.

2nd Sephadex G-200 chromatography

About 1 mL of enzyme solution obtained from DEAE-Sephadex A50 chromatography was loaded carefully onto 2nd Sephadex G-200 column (2.0×85 cm) preequilibrated with buffer C. The column was run at a flow rate of 0.1 mL/min. 2 mL fractions with alkaline invertase activity were pooled and concentrated by Amicon ultracentrifugation.

Con A-Sepharose 4B chromatography

1 mg (1 mL) of purified enzyme obtained from 2nd Sephadex G-200 chromatography was applied to the Con A-Sepharose 4B column (1.5×8 cm) previously equilibrated with buffer C. The column was washed with the same buffer until the A280 was decreased to minimum level, and then eluted with 0.2 M methyl-α-D-mannopyranoside in buffer C at a flow rate of 0.5 mL/min. Samples around A_{280} peak fractions in both wash and elution fractions were assayed for alkaline invertase activity.

Native molecular weight estimation

The native molecular weight of the purified enzyme was determined by using Sephadex G-200 chromatography (2.0×85 cm). 150 μg of 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 alkaline 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 were myosin (200 kD), galactosidase (116.3 kD), phoshorylase (97.4 kD), BSA (66.3 kD), glutamic dehydrogenase (31 kD), trypsin inhibitor (21.5 kD), lysozyme (14.4 kD), apotinin (6 kD) and insulin B chain (3.5 kD).

Glycoprotein staining of SDS-PAGE gels was performed by the periodic acid-Schiff method (Segresi and Jackson, 1973).

Nondenaturing PAGE was performed in a similar way to SDS-PAGE except that SDS and β-mercaptoethanol were not added to the gels. Proteins were separated on a 8% resolving gel at 100 V.

RESULTS

Purification of alkaline invertase

The protocol for the purification of alkaline invertase from mung bean hypocotyls is summarized in Table 1. Both alkaline and acid invertases occurred in crude soluble extracts of mung bean hypocotyls. Alkaline invertase could be separated from acid invertase by ammonium sulfate fractionation. The enzyme activity measured at pH 7.0 mainly exhibited from 20 to 40% ammonium sulfate saturation while the activity measured at pH 5.0 mainly exhibited from 50 to 70% ammonium sulfate saturation. The similar separations have achieved in other plant tissues such as soybean hypocotyls (Chen and Black, 1992). Using the 20-40% ammonium sulfate precipitates from mung bean hypocotyls, alkaline invertase was purified by consecutive step using DEAE-cellulose anion exchange, first Sephadex G-200, DEAE-Sephadex A50 and second Sephadex G-200 chromatography. The overall purification of alkaline invertase was about 77-fold with a yield of about 6% from crude extracts (Table 1). The finally purified enzyme had a specific activity of approximately 48 μmol of glucose produced mg^{-1} protein min^{-1} at pH 7.0, and showed a single band in nondenaturing PAGE as indicated by silver staining (Fig. 1A). The purified protein was composed of two types of subunits by SDS-PAGE (Fig. 1B).

The elution profile of alkaline invertase after chromatography on DEAE-cellulose anion exchange

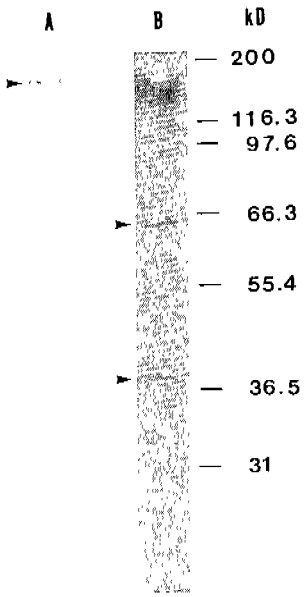

Fig. 1. Electrophoretic proof of the purified alkaline invertase from the hypocotyls of *P. radiatus*. (A) Nondenaturing PAGE of the purified alkaline invertase (indicated by the arrow) obtained from 2nd Sephadex G-200 chromatography. The gel was stained by Coomassie brilliant blue. (B) SDS-PAGE of the purified alkaline invertase (indicated by the arrow) obtained from 2nd Sephadex G-200 chromatography. The gel was stained by silver. 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).

Table 1. Purification protocol of alkaline invertase in the hypocotyls of *P. radiatus*[a]

Steps	Total protein (mg)	Total activity (U)	Specificactivity (U mg^{-1} protein)	Purification (fold)	Yield (%)
Crude extract	631	390	0.62	1	100
20-40% ammonium sulfate precipitate	80.3	139	1.73	2.8	35.6
DEAE-cellulose chromatography	31.5	89	2.83	4.6	22.8
1st Sephadex G-200 chromatography	12.6	51	4.05	6.5	13.1
DEAE-Sephadex chromatography	1.7	28.9	17	27.4	7.4
2nd Sephadex G-200 chromatography	0.48	22.9	47.71	77	5.9

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 7.0.

[a]From about 500 g of elongating hypocotyls.

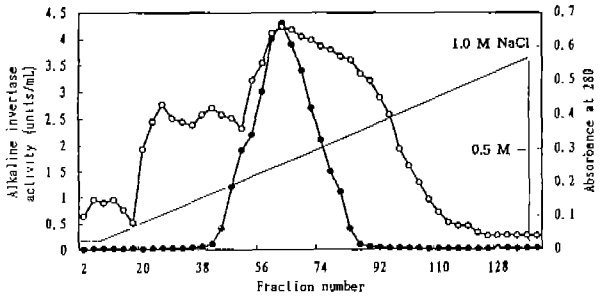

Fig. 2. DEAE-cellulose anion exchange chromatography of alkaline invertase in the hypocotyls of *P. radiatus*, with elution with a linear gradient from 0 to 1.0 M NaCl at a flow rate of 0.8 mL/min. Fractions (8 mL) were assayed for alkaline invertase activity (-●-) and protein content (-○-) by A_{280}.

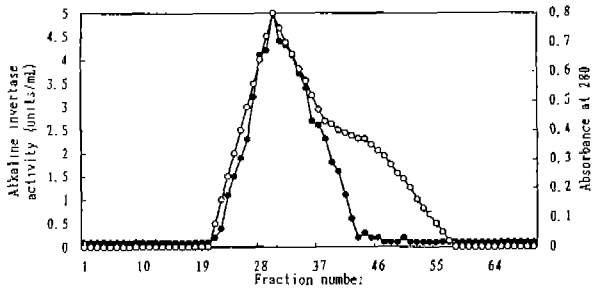

Fig. 3. 1st Sephadex G-200 chromatography of alkaline invertase in the hypocotyls of *P. radiatus*, with elution at a flow rate of 0.2 mL/min. Fractions (4 mL) were assayed for alkaline invertase activity (-●-) and protein content (-○-) by A_{280}.

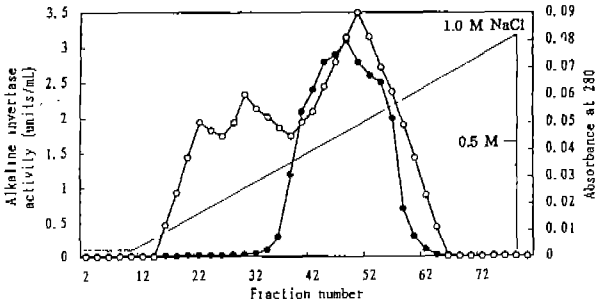

Fig. 4. DEAE-Sephadex A50 anion exchange chromatography of alkaline invertase in the hypocotyls of *P. radiatus*, with elution with a linear gradient from 0 to 1.0 M NaCl at a flow rate of 0.5 mL/min. Fractions (5 mL) were assayed for alkaline invertase activity (-●-) and protein content (-○-) by A_{280}.

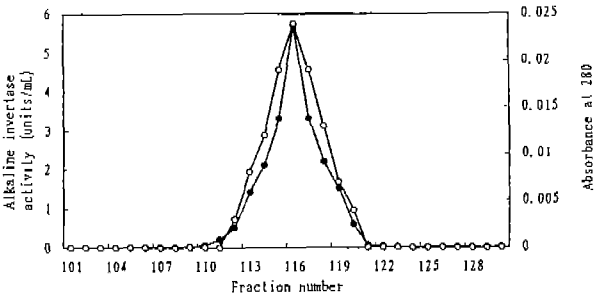

Fig. 5. 2nd Sephadex G-200 chromatography of alkaline invertase in the hypocotyls of *P. radiatus*, with elution at a flow rate of 0.1 mL/min. Fractions (4 mL) were assayed for alkaline invertase activity (-●-) and protein content (-○-) by A_{280}.

column is shown in Fig. 2. The alkaline invertase was bound more tightly to DEAE matrix, as compared to the acid invertase, and the enzyme was eluted with NaCl gradient ranging from 0.30-0.55 M. The chromatography was an effective step to remove contaminating proteins, because the contaminating proteins were weakly bound to DEAE matrix and washed through the column with buffer B.

The pooled fractions containing alkaline invertase activity were precipitated to 40% saturation with ammonium sulfate, and then the precipitates desalted with Sephadex G-25 column were subjected to gel filtration on Sephadex G-200 column (Fig. 3). Gel filtration on Sephadex G-200 column resulted in small increase in purification with good yield of the alkaline invertase activity.

The fractions with alkaline invertase activity obtained from Sephadex G-200 chromatography were loaded onto DEAE-Sephadex A50 column. The alkaline invertase was eluted with NaCl gradient ranging from 0.40-0.55 M (Fig. 4). The DEAE-Sephadex A50 anion exchange chromatogrphy, which induced about 27.4-fold purification with 7.4% of activity recovery overall, was a major step in the purification of the enzyme.

There were some impurities in the pooled DEAE-Sephadex A50 fractions. To remove the impurities the fractions were subjected to the second gel filtration on Sephadex G-200 column (Fig. 5). The chromatography increased the purification to 77-fold with a yield of about 6%. The alkaline invertase, which was finally separated from impurities, represented a single protein as shown by nondenaturing PAGE (Fig. 1A).

Molecular weight determination

The native molecular weight determination by gel

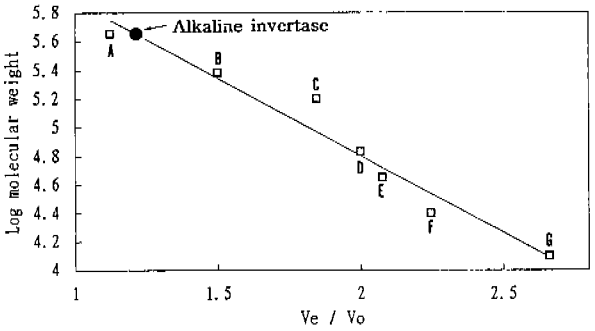

Fig. 6. Determination of molecular weight of native alkaline invertase by gel filtration in the hypocotyls of *P. radiatus*. Standard proteins (□) and alkaline invertase (●) eluted from Sephadex G-200 column at a flow rate of 0.1 mL /min. Fractions (1 mL) were assayed for alkaline invertase. A, ferritin (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).

filtration on a Sephadex G-200 column with protein standards showed that purified alkaline invertase had a molecular weight of about 450 kD (Fig. 6). SDS-PAGE of purified alkaline invertase gave two bands which estimated molecular weight of about 63 kD and 38 kD on the basis of their mobility relative to those of standard proteins (Fig. 1B). These results indicate that the alkaline invertase is composed of two types of subunits, thus seems to be a heteromultimeric protein of 63 kD and 38 kD polypeptides.

Glycoprotein status of alkaline invertase

To elucidate glycosylation status of alkaline invertase, we performed glycoprotein staining of purified alkaline invertase from mung bean hypocotyls. The enzyme was not stained by the periodic acid-Schiff stain (data not shown). Based on Con A, a lectin that binds specifically to α-D-glucose residues of the carbohydrate chains, we also performed affinity chromatogrphy on Con A-Sepharose 4B. But alkaline invertase was not bound to Con A in contrast to soluble acid invertase (data not shown) (Lee and Kim, 1995). Therefore, it is suggested that alkaline invertase from mung bean hypocotyls is not a glycoprotein.

pH and substrate specificity

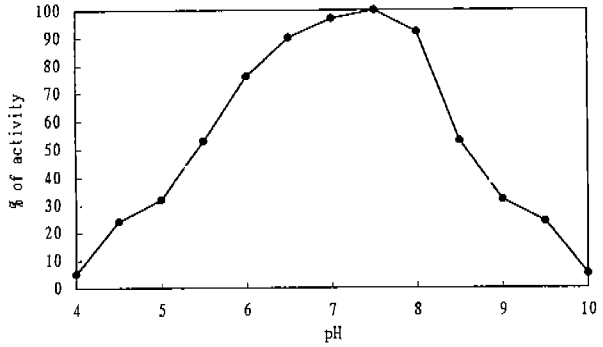

Fig. 7. The pH profile of alkaline invertase activities of *P. radiatus*. The buffers used included acetate (pH 4.0-5.0), sodium citrate (pH 5.5-6.5), HEPES (pH 7.0-8.0) and glycine (pH 8.5-10). The reactions were carried out at 25℃ for 60 min.

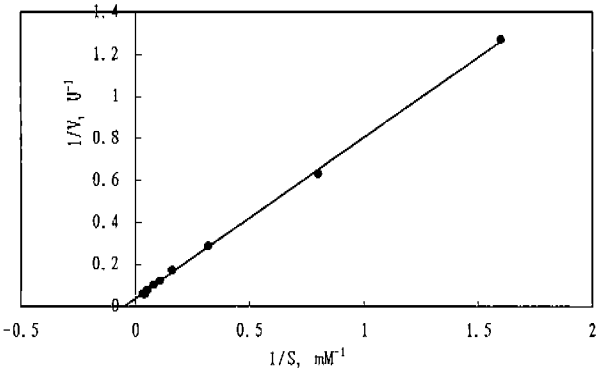

Fig. 8. Kinetics of purified alkaline invertase activities of *P. radiatus* versus sucrose concentration (3 to 30 mM) obtained from Lineweaver-Burk double reciprocal plots. The reactions were carried out for 60 min at 25℃, pH 7.0.

The alkaline invertase exhibited maximum activity at pH 7.5, as shown in some plants such as suspension cultured carrot cells (Stommel and Simon, 1990) (Fig. 7). The purified enzyme activity for sucrose concentration fit Michaelis-Menten kinetics and the Km value for sucrose obtained from Lineweaver-Burk double reciprocal plots was about 19.7 mM (Fig. 8). The enzyme was most active with sucrose compared to raffinose, cellobios, lactose and maltose. On the other hand, it had a little enzyme activity with raffinose and cellobiose, and no hydrolytic activity was detected with lactose and maltose (Table 2). Therefore, the alkaline invertase seems to be a β-fructofuranosidase having the same substrate specificity with soluble acid invertase (Lee and Kim, 1995).

Table 2. Substrate specificity of the purified alkaline invertase from the hypocotyls of *P. radiatus*

Substrate	Invertase activity (% with sucrose)
Sucrose	100
Raffinose	16
Cellobiose	19
Maltose	0
Lactose	0

DISCUSSION

Both alkaline and acid invertase coexisted in crude soluble extracts of mung bean hypocotyls, and the activity of alkaline invertase was lower than that of acid invertase. To separate these two enzymes, alkaline invertase was fractionated with ammonium sulfate ranging from 20-40%, while acid invertase was fractionated with ammonium sulfate ranging from 50-70%. The similar separations have achieved in other plant tissues such as soybean hypocotyls and mature sugar beet leaves (Masuda *et al.*, 1988; Chen and Black, 1992). Alkaline invertase was subsequently purified by consecutive step of DEAE-cellulose anion exchange, first Sephadex G-200, DEAE-Sephadex A50 anion exchange and second Sephadex G-200 chromatography. The overall purification of alkaline invertase was about 77-fold with a yield of about 6% from starting materials (Table 1). Second Sephadex G-200 chromatography exhibited a good increase in the specific activity, suggested that the chromatography would be an effective step in removal of an endogeneous alkaline invertase inhibitor. The results are similar to prior reports obtained from potato invertase (Bracho and Whitaker, 1990). The finally purified alkaline invertase exhibited a specific activity of about 48 μmol of glucose produced mg^{-1} protein min^{-1} at pH 7.0, which was comparable to that of soybean hypocotyls alkaline invertase (Chen and Black, 1992). The specific activity was higher than that of soybean nodules (Morell and Copeland, 1984), sugar beet leaves (Masuda *et al.*, 1988) and carrot culture cells (Stommel and Simon, 1990). But the specific activity of the enzyme was significantly lower than that of acid invertase purified in the same plant tissues (Lee and Kim, 1995). After second Sephadex G-200 chromatogrphy, alkaline invertase showed a single band in nondenaturing PAGE (Fig. 1A).

The purified alkaline invertase had a native molecular weight of about 450 kD by gel filtration on Sephadex G-200 column (Fig. 6). SDS-PAGE of the enzyme gave two bands of about 63 kD and 38 kD on the basis of their mobility relative to those of standard proteins (Fig. 1B), indicating that alkaline invertase of mung bean hypocotyls was composed of two types of subunits. Therefore, it is suggested that the alkaline invertase may be a heteromer. The results are consistent with that the invertase from washed discs of strorage roots of red beet is composed of two types of polypeptides of 65 kD and 46 kD (Milling *et al.*, 1993), althrough the enzyme is composed of single type of subunits in many plant tissues, for example, a monomer in barley leaves (Obenland *et al.*, 1993), a dimer in soybean hypocotyls (Chen and Black, 1992), a tetramer in wheat coleoptiles and a heptamer in *Ricinus communis* (Prado *et al.*, 1985). The native molecular weight of alkaline invertase from mung bean hypocotyls is similar to that from *Lilium* pollens, 450 kD (Singh and Knox, 1984). However, plant invertase showed comparable variability in their native molecular weight ranging from 48.5 kD for radish seedlings (Fay *et al.*, 1881) to 750 kD for maize kernels (Doehlert and Felker, 1987). The variability may be attributed to different degrees of oligomerization of the enzyme subunits, althrough accurate reasons for the variability of the molecular weight are not clear yet. On the other hand, the subunits molecular weight of the enzyme obtained from SDS-PAGE is consistant with that from carrot cells, 63 kD (Sturm and Chrispeels, 1990), and it is also comparable to that from barley leaves, 64 kD (Obenland *et al.*, 1993), that from the storage roots of red beet, 65 kD (Milling *et al.*, 1993) and that from potato tubers, 30 kD (Bracho and Whitaker, 1990). It is apparent that further researches should be directed toward an understanding of accurate molecular configuration of alkaline invertase from mung bean hypocotyls.

Soluble acid invertase is shown to be glycoproteins in most plants (Anderson and Ewing, 1978; Fahrendorf and Beck, 1990). Avigad (1982) has proposed that the glycosylation of the enzyme would be necessary for its transportation. To elucidate glycosylation status of alkaline invertase we performed

glycoprotein staining and Con A-Sepharose 4B chromatography. Our finding that alkaline invertase of mung bean hypocotyls is likely not a glycoprotein in contrast to acid invertase, is in general agreement with prior report obtained from the study using soybean hypocotyls (Chen and Black, 1992).

The alkaline invertase from mung bean hypocotyls had maximum activity around pH 7.5 (Fig. 7). The pH optimum is consistent with that of suspension cultured carrot cells (Stommel and Simon, 1990), and it is comparable to that of soybean nodules, pH 7.6 (Morell and Copeland, 1984), and that of soybean hypocotyls, pH 7.0 (Chen and Black, 1992). The purified enzyme activity response versus sucrose concentration fit Michaelis-Menten kinetics, and the enzyme showed a monophasic curve in Lineweaver-Burk double reciprocal plots (Fig. 8). The monophasic curve in double reciprocal plots has been also showed in the enzyme of soybean nodules and soybean hypocotyls (Morell and Copeland, 1984; Chen and Black, 1992). In contrast, it has been reported that the alkaline invertase from sweet potato displays non-Michaelis-Menten kinetics in the activity response to sucrose concentration, thus the enzyme exhibits a biphasic curve in double reciprocal plots (Matsushita and Uritani, 1974). Chen and Black (1992) has proposed that the enzyme shows a biphasic kinetics because of the impurity of enzyme preparation. Alkaline invertase from mung bean hypocotyls had a Km for sucrose of about 19.7 mM. The value is similar to that of alkaline invertase obtained from sweet potato, 16 mM (Matsushita and Uritani, 1974), but higher than those of the enzyme from soybean nodules and soybean hypocotyls, 10 mM (Morell and Copeland, 1984; Chen and Black, 1992). It has been reported that the Km values are in the range from 10 mM to 65 mM for alkaline invertase (Copeland, 1990). The enzyme was more specific for sucrose with less activity for raffinose and cellobiose, and with no activity for maltose and lactose (Table 2). Therefore, the enzyme seems to be a β-fructofuranosidase having the same substrate specificity with soluble acid invertase purified in the same plant tissues (Lee and Kim, 1995). It will be great interest to elucidate relations between multiple forms of invertase, and their gene structure and regulation mechanism of sucrose metabolism. Therefore, further studies are needed on the purification of cell wall-bound forms in the same tissues as well as their regulation mechanisms by environmental stimuli using antibody obtained from the purified alkaline invertase.

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綠豆의 下胚軸에서 分離한 Alkaline Invertase의 정제와 特性

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녹두(*Phaseolus radiatus* L.)의 하배축에서 분리한 alkaline invertase를 DEAE-cellulose 음이온교환, 1차 Sephadex G-200, DEAE-Sephadex A50, 그리고 2차 Sephadex G-200 크로마토그라피의 순서로 정제하였다. 본 효소는 정제과정을 통하여 약 6%의 회수율과 약 77배의 정제배율로 정제되었다. 정제된 효소는 pH 7.0에서 약 48 μmol of glucose produced mg^{-1} protein min^{-1}의 specific activity를 가졌으며, nondenaturing PAGE로 조사한 결과 단일 밴드를 가졌다. 본 효소의 분자량은 450 kD이었고, SDS-PAGE로 조사한 결과 두 종류의 소단위의 분자량은 63 kD와 38 kD이었다. 따라서 본 효소는 heteromultimeric protein인 것으로 추측된다. 한편 본 효소가 Con A 칼럼에 흡착되지 않으며 당단백질 염색이 되지 않는 것으로 보아 당단백질이 아닌 것으로 보인다. 서당에 대한 Km은 19.7 mM이고 최적활성 pH는 7.5이었다. Sucrose, raffinose, cellobiose, maltose 그리고 lactose 등 여러 가지 기질에 대한 특이성을 조사해 본 결과, 본 효소는 sucrose에 대하여 최적 활성을 나타내었다. 따라서 본 효소는 β-fructofuranosidase인 것으로 추측된다.

주요어 : alkaline invertase, 정제, 녹두, 하배축

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