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Separation and Some Properties of Two Intracellular β-Glucosidases of *Sporotrichum (Chrysosporium) thermophile*

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Intracellular, inducible β-glucosidase from the cellulolytic fungus *Sporotrichum (Chrysosporium) thermophile* (ATCC 42464) was fractionated by gel chromatography or isoelectric focusing into components A and B. Enzyme A (molecular weight 440,000) had only aryl-β-glucosidase activity, whereas enzyme B (molecular weight 40,000) hydrolyzed several β-glucosides but had only low activity against *o*-nitrophenyl-β-D-glucopyranoside (ONPG). Both enzymes had temperature optima of about 50°C. The pH optimum was 5.6 for enzyme A and 6.3 for enzyme B, respectively. The K_m (ONPG) value for enzyme A was 0.5 mM, and the corresponding values for enzyme B were 0.18 mM (ONPG) and 0.28 mM (cellobiose). Enzyme B, when tested with ONPG, showed substrate inhibition at a substrate concentration above 0.4 mM which could be released by cellobiitol and other alditols. Enzyme A was isoelectric at pH 4.48, and enzyme B was isoelectric at pH 4.64. Several inhibitors were tested for their action on the activity of enzymes A and B. Both enzymes were found to be concomitantly induced in cultures with either cellobiose or cellulose as carbon source.

In a recent publication (4) the induction and synthesis of an intracellular β-glucosidase in *Sporotrichum (Chrysosporium) thermophile*, a cellulolytic deuteromycete, were described. Cell extracts from this strain showed hydrolytic activity against *o*-nitrophenyl-β-D-glucopyranoside (ONPG) as well as against cellobiose, laminaribiose, and sophorose. No physiological evidence was, however, found in favor of the presence of more than one β-glucosidase species in this fungus.

With respect to their number (7, 9, 20), location (7, 9), induction (4, 7, 15), and substrate specificity (9, 23), microbial β-glucosidases show remarkable differences which, in some cases, may be correlated with their different functions, for example, morphogenesis, intra-, or extracellular β-glucoside hydrolysis. On the other hand these differences may simply reflect some natural (e.g., aging [27, 30]) or artificial (e.g., biochemical purification) secondary modifications.

In the present study it was shown that the aryl-β-glucosidase activity from *Sporotrichum thermophile* was associated with two distinct enzymes of which only one hydrolyzes disaccharides such as cellobiose, sophorose, or laminaribiose.

MATERIALS AND METHODS

Organism. The organism used in this study was a strain of *S. thermophile* originally isolated from a paper compost (T. G. Barnes, Ph.D. thesis, Aston University, Birmingham, Great Britain 1974). The strain has now been added to the American Type Culture Collection (no. 42464).

Growth techniques. The fungus was maintained and grown as previously described (3). Large amounts of mycelium were produced in a 14-liter fermentor (New Brunswick Scientific Co., New Brunswick, N.J., model MGF-14).

Enzyme extraction and purification. The harvested mycelium was homogenized by sonication (4). β-Glucosidase enrichment and partial purification were achieved as follows. The protein content of the cell-free extract (50,000 × *g* supernatant, 20 min) was adjusted to about 5 mg/ml with 0.05 M acetate buffer (pH 5.75); ammonium sulfate powder was then slowly added with constant stirring to 20% saturation at about 0°C, and after 4 h in the cold the precipitate was removed by centrifugation (50,000 × *g*, 15 min) and discarded. The ammonium sulfate concentration of the supernatant was adjusted to 75% saturation and left overnight in the cold. The precipitate, which contained most of the β-glucosidase, was collected by centrifugation (50,000 × *g*, 15 min) and dissolved in a minimum amount of buffer. Ammonium sulfate was removed by dialysis in a collodion tube (SM 13200; Sartorius, Goettingen, Federal Republic of Germany) against distilled water and finally against 0.05 M tris-(hydroxymethyl)aminomethane-hydrochloride buffer (pH 8.0). The enzyme sample was concentrated with immersible molecular separators with a nominal exclusion limit of 10,000 molecular weight (Millipore Corp., Bedford, Mass.). Ion-exchange chromatography was carried out on a column (420 by 45 mm inside di-

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ameter) of diethylaminoethyl-Sephacel (Pharmacia, Uppsala, Sweden) by step-wise elution with tris-(hydroxymethyl)aminomethane-hydrochloride buffer (0.05, 0.1, and 0.5 M) at pH 8.0 and 1°C. The β-glucosidase was eluted as a single sharp peak with 0.5 M buffer. The fractions containing the β-glucosidase were concentrated and dialyzed against 0.05 M sodium acetate buffer (pH 5.75). The material was finally chromatographed on a column (430 by 25 mm inside diameter) of Sephadex G-100 (Pharmacia) equilibrated with 0.05 M sodium acetate buffer (pH 8.0) at 1°C to give two fractions containing β-glucosidase activity.

Isoelectric focusing. Isoelectric focusing was carried out in tubes (125 by 15 mm inside diameter) at 1°C. The gels contained 7.5% polyacrylamide (Cyanogum 41; BDH Chemicals, Poole, Great Britain) and 2.7% ampholyte (Servalit; Serva, Heidelberg, Federal Republic of Germany) to give a pH gradient from pH 4 to 6. The initial current was 4 mA per tube, and the isoelectric focusing was continued for 12 h at constant power. The gels were cut into slices of thickness 1 to 2 mm, and each slice was left standing at room temperature in 0.5 ml of boiled distilled water. After 3 h the pH of each solution was measured and then adjusted to pH 5.75 with 0.1 M sodium acetate buffer before determining the β-glucosidase activity.

Molecular weight. Molecular weight estimation was carried out by gel filtration on Sephadex G-200 (2).

Enzyme assays. Endo-cellulase (carboxymethylcellulase; EC 3.2.1.4) was assayed viscometrically and expressed in arbitrarily defined units as previously described (3).

β-Glucosidase (EC 3.2.1.21) activity was assayed at 30°C (unless otherwise indicated) with either ONPG or cellobiose as previously reported (4). Enzyme activity was expressed as nanomoles of substrate hydrolyzed in unit time. *o*-Nitrophenol concentration was calculated by applying a molar extinction coefficient = 19.7×10^3. (Due to a deteriorated sample of *o*-nitrophenol, the calculated molar extinction coefficient applied in reference 4 was found to be smaller; the values therein reported should be corrected by a factor = 0.25.) To avoid substrate inhibition by ONPG with enzyme B, the concentration of the former in the test solution was reduced to 0.3 mM.

Transglycosylation. Glucosyl-transferase activity of the β-glucosidase was tested by incubating the enzyme solution (0.1 ml) with 110 mM cellobiose (1 ml) for 2 h at 40°C. The reaction products were examined by thin-layer chromatography on silica gel (Kieselgel G; Merck, Darmstadt, Federal Republic of Germany) by double development with acetone-water (88:12, vol/vol) at 45°C. The products were detected by spraying the plates with naphthol-1-concentrated sulfuric acid and heating at 110°C.

Protein determination. Protein was estimated by the method of Lowry et al. (19) using bovine serum albumin as standard.

Glucose determination. Glucose was estimated using glucose-oxidase (GOD-Perid method; Boehringer, Mannheim, Federal Republic of Germany). As reported previously (4), the contaminating β-glucosidase present in the commercial enzyme preparation was inhibited with tris(hydroxymethyl)aminomethane at pH 8.5; interference due to hydrolysis of cellobiose was then negligible.

Biomass estimation. Biomass was determined by weighing washed mycelium samples using the conventional filter paper technique.

Chemicals. All reagents were of analytical grade. Cellobiose and sophorose were purchased from Senn Chemicals (Switzerland); ONPG, glucono-δ-lactone, and sodium *p*-hydroxymercuribenzoate were from Fluka (Switzerland). Cellobionic acid was prepared from cellobiose by chlorite oxidation, and cellobiitol was prepared by reduction of cellobiose with $NaBH_4$ as previously described (3).

RESULTS

β-Glucosidase enrichment and purification. A summary of the purification scheme for the β-glucosidase from *S. thermophile* is given in Table 1. The cell-free extract from the mycelium harvested from a 10-liter culture on cellobiose (0.15%) as carbon source was partially purified by ammonium sulfate precipitation and ion-exchange chromatography on diethylaminoethyl-cellulose. Subsequent gel filtration on Sephadex G-100 gave two distinct protein fractions with β-glucosidase activity (Fig. 1), indicating that the two enzymes differ in molecular size. These β-glucosidases are hereafter referred

TABLE 1. *Purification scheme for the β-glucosidase from S. thermophile*

Purification step	Total protein (mg)	Total aryl-β-glucosidase activity[a] (nmol/min)	Sp act (nmol/min per mg)	Purification factor	Yield (%)
Supernatant after centrifugation of the cell-free extract (50,000 × *g*, 20 min)	5,200	217,142	42		100
$(NH_4)_2SO_4$ precipitation between 20% and 75% saturation at 0°C	1,270	210,476	165	4	97
Diethylaminoethyl-Sephacel chromatography	230	88,095	383	9	41
Sephadex G-100 chromatography					
Enzyme A	30	17,857	595	14	
Enzyme B	50	64,761	1,295	31	

[a] The activity was routinely determined as aryl-β-glucosidase with ONPG at 45°C.

to as enzyme A and enzyme B, respectively.

Fractionation of the β-glucosidase activity into the two components could also be achieved in one step by gel chromatography or isoelectric focusing but was less efficient. The elution profile from a Sephadex G-100 column was essentially similar to that shown in Fig. 1. Isoelectric focusing of the crude mycelium extract in a pH gradient of 4.0 to 5.3 gave two β-glucosidase components isoelectric at pH 4.48 and 4.64, respectively (Fig. 2). It has subsequently been shown that these values correspond to enzyme A and enzyme B, respectively. Molecular weight estimations of both protein species, obtained by gel filtration on Sephadex G-200 (Fig. 3), showed that enzyme A was larger (about 11 times or 440,000 molecular weight) than enzyme B (40,000 molecular weight).

Enzyme characteristics. Both enzymes were tested for their specificity towards several β-glucosides. Table 2 shows that enzyme A was specific for ONPG whereas enzyme B displayed a broader specificity and could hydrolyze *inter alia* β-linked oligosaccharides such as cellobiose, laminaribose, and sophorose and to a much smaller extent ONPG. It is interesting to note that, whereas the ability of a crude cell extract to hydrolyze laminaribiose was retained during the various purification steps, the ability to hydrolyze sophorose was substantially lost (cf. 4).

The effect of various buffers on the hydrolytic activity of enzyme B is shown in Table 3. Tris(hydroxymethyl)aminomethane buffer considerably decreased (50% inhibition) the hydrolytic activity of enzyme B but had a smaller effect on enzyme A. Results with phosphate buffer gave no evidence for the presence of any additional phosphorolytic activity. In all of the buffers tested, no activity other than aryl-β-glucosidase could be ascribed to enzyme A.

Different substances were tested for their inhibition of the activity of both enzymes A and B (Table 4). It can be seen that glucono-δ-lactone, known to specifically inhibit β-glucosidases (21,

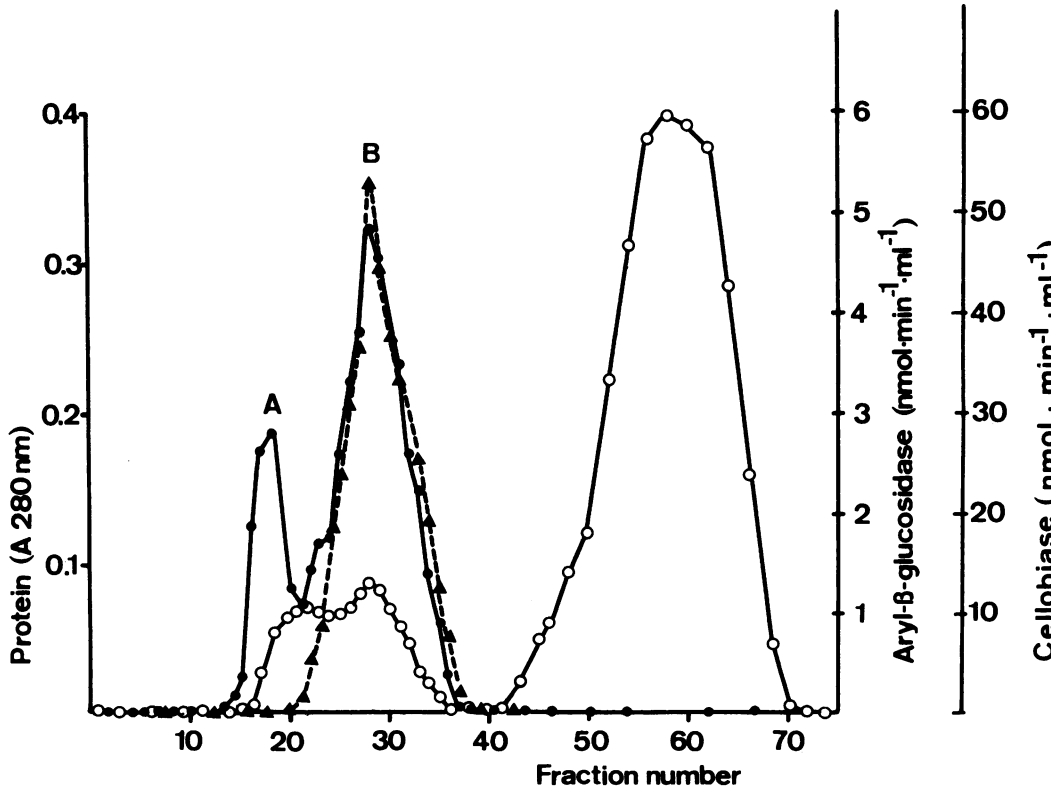

FIG. 1. *Distribution of protein, aryl-β-glucosidase, and cellobiase activity after chromatography on Sephadex G-100. The pooled β-glucosidase (ONPG) fractions after chromatography on diethylaminoethyl-Sephacel were applied to a Sephadex G-100 column (25 by 430 mm inside diameter). Enzyme solution was dialyzed and concentrated before application as described in the text. The enzymes were eluted with 0.05 M sodium acetate buffer (pH 5.75) at 57 ml/h. The fraction volume was 4 ml. Symbols:* ○——○, *protein;* ●——●, *aryl-β-glucosidase activity;* ▲- - -▲, *cellobiase activity.*

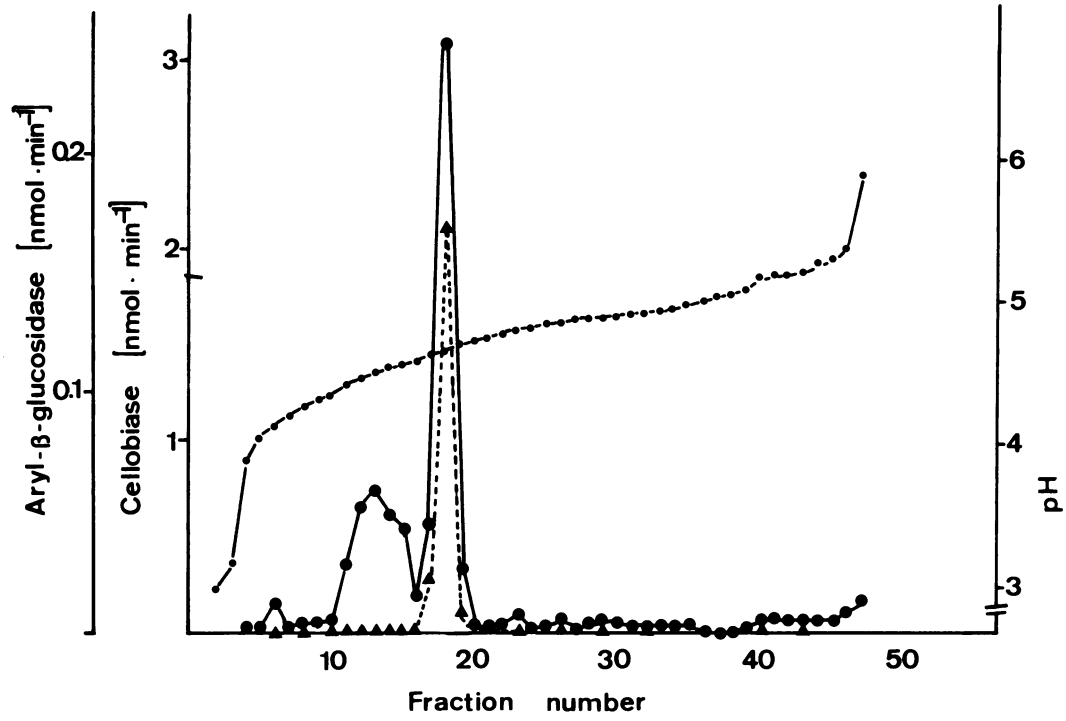

FIG. 2. *Isoelectric focusing of a cell-free extact of S. thermophile. Symbols:* ●——●, *aryl-β-glucosidase activity;* ▲- - -▲, *cellobiase activity;* ●——●, *pH.*

22), affected enzymes A and B to a similar extent. Heavy metal ions and the thiol reagent 4-hydroxy-mercuribenzoate were significantly less efficient against enzyme A than enzyme B. Cellobiitol, which at a high substrate concentration was found to suppress substrate inhibition of ONPG with enzyme B, inhibited the hydrolysis of cellobiose by the same enzyme.

K_m values for both enzymes were determined by the double-reciprocal plot method of Lineweaver-Burk. Only with enzyme A (ONPG as substrate) was a linear relationship observed. Enzyme B showed substrate inhibition with either ONPG or cellobiose; this inhibition was apparent at substrate concentrations above 4×10^{-4} M for ONPG and 3.5×10^{-3} M for cellobiose. The inhibition at a high substrate concentration (3.3 mM) was completely suppressed by cellobiitol in the concentration range 100 to 200 mM (Fig. 4), less efficiently suppressed by other alditols such as xylitol, glucitol, mannitol, or galactitol, and not at all suppressed by maltitol or lactitol (results not given). Figure 5 shows the inhibition effect of cellobiose on the hydrolysis of ONPG by enzyme B. The effect was observed at cellobiose concentrations between 1 and 100 mM and found to be more pronounced at high (3 mM) rather than low (0.3 mM) substrate (ONPG) concentration.

Table 5 gives a summary of the properties of the partially purified enzymes. The pH optima for hydrolysis for enzymes A and B were 5.6 and 6.2 to 6.3, respectively, but both enzymes had a temperature optimum of 50°C. At this temperature some heat deactivation could be observed. Both enzymes were stable at temperatures less than 40°C, and heat sensitivity increased rapidly between 40 and 50°C. The K_m values (ONPG) for enzymes A and B were 5×10^{-4} M and 1.8×10^{-4} M, respectively, and the K_m value (cellobiose) for enzyme B was 2.8×10^{-4} M.

Only enzyme B showed glucosyl-transferase activity when incubated with cellobiose (100 mM) as substrate. At least three transglycosylation products (with mobilities corresponding to di- and trisaccharides on thin-layer chromatography) were detected after incubation for 2 h at 40°C.

β-Glucosidase formation. The intracellular aryl-β-glucosidase and the cellobiase activities appear together in this organism when grown on cellobiose or cellulose (4). In an additional experiment, the induction profiles of enzymes A and B were obtained by fractionation on Sephadex G-100 of cell extracts prepared at different growth intervals during the initial growth period on cellobiose. The results are given in Fig. 6. It can be seen that both enzymes A and B were

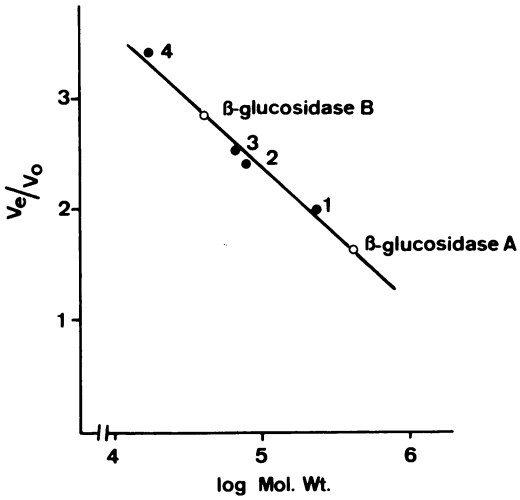

FIG. 3. *Estimation of the molecular weight of enzymes A and B by Sephadex G-200 gel chromatography. A cell-free extract (0.5 ml) was loaded onto the top of a Sephadex G-200 column (265 by 14 mm inside diameter) previously equilibrated in the cold (2°C) with 0.05 M sodium acetate buffer (pH 5.75). Subsequent elution (4.5 ml/h) was with the same buffer, and fractions (1.1 ml) were collected and analyzed for aryl-β-glucosidase as well as cellobiase activity. V_0 was determined with Blue Dextran 2000. The following proteins were used for calibration: 1, catalase (molecular weight 240,000); 2, human transferrin (molecular weight 80,000); 3, bovine serum albumin (molecular weight 68,000); 4, myoglobin (molecular weight 17,800).*

TABLE 2. *Hydrolytic activity towards some β-glucosides of partially purified β-glucosidases A and B of S. thermophile grown on cellobiose*[a]

Substrate	Relative activity (%)	
	Enzyme A	Enzyme B
Cellobiose	0	100
Laminaribiose	8	98
ONPG	100	9
Sophorose	4	52
Cellobionic acid	0	4
Gentiobiose	0	4
Cellobiitol	0	2

[a] Activities against cellobiose and ONPG were determined as described in the text. Activities against the other substrates were determined as for cellobiose by replacing the latter with the corresponding substrate (3.3 mM).

induced simultaneously and that their activity ratios were more or less constant throughout the period of observation. An essentially similar result was obtained (results not given) when the organism was grown on crystalline cellulose (Whatman CC31). With cellulose as substrate, however, the growth of the organism, as reflected in the cellulase induction phase (4), was retarded.

TABLE 3. *Hydrolytic activity of enzymes A and B against ONPG and cellobiose in different buffers at pH 6.0*[a]

Buffer	Enzyme A		Enzyme B	
	Aryl-β-glucosidase	Cellobiase	Aryl-β-glucosidase	Cellobiase
PIPES	100	2	100	90
Sodium acetate	96	1	89	96
McIlvaine	96	1	92	100
Phosphate (Sørensen)	94	1	91	100
Tris-hydrochloride[b]	87	0	49	51

[a] An adequately diluted enzyme sample (0.1 ml) was mixed with buffer (0.9 ml), and the activity was determined as described in the text.

[b] Tris, Tris(hydroxymethyl)aminomethane.

TABLE 4. *Effect of different inhibitors on the hydrolytic activity of β-glucosidases A and B*[a]

Inhibitors	Actual inhibitor concn at which a 50% inhibition of β-glucosidase activity was observed		
	β-Glucosidase A: ONPG	β-Glucosidase B	
		ONPG	Cellobiose
Gluconolactone	4×10^{-4} M	3×10^{-4} M	2×10^{-3} M
$CuCl_2$	2×10^{-2} M	7×10^{-4} M	7×10^{-5} M
$Pb(NO_3)_2$	2×10^{-2} M	8×10^{-4} M	8×10^{-3} M
4-Hydroxy-mercuribenzoate	—[b]	3×10^{-5} M	7×10^{-6} M
Cellobiitol	—	—	4×10^{-2} M

[a] To 0.9 ml of enzyme solution, 0.1 ml of the inhibitor solution was added and preincubated at 30°C for 10 min.

[b] —, ONPG hydrolysis by enzyme A was unaffected at 5×10^{-4} inhibitor concentration.

DISCUSSION

The results obtained after gel chromatography or isoelectric focusing of *S. thermophile* cell-free extracts indicate the presence of two β-glucosidases, one of which, enzyme A, is able to efficiently hydrolyze only the aryl glucoside ONPG. A similar restricted specificity has been reported for β-glucosidases from different sources (cf. 23). Since the normal substrate is unknown, the metabolic role of these enzymes is not clear. They may represent an inactive cellobiase precursor or have lost their full catalytic activity during the purification procedure or even, in the case of extracellular enzymes, during secretion. Some exo-β-glucanases (5) can also hydrolyze ONPG, but very little β-1,3-glucanase (laminaranase) has been found in cell-free extracts from *S. thermophile* (4).

The second enzyme, enzyme B, which in addition to ONPG also hydrolyzes cellobiose, lam-

inaribiose, and sophorose, has no action on gentiobiose and is hereby similar to the β-glucosidase of *Alicaligenes faecalis* (12).

The values for the temperature and pH optima for hydrolysis and the pI values of both enzymes of *S. thermophile* are similar to those reported for other β-glucosidases from microorganisms (1, 7, 10, 11, 16, 32). Both β-glucosidases are not particularly thermoresistant, being inactivated at a temperature of about 50°C. Although a temperature optimum as high as 75°C has been reported for the extracellular enzyme from *Lenzites trabea* (13), the values are normally much lower.

Among the different inhibitors studied, which were specific for β-glucosidases, glucono-δ-lactone was the most efficient. Such inhibition has been explained by steric similarities between the lactone and the enzyme-bound substrate (7, 10, 21, 29). Some yeast β-glucosidases (8, 14, 15) as well as the β-glucosidase of *Botryodiplodia theobromae* (27) have been reported to be inhibited by thiol reagents like indoacetamide or *p*-chloromercuribenzoate. Enzyme B was strongly inhibited by *p*-chloromercuribenzoate, whereas enzyme A was unaffected even at a 10-fold inhibitor concentration. Although to a lesser extent a differential inhibition was also observed with Pb^{2+} and Cu^{2+} ions, these effects are indic-

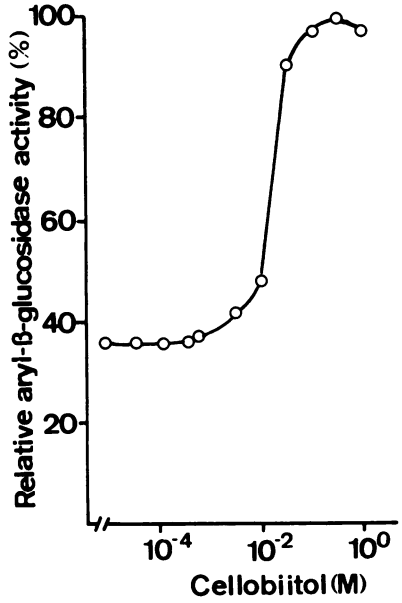

FIG. 4. *Effect of cellobiitol on aryl-β-glucosidase activity of enzyme B. Relative activities are given as percent of the maximum activity obtained. Inhibiting amounts of ONPG (3.3 mM) were added to enzyme B, and the activity was measured in the presence of different amounts of cellobiitol.*

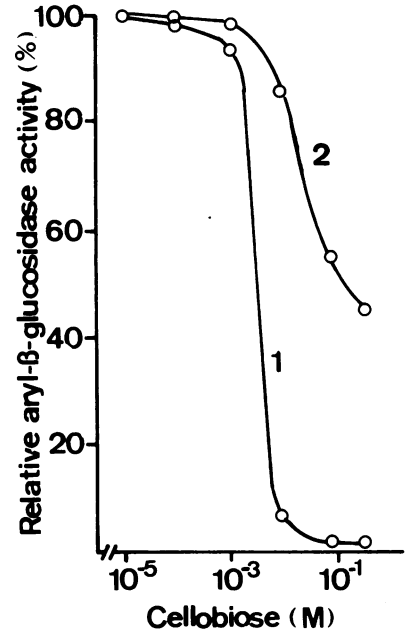

FIG. 5. *Effect of cellobiose on the aryl-β-glucosidase activity of enzyme B. Relative activities are expressed as in Fig. 4. Enzyme activity was determined at high (3.3 mM; graph 1) and low (0.3 mM; graph 2) ONPG concentration in the presence of different amounts of cellobiose.*

TABLE 5. *Summary of salient characteristics of β-glucosidases A and B*

Characteristic	Enzyme A: aryl-β-glucosidase	Enzyme B	
		Aryl-β-glucosidase	Cellobiase
Mol wt	440,000	40,000	40,000
pH optium	5.6	6.2	6.3
Temp optimum	~50°C	~50°C	~50°C
K_m	5×10^{-4} M	1.8×10^{-4} M	2.8×10^{-4} M
Substrate inhibition	—	4×10^{-4} M	3.5×10^{-3} M
50% Inhibition by 4-hydroxymercuribenzoate	—	10^{-5} M	10^{-6} M
Thermolability[a]	47–48°C	46–47°C	47–48°C

[a] Enzyme solutions were held at different temperatures for 15 min, and then the activity was determined as described in the text. The given values represent the temperature at which 50% of the activity was lost.

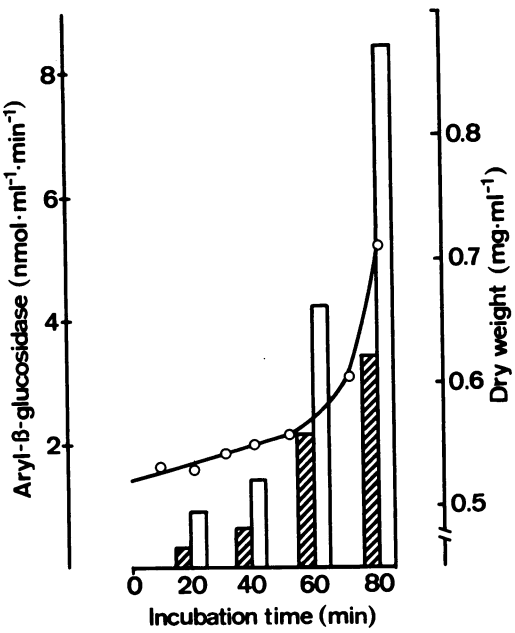

FIG. 6. *Induction pattern of enzyme A (dashed bars) and enzyme B (open bars) during growth on cellobiose. After spore germination in basic medium without sugar (inoculated with 4 × 10^6 conidia per ml; 8 h at 44°C), the young mycelium was filtered off, washed, and used to inoculate the basic medium supplemented with 0.25% (wt/vol) cellobiose. After 20, 40, 60, and 80 min of incubation at 44°C, samples were withdrawn, from which cell-free extracts were prepared. Each sample was then fractionated on a Sephadex G-100 column (10 by 480 mm inside diameter) equilibrated with 0.05 M sodium acetate buffer (pH 5.75). Elution was as described under Fig. 1.*

ative of sulfhydryl groups being involved in the catalytic action of enzyme B.

Substrate inhibition has been reported for the β-glucosidases from *Aspergillus phoenicus* (26) and *B. theobromae* (28); the latter organism produces, under senescence conditions, four β-glucosidases of different molecular weight which are derived by dissociation from the largest enzyme species. Only the smallest molecule shows substrate inhibition, whereas the Michaelis constant (K_m, ONPG) and heat stability increase with increasing molecular complexity. The strong substrate inhibition observed here for enzyme B with either ONPG or cellobiose (inhibition by cellobiose was smaller; see Table 5) is efficiently suppressed by cellobiitol at 400 mM and some other alditols at quite high concentrations (results not given), whereas reducing sugars are inefficient or even inhibit as in the case of cellobiose during the hydrolysis of ONPG. This cellobiose inhibition, which is particularly strong when ONPG hydrolysis is carried out at high substrate (ONPG) concentration, is likely to be of competitive nature, but no work has been undertaken to elucidate this point. The relief from ONPG (substrate) inhibition by cellobiitol could be explained by its glucosyl acceptor nature at high concentration. For the β-glucosidase from *B. theobromae*, a similar effect was observed with *p*-nitrophenyl-glucoside as donor and glycerol (at 100 mM) as receptor (28). In this instance, the involvement of glucosyltransferase activity was directly demonstrated. The Michaelis constants for both the β-glucosidases from *S. thermophile* show values which are situated in a fairly common range of about 10^{-4} M as compared with β-glucosidases from other source (1, 7).

Estimations of the molecular weights of the two enzymes by gel filtration show that enzyme B is about 11 times smaller than enzyme A. Umezurike (27, 28) found that the β-glucosidase of *B. theobromae* (molecular weight 350,000 to 380,000) is built up of eight subunits (molecular weight 45,000 to 47,000), which in turn represent the association of four identical noncatalytic polypeptides. For the enzymes from *S. thermophile*, no evidence was found for a similar multimeric association. Moreover, the ratio between the two protein species was constant from the very beginning of the induction phase. Enzyme B (molecular weight 40,000) is quantitatively more abundant and possesses a broader specificity than the high-molecular-weight enzyme A (molecular weight 440,000). With respect to its molecular weight, enzyme B does not fit the general observation that fungal, and particularly yeast, β-glucosidases are very large molecules in the molecular weight range 76,000 to 440,000 (8, 17, 18, 25). Recently, however, a β-glucosidase from *Candida guilliermondii* has been reported to have molecular weight of 48,000 (24).

Transglucosylase activity is common to many β-glucosidases and has been reported by several authors (6, 29). Vaheri et al. (31) demonstrated a definite glucosyl-transferase activity in the culture filtrate of *Trichoderma reesei* and identified laminaribiose, sophorose, gentiobiose, and additional trisaccharides as transglycosylation products. They believe that this activity is most likely due to the intracellular β-glucosidase leaking into the medium as the culture ages. Enzyme B and also cell-free extracts from *S. thermophile*, when incubated with cellobiose (100 mM), produce at least three new unidentified products. Obviously the direct involvement of a transglucosylation reaction in the intracellular (cellulase) induction process is, on the basis of these simple findings, only speculative.

Since enzymes A and B are induced simultaneously in cultures on cellobiose or cellulose and their ratio remains more or less constant, it is possible that both are regulated by the same

mechanism of induction. The evidence for the involvement of either of the β-glucosidases in the cellulolytic metabolism of *S. thermophile* is for the moment only circumstantial: (i) the enzymes are synthesized together with endo-cellulose, albeit with a distinctly delayed synthesis of the latter (4); (ii) the metabolic fate of cellobiose in this organism is at present unknown, and the proposition of simple intracellular cleavage of cellobiose into glucose is somewhat countered by the fact that glucose exerts catabolic repression on enzyme formation (β-glucosidase and endo-cellulase); (iii) transglucosylation reactions do not necessarily occur intracellularly in vivo as in vitro to produce specifically inducing substances.

These considerations warrant further studies to elucidate the function of the β-glucosidases in the whole process of cellulolysis in this organism.

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