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Purification and Characterization of High-Molecular-Weight β-Glucosidase from *Trichoderma koningii*

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*Trichoderma koningii*가 생성하는 고분자량 β-Glucosidase의 정제 및 특성

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Abstract: High-molecular-weight β-glucosidase (EC 3.2.1.21) was purified from the culture filtrate of *Trichoderma koningii* through a four-step procedure including chromatography on Bio-Gel P-150, DEAE-Sephadex A-50 and SP-Sephadex C-50 ; and chromatofocusing on Polybuffer exchanger PBE 94. The molecular weight of the enzyme was determined to be about 101,000 by SDS-polyacrylamide gel electrophoresis, and the isoelectric point was estimated to be 4.96 by analytical isoelectric focusing. The temperature optimum for activity was about 55 ℃, and the pH optimum was 3.5. The enzyme was considerably thermostable, for no loss of activity was observed when the enzyme was preincubated at 60 ℃ for 5 h. Km values for cellobiose, gentiobiose, sophorose, salicin and p-nitrophenyl-β-D-glucoside were 99.2, 14.7, 7.09, 3.15 and 0.70 mM, respectively, which indicates that the enzyme has much higher affinity towards p-nitrophenyl-β-D-glucoside than towards the other substrates, especially cellobiose. Substrate inhibition by p-nitrophenyl-β-D-glucoside and salicin was observed at the concentrations exceeding 5 mM. Gluconolactone was a powerful inhibitor against the action of the enzyme on p-nitrophenyl-β-D-glucoside (K_i 37.9 μM), wherease glucose was much less effective (K_i 1.95 mM). Inhibition was of the competitive type in each case. Transglucosylation activity was detected when the reaction products formed from p-nitrophenyl-β-D-glucoside by the enzyme were analysed using high-performance liquid chromatography.

Key words: *Trichoderma koningii*, β-glucosidase, kinetics, transglucosylation.

The enzyme β-glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21), which is known to be distributed in plants, filamentous fungi, yeasts, bacteria and animals, can catalyse the hydrolysis of alkyl- and aryl-β-D-glucoside as well as glycosides containing only carbohydrate residues (Woodward and Wiseman, 1982). In various cellulolytic microorganisms, β-glucosidase is an essential component of their cellulase enzyme system. Cellulose is firstly hydrolysed to collobiose and small cellooligosaccharides by the combined action of endo-1,4-β-glucanase (1,4-β-D-glucan glucanohydrolase, EC 3.2.1.4) and exo-1,4-β-glucanase (1,4-β-D-glucan cellobiohydrolase, EC 3.2.1.91). Then β

-glucosidase hydrolyses these products to glucose. Moreover, β-glucosidase effectively enhances the rate of cellulose hydrolysis by removing cellobiose, the strong end-product inhibitor of both endo- and exo-1, 4-β-glucanase (Enari, 1983).

Although *Trichoderma reesei* is regarded as the best source of cellulolytic enzymes, the low β-glucosidase activity in the secreted enzyme complex appears to be the rate-limiting factor if glucose is the desired product (Sternberg, 1976). Thus the possibility of using β-glucosidases from other sources to supplement the *T. reesei* enzymes has been investigated. Fungi, including *Aspergillus phoenicis* (Sternberg *et al.*, 1977), *Aspergillus wentii* (Legler *et al.*, 1972), *Botryodiplodia theobromae* (Umezurike, 1971) and *Sporotrichum pulverulentum* (Deshpande *et al.*, 1978) have been studied as potential β-glucosidase sources.

Trichoderma koningii, together with *T. reesei*, is also a potent producer of cellulolytic enzymes (Halliwell and Riaz, 1970; Wood and McCrae, 1972). Two β-glucosidase components of identical molecular weight (39, 800) have been purified from this organism by Wood and McCrae (1982). In the present research, another β-glucosidase component of which the molecular weight is much larger than that of the former components was isolated from this fungus. This paper describes the purification and characterization of the high-molecular-weight β-glucosidase.

MATERIALS AND METHODS

Preparation of crude enzyme

The crude enzyme was prepared from the cell-free culture filtrate of *T. koningii* ATCC 26113 as described by Hong *et al.* (1986).

Substrates

Carboxymethylcellulose (CMC) with degree of substitution of approximate 0.6 was purchased from Wako Pure Chemical (Osaka, Japan). Microcrystalline cellulose (Aviel) and cellobiose were supplied by E. Merck (Darmstadt, FRG). p-Nitrophenyl-β-D-glucoside (PNPG) and gentiobiose were obtained from Sigma Chemical (St. Louis, MO, USA), sophorose from Koch-Light Laboratories (Colnbrook, Bucks, UK), and salicin from Difco Laboratories (Detroit, MI, USA), respectively.

Enzyme assay

The enzyme activity towards CMC or Avicel was measured according to the method previously described by Hong *et al.* (1986).

β-Glucosidase activity of column effluents and purified enzyme solution was assayed by measuring the amount of p-nitrophenol (PNP) liberated from PNPG. The reaction mixture was composed of 400 μl of 2 mM PNPG solution, 400 μl of 0.1 M citrate buffer, pH 3.5, (or 0.1 M acetate buffer, pH 5.0, in the cases of culture filtrate, dialysed $(NH_4)_2SO_4$ precipitate and column eluates), and 200 μl of enzyme solution (or ten-fold diluted enzyme solution, when necessary). After incubation at 40℃ for 30 min, 2 ml 1 M sodium carbonate solution were added to the mixture. The mixture was then diluted with 10 ml of distilled water, and the absorbance at 420 nm was measured.

β-Glucosidase activity of purified enzyme towards the substrates other than PNPG was assayed by measuring the amount of glucose liberated from the substrates. The reaction mixture contained 400 μl of substrate solution, 400 μl of 0.1 M citrate buffer, pH3.5, and 200 μl of enzyme solution. After incubation at 40℃ for 30 min, the reaction was stopped by boiling the mixture for 1 min. Added to the reaction mixture was 10 ml of glucose oxidase-peroxidase reagent (Sigma) prepared by mixing: 100 ml of enzyme solution glucose oxidase from *Aspergillus niger* (500 International units), peroxidase from horseradish (100 Purpurogallin units) and buffer salts in distilled water; and 1.6 ml of 0.25 % o-dianisdine dehydrochloride-solution in distilled water. The whole mixture was then incubated at 37℃ for 30 min, followed by immediate measurement of the absorbance at 450 nm.

One unit of enzyme activity was defined as the amount of enzyme producing 1 μmol min^{-1} of PNP or glucose under the conditions of the assay.

Determination of protein concentration

Protein concentrations were determined by the method of Lowry *et al.* (1951), with bovine serum albumin (Sigma) as standard. Protein concentrations of column effluents were estimated from the absorbance at 280 nm.

Enzyme purification

The crude enzyme preparation was fractionated by gel filtration on Bio-Gel P-150, 100-200 mesh (Bio-Rad Laboratories, Richmond, CA, USA) as described in the previous report (Hong *et al.*, 1986).

The high-molecular-weight β-glucosidase fraction (F-I, 140 m*l*) from the Bio-Gel P-150 column was concentrated ten-fold by ultrafiltration through a Diaflo membrane, type PM 10 (Amicon, Lexington, MA, USA). The ultrafiltrate was diluted ten-fold with 0.02 M phosphate buffer, pH 6.9, and concentrated again. This procedure was repeated once more. Further fractionation of the concentrated enzyme solution was carried out on a DEAE-Sephadex A-50 (Pharmacia Fine Chemicals, Uppsals, Sweden) column (2.5×50 cm) equilibrated with 0.02 M phosphate buffer, pH 6.9. The column was eluted with a linear NaCl concentration gradient, 0-0.4 M. The fraction volume was 4 m*l*.

The β-glucosidase fraction (F-I-I, 76 m*l*) from the DEAE-Sephadex column was concentrated to 7 m*l* by ultrafiltration. The buffer system of the enzyme solution was exchanged for 0.02 M acetate buffer, pH 5.0, by twice repeated dilution and ultrafiltration. The concentrate was applied to an SP-Sephadex C-50 (Pharmacia) column (2.5×50 cm) equilibrated with 0.02 M acetate buffer, pH 5.0. The column was eluted with a linear NaCl concentration gradient (0-0.4 M) at a flow rate of 20 m*l* h^{-1}. The fraction volume was 4 m*l*.

The β-glucosidase fratction from the SP-Sephadex C-50 column (F-I-I-I, 60 m*l*) was concentrated by ultrafiltration. The buffer of the enzyme solution was exchanged for 0.025 M histidine-HCl buffer, pH 5.5, by the same procedure as described above. A 2 m*l* aliquot of the concentrated enzyme solution was further purified through a Polybuffer exchanger PBE 94 (Pharmacia) chromatofocusing column (0.9×35 cm) equilibrated with 0.025 M histidine-HCl buffer, pH 5.5. The column was eluted with 250 m*l* of ten-fold diluted Polybuffer 74 (Pharmacia)-HCl buffer, pH 4.0, at a flow rate of 9 m*l* h^{-1}. The fraction volume was 2 m*l*.

Polyacrylamide gel electrophoresis

Detailed procedures for disc-gel electrophoresis on polyacrylamide and SDS-polyacrylamide gel slabs were the same as described by Hong *et al.* (1986).

Analytical isoelectric focusing

Analytical isoelectric focusing was performed on a T= 5%, C= 3% gel slab containing 6.25 % Pharmalyte (Pharmacia) in the pH range 4-6.5 as described in the previous report (Hong *et al.*, 1986).

Separation of reaction products by HPLC

For the analysis of the reaction products formed from PNPG by the enzyme, the reaction mixture containing 150 μ*l* of 5 mM PNPG solution in 0.02 M cirtrate buffer, pH 3.5, and 100 μ*l* of the enzyme solution was incubated at 40 ℃. Samples were removed at intervals and analysed with a high-performance liquid chromatography (HPLC) system containing a Model 6000 A pump (Waters Associates, Milford, MA, USA) and a Partisil PXS-10/25 PAC column (Whatman Inc., Clifton, NJ, USA). The solvent was 23 % (in volume) water in acetonitrile, and the flow rate was 1.0 m*l* min^{-1}. The reaction products were detected with a Waters Model 440 absorbance detector at 280 nm. For the quantification of the products, a Waters Model 730 data module was used.

RESULTS AND DISCUSSION

Enzyme purification

A high-molecular-weight β-glucosidase

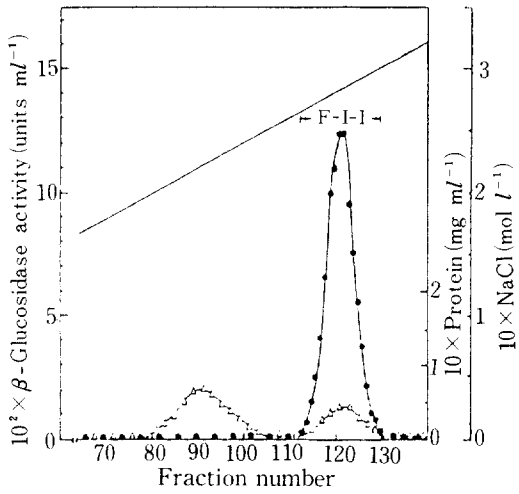

Fig. 1. *Ion exchange chromatography of F-I on DEAE-Sephadex A-50.*

Column dimensions: 2.5×50 cm. Fraction volume: 4 ml. Observed values: △, protein; ●, β-glucosidase activity.

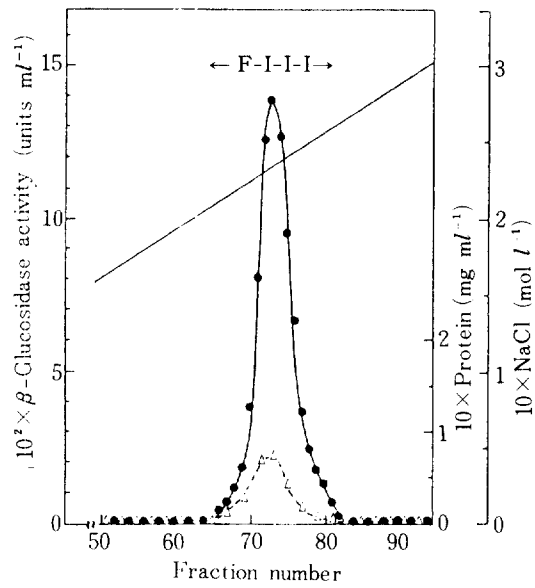

Fig. 2. *Ion exchange chromatography of F-I-I on SP-Sephadex C-50.*

Column dimensions: 2.5×50 cm. Fraction volume: 4 ml. Observed values: △, protein; ●, β-glucosidase activity.

fraction (F-I) was effectively separated from the other components by gel filtration chromatography on a Bio-Gel P-150 column as described by Hong *et al.* (1986). Further purification of F-I was achieved by ion exchange chromatography on a DEAE-Sephadex A-50 column. As shown in Figure 1, a β-glucosidase fraction, F-I-I, was separated from the other contaminating protein fraction. The result of ion exchange chromatography of F-I-I on an SP-Sephadex C-50 column is presented in Figure 2, which shows a single β-glucosidase peak, F-I-I-I. Figure 3 exhibits the result of chromatofocusing of F-I-I-I fraction on a Polybuffer exchanger PBE 94 column. β-Glucosidase activity was detected in a symmetrical peak, F-I-I-I-I.

A quantitative evaluation of the results obtained from the consecutive purification steps is given in Table 1. The specific activity of the purified enzyme was estimated to be 1.5 units (mg protein)$^{-1}$, which is about fifteen-fold higher than that of the starting culture filtrate. The final yield of enzyme activity was approximately 0.7 % of the culture filtrate.

Enzyme purity

Analytical polyacrylamide gel electrophor-

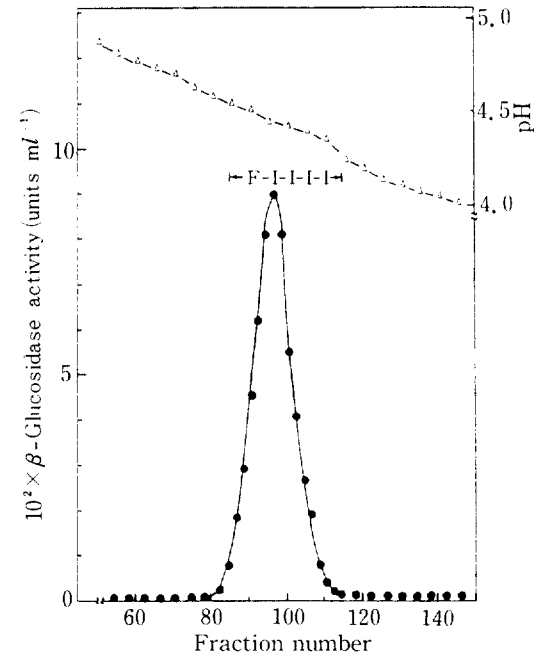

Fig. 3. *Chromatofocusing of F-I-I-I on Polybuffer exchanger PBE K94*

Column dimensions: 0.9×35 cm. Fraction volume: 2 ml. Observed values: △, pH; ●, β-glucosidase activity.

Table 1. *Purification of β-glucosidase from T. koningii.*

Fraction	Total protein (mg)	β-glucosidase		
		Total activity (units)	Specific activity (units (mg protein)$^{-1}$)	Yield (%)
Culture filtrate	3,150	336	0.1	100
Dialysed $(NH_4)_2SO_4$ precipitate	1,100	267	0.2	79
Bio-Gel P-150 eluate (F-I)	47	20	0.4	6.0
DEAE-Sephadex A-50 eluate (F-I-I)	3.5	3.7	1.1	1.1
SP-Sephadex C-50 eluate (F-I-I-I)	2.2	3.3	1.5	1.0
Chromatofocusing eluate (F-I-I-I-I)	1.5	2.3	1.5	0.7

esis was used to examine the purity of the enzyme. As shown in Figure 4. a., the electrophoretogram of the purified β-glucosidase displayed a single band. The electrophoretogram of the enzyme in SDS-polyacrylamide also demonstrated a single band(Figure 4. b) Analytical isoelectric focusing was also used as a criterion for purity. A single protein band was observed with this method, which indicates the high purity of the enzyme(Figure 4. c)

Molecular weight

The molecular weight of the purified enzyme was estimated by SDS-polyacrylamide gel electrophoresis with standard proteins (Figure 4. b). A linear relationship was obtained when the relative mobilities of the standard proteins were plotted against the logarithmic values of the molecular weights. The molecular weight of the enzyme was estimated to be about 101,000 (Figure 5). This value is larger than those of *T. reesei* (76,000 and 47,000; Li *et al.*, 1965; Berghem and Pettersson, 1974), but is smaller than those of *Fusarium solani* (400,000; Wood, 1971), *A. wentii* (170,000; Legler *et al.*, 1972) and *S. pulverulentum* (165,000-182,000; Deshpande *et al.*, 1978). Wood and McCrae (1982) have isolated two β-glucosidase components of identical molecular weight (39,800) from *T. koningii*. Thus it appears that the β-glucosidase purified in the present research is quite a different component from the previously isolated ones.

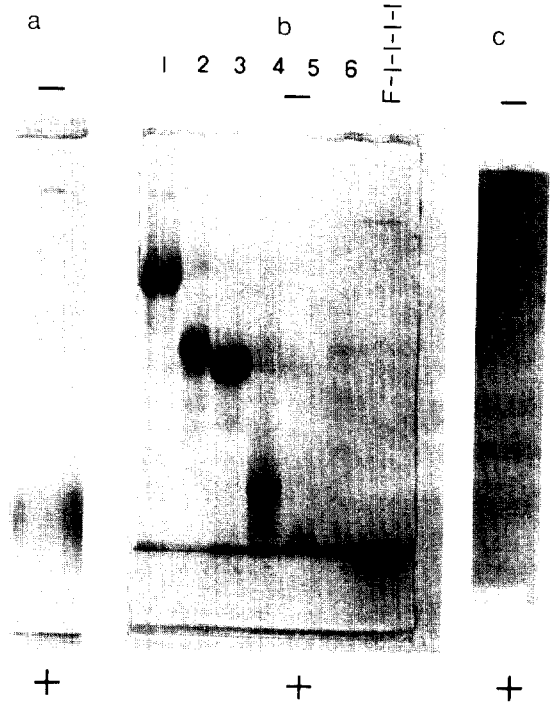

Fig. 4. **a.** *Polyacrylamide gel electrophoresis of F-I-I-I-I*
Gel composition: T=10 %, C=2.7 %.
b. *SDS-polyacrylamide gel electrophoresis of F-I-I-I-I*
Gel composition: T=10 %, C=2.7 %. Molecular weight Markers: 1, bovine serum albumin (66,000)+bovine serum albumin dimer (132,000), trimer (198,000), tetramer (264,000); 2, ovalbumin (45,000); 3, pepsin (34,000); 4, trypsinogen (24,000); 5, β-lactoglobulin 18,400); 6, lysozyme (14,300).
c. *Analytical isoelectric focusing of F-I-I-I-I*
Gel composition: T=5%, C=3 %, 6.25 % Pharmalyte, pH4-6.5.

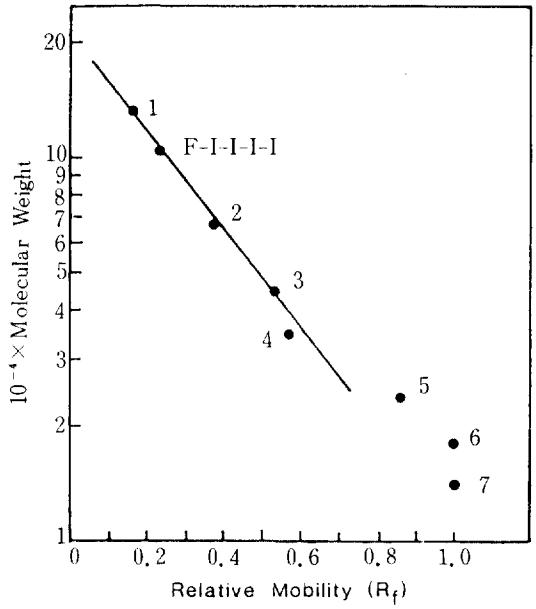

Fig. 5. *Molecular weight estimation of F-I-I-I-I by SDS-polyacrylamide gel electrophoresis.*

Molecular weight markers: 1, bovine serum albumin dimer (132,000); 2, bovine serum albumin (66,000); 3, ovalbumin (45,000); 4, pepsin (34,000), 5, trypsinogen (24,000); 6, β-lactoglobulin (18,400); 7, lysozyme (14,300).

Isoelectric point

The isoelectric point of the enzyme was determined to be 4.96 by analytical isoelectric focusing (Figure 4.c). Although the apparent pI value of the enzyme appeared to be about 4.45 in chromatofocusing (Figure 3), this value is not credible. In chromatofocusing, cations, including hydrogen ions, are repelled by the Polybuffer exchanger, since the matrix carries a positive charge. As a result, the pH inside the matrix is higher than that of the eluent immediately outside. Therefore the eluent in which the protein finds itself when it elutes will be at a lower pH than the pI of the protein, and thus the apparent pI is less than that measured by isoelectric focusing (Pharmacia Fine Chemicals).

Effect of temperature on the enzyme activity

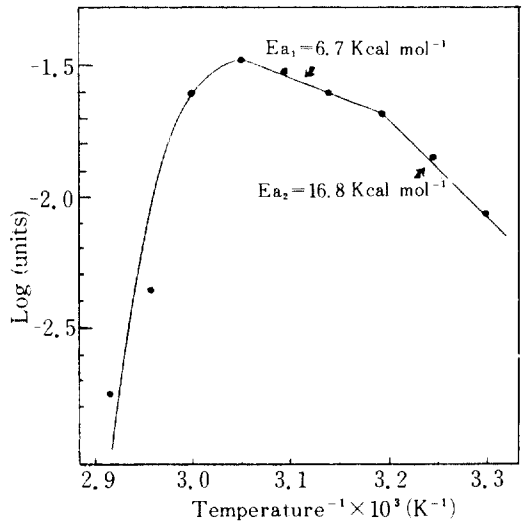

Fig. 6. *Effect of temperature on the activity of F-I-I-I-I*

In order to study the effect of temperature on the activity of the purified enzyme, the activity was assayed at various temperatures between 30 and 70 ℃. The activation energy for the enzymatic reaction was calculated from the Arrhenius equation (Segel, 1975).

As shown in Figure 6, the optimal temperature for the enzyme activity was about 55 ℃. The Arrhenius plot indicates an activation energy of 6.7 Kcal mol^{-1} from 40 to 55 ℃. Below 40℃, there is an abrupt change to 16.8 Kcal mol^{-1}. This sharp change at 40 ℃

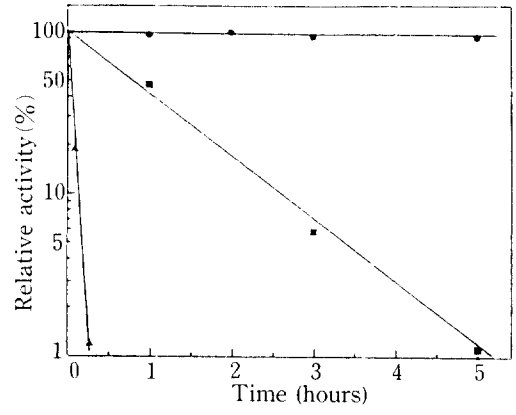

Fig. 7. *Thermal stability of F-I-I-I-I.*

The enzyme was preincubated in acetate buffer, pH 5.0, at: ●, 60 ℃; ■, 65 ℃; ▲, 70 ℃.

(transition temperature) might result from the change of the rate-limiting step from one step to another (Segel, 1975).

Thermal stability

The thermal stability of the purified enzyme was examined by incubating the enzyme at various temperatures in acetate buffer, pH 5.0, in the absence of substrate followed by measuring the residual activity at intervals under the standard assay conditions.

As shown in Figure 7, no loss of activity was observed when the enzyme was incubated at 60℃ for 5h. The enzyme lost 50 and 99 % of its activity after 1 and 5 h incubation at 65℃, respectively. The stability fell drastically at 70℃, thus almost complete inactivation of the enzyme was observed within 15 min at this temperature. On the other hand, the β-glucosidase components, β-glucosidase$_1$ and β-glucosidase$_2$, purified by Wood and McCrae (1982) from the same fungus were denatured rapidly (with 30 min) at 65℃ and pH 5.0. Therefore it turns out that the β-glucosidase component, F-I-I-I-I, is much more thermostable than the other two components.

Effects of pH on the enzyme activity

The enzyme activity was assayed at various pH values to examine the effect of pH on the activity of the purified enzyme. The buffer solutions used in this experiment were 0.1 M maleate buffer (pH 2.5-2.8), 0.1 M citrate buffer (pH 3.0-4.0), 0.1 M acetate buffer (pH 4.5-5.5), 1.0 M phosphate buffer (pH 6.0-7.5) and 0.1 M Tris-HCl buffer (pH 8.0-9.0).

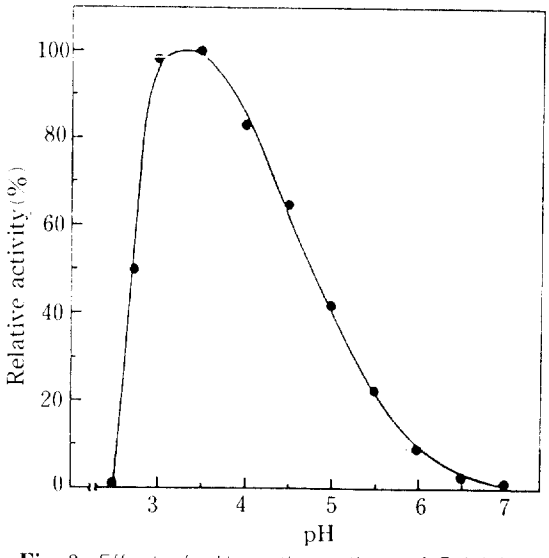

Fig. 8. *Effect of pH on the activity of F-I-I-I-I*

As shown in Figure 8, the enzyme showed its maximal activity at pH 3.5, and almost the same activity was observed at pH 3.0. No activity was detected at pH 2.5 and 7.0. Generally, the values of pH optima for fungal β-glucosidase range over pH 4.0-6.8 (Woodward and Wiseman, 1982). Thus it is noticeable that this enzyme has considerably lower pH optimum for activity than the other fungal β-glucosidases.

Kinetic study

For the determination of K_m and V_{max} values of the purified β-glucosidase for several substrates (PNPG, cellobiose, salicin, gentiobiose and sophorose), the enzyme activity was assayed at various concentrations of the substrates. The substrate concentrations were ranged over 0.3-30 mM for PNPG, 5-120 mM for cellobiose, and 5-100 mM for salicin, gentiobiose and sophorose, respectively.

K_m and V_{max} values were determined using Lineweaver-Burk plot (Figure 9). The K_m and V_{max} values for cellobiose, gentiobiose, sophorose, salicin and PNPG were calculated to be 99.2, 14.7, 7.09, 3.15 and 0.70 mM; 4.34, 1.82, 1.14, 0.88 and 2.30 units (mg protein)$^{-1}$, respectively. Comparing the K_m values for the substrates with each other, it appears that this enzyme has about 140-fold, 20-fold and 10-fold higher affinity towards PNPG than towards cellobiose, gentiobiose and sophorose, respectively. On the other hand, the K_m values of previously isolated β-glucosidase from *T. koningii* towards cellobiose and o-nitrophenyl-β-glucoside (ONPG) were 1.18 and 0.86 mM (β-glucosidasei); 0.37 and 0.85 mM (β-glucosidasei), respectively (Wood and McCrae, 1982). It is therefore clear that the β-glucosidase, F-I-I-I-I, has extremely lower affinity towards cellobiose than the other two

components, β-glucosidasei and β-glucosidasei. It is noteworthy that this enzyme has much lower affinity towards cellobiose even than towards gentiobiose and sophorose.

As seen in Figure 9. a and 9. b, the β-glucosidase was subjected to substrate inhibition by PNPG and salicin at the cocentrations higher than 5.0 mM, while no substrate inhibition occurred in the cases of cellobiose, gentiobiose and sophorose (Figure 9. c). On the other hand, the activity of β-glucosidase$_1$ and β-glucosidase$_2$ was inhibited by both cellobiose and ONPG at the concentrations exceeding 1.0 and 0.8 mM, respectively (Wood and McCrae, 1982). Most substrate inhibitions result from the combination of a substrate with wrong enzyme form and, in general, are apparent only at high substrate concentrations and/or when the reaction is studied in the nonphysiological direction. In these cases, a molecule of substrate binds to one site on the enzyme, and then another molecule of substrate binds to a separate site on the enzyme to form a dead-end complex. This can be regarded as form of uncompetitive inhibition, the extra substrate molecule being the inhibitor (Palmer, 1981).

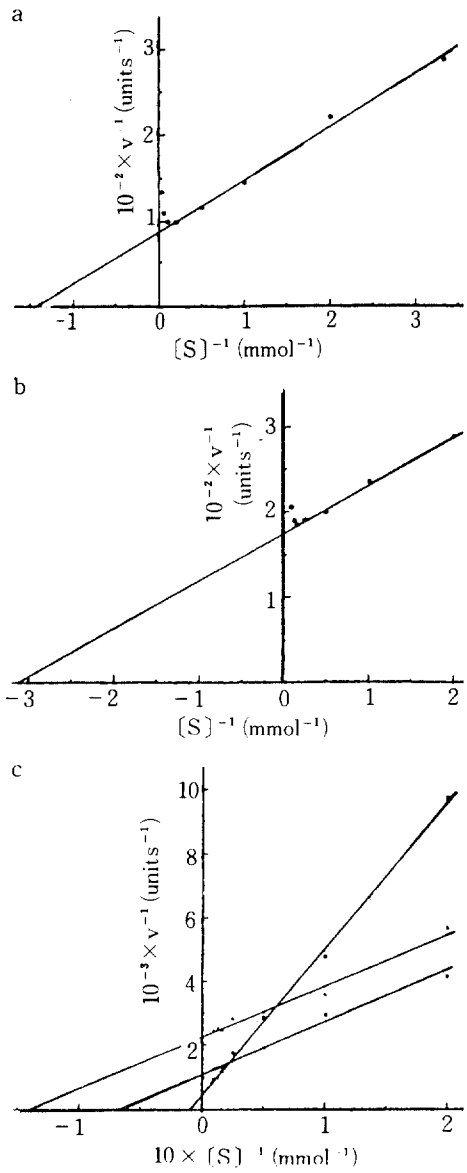

Fig. 9. **a.** *Lineweaver-Burk plot for PNPG.*
b. *Lineweaver-Burk plot for salicin.*
c. *Lineweaver-Burk plot for cellobiose, gentiobiose and sophorose.*
Substrates: ●, cellobiose; ■, gentiobiose; ▲, sophorose

The effects of two inhibitors, glucose and gluconolactone, on the activity of the purified enzyme towards PNPG were studied at various concentrations of the inhibitors, and the K_i values were determined. The concentrations of glucose and gluconolactone were ranged over 1.5-12 mM and 15-120 μM, respectively. The concentration of PNPG was varied from 0.3 to 5 mM.

As shown in Figure 10. a and 10. b, the β-glucosidase was competitively inhibited by both of the inhibitors. The inhibition constants (K_i) for glucose and gluconolactone with PNPG as a substrate were 1.95 mM and 37.9 μM, respectively (Figure 11. a and 11. b). Thus gluconolactone was proved to be a much more powerful inhibitor than glucose. Glucose and gluconolactone have been also reported to be competitive inhibitors of both β-glucosidase$_1$ and β-glucosidase$_2$ (Wood and McCrae, 1982). The inhibition constants for gluconolactone with ONPG as a substrate were 1.8 μM in the case of β-glucosidase$_1$, and 1.17 μM in the case of β-glucosidase$_2$, respectively. Glucose was less inhibitory, values of K_i

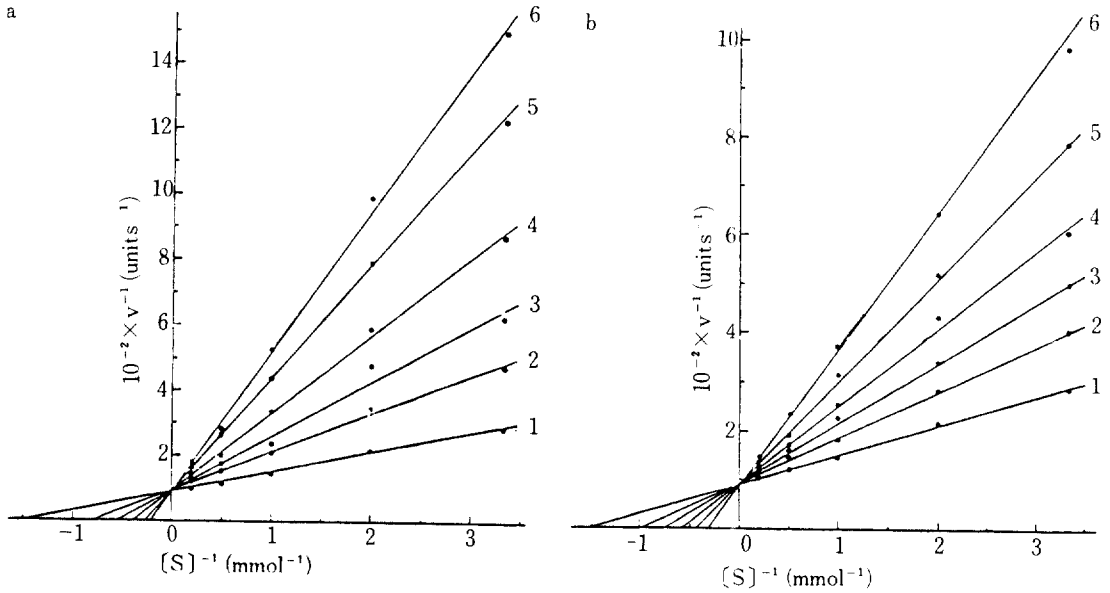

Fig. 10. a. *Effect of glucose on the activity of F-I-I-I-I.*
Glucose concentration: 1, 0.0 mM; 2, 1.5 mM; 3, mM; 4, 6.0 mM; 5, 9.0 mM; 6, 12.0 mM.
b. *Effect of gluconolactone on the activity of F-I-I-I-I.*
Gluconolactone concentration: 1, 0 μM; 2, 15 μM; 3, 30 μM; 4, 60 μM; 5, 90 μM; 6, 120 μM.

being 1.05 and 0.66 mM, respectively. The β-glucosidases of *Lenzites trabea* (Herr *et al.*, 1978), *Aspergillus terreus* (Workman and Donal, 1982) and *Alcaligenes faecalis* (Han and Srinivasan, 1969) had K_i values of 2.7, 3.5 and 3.0 mM, respectively, indicating that glucose has less effect on these enzyme than on β-glucosidase, F-I-I-I-I. Glucose inhibition for the β-glucosidases from *A. terreus* and *A. faecalis* is competitive, while it is non-competitive in the case of *L. trabea*. It seems that the potent inhibition by gluconolactone resulted from its half-chair conformation in an aqueous solution. This is consistant with the postulation that the mechanism of the action of β-glucosidases involves an initial general-acid

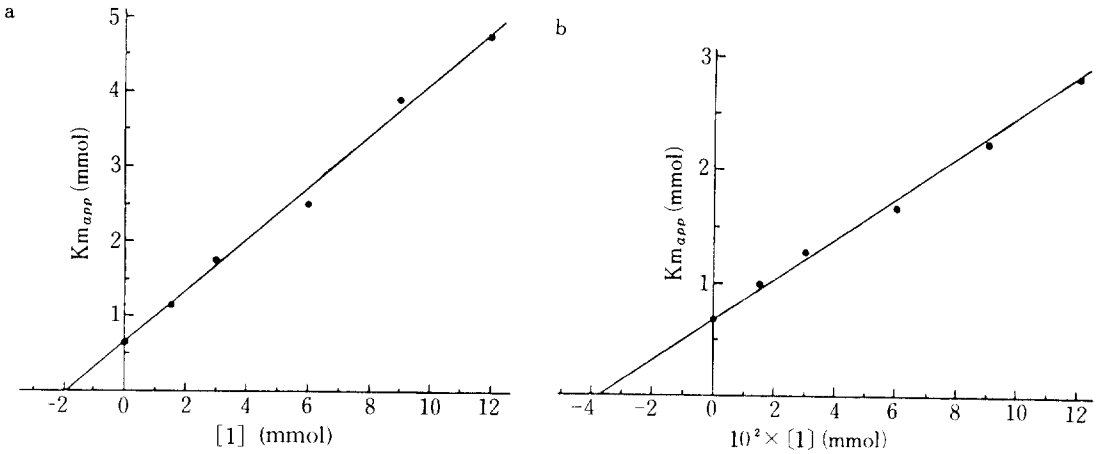

Fig. 11. **a.** *Determination of K_i value of glucose* **b.** *Determination of K_i value of gluconolactone.*

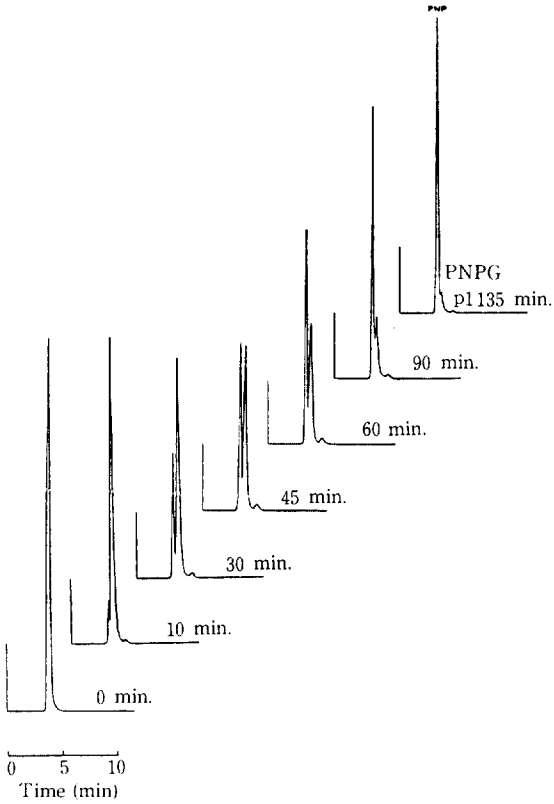

Fig. 12. *Separation of reaction products from PNPG using HPLC.*

catalysis by an acidic group on the enzyme to generate a glucosyl cation that is stabilized by an ionized group (Umezurike, 1981).

Transglucosylation

The reaction products from PNPG by the action of the purified β-glucosidase were analysed by HPLC. As shown in Figure 12, the enzyme readily attacked PNPG to accumulate PNP as a product. Although the chromatograms do not show any peaks for glucose which has no UV absorbance, it is resonable to assume that the amount of glucose produced from PNPG was similar to that of PNP. PNPG was almost completely hydrolysed after the reaction mixture was incubated for 135 min. A minor reaction product (P_1) was also detected throughout the reaction. Although this product could not be identified because of the lack of standard PNP-derivatives, it seems that this product is a transglycosylation procuct formed by the addition of a glucosyl residue to a PNPG molecule. It has been known that β-glucosidases from various organisms frequently exhibit transglucosylation activity. Several reports have described the transglucosylation capacity of the β-glucosidases from *Irpex lacteus* (Nisizawa and Hashimoto, 1959), *B. theobromae* Pat. (Umezurike, 1971), *Geotrichum candidum* (Kozlovskaya *et al.*, 1981), *Aspergillus nidulans* (Jung *et al.*, 1983) and *Aspergillus foetidus* (Gusakov *et al.*, 1984). Gusakov *et al.* (1984) analysed the products of enzymatic cellobiose hydrolysis using HPLC, and reported that the basic transglycosylation products were isocellotriose and gentiobiose. They thus concluded that in the transglucosylation reaction catalysed by the β-glucosidase from *A. foetidus*, the transfer of a glucosyl residue to an acceptor molecule occurs with the formation of, not a β-1, 4-glycosidic linkage as in cellobiose and cellulose molecules, but a β-1, 6-glycosidic linkage.

적 요

*Trichoderma koningii*의 배양액으로부터 고분자량 β-glucosidase (EC 3.2.1.21)가 Bio-Gel P-150, DEAE-Sephadex A-50 및 SP-Sephadex C-50에서의 chromatography와 Polybuffer exchanger PBE 94에서의 chromatofocusing 등 4 단계의 분리 과정을 통하여 정제되었다. 이 효소의 분자량은 SDS-polyacrylamide gel 전기영동에서 약 101,000으로 측정되었으며, 등전점은 analytical isoelectric focusing에서 4.96으로 나타났다. 최적 반응 온도는 약 55 ℃, 최적 반응 pH는 3.5였다. 이 효소는 60 ℃에서 5시간 동안 처리하여도 그 활성이 전혀 떨어지지 않았으므로, 열에 상당히 안정한 것으로 밝혀졌다. Cellobiose, gentiobiose, sophorose, salicin및 p-nitrophenyl-β-D-glucoside에 대한 이 효소의 K_m 값은 각각 99.2, 14.7, 7.09, 3.15 및 0.70 mM이었다. 따라서, 이 효소는 다른 기질들, 특히 cellobiose, 보다 p-nitrophenyl-β-D-glucoside에 훨씬 더 큰 친화력을 나타낸다는 것이 밝혀

졌다. p-Nitrophenyl-β-D-glucoside와 salicin을 기질로 사용하였을 경우, 그들의 농도가 5 mM을 초과하는 범위에서 이 효소는 기질방해를 받는 것으로 나타났다. Gluconolactone은 이 효소가 p-nitrophenyl-β-D-glucoside 에 작용하는 데 있어서 매우 강력한 방해를 나타낸 반면(K_i 37.9 μM), glucose는 훨씬 약한 방해를 보여 주었다(K_i 1.95 mM). 이 때, 방해의 유형은 모두 경쟁방해의 양상을 나타내었다. 이 효소를 p-nitrophenyl-β-D-glucoside 에 작용시킨 후 그 산물을 high-performance liquid chromatography로 분석한 결과, 이 효소는 transglucosylation 활성을 가지고 있음이 확인되었다.

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