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Purification and Properties of β-Glucosidase from *Aspergillus terreus*

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A β-glucosidase (EC 3.2.1.21) from the fungus *Aspergillus terreus* was purified to homogeneity as indicated by disc acrylamide gel electrophoresis. Optimal activity was observed at pH 4.8 and 50°C. The β-glucosidase had K_m values of 0.78 and 0.40 mM for *p*-nitrophenyl-β-D-glucopyranoside and cellobiose, respectively. Glucose was a competitive inhibitor, with a K_i of 3.5 mM when *p*-nitrophenyl-β-D-glucopyranoside was used as the substrate. The specific activity of the enzyme was found to be 210 IU and 215 U per mg of protein on *p*-nitrophenyl-β-D-glucopyranoside and cellobiose substrates, respectively. Cations, proteases, and enzyme inhibitors had little or no effect on the enzyme activity. The β-glucosidase was found to be a glycoprotein containing 65% carbohydrate by weight. It had a Stokes radius of 5.9 nm and an approximate molecular weight of 275,000. The affinity and specific activity that the isolated β-glucosidase exhibited for cellobiose compared favorably with the values obtained for β-glucosidases from other organisms being studied for use in industrial cellulose saccharification.

Cellulose may be hydrolyzed enzymatically by the combined action of at least three enzymes: exoglucanases, endoglucanases, and β-glucosidases (8, 16, 33). The exoglucanases and endoglucanases, through mechanisms not fully understood, hydrolyze cellulose to the disaccharide cellobiose. Cellobiose is in turn hydrolyzed to glucose by a β-glucosidase.

The cellulolytic enzymes of *Trichoderma reesei* have been investigated thoroughly (2, 21, 22) and are considered by many to show commercial potential, but the low β-glucosidase activity in the secreted enzyme complex appears to be the rate-limiting factor if glucose is the desired product (14, 27). Cellobiose exerts a product inhibition on both the exo- and endoglucanases of *T. reesei* (8). The β-glucosidase not only produces glucose, but also reduces the cellobiose inhibition, allowing the cellulolytic enzymes to function more efficiently.

The possibility of using β-glucosidases from other sources to supplement the *T. reesei* enzymes is being investigated. Fungi, including *Aspergillus phoenicis* (28), *Botryodiplodia theobromae* (32), *Lenzites trabea* (13), and *Sclerotium rolfsii* (25), have been studied as potential β-glucosidase sources. Currently, attention has turned towards the use of *A. phoenicis* β-glucosidase in an immobilized form (3, 29).

Aspergillus terreus is a common soil microorganism that produces cellulolytic enzymes. Fermentation studies with cellulose as substrate have been conducted with *A. terreus* (9), but information on the cellulolytic enzymes is lacking. The purification and characterization of the *A. terreus* β-glucosidase, as well as a comparison of this enzyme with other β-glucosidases, is presented in this report.

MATERIALS AND METHODS

Chemicals. Acrylamide was obtained from Miles Laboratories, Inc. (Elkhart, Ind.). Gel filtration marker proteins were obtained from Pharmacia Fine Chemicals, Inc. (Piscataway, N.J.). Carboxymethyl cellulose (CMC) and the protein assay kit were purchased from Bio-Rad Laboratories (Richmond, Calif.). Enzyme substrates, protease, Sepharose 6B, agarose-bound concanavalin A (ConA), DEAE-cellulose, *N,N*-methylene-bisacrylamide, and the glucose assay kit (glucose oxidase; kit no. 510-A) were obtained from Sigma Chemical Co. (St. Louis, Mo.). All other chemicals used were commercially available reagent grade.

Organism. *A. terreus* was a gift from V. R. Srinivasan (Louisiana State University, Baton Rouge). The stock cultures were maintained at room temperature on potato dextrose agar (Difco Laboratories, Detroit, Mich.) plates.

Media. The spores for the fermentor inoculum were allowed to germinate in a modified preparation of Czapek-Dox medium (30). The medium consisted of 35 mM sodium nitrate–6.7 mM potassium chloride–5.7 mM dipotassium phosphate–2.0 mM magnesium sulfate–0.04 mM ferrous sulfate–28 mM glucose in distilled water.

The concentrated citrate medium used for enzyme production contained 70 mM sodium nitrate–13 mM potassium chloride–11 mM dipotassium phosphate–4 mM magnesium sulfate–0.43 mM ferrous sulfate–140 mM potassium citrate in distilled water; the pH was adjusted to 4.5.

Enzyme production. Spores from one potato dextrose agar plate were harvested by adding 25 ml of a 2% (vol/vol) Tween 80 solution to the plate and suspending the spores by scraping the plate. The spore suspension was then transferred to a 16-liter fermentation vessel containing 4 liters of the modified Czapek-Dox preparation containing 28 mM glucose (pH 7.3). The culture was maintained at 35°C with gentle aeration (0.9 liters/min) and vigorous agitation (3,600 rpm) with an overhead stirrer. After 2 days of incubation citrate was fed into the fermentor containing the 4-liter mycelial inoculum such that the citrate concentration increased linearly from none to 140 mM citrate. A gradient maker of 12-liter capacity which mixed linearly 6 liters of the concentrated citrate medium with 6 liters of sterile water was used to produce the gradient, which was fed into the fermentor at a rate of 4.2 ml/min for 2 days. Temperature, aeration, and agitation remained constant. After the gradient was complete (48 h), the cells were harvested by filtration on Whatman no. 1 filter paper and then resuspended in 400 ml of 0.05 M potassium phosphate buffer, pH 6.0. The cells were frozen at −20°C for future use.

Cell breakage. The frozen cells were thawed, and 100 ml of the resulting cell slurry was combined with 100 ml of 0.05 M potassium phosphate buffer, pH 6.0. The cell suspension was sonicated at 4°C for 10 min at 60% of maximum output of the sonicator (Fisher sonic dismembrator, with 0.5-in. [1.27-cm] tip; Artek Systems Corp., Farmingdale, N.J.). The suspension was then centrifuged at 13,000 × *g* for 10 min. The packed cells and debris were resuspended in 3 volumes of 0.05 M potassium phosphate buffer (pH 6.0), sonicated, and centrifuged as described previously. The procedure was repeated for a third time. The supernatants from each centrifugation were combined and filtered through glass wool. This fraction is referred to as crude enzyme.

Enzyme assays. The β-glucosidase activity was routinely determined with *p*-nitrophenyl-β-D-glucopyranoside (PNPG) as the substrate, using a modification of the assay described by Hagerdal et al. (11). A 2-ml portion of a 10 mM PNPG solution in 0.05 M sodium citrate buffer, pH 4.8, was heated to 50°C. Then, 25 μl of the enzyme solution was added to the substrate, and the solution was incubated for 15 min. After incubation, 3 ml of 1.0 M sodium carbonate was added to stop the reaction and develop the color. The absorbance was read at 400 nm, and the amount of *p*-nitrophenol liberated was determined. The time of enzyme preparation tested. A unit (international unit) of activity was defined as the amount of enzyme required to liberate 1 μmol of *p*-nitrophenol per min from the substrate.

The activity on cellobiose was determined by heating 0.4 ml of the substrate, 12.5 mM cellobiose in 62.5 mM sodium citrate buffer (pH 4.8), to 50°C, adding 0.1 ml of preheated enzyme solution and then incubating for 1.5 min. The reaction mixture was placed in boiling water for 30 s to stop the reaction and then immediately cooled in an ice bath. This mixture was added to 5 ml of the glucose oxidase reagent from a glucose assay kit (Sigma Chemical Co.), and the amount of glucose produced from cellobiose hydrolysis was determined spectrophotometrically. A unit of cellobiose activity was defined as the amount of enzyme that produced 1 μmol of glucose per min from cellobiose.

Protein assay. Protein was measured with a commercial protein assay kit (Bio-Rad Laboratories). Bovine serum albumin was used as the standard protein.

Disc gel electrophoresis. Disc gel electrophoresis was performed by using a modification of the technique of Brewer and Ashworth (4).

Gels to be stained for protein were fixed at room temperature in 20% (wt/vol) sulfosalicylic acid for 2 h and then immersed in stain. The protein was stained by exposure to 0.2% Coomassie brilliant blue solution for 8 h. Gels were stored in 7% acetic acid.

Gels to be stained for carbohydrate were fixed and stained by a modification of the periodic acid-Schiff base technique described by Leach et al. (18) for sodium dodecyl sulfate-disc gel electrophoresis.

The gels were stained for β-glucosidase activity by immersing unfixed gels in 10 mM PNPG in 0.05 M sodium citrate buffer, pH 4.8, at room temperature for 2 h. The formation of a yellow band in the gel indicated the liberation of *p*-nitrophenol.

The presence of protein on the gel was verified by scanning the gel at 280 nm before staining (18). The gels were scanned through a 0.1-mm slit at a wavelength of 550 nm for gels stained with Coomassie brilliant blue and at 560 nm for periodic acid-Schiff base-stained gels, using a Gilford gel scanner 2520 (Gilford Instruments, Oberlin, Ohio).

Chromatography. (i) DEAE-CMC combined ion-exchange column. A DEAE-cellulose column (15 by 2.5 cm) and a CMC column (12 by 1.5 cm) were prepared and equilibrated with 50 mM potassium phosphate buffer (pH 6.0). The two columns were then connected in series such that a solution would pass through the DEAE-cellulose column and then through the CMC column.

(ii) ConA-agarose affinity column. An agarose-bound ConA column (8.5 by 1 cm) that contained 100 mg of bound ConA was prepared. The column was equilibrated with 20 mM potassium phosphate buffer (pH 6.0) containing 1.0 mM $MnCl_2$–1.0 mM $MgCl_2$–1.0 mM $CaCl_2$–0.5 M NaCl.

(iii) DEAE-cellulose ion-exchange column. A DEAE-cellulose column (4.5 by 1 cm) was equilibrated with 20 mM Tris-hydrochloride buffer (pH 8.5). After the enzyme had been absorbed to the column, a 200-ml gradient, 0.05 to 0.2 M KCl in 20 mM Tris-hydrochloride buffer (pH 8.5), was passed through the column at a flow rate of 0.8 ml/min, and 1.2-ml fractions were collected.

Determination of carbohydrate content. The carbohydrate content of the enzyme was determined by the phenol-sulfuric acid assay for total carbohydrate (7) on a sample of known protein concentration. The enzyme solution used for the determination was pure and free of contaminating carbohydrate. After the enzyme was adsorbed to DEAE-cellulose, the column was washed thoroughly, and the enzyme was eluted from the column with a salt gradient and then dialyzed against water. Glucose was used as the standard.

Protease inhibition. The effects of proteases from *Streptomyces griseus* (type XIV), ficin, and trypsin

(bovine pancreas) on β-glucosidase activity were determined. Stock solutions of the proteases were prepared as follows: (i) protease from *S. griseus*—0.2 mg/ml (1.08 U/ml) in 0.1 M potassium phosphate buffer (pH 7.5); (ii) ficin (crude enzyme)—0.2 mg/ml (0.02 U/ml) in 0.1 M potassium phosphate buffer (pH 7.0); (iii) trypsin (bovine pancreas)—0.2 mg/ml (2,400 Na-benzoyl-L-arginine ethyl ester U/ml) in 0.1 M potassium phosphate buffer (pH 7.6).

Equal volumes of the protease stock solutions and the enzyme (in distilled water) were mixed and incubated at the optimum temperature for the protease (*S. griseus* protease, 37°C; ficin, 37°C; and trypsin, 25°C). A 25-μl sample was withdrawn from each reaction at 0.5, 2.0, 5.5, and 8.0 h and assayed for β-glucosidase activity, using the previously described PNPG assay.

Effect of cations on activity. The effect of cations on activity was determined by adding the cations in a 10 mM concentration to the PNPG substrate, which was made with 0.05 M sodium acetate buffer (pH 4.8) instead of citrate buffer to prevent chelation; Ca^{2+}, Ni^{2+}, Hg^{2+}, Co^{2+}, Fe^{3+}, and K^{+} were all added as chlorides, and Cu^{2+}, Mg^{2+}, Fe^{2+}, and Zn^{2+} were added as sulfates.

Determination of the Stokes radius. A gel filtration column of Sepharose 6B (42 by 2.5 cm) was prepared. The gel was equilibrated with 50 mM potassium phosphate buffer (pH 6.0) and run with reverse flow at 0.15 ml/min. Fractions (2.2 ml) were collected. Protein markers of known Stokes radii and molecular weights were used to calibrate the column. Aldolase, catalase, and thyroglobulin were used as protein markers. Using the absorbance at 280 nm to identify the protein peaks, a plot of absorbance at 280 nm versus the fraction number was used to calculate the elution volume (V_e) of the different protein species. The β-glucosidase peak was identified by activity, using the previously described PNPG assay. The K_{av} was determined for each protein as follows: $K_{av} = (V_e - V_0)/(V_t - V_0)$ (17). A plot of $\sqrt{-\log (K_{av})}$ versus the Stokes radius (R_s) was used to determine the Stokes radius of the β-glucosidase.

Effect of reducing agents. Dithiothreitol and β-mercaptoethanol were used to determine whether either had an inhibitory effect on the enzyme. Dithiothreitol was prepared at a 10 mM concentration in 0.05 M sodium citrate buffer, pH 4.8, and β-mercaptoethanol was prepared at a 1% concentration in the same buffer. One milliliter of the inhibitor and 25 μl of enzyme were incubated at 37°C for 20 min. Then, 1 ml of 20 mM PNPG was added and the assay was done as previously described.

RESULTS

Enzyme purification. For the first step of purification, the crude enzyme was passed through two columns connected in series filled with DEAE-cellulose and CMC. When the columns were run separately at pH 6.0, 53% of the protein in the crude enzyme was absorbed by the DEAE-cellulose and 9% was absorbed by the CMC. The β-glucosidase was not absorbed by either ion exchanger at pH 6.0. The ability of DEAE-cellulose and CMC to absorb the β-glucosidase was tested at pH 7.5 and 4.5, respectively. The enzyme was not absorbed in either case.

The two columns were interconnected such that the crude enzyme solution first passed through the DEAE-cellulose column and then through the CMC column. The columns could be used to purify 1 liter of the crude enzyme solution before they had to be repacked with fresh exchanger. After passing 1 liter of the crude enzyme solution through the columns, the columns were washed with 50 ml of 50 mM potassium phosphate buffer (pH 6.0), and the wash was combined with the column filtrate.

After 2 liters of the enzyme solution had been collected, it was concentrated to 50 ml, using a hollow fiber concentrator (cutoff, 5,000 molecular weight). During concentration a precipitate formed which was removed by centrifugation at 13,000 × *g* for 10 min. The concentrated enzyme was dialyzed against 20 mM potassium phosphate buffer containing 1 mM $MnCl_2$–1 mM $MgCl_2$–1 mM $CaCl_2$–0.5 M NaCl, which was adjusted to pH 6.0 (ConA eluting buffer). A faint white precipitate formed which was removed by centrifugation at 13,000 × *g* for 10 min.

The second step in the purification utilized affinity chromatography on agarose-bound ConA in a modification of the technique described by Gong et al. (10). A ConA-agarose column was prepared and equilibrated with eluting buffer. The 50 ml of dialyzed enzyme solution was then absorbed to the column, and the column was washed with 50 ml of the eluting buffer to remove unbound protein. The filtrate and wash were discarded. The column was then eluted with 100 ml of eluting buffer that contained 0.1 M α-methyl-D-mannopyranoside.

The wash containing the enzyme was dialyzed against 0.02 M Tris-hydrochloride buffer, pH 8.5. A faint white precipitate formed which was removed by centrifugation at 13,000 × *g* for 10 min.

The final step of the purification was the separation of the β-glucosidase, using a DEAE-cellulose column equilibrated with 0.02 M Tris-hydrochloride buffer, pH 8.5. After the β-glucosidase was adsorbed, the DEAE-cellulose column was washed with 25 ml of the 0.02 M Tris-hydrochloride buffer (eluting buffer) to remove unbound protein (Fig. 1). A 200-ml gradient was passed through the DEAE-cellulose column as described in Materials and Methods. Protein peaks from the column were monitored by measuring the absorbance at 280 nm. The enzyme location was determined by the PNPG assay. The fractions containing the enzyme were pooled and determined to be homogeneous by electrophoresis. A summary of the purification is given in Table 1.

Temperature stability and pH optimum. The

TABLE 1. Purification of the β-glucosidase of *A. terreus*

Treatment	Total enzyme (IU)[a]	Total protein (mg)	Sp act (IU/mg)	Enzyme yield (%)	Fold purification
Crude extract	500	319	1.6	100	1.0
DEAE-CMC column	504	122	4.1	100	2.6
First dialysis	377	63	5.9	75	3.7
ConA-agarose column	125	3	41.7	25	26.0
Second dialysis	116	3	38.7	23	24.2
DEAE-cellulose column	30	0.15	200.0	6	125.0

[a] Activity was measured with PNPG as substrate.

enzyme was found to be stable up to 20 min at 55°C (Fig. 2), but after 30 min a slight inactivation of the enzyme was observed. For this reason all assays were done at 50°C. The enzyme was found to exhibit its highest activity at pH 4.8.

Determination of the Michaelis constant. An average K_m of 0.78 mM was obtained for PNPG on three separate trials, using a Lineweaver-Burk plot. The maximum velocity, as determined from the graph, was 210 IU/mg of protein with PNPG.

An average K_m of 0.4 was obtained with cellobiose as the substrate on three separate trials (Fig. 3). The average velocity was 215 U/mg of protein.

Inhibitory effect of glucose. Glucose was found to be a competitive inhibitor of the enzyme as shown by a Lineweaver-Burk plot in the presence of various concentrations of glucose. As the glucose concentration increased the apparent K_m for the PNPG decreased, whereas the maximum velocity remained the same. The graphical method of Dixon (6) was used to determine the K_i, the dissociation constant, of glucose (Fig. 4). The K_i was found to be 3.5 mM for glucose when PNPG was used as the substrate.

Determination of the carbohydrate content. Because the enzyme bound to the agarose-bound ConA, it was assumed that it was a glycoprotein. This was confirmed by staining electrophoresis gels for carbohydrate (Fig. 5). The enzyme was found to contain 65% carbohydrate by weight as determined by the phenol-sulfuric acid assay.

Protease inactivation. None of the proteases tested had any effect on the activity of the enzyme when the protease and enzyme were preincubated together under conditions optimal for the protease.

Effect of cations on activity. The salts of the cations tested were included in the PNPG sub-

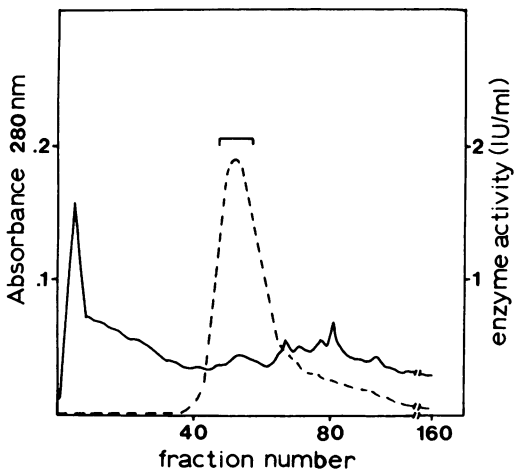

FIG. 1. Typical DEAE-cellulose column profile for *A. terreus* β-glucosidase. Protein (solid line) and enzyme (broken line) are plotted against fraction number. Brackets indicate the pooled fractions.

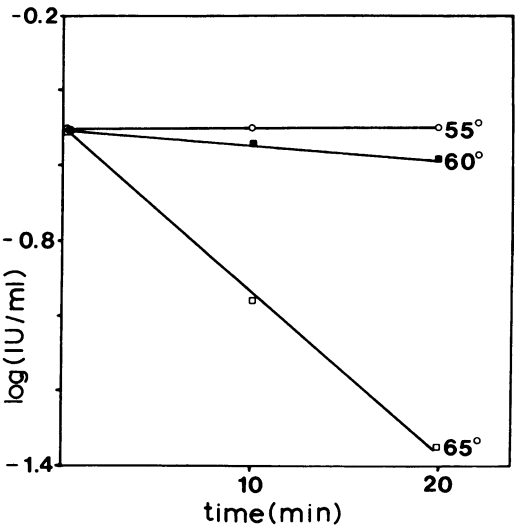

FIG. 2. Log of units per milliliter plotted versus time of preincubation at the following temperature: 55°C (○); 60°C (■); 65°C (□). Temperature stability was determined by incubating the enzyme at the specified temperature and assaying remaining activity at various times, using the standard PNPG assay.

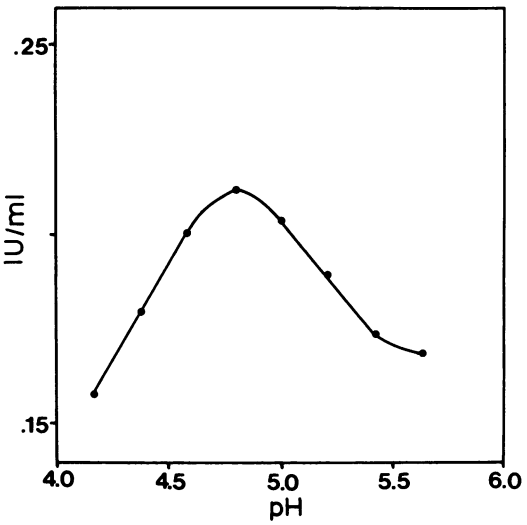

FIG. 3. pH optimum of the enzyme measured in 0.05 M sodium citrate buffer with PNPG as substrate.

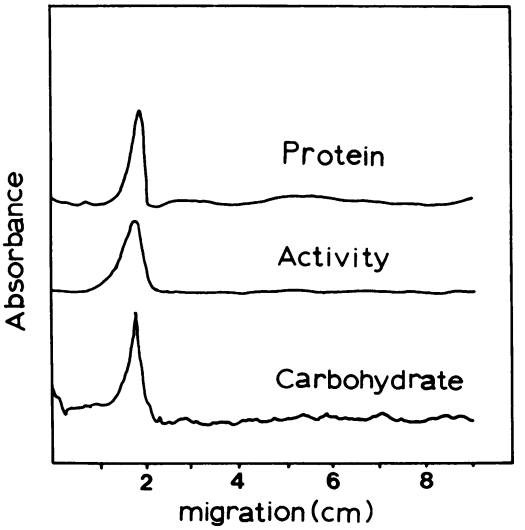

FIG. 5. Typical gel scan of absorbance versus migration distance on duplicate gels stained for protein (upper tracing) activity (middle tracing) and carbohydrate (lower tracing).

strate at a 1.0 mM concentration to determine what effect they had on enzyme activity. The sodium citrate buffer was replaced by sodium acetate buffer to prevent chelation of the ions; otherwise the assays were done as described in Materials and Methods. The greatest inhibition was exhibited by Cu^{2+}, which inhibited the enzyme by 14%. This degree of inhibition was not considered significant. All other cations tested had neither an inhibitory nor a stimulatory effect on the enzyme.

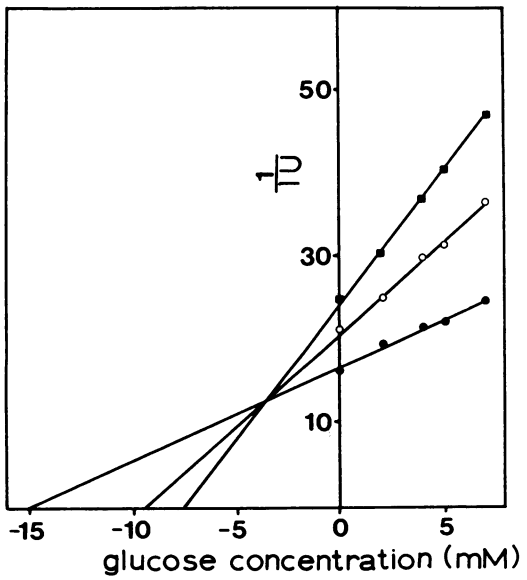

FIG. 4. Dixon plot carried out on assays run in the presence of 2.00 mM PNPG (●), 1.00 mM PNPG (○), and 0.67 mM PNPG (■).

Determination of the Stokes' radius. The size of the enzyme was determined by gel filtration. Because the enzyme contains a large percentage of carbohydrate, it is difficult to obtain an accurate molecular weight. The more accurate measure of the size of the molecule was the Stokes radius (R_s). A plot of $\sqrt{-\log (K_{av})}$ versus R_s was prepared with aldolase, catalase, and thyroglobulin (Fig. 6). The Stokes radius of the β-glucosidase (from Fig. 6) was 5.9 nm. This corresponds to a molecular weight of 275,000. The molecular weight is provided for comparison with other enzymes and is not intended to be taken as the true molecular weight.

DISCUSSION

Because of its possible usefulness in the saccharification of cellulose, *A. terreus* was selected for studies to determine whether it might be an important source of β-glucosidase.

When the crude enzyme was passed through the DEAE-CMC column, 100% of the β-glucosidase was recovered. If the enzyme was at or near its isoelectric point, it would not be unusual for it not to bind to either DEAE-cellulose or CMC. However, the enzyme did not stick to either a CMC column at pH 4.5 or a DEAE column at pH 7.5. This suggests that the enzyme has a minimal charge such that it does not bind strongly to an ion exchanger between the pH range of 4.5 to 7.5 or that the eluting buffer has

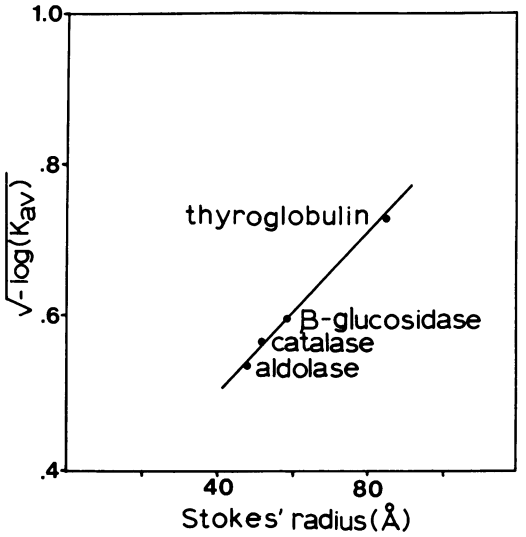

FIG. 6. Plot of $\sqrt{-\log (K_{av})}$ versus the Stokes radius of the protein standards used to determine the Stokes radius of the β-glucosidase.

an ionic strength high enough to displace the enzyme from the columns.

During the first dialysis (Table 1) 25% of the activity was lost. We determined that this was not due to the concentration step before the dialysis. It may have been due to the overnight dialysis against the ConA eluting buffer because of instability to long-term exposure to buffers of high ionic strength.

The β-glucosidase, being a glycoprotein, was further purified by affinity chromatography, using the lectin ConA in an immobilized form, suggesting that the enzyme's carbohydrate moiety contains glucose or mannose or their derivatives.

The β-glucosidase was 65% carbohydrate by weight so it may have been bound very strongly by the ConA, making elution difficult. It was assumed that the loss of activity during affinity chromatography was due to some of the enzyme remaining on the ConA column, although the possibility of isozymes existing with only one species eluting from the column cannot be dismissed.

The pH optimum was 4.8 and the enzyme was stable at 50°C. This is significant because it is desirable to use this enzyme to supplement the cellulase from *T. reesei* in an industrial saccharification. The pH and temperature optima for *Thermoactinomyces* sp. β-glucosidase are pH 6.5 and 50 to 55°C (11); for *Humicola insolens*, pH 5.0 and 50°C (34); for *A. phoenicis*, pH 4.3 and 50°C (28). The pH optimum and temperature stability for *Trichoderma* cellulase are pH 4.8 and 50°C (28).

The K_m for PNPG was found to be 0.78 mM, which is in the same range as the K_m values obtained for the β-glucosidases of *Alcaligenes faecalis* (12), *B. theobromae* (31), and *Clostridium thermocellum* (1). The specific activity of the β-glucosidase, using PNPG, was 210 IU/mg of protein, which is high compared with other sources of β-glucosidase (1, 5, 19, 23, 24). The β-glucosidase had a K_m of 0.4 mM for cellobiose. Its affinity was higher than those from other organisms suggested for industrial saccharification (1, 15, 28, 31). A low K_m for the cellobiase is important in an industrial saccharification because during the saccharification it is desirable to reduce the product inhibition cellobiose exerts on the other enzymes in the system. A specific activity of 215 U/mg of protein was obtained when cellobiose was the substrate. This is very high compared with other purified β-glucosidases (15, 19, 28).

Only one other β-glucosidase, that of *H. insolens* (34), has been reported to have a higher specific activity than that observed for *A. terreus*, although the *A. terreus* enzyme was found to have a higher affinity than that of *H. insolens*.

The β-glucosidase was inhibited by glucose, as are most β-glucosidases from other sources. The dissociation constant (K_i) was found to be 3.5 mM when PNPG was the substrate. The β-glucosidases of *Phanerochaete chrysosporium* (26), *L. trabes* (13), and *Alcaligenes faecalis* (12) had K_i values of 0.5, 2.7, and 3.0 mM, respectively, indicating that glucose had a greater effect on these enzymes than on the β-glucosidase of *A. terreus*. Glucose inhibition of the β-glucosidase of *A. terreus*, *P. chrysosporium*, and *Alcaligenes faecalis* was competitive. Glucose exerted a noncompetitive inhibition on *L. trabea* β-glucosidase. Even though the K_i for glucose obtained was high compared with other β-glucosidases, high levels of this enzyme would be required to obtain maximal rates of hydrolysis in the presence of elevated levels of glucose. It should also be noted that the K_i was determined with an analog substrate and more meaningful data would be obtained if cellobiose were used as the substrate.

Proteases had no effect on the enzyme activity. Possibly this was due to the large carbohydrate moiety protecting the protein moiety by steric hindrance, preventing the protease from coming in contact with the protein. Cations also had little or no effect on the activity of the enzyme. Dithiothreitol and β-mercaptoethanol had no effect on the enzyme activity, suggesting either that sulfhydryl groups play a minor, if any, role in the enzyme activity or that they are protected.

The β-glucosidase of *A. terreus* shows potential as an industrial source of β-glucosidase. It

has a high affinity for cellobiose and one of the highest specific activities ever reported for cellobiase activity. It is affected only slightly by glucose compared with other fungal β-glucosidases. The enzyme is extremely stable and is not affected significantly by proteases or cations. One of its most important attributes is that its pH and temperature optima match those reported for *Trichoderma* cellulase, indicating a potential for supplementing *Trichoderma* cellulase with β-glucosidase from *A. terreus*.

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