J. Food Sci. Nutr.
Vol. 3, No. 2, p. 152~156 (1998)

Partial Purification and Characterization of a Soluble β-Fructofuranosidase from Onion (*Allium cepa*)

Yong-Eok Lee and Jin-Young Yoo*†

Dept. of Biochemistry, Dongguk University, Kyungju 780-714, Korea
**Div. of Food Biochemistry and Biotechnology, Korea Food Research Institute, Kyonggido 463-600, Korea*

Abstract

A fructan-degrading enzyme was partially purified from onion (*Allium cepa*) bulbs by a combination of ammonium sulfate precipitation, concanavalin-A affinity chromatography, and ion-exchange and gel-filtration chromatography. The enzyme hydrolyzed sucrose more effectively than inulin and was identified as a β-fructofuranosidase (invertase). The optimum pH and temperature were pH 5.5 and 35°C, respectively. The enzyme hydrolyzed sucrose with a K_m of 1.2 mM. The soluble β-fructofuranosidase is likely glycoprotein based on its ability to bind the lectin concanavalin-A. The enzyme was heatlabile, with most activity being lost at 50°C in 1 hr of incubation. The onion β-fructofuranosidase was partially inhibited by $ZnCl_2$, $HgCl_2$ and $CuSO_4$.

Key words: onion, purification, β-fructofuranosidase

INTRODUCTION

Inulin belongs to a class of fructose-based, highly soluble polysaccharides collectively called fructans. Fructans are the major non-structural carbohydrates in many plant species, particularly in the prevalent and evolutionarily advanced orders of *Asterales*, *Liliales* and *Poales* (e.g. chicory, onions, wheat) (1). Fructans are deposited in vacuoles and play an important role as carbohydrate reserves in addition to or as an alternative to starch (2, 3). They are also involved in osmoregulation (4) and are believed to function as protectants against drought and cold stress (1,5,6). Enzymatic hydrolysis of fructans has been found to yield syrups with a high fructose content which are suitable for further uses (7,8). There is a great potential for the use of fructans as a raw material in a number of interesting food and non-food applications (9,10). Despite the widespread occurrence of fructans, they have been studied very little in comparison to starch, the other major non-structural carbohydrate polymer found in plants.

β-Fructofuranosidase (Invertase; EC 3.2.1.26) is an exo-acting enzyme which hydrolyses sucrose and inulin. Plants contain multiple forms of invertase which can be distinguished on the basis of pH optimum, glycosylation state and subcellular location. Functions of the different β-fructofuranosidases are not clear. It has been proposed that cell-wall β-fructofuranosidase plays an important role in the ripening of fruits (11,12) and in stress response (13). It has been suggested that the soluble β-fructofuranosidases participate in the regulation of the hexose level in mature tissues (14) and in the utilization of sucrose stored in vacuoles (15). Whether the different β-fructofuranosidases are the products of a single gene or not is not known.

Numerous reports on invertase purification and properties dealt with invertases of microorganisms, especially fungi and bacteria. However, very little information is available for purified invertases from higher plants. Onion (*Allium cepa* L.) bulbs contain considerable quantities of fructans (up to 65% dry weight) (16). Although there have been reports of fructan-synthesizing enzymes in onion (17), no detailed characterization of fructan-degrading enzymes has been attempted. The present study is a part of the investigation aimed at characterizing fructan- degrading enzymes in onion bulbs.

MATERIALS AND METHODS

Plant material

Onion (*Allium cepa* L. cv Changnyeong-Daego) bulbs

†Corresponding author

were obtained from Changnyeong Onion Experiment Station, Gyeongsangnamdo.

Extraction of plant material

Onion bulbs (approximately 2 kg fresh wt) were homogenized in a blender with 500 ml of ice-cold extraction buffer [50 mM sodium acetate buffer, pH 5.5, containing 0.5% 2-mercaptoethanol, 100 μM phenylmethylsulfonyl fluoride (PMSF) and 100 mg/ml insoluble PVP]. The homogenate was squeezed through two layers of cotton cloth and the collected filtrate was centrifuged at 12,000×g for 30 min. The turbid yellowish supernatant was used for further protein purification.

Enzyme assay

β-Fructofuranosidase activity was assayed by incubating an aliquot of enzyme solution at 35°C in 50 mM sodium acetate buffer (pH 5.5) containing 100 mM sucrose. The reducing sugars produced were assayed using 3,5-dinitrosalicylic acid (DNS) according to the method of Miller (18). One unit of enzyme activity was defined as the amount of enzyme which hydrolysis 1 μmol sucrose per minute under the assay conditions.

Purification of β-fructofuranosidase

All the steps were performed at 4°C unless specified otherwise. Ammonium sulfate was added to the filtrate to 75% saturation and the mixture was stored overnight at 4°C. The precipitate obtained by centrifugation was dissolved in 50 mM sodium acetate buffer (pH 5.5) and the solution was dialyzed against the same buffer overnight. The dialyzed solution was loaded onto a column (2.5 cm×18 cm) of Q-Sepharose (Pharmacia LKB Biotechnology, Uppsala, Sweden) equilibrated with the same buffer. Gradient elution was performed with increasing NaCl concentration from 0 to 0.5 M. The active fractions were collected and concentrated by ultrafiltration using YM10 membrane (M.W. cutoff 10 kDa; Amicon). The concentrated enzyme was dialyzed against concanavalin A (ConA) buffer (50 mM sodium acetate buffer, pH 5.5, containing 1 mM $MgCl_2$, 1 mM $MnCl_2$, 1 mM $CaCl_2$, 0.1 mM PMSF). The dialyzed enzyme was applied on a column of ConA-Sepharose 4B (Sigma Chemicals) which was equilibrated with ConA buffer. The column was washed with ConA buffer and subsequently eluted with ConA buffer containing 0.5 M α-methylmannoside. The active fractions were collected and concentrated by ultrafiltration The concentrated protein solution was loaded onto a gel-filtration column (1.5 cm×100 cm) of Sephacryl S-200 (Pharmacia LKB) equilibrated with 50 mM sodium acetate buffer (pH 5.5) and eluted with the same buffer at a flow rate of 10 ml/h.

Protein determination and SDS-PAGE

Protein was measured by the method of Lowry (19) using bovine serum albumin (Sigma) as the standard. SDS-PAGE was carried out using 12% acrylamide with the Bio-Rad Mini-Protein apparatus according to the method of Laemmli (20). Gels were stained with 0.1% Coomassie Brilliant Blue R-250 in methanol : acetic acid : water (45 : 10 : 45, v/v) and destained in methanol : acetic acid : water (10 : 10 : 80, v/v).

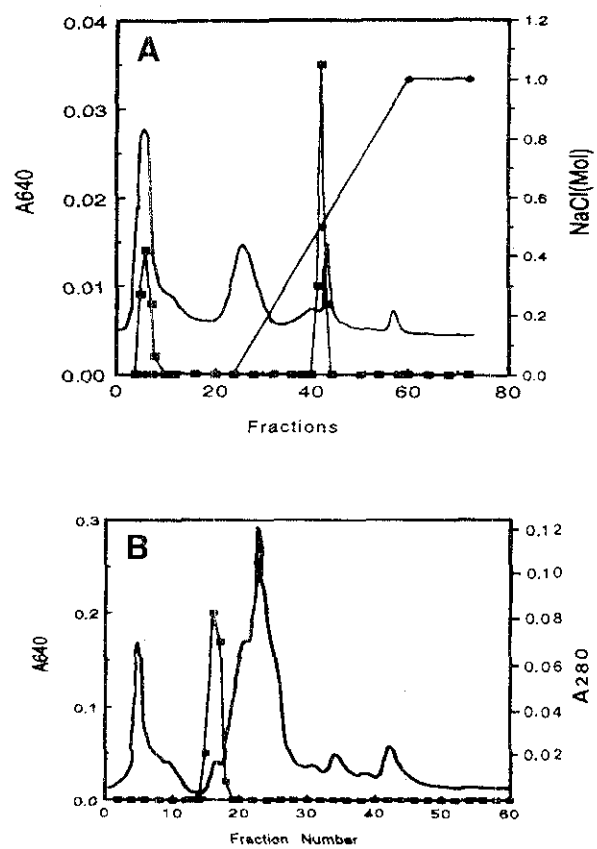

Fig. 1. Q-Sepharose ion-exchange profile (A) and Sephacryl S-200 profile (B) of onion β-fructofuranosidase.
——, absorbance at 280nm; — ● —, NaCl gradient; — ■ —, β-fructofuranosidase activity (A_{640}).

Table 1. Purification of β-fructofuranosidase from onion

Purification step	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Activity yield (%)	Purification (-fold)
Initial extract	973.1	7993	8.2	100	1.0
75% $(NH_4)_2SO_4$	399.4	3366	8.4	42	1.0
Q-Sepharose	14.0	972	69.4	12	8.5
ConA-Sepharose	1.7	711	418.2	9	51.0
Sephacryl S-200	0.2	184	920.0	2	112.2

RESULTS AND DISCUSSION

Purification of β-fructofuranosidase

Soluble β-fructofuranosidase was partially purified from the crude extract of onion bulbs by successive use of ammonium sulfate precipitation, anion-exchange chromatography, ConA chromatography, and gel-filtration chromatography. The elution profiles of the onion β-fructofuranosidase by Q-Sepharose and Sephacryl S-200 chromatographies are shown in Fig. 1. Results of the enzyme purification are summarized in Table 1. The overall purification was 140-fold in the specific activity, and the recovery of the β-fructofuranosidase activity was 2.3%. All the soluble β-fructofuranosidase activity was bound to ConA-Sepharose, indicating that the soluble β-fructofuranosidase of onion bulb was glycosylated. β-Fructofuranosidases are present in most plant tissues in multiple forms (21,22). All plant β-fructofuranosidases appear to be N-glycosylated, but the multiple forms differ in pH optima and isoelectric points, as well as their subcellular locations. Fig. 2 shows SDS-PAGE analysis of protein fractions obtained during each purification step.

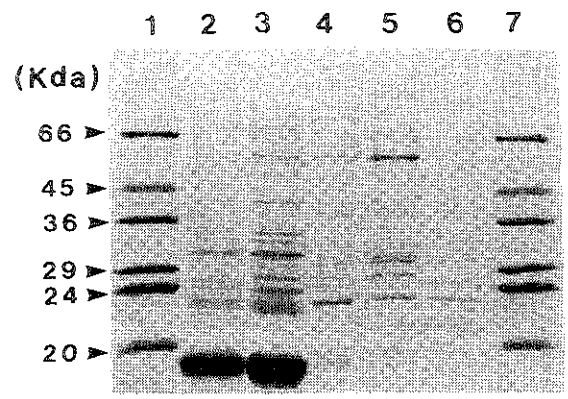

Fig. 2. SDS-PAGE of protein fractions obtained during the purification of β-fructofuranosidase.
Lane 1, 7: protein molecular weight markers. Lane 2: protein extract from onion bulbs. Lane 3: fraction after ammonium sulfate precipitation. Lane 4: fraction after Q-Sepharose. Lane 5: fraction after Con-A Sepharose. Lane 6: fraction after Sephacryl S-200.

Effects of pH and temperature on enzyme activity

The effect of pH on partially purified β-fructofuranosidase activity was investigated by measuring the activities in the pH range from 2.5~7.5 using MacIlvaine buffer at 35°C. As illustrated in Fig. 3A, the enzyme

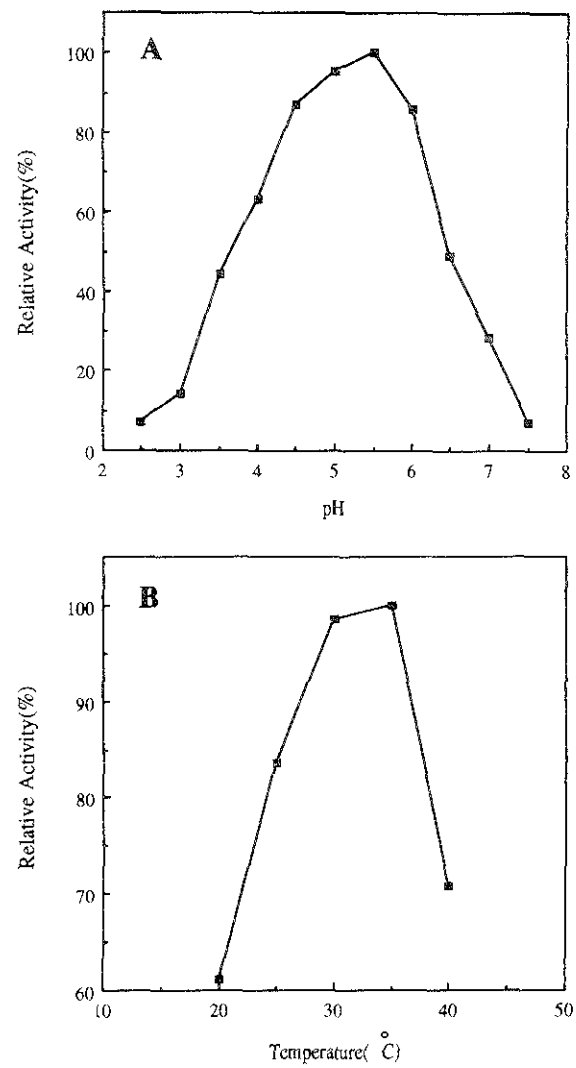

Fig. 3. Effects of pH (A) and temperature (B) on onion β-fructofuranosidase activity.

displayed high β-fructofuranosidase activity over a broad range, pH 4.5-6.0, with optimum activity at pH 5.5. Soluble β-fructofuranosidase with an acidic pH optimum has been characterized from several plant species (12,21,23). All soluble β-fructofuranosidases characterized hydrolyze sucrose at a pH optimum between 4.5 and 5.5. The effect of temperature on invertase activity at pH 5.5 was assayed in the temperature range of 20~40°C. As shown in Figure 3B, the enzyme has the highest activity at 35°C.

The enzyme solutions were kept at different temperature for 1 h, pH 5.5, and the remaining activities were determined by sampling at 10 min intervals. The activity was fairly stable at 30°C and was completely lost after incubation at 50°C for 1 h (Fig. 4). This showed that the onion β-fructofuranosidase was labile to heat treatment.

Substrate specificity

The partially purified enzyme was tested for activity on a number of oligosaccharide substrates by monitoring the release of reducing sugars from the appropriate carbon source. The onion bulb β-fructofuranosidase catalyzed the hydrolysis of sucrose, raffinose and inulin. The enzyme was most active with sucrose as a substrate and exhibited considerable activity with raffinose. The relative activity for raffinose was 76% of that for sucrose. It hydrolyzed inulin weakly. No hydrolysis of substituted β-fructofuranosides was observed. The K_m and V_{max} values for sucrose were determined as 1.2 mM and 537 μmol/min/ml, respectively. This enzyme hydrolyzed raffinose with a K_m of 1.4 mM and a V_{max} of 494 μmol/min/ml. These results indicate that the purified enzyme is likely to be a β-fructofuranosidase, similar to those found in higher plants.

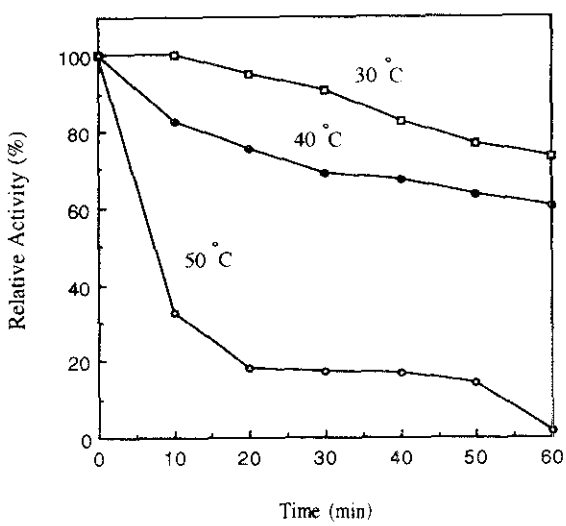

Fig. 4. Thermal stability of onion β-fructofuranosidase.

Table 2. Effect of metal ions and EDTA on activity of onion invertase.

Compound	Final conc. (mM)	Relative activity (%)
None	-	100
$CaCl_2$	1	97
$HgCl_2$	1	40
$ZnCl_2$	1	57
$AgNO_3$	1	84
$CuSO_4$	1	51
$MnCl_2$	1	117
$FeSO_4$	1	114
EDTA	1	83

Results are expressed as relative activity (%)

Effects of metal ions and EDTA on enzyme activities

The effects of various metal ions and EDTA on the activities of the onion β-fructofuranosidase were examined. The enzymes were preincubated with each ion or EDTA in 50 mM acetate buffer (pH 5.5) at 4°C for 30 min and then the residual activities were assayed. The results are shown in Table 2. Soluble β-fructofuranosidase activities were partially inhibited by $HgCl_2$, $AgNO_3$, $ZnCl_2$, and $CuSO_4$. Inhibition of β-fructofuranosidase activity by Hg^{2+} and Ag^{+} ions has been reported for other invertases (23-25). The inhibition of β-fructofuranosidase by Hg^{2+} and Ag^{+} suggests the involvement of sulfhydryl groups in catalytic activity.

ACKNOWLEDGEMENTS

We give special thanks to Dr. Jun Kyu Suh, Changnyeong Onion Exp. Station, for kindly providing the onion bulbs. We thank Ms. Yoo Ree Kim and Mr. Jong Sung Park for technical assistances.

REFERENCES

1. Hendry, G. A. F. : Evolutionary origins and natural functions of fructans: A climatological, biogeographic and mechanical appraisal. *New Phytol.*, **123**, 3 (1993)
2. Housley, T. L. and Daughtry, C. S. T. : Fructan content and fructosyl transferase activity during wheat seed growth. *Plant Physiol.*, **83**, 4 (1987)
3. Lewis, D. H. : Nomenclature and diagrammatic repre-

sentation of oligomeric fructans. *New Phytol.*, **124**, 583 (1993)
4. Bieleski, R. L. : Fructan hydrolysis drives petal expansion in the ephemeral daylily flower. *Plant Physiol.*, **103**, 213 (1993)
5. Pilon-Smits, E. A. H., Ebskamp, M. J. M., Paul, M. J., Jeuken, M. J. W., Weisbeek, P. J. and Smeekens, S. C. M. : Improved performance of transgenic fructan-accumulating tobacco under drought stress. *Plant Physiol.*, **107**, 125 (1995)
6. Pontis, H. G. : Fructans and cold stress. *Plant Physiol.*, **134**, 148 (1989)
7. Fleming, S. E. and Grootwassink, J. W. D. : Preparation of high fructose syrup from the tubers of the Jerusalem artichoke (*Helianthus tuberous* L.). *Crit. Rev. Food. Sci. Nutr.*, **12**, 1 (1979)
8. Yun, J. W. : Fructooligosaccharides-Occurrence, preparation, and application. *Enzyme Microb. Technol.*, **19**, 107 (1996)
9. Fuchs, A. : Current and potential food and non-food applications of fructans. *Biochem. Soc. Trans.*, **19**, 555 (1991)
10. Hidaka, H., Hirayama, M. and Yamada, K. : Fructooligosaccharides: Enzymatic preparation and biofunctions. *J. Carbohydr. Chem.*, **10**, 509 (1991)
11. Miron, D. and Schaffer, A. A. : Sucrose phosphoate synthase, sucrose synthase, and invertase activities in developing fruit of *Lycopersicon esculentum* Mill. and the sucrose-accumulating *Lycopersicon hirsutum* Humb. and Bonpl. *Plant Physiol.*, **95**, 623 (1991)
12. Yelle, S., Chetelat, R. T., Dorais, M., DeVerna, J. W. and Bennett, A. B. : Sink metabolism in tomato fruit. *Plant Physiol.*, **95**, 1026 (1991)
13. Sturm, A. and Chrispeels, M. J. : cDNA cloning of carrot extracellular β-fructosidase and its expression in response to wounding and bacterial infection. *Plant Cell*, **2**, 1107 (1990)
14. Ricardo, C. P. P. : Alkaline β-fructofuranosidases of tuberous roots: possible physiological function. *Planta*, **118**, 333 (1974)
15. Leigh, R. A., Ap Rees, T., Fuller, W. A. and Banfield, J. : The location of acid invertase activity and sucrose in the vacuoles of storage roots of beetroot (*Beta vulgaris*). *Biochem. J.*, **178**, 539 (1987)
16. Darbyshire, B. and Henry, R. J. : The distribution of fructans in onions. *New Phytol.*, **81**, 29 (1978)
17. Henry, R. J. and Darbyshire, B. : Sucrose:sucrose fructosyltransferase and fructan:fructan fructosyltransferase from *Allium cepa*. *Phytochemistry*, **19**, 1017 (1980)
18. Miller, G. L. : Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.*, **31**, 331 (1959)
19. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. : Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265 (1951)
20. Laemmli, U. K. : Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680 (1970)
21. Lauriere, C., Lauriere, M., Sturm, A., Faye, L. and Chrispeels, M. J. : Characterization of β-fructosidase, an extracellular glycoprotein of carrot cells. *Biochimie* (Paris), **70**, 1483 (1988)
22. Singh, M. B. and Knox, R. B. : Invertases of *Lilium* pollen: characterization and activity during *in vitro* germination. *Plant Physiol.*, **74**, 510 (1984)
23. Krishnan, H. B., Blanchette, J. T. and Okita, T. W. : Wheat invertases: characterization of cell wall bound and soluble forms. *Plant Physiol.*, **78**, 241 (1985)
24. Masuda, H., Takahashi, T. and Sugawara, S. : The occurrence and properties of alkaline invertase in mature roots of sugar beets. *Agric. Biol. Chem.*, **51**, 2309 (1987)
25. Ranwala, A. P., Iwanami, S. and Masuda, H. : Acid and neutral invertases in the mesocarp of developing muskmelon (*Cucumis melo* cv. Prince) friuits. *Plant Physiol.*, **96**, 881 (1991)

(Received April 4, 1998)