

β -Glucosidase Induction and Repression in the Cellulolytic Fungus, *Trichoderma reesei*

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STERNBERG, D., AND MANDELS, G. R. 1982. β -Glucosidase induction and repression in the cellulolytic fungus, *Trichoderma reesei*. *Experimental Mycology* 6, 115-124. β -Glucosidase in *Trichoderma reesei* (QM 6a) can be induced by methyl- β -glucoside and less effectively by gentiobiose; other glycosides tested, including cellobiose, did not induce this enzyme. Sophorose (a β -1,2 dimer of glucose) at sub-micromolar concentrations repressed β -glucosidase, repression being only partially reversed after sophorose was removed. β -Glucosidase induction has no well-defined pH optimum, although in citrate buffer it is sharply enhanced around pH 3. The optimum temperature for induction is 28°C (at pH 3.0) and response to inducer concentration is hyperbolic. β -Glucosidase (1) is tightly associated with mycelium, (2) is produced with no detectable lag between the time inducer is absorbed and induction starts, and (3) is produced constitutively at low levels. The low, constitutive activity of β -glucosidase is more than adequate to theoretically account for respiration of the fungus on cellobiose.

INDEX DESCRIPTORS: cellulose; cellulolytic fungi; β -glucosidase; induction; methyl- β -glucoside; repression; sophorose; *Trichoderma reesei*.

The fungus *Trichoderma reesei* (Simmons, 1977) has been used extensively in studies on the enzymatic saccharification of cellulosic materials to glucose. The primary characteristic of this fungus which distinguishes it from other known organisms, some of which may be more active degraders of cellulose, is its secretion of an active extracellular cellulolytic system. The cellulolytic systems of *Trichoderma* species have been studied both for the nature of the various enzymes involved in the hydrolysis of crystalline cellulose and for the production of highly active culture filtrates for practical saccharifications. The *Trichoderma* cellulase enzymes are of two types, differentiated by the site of hydrolysis of cellulose: random acting (*endo*- β -1,4-glucanoglucanase, EC 3.2.1.4) and terminal acting cellobiohydrolase, EC 3.2.1.91). The major soluble product of the cellulase enzymes is cellobiose (4-*O*- β -glucopyranosyl-D-glucose) which is hydrolyzed to glucose by the noncellulolytic enzyme, β -glucosidase (EC 3.2.1.21). The whole sub-

ject has been reviewed recently (Ryu and Mandels, 1980).

Most studies of *Trichoderma* fermentations have focused on the cellulase enzymes which represent about 95% of the extracellular protein (Bissett, 1979). β -Glucosidase represents only about 1% of the cellulase complex protein. However, it has a much higher specific activity on cellobiose than does cellulase acting on cellulose. Hence, the amount of β -glucosidase produced during growth on cellulose is adequate to hydrolyze cellobiose during growth on cellulose where soluble sugars do not accumulate. In a saccharification reaction β -glucosidase activity is greatly reduced by glucose inhibition, and higher β -glucosidase activities are required for efficient conversion of cellulose (Sternberg *et al.*, 1977).

The induction of cellulase has been the subject of several investigations (Gritzali and Brown, 1979; Mandels *et al.*, 1962; Nisizawa *et al.*, 1971; Sternberg and Mandels, 1979). The present paper reports on

the induction of β -glucosidase in *T. reesei* and points out some fundamental differences from cellulase induction by sophorose and from β -glucosidase induction in other fungi.

MATERIALS AND METHODS

Cultures. The organism used was a wild-type strain of *T. reesei* QM 6a. Spores for inoculum were produced on an agar medium as previously described (Sternberg and Mandels, 1979). Early postexponential mycelia were prepared by culturing on 0.3% glucose minimal medium (Mandels and Weber, 1969; Sternberg and Mandels, 1979) buffered with 0.05 M K-citrate at pH 4.4 for 24 h by which time all glucose had disappeared. Mycelia were centrifuged and washed two times with distilled water immediately prior to induction studies. Autolysis of mycelium sufficient to interfere with experiments or assays did not occur.

Induction. Mycelial suspensions were made with 0.25-strength *Trichoderma* salts (Sternberg and Mandels, 1979) lacking urea and KH_2PO_4 in 0.05 M K-citrate at pH 3.0 (unless specified otherwise) and the appropriate concentration of inducer in a total of 12 ml reaction volume. Growth does not occur in this system since nitrogen and phosphate are lacking and the inducer is at too low a concentration. The final mycelial dry weight in the induction medium was ca. 1.5 (1.2–1.9) mg/ml. Incubation was carried out in covered 50-ml flasks on a reciprocal shaker at 28°C except where noted. The entire contents of each flask were harvested at appropriate times and centrifuged for each sample. The mycelial pellet was washed two times and stored at –15°C. Absorption of inducers was determined in the supernatant fluids by measuring the change in concentration from zero time to that at harvest. (Numerous experiments have established this as a reliable way to determine kinetics of absorption for purposes reported here.)

Assays. For determining mycelial as-

sociated β -glucosidase activity, the mycelial pellets were thawed, suspended in 12 ml of 0.05 M Na-citrate buffer, pH 4.8, and blended in a VirTis "45" homogenizer (VirTis Research Equipment, Gardiner, N.Y.) at medium speed for 30 s; the blended mycelium was pipetted into reaction tubes. Extramycelial β -glucosidase was determined by using the supernatant solutions collected at the time of harvest. β -Glucosidase activity was measured with 7.5 mM cellobiose (Eastman Kodak Co., Rochester, N.Y.) as substrate (Sternberg *et al.*, 1977). Carboxymethyl cellulase was measured by following the increase in reducing power in a 0.5% solution of carboxymethyl cellulose (Mandels and Weber, 1969). Enzyme assays were incubated at 50°C in 0.05 M Na-citrate buffer, pH 4.8, for 30 min; reactions were stopped by immersion in a boiling water bath for 5 min. A unit (U) of activity is expressed as the number of micromoles glucose (or glucose equivalents) produced per minute. Residual methyl- β -glucoside (M β G)¹ was measured by the phenolsulfuric acid method (Dubois *et al.* 1956). The treatment of mycelium (freezing) and the conditions of assay (50°C) were such that assimilation of hydrolytic products did not interfere with the enzyme assays by metabolism of glucose released during the assay.

Respirometric methods. Typical methods were used, employing a Gilson differential respirometer (Gilson Medical Electronic, Middletown, WI) at 30°C; 2 ml of mycelial suspension was added to the vessel, and 0.5 ml of substrate solution to the sidearm. Results were expressed as Q_{O_2} = microliters of O_2 consumed per hour per milligram dry weight of mycelium.

RESULTS

Induction

(1) *Specificity of inducing compounds.* A wide variety of glycosides and other carbon

¹ Abbreviation used: M β G, methyl- β -glucoside.

sources was tested for their activity as inducers (Table 1). Methyl- β -glucoside (M β G), gentiobiose (6-*O*- β -D-glucopyranosyl-D-glucose), and 5-thioglucoase gave appreciable induction, the latter two being less than half as effective as M β G. Other β -glucosides did not induce β -glucosidase, while a number of compounds had little or no inducing ability by themselves or had questionable effects, but when used at a low concentration (100 μ g/ml) enhanced induction by M β G at 500 μ g/ml). M β G, the most active inducer, was used for further studies.

(2) *Temperature and pH optima for induction.* Similar to the induction of cellulase by sophorose (Sternberg and Mandels, 1979), the optimum induction response was at 28°C with a slower induction and slightly lower yield at 24°C. At 34°C the induction response was rapid but the M β G was rapidly consumed and induction ceased with enzyme at a lower level than at 28°C. Only constitutive levels (ca. 0.3 U/mg) of β -glucosidase were produced at 40°C.

The effect of pH on induction depended on the kind of buffer used (Fig. 1). At pH 4.5 the effects of KPO₄ and K-citrate buffers were comparable; at higher pH values with citrate the response was constant up to pH 7.0. However, below pH 4.5 the response with citrate buffer increased to a maximum at pH 3.0, whereas with PO₄ buffer the yield declined. The discrepancy between the two buffer systems appeared to be due to a positive effect of citrate rather than a deleterious effect of the K-phosphate because: (1) Using 0.05 M K-phosphate with 0.05 M citrate at pH 3.0 in the induction medium gave the same yield of β -glucosidase as citrate alone. (2) When induction was carried out in a fermentor without buffer and with the pH automatically controlled at 3.0 (by the addition of dilute H₂SO₄), the amount of β -glucosidase induced was similar to that using phosphate buffer in flasks. (3) When 0.05 M citrate was included in the fermentor medium, full induction occurred. The citrate effect probably was not due to its complexing with met-

als in the medium because induction was the same with or without the nutrient salts to which trace metals had been added. Other buffers (acetate, glutamate, phthalate, and succinate) did not increase β -glucosidase induction above that supported by K-phosphate. Perhaps the favorable effect of citrate was related to its metabolic utilization which can occur at very slow rates although respiration in the pH range is essentially the same in PO₄ and citrate buffers. At any rate, we were unable to establish the cause of the effect without doing unjustifiably more extensive experimentation.

(3) *Effect of M β G concentration.* The rate of absorption of M β G was concentration independent (Fig. 2A). Similar to cellulase induction by sophorose, the amount of β -glucosidase induced was a hyperbolic function of M β G concentration (Fig. 2B) being characterized by a linear relationship in a double-reciprocal plot (Fig. 2C). Half-maximal induction occurred at 2.9 mM (560 μ g/ml) M β G and the maximum activity inducible in this particular mycelial preparation was 2.8 U/mg. The induction of cellulase by sophorose is more sensitive with half-maximal induction at about 0.15 mM (Sternberg and Mandels, 1979).

(4) *Influence of age and developmental stage of mycelium.* The spores of *T. reesei* had a constitutive level of β -glucosidase equal to that of the mycelium, i.e., 0.2 to 0.3 U/mg, but were not subject to induction by M β G (Fig. 3) even though substantial amounts of the inducer were absorbed over a 24-hour period (data not shown). Swollen, but ungerminated, spores (incubated for 4 hours) also were not induced. Induction occurred after germination and reached its full potential in mycelium at the time of glucose exhaustion in the growth medium (24 hours old). In contrast, with *Schizophyllum commune*, maximum induction of β -glucosidase occurs in the germlings (just a few hours old) and relatively little induction in the mycelial stage (Wilson and Niederpruem, 1967). As the *T. reesei* culture starved in the

growth medium, inducibility declined. The number of cell divisions the mycelia underwent prior to induction had little or no effect on the amount of β -glucosidase induced. This was tested by transferring varying amounts of mycelium from one growth medium to another so that from 5 to 11 generations had been completed. In all cases amount of induction was about the same (data not shown).

(5) *Time study.* β -Glucosidase activity began increasing before absorption of M β G could be detected, i.e., there was no lag between inducer uptake and enzyme induction (Fig. 4). At pH 3.0 the consumption

of M β G lagged behind the induction response on a relative basis. When induction was at pH 3.5 or above, the uptake and induction curves were superimposable. In contrast, when cellulase was induced by sophorose, induction lagged several hours behind sophorose absorption. Another difference between these two systems was the low, but significant constitutive level of β -glucosidase in the mycelium, which slowly increased with time. No constitutive cellulase activity could be detected. The inducer (M β G) must be present for continued synthesis of β -glucosidase since washing the mycelium at any stage during induction

TABLE 1
Carbohydrates and Related Compounds as Inducers^a or Repressors^a of β -Glucosidase^b

Compound	Concn (μ g/ml)	β -Glucosidase (U/mg myc)		Source ^c
		-M β G	+M β G	
Induction control	—	0.24	1.15	—
Monosaccharides				
Sorbose	100	0.33	0.63	7
Mannose	100	0.32	1.41	8
Glucose	100	0.33	1.48	5
	500	0.22	0.66	
Fructose	100	0.28	1.50	9
α -Linked disaccharides				
Maltose	100	0.43	1.22	9
α -Trehalose ²	100	0.35	1.29	7
Isomaltose	100	0.24	1.40	12
Kojibiose	100	0.28	1.50	12
β -Linked disaccharides				
Sophorose	0.03	NT ^d	1.00	10
	0.3	NT	0.58	
	3.0	NT	0.26	
	100	0.10	0.11	
Laminaribiose	100	0.23	0.89	12
Lactose	100	0.25	0.89	8
Cellobiose	100	0.27	0.94	7
	500	0.32	0.77	
$\beta\beta$ -Trehalose	100	0.41	0.98	12
Sucrose	100	0.28	1.33	2
Gentiobiose	50	0.51	NT	1
	100	0.61	1.42	
	500	0.79	NT	
Aryl- β -glycosides				
Amygdalin	500	0.44	0.39	1
1- <i>p</i> -NO ₂ -Phenyl- β -galactoside	500	0.08	0.54	1
Esculin	500	0.44	0.63	4
Salicin	500	0.46	0.93	7
Phenyl- β -glucoside	500	0.51	1.05	9

TABLE 1—Continued

Compound	Concn ($\mu\text{g/ml}$)	β -Glucosidase (U/mg myc)		Source ^c
		-M β G	+M β G	
Alkyl-glycosides				
Methyl- β -thiogalactoside	500	0.17	0.79	3
Methyl- α -glucoside	500	0.24	0.91	9
Methyl- β -cellobioside	100	0.23	1.16	12
Methyl- β -galactoside	100	0.21	1.41	12
Methyl- β -thioglucoside	100	0.24	1.45	6
	500	0.22	NT	
<i>n</i> -Butyl- β -glucoside	100	0.52	1.45	12
Miscellaneous				
Nojirimycin	5	0.26	1.06	11
	100	NT	0.15	
Cellulose hydrolysate	100	0.16	0.57	13
2-Deoxyglucose	100	0.27	0.57	8
Cellobiitol	500	0.53	1.05	12
Glycerol	100	0.32	1.47	5
5-Thioglucose	100	0.76	1.49	1
	500	0.55	NT	

^a Induction determined in the absence of M β G; repression determined using 500 $\mu\text{g/ml}$ M β G as inducer. Statistical analysis of significance not possible, but experiments were repeated with all compounds reported as being active.

^b Standard mycelial preparation (1.5–1.9 mg/ml) incubated ca. 20 h with test compound; 0.05 M citrate buffer, pH 3.0.

^c Sources: 1—Aldrich Chemical Co.; 2—J. T. Baker Chemical Co.; 3—Calbiochem; 4—Difco; 5—Fisher Scientific Co.; 6—Mann Research Laboratory; 7—Nutritional Biochem. Corp.; 8—Pfanstiehl Labs.; 9—Sigma Chem. Co.; 10—D. H. Ball; 11—S. Inouye; 12—E. T. Reese; 13—A. Allen.

^d Not tested.

stops the process (Fig. 5). This was similar to the deinduction of cellulase by removal of sophorose (Sternberg and Mandels, 1979).

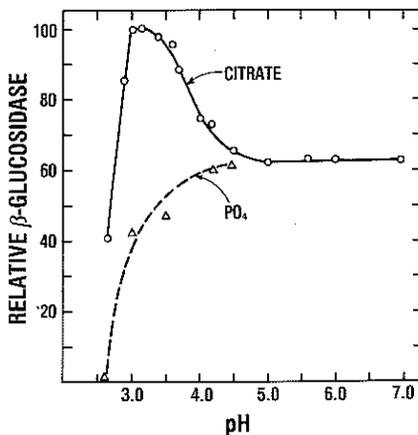


FIG. 1. Effect of pH on induction of β -glucosidase by M β G. M β G, 500 $\mu\text{g/ml}$; 0.05 M buffers—potassium citrate (O); potassium phosphate (Δ).

Repression

Sophorose was by far the most active repressor of induction by M β G (Table 1). Glucose at 500 $\mu\text{g/ml}$ repressed induction of β -glucosidase by M β G, but was stimulatory

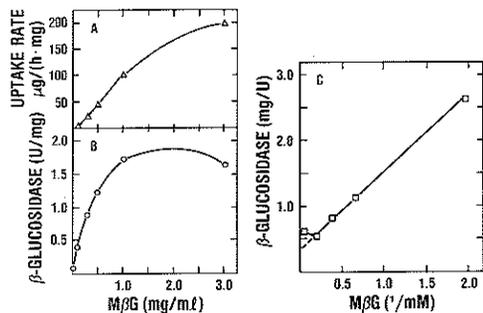


FIG. 2. Effect of M β G concentration on rate of uptake per milligram of mycelium (A) and on induction of β -glucosidase in units per milligram of mycelium (linear plot—B; double reciprocal plot—C). Citrate buffer, pH 3.0.

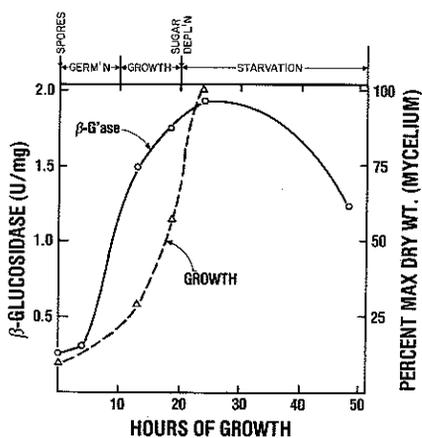


FIG. 3. Induction of β -glucosidase (\circ) in mycelium at different stages in life cycle: in resting spores and in mycelium at exponential, stationary, and starvation phases. Mycelium at different stages in standard growth medium, washed and transferred to induction medium ($500 \mu\text{g}$ M β G/ml in citrate buffer, pH 3.0). Relative inoculum and mycelial weight during growth on right-hand axis (Δ). Weight of mycelium adjusted to ca. 1.5 mg/ml in each induction assay.

at $100 \mu\text{g/ml}$. Several compounds reduced the amount of induction when added at $100 \mu\text{g/ml}$ or less. Nojirimycin, an effective inhibitor of both α - and β -glucosidase (Reese *et al.*, 1971), prevented induction at $100 \mu\text{g/ml}$ presumably because at this concen-

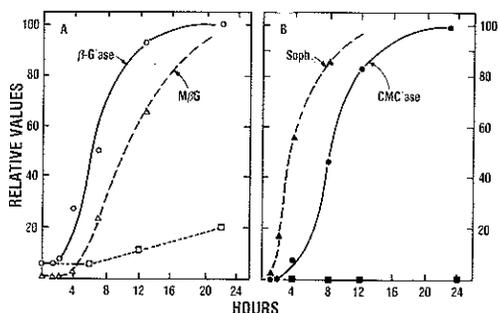


FIG. 4. Kinetics of β -glucosidase induction (\circ) and M β G absorption (Δ) (A) compared with carboxymethyl cellulase induction (\bullet) and sophorose absorption (\blacktriangle) (B). M β G at $500 \mu\text{g/ml}$ in citrate buffer, pH 3.0; sophorose at $300 \mu\text{g/ml}$ in citrate buffer, pH 2.8. Constitutive level of β -glucosidase (\square) and of CMCcase (\blacksquare) relative to maximum induced levels.

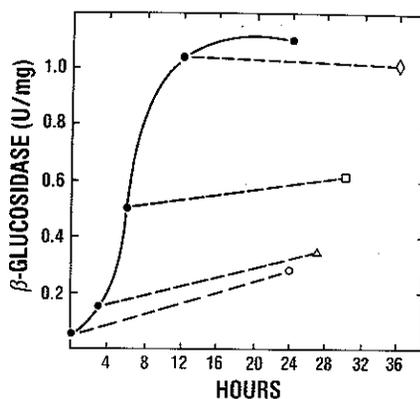


FIG. 5. Reversal of M β G induction of β -glucosidase. Mycelium incubated with M β G ($500 \mu\text{g/ml}$) for 0 (--- \circ), 3 (--- Δ), 6 (--- \square), or 12 (--- \diamond) h, washed, suspended in fresh medium and incubated an additional 24 h for determination of maximum β -glucosidase activity. The mycelium incubated 24 h with M β G (\bullet) received no additional treatment. The validity of the linear extrapolation (—) is based on other experimental data.

tration it completely blocked the absorption of M β G. Sorbose and deoxyglucose at $100 \mu\text{g/ml}$ substantially reduced induction as did all of the β -linked disaccharides except sucrose and gentiobiose. At $500 \mu\text{g/ml}$ the aryl- β -glycosides were all somewhat repressive. Sophorose was unique in that at $100 \mu\text{g/ml}$, β -glucosidase induction was repressed below the constitutive level and total repression of induction was observed at $3 \mu\text{g/ml}$. More than 50% repression occurred at $0.3 \mu\text{g/ml}$. Sophorose thus has a dual role in *Trichoderma* as a potent inducer of cellulase and as a repressor of β -glucosidase (Sternberg and Mandels, 1980). The repressive effect of sophorose was partially reversible (Fig. 6). Washed mycelia were incubated for 3 and 6 hours in sophorose, the sophorose was washed out, and the mycelium was resuspended in fresh inducing medium containing M β G. The control was not preincubated in sophorose. Although β -glucosidase was induced after sophorose pretreatment, there was a lag before induction started (although M β G was being absorbed as fast as in the control) and the final enzyme yield was 50 to 70% of

the control. The persistence of sophorose repression was further indicated when mycelia were preincubated in 3 μ g sophorose/ml, washed, starved from 1 to 5 hours in a buffered salts medium, and then induced for β -glucosidase by adding M β G. In all cases the induction in the sophorose-preincubated mycelia lagged behind that of the controls and gave lower yields. Contrary to the abrupt almost linear decline of cellulase induction of sophorose at pH values from 3 to 5 (Sternberg and Mandels, 1979), the repressive effects of sophorose on β -glucosidase synthesis were essentially unchanged in this pH range. At the sophorose concentration used (30 μ g/ml) the β -glucosidase activity was reduced close to the constitutive level.

Some Characteristics of Mycelial β -Glucosidase

The β -glucosidase activity in these studies was associated with the mycelium and was not released into the medium upon prolonged incubation (48 hours) nor by various extraction procedures such as 18 hours incubation at room temperature (pH 4.8) with 2 M NaCl, 0.6% Tween 80, or 6 units β -1,3-glucanase/ml (from *Sporotrichum dimorphosporum*). Even at 50°C the β -1,3-glucanase effected no release of mycelial associated β -glucosidase, although such treatment released more than 90% of the mycelial β -glucosidase of *Aspergillus phoenicis* (Allen and Sternberg, 1980). About 20% of the β -glucosidase activity was found in the soluble fraction after vigorous grinding in a mortar and pestle with sand. Although the cellular location of β -glucosidase of *T. reesei* is not known, it appeared to be tightly associated with the mycelium and was not removed by treatments which have been found to release enzymes from the periplasmic spaces of fungi (Lisker *et al.*, 1975). Further investigation is needed to establish the location and nature of binding with the cells as well as to determine whether differences exist in the

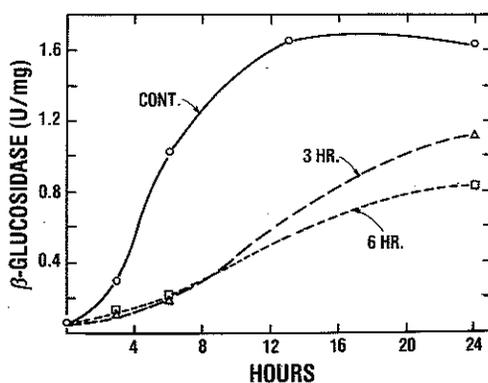


FIG. 6. Reversal of sophorose repression. Mycelium preincubated with sophorose (30 μ g/ml) for 3 (Δ --- Δ) or 6 (\square --- \square) h, washed, and suspended in fresh medium containing M β G (500 μ g/ml), and β -glucosidase induction was determined for the next 24 h. Control (\circ --- \circ) was not preincubated in sophorose. M β G was absent from the preincubation medium.

binding of constitutive and induced enzyme. While methods for solubilizing the enzyme can probably be found, the fact remains that the enzyme is associated with the cells.

The pH optimum for mycelial β -glucosidase was 4.5 to 4.7 and the enzyme was relatively active at low pH, e.g., 40% of the maximum activity of pH 3.0.

β -Glucosidases from fungi often have a broad specificity for many β -glucosides (Reese *et al.*, 1968). The β -glucosidase of *T. reesei* hydrolyzed all β -glucosides tested: salicin (an aryl- β -glucoside), gentiobiose, cellobiose, sophorose, and methyl- β -glucoside. It was not demonstrated that a single enzyme responsible for activity against all of these substrates was induced by M β G and repressed by sophorose by the same relative amount. $\alpha\alpha$ -Trehalase induction was not affected in these ways by M β G or sophorose.

The K_M values, using mycelial associated enzyme, for cellobiose and sophorose were 1.2 and 1.4 mM, respectively (Sternberg and Mandels, 1979), and for methyl- β -glucoside, 9.5 mM. The maximum velocities of hy-

drolisis by induced mycelia were typically around 1.0 U/mg mycelium for cellobiose and sophorose and 0.6 for M β G.

Respiratory Studies

To examine the relation of β -glucosidase activity to metabolism of β -glucosides, respiration of mycelia having constitutive or induced levels of β -glucosidase was measured (Fig. 7). Citrate was not respired at appreciable rates over any of the pH ranges used, the response being the same as with phosphate. Responses to glucose by constitutive or induced mycelium were identical, but differences occurred with cellobiose. In the control mycelium, the initial rate on cellobiose approximated the endogenous rate, the linear increase then proceeding at a rate lower than that on glucose. Induced mycelium responded to cellobiose more rapidly, the rate being essentially identical with that of glucose.

To determine if β -glucosidase activity was adequate to account for the respiration on cellobiose, it was assumed the enzyme had a Q_{10} of 2 over the range of 30 to 50°C and that 50% of the sugar absorbed was oxidized to CO₂ and H₂O. On this basis the constitutive activity, 0.144 U/mg dry mycelium, was adequate to account for a Q_{O_2} of 144, whereas an induced level of 0.99 U/mg mycelium would have been sufficient to support a Q_{O_2} of 1000. Thus, respiration on cellobiose does not appear to be limited by β -glucosidase since the experimental Q_{O_2} for control as well as induced mycelium was significantly below 100 (Fig. 7).

DISCUSSION

The major soluble product of the cellulase enzymes is cellobiose, which must be cleaved before it enters the metabolic system. β -Glucosidase is thus considered as part of the cellulolytic system, although it has no activity against cellulose. As in the case of cellulase induction, synthesis of β -glucosidase can occur at the expense of endogenous reserves. No extracellular nitro-

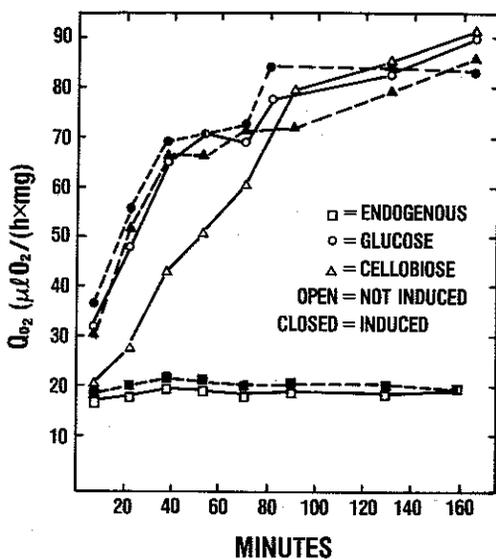


FIG. 7. Respiration of noninduced and induced mycelium on glucose (○), on cellobiose (△), or without exogenous substrate (□). Mycelium preincubated 16 h with (closed symbols) or without (open symbols) M β G (500 μ g/ml) at pH 4.4 in citrate buffer, washed, and resuspended in citrate, pH 4.4. Sugars, 2 mg/ml; mycelial dry weight, 1.44 mg/ml.

gen or carbon sources are required unless the inducers satisfy this latter requirement, which seems unlikely at the low concentrations used. In a previous report we presented some information on regulation of the cellulase system by sophorose (Sternberg and Mandels, 1979, 1980).

It appears that cellulase and β -glucosidase induction systems are under separate control since different inducers are required. Furthermore cellulases are actively secreted enzymes, appearing free in the medium, whereas β -glucosidase under comparable conditions is tightly associated with the mycelium. However, in actual cellulose fermentations about 70% of the β -glucosidase activity is extramycelial (unpublished data). A similar effect of cellulose on the distribution of β -glucosidase has been found in other fungi (Smith and Gold, 1979; Wilson and Niederpruem, 1967). Unlike cellulase, β -glucosidase is produced

constitutively, low activity (0.05 and 0.30 U/mg) being associated with mycelia and spores. As mycelia age in a starvation medium the constitutive level of β -glucosidase gradually increases to a maximum of around 0.3 U/mg.

Additional differences between cellulase and β -glucosidase relate to absorption of the inducers. β -glucosidase activity is induced as soon as M β G absorption is initiated as detected by our methods. With cellulase, nearly 20% of the sophorose was absorbed before enzyme induction could be detected and cellulase production lagged behind uptake of sophorose. Whereas cellulase induction by sophorose is dependent on pH and a well-defined optimum at ca. 3 (Sternberg and Mandels, 1979), induction of β -glucosidase is unaffected from about pH 4.5 to above 7.0. With citrate buffer, however, there is an optimum at pH 3. These data imply that the rate-limiting mechanism in β -glucosidase induction is interior to the cell membrane whereas cellulase induction is more affected by phenomena at the external cell surface. This difference may be of additional significance with respect to induction mechanisms, in that β -glucosidase is retained within the cells, whereas cellulase is secreted.

Whether M β G or sophorose molecules as such are the specific activators of induction or repression, or whether metabolic transformations occur prior to induction is not known. Nor do we know whether the constitutive and induced enzymes are isozymes having similar characteristics and locations within the cell.

Our findings of distinct dual roles for sophorose in the synthesis of the cellulase system are qualitatively different from other reports (Gritzali and Brown, 1979; Nisizawa *et al.*, 1971), which indicate sophorose to have no repressive effect but rather to be an inducer of β -glucosidase as an extracellular enzyme as opposed to our demonstration of repression and mycelial association. However, major differences in

methodology were used. For example, our substrate for assay was cellobiose, whereas other workers used *p*-nitrophenyl- β -glucoside and hence were measuring aryl- β -glucosidase activity. Other differences included pH, growth conditions, physiological status of the mycelium, and possibly of greatest importance, different strains. Elaboration of the reasons for such diverse conclusions, and the extent to which environmental conditions can control the mechanism, would require a good deal of additional experimental evidence to be of any value and should be the subject of a separate investigation.

The induced activity of β -glucosidase based on mycelial weight in *T. reesei* is comparable to activities reported in the black aspergilli, which were the most active of many fungal species tested (Sternberg *et al.*, 1977). However, in contrast to *T. reesei*, *A. phoenicis*, the best producer appears to synthesize high levels of β -glucosidase constitutively (Allen and Sternberg, 1980).

The function of the high, induced levels of β -glucosidase in *T. reesei* is not clear. The amount of β -glucosidase produced by *T. reesei* in cellulose culture is at the constitutive level. The only effective inducer found was M β G. Cellobiose, the β -glucoside most probably encountered and metabolized by *Trichoderma* in nature, does not induce β -glucosidase under the conditions used here. Furthermore, sophorose is a potent repressor of β -glucosidase induction, totally blocking β -glucosidase synthesis at concentrations which are at the threshold for cellulase induction. It has been demonstrated that the higher levels of β -glucosidase (induced by M β G) reduced cellulase induction by sophorose, probably by hydrolyzing the inducer (Sternberg and Mandels, 1980). Repression of β -glucosidase may be a mechanism for preventing the destruction of a cellulase inducer. This may explain why only constitutive levels are found in cellulose

cultures. The constitutive level of β -glucosidase is more than sufficient to account for the respiration rates found on cellobiose, and the induced level is about 10 times higher than required. One situation in which high β -glucosidase activity would be of value is if β -glucosides became available to the organism at concentrations substantially below their effective K_m , but whether such a condition would lead to induction is untested.

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