

Induction of Cellulolytic Enzymes in *Trichoderma reesei* by Sophorose

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Sophorose (2-*O*- β -glucopyranosyl-D-glucose) induces carboxymethyl cellulase in *Trichoderma reesei* QM6a mycelium within 1.5 to 2 h. The induction response to sophorose concentration, although complicated by the metabolism of sophorose, shows saturation kinetics. Most of the cellulase appears after most of the sophorose has been taken up, but the presence of an inducer is required to maintain cellulase synthesis because enzyme production ceases after separation of the mycelium from the induction medium. Cellulase appears simultaneously in the medium and in the mycelium, and no appreciable levels accumulate in the mycelium. Response to pH suggests either that synthesis and secretion of the enzyme are closely associated or concurrent events affected by surface interactions with the medium. Effects of temperature and pH on cellulase induction by sophorose are similar to those reported for induction by cellulose. The kinetics of absorption by mycelium differs from that of other β -linked saccharides and glucose, the uptake of sophorose being much slower. Under our cultural conditions, sophorose appears to induce an incomplete array of cellulase enzymes, as indicated by enzymatic and electrophoretic studies.

The natural inducer for cellulase enzymes produced while a microorganism is growing on cellulose is unknown. The assumption among most researchers in this area is that low, constitutive levels of cellulase react with cellulose to produce a soluble molecule which enters the cell and effects induction. It must be recognized, however, that spores of many fungi, including *Trichoderma*, contain carbohydrate reserves which might act as inducers, thus permitting initiation of growth on cellulose. Subsequently, inducers are produced at some point in the hydrolysis and assimilation of cellulose. Binder and Ghose (3) claim that actual contact between hyphae and cellulose is essential for cellulase induction by cellulose. Their data are not conclusive, however, since the distances between cell and substrate, where separated, were of a magnitude such that diffusion of small molecules away from the mycelium would prevent, minimize, or delay cellulase production. Mandels et al. (17) discovered that sophorose (2-*O*- β -glucopyranosyl-D-glucose), a trace contaminant in cerelese (glucose prepared by the acid hydrolysis of starch), was a potent inducer of cellulase in *T. reesei* QM6a. D. E. Brown (5) found cellulase production by *T. reesei* QM9123 with glucose (cerelese) as a carbon source in fermentor cultures. Cellulase production on cerelese does not commence until glucose is consumed, and then the remain-

ing sophorose induces the enzyme. Sophorose is the most potent of known cellulase inducers. Both cellobiose and lactose can serve as inducers but must be present at high initial concentrations to be effective. During growth on cellobiose, products of glucosyl transfer are found in the medium, and these are reported to be better inducers than cellobiose (18, 23). Fungal β -glucosidase is a transferase; it has been shown in vitro that this enzyme in the presence of cellobiose and glucose can form all the β -linked disaccharides, including sophorose (6). If such transferase activity occurs in vivo, sophorose might be a naturally occurring inducer of cellulase. Such a system would have some similarities to β -galactosidase induction in *Escherichia coli* in which allolactose, the natural inducer, is formed by the transgalactosylic action of β -galactosidase (11).

Nisizawa et al. have shown that sophorose induction of cellulase in *T. viride* requires de novo protein synthesis and that induction is subject to catabolite repression (20, 21). Loewenberg and Chapman (12) studied catabolic and inductive pathways of sophorose utilization in *T. reesei*. They suggest that the effectiveness of sophorose as an inducer may be related to a higher affinity of the inductive system for sophorose, the catabolic system having a higher capacity but lower affinity. Although sophorose

induction of cellulase was reported to be restricted to *Trichoderma* (17), Eriksson and Hamp (10) have reported its effectiveness in *Sporotrichum pulverulentum*. Suzuki et al. discovered that earlier reports of cellulase induction by sophorose in *Pseudomonas* were due to the constitutive nature of cellulase in this bacterium and that because sophorose is consumed more slowly than other sugars, cellulase levels are increased, or derepressed, in the presence of sophorose (27).

The cellulase-producing system of *Neurospora crassa* (8) is distinctly different from that of *T. reesei* with respect to the nature of the inducer, the conditions for induction, and the quantity of enzyme produced.

Reported here is a study on the influence of physiological conditions on induction and deinduction of cellulase by sophorose, as well as a comparison with induction in cellulose cultures. Included also are studies on some relevant aspects of uptake and metabolism of sugars.

MATERIALS AND METHODS

Cultures. The organism used was a wild-type strain of *T. reesei* (formerly *T. viride*) QM6a. Spores for inoculum were obtained by culturing at 28°C in 250-ml culture flasks with Morton closures containing 50 ml of *Trichoderma* salts solution (19) plus maltose (practical grade; Matheson, Coleman and Bell) and 1.5% agar (Difco Laboratories, Detroit, Mich.). After 3 to 10 weeks of incubation, the spores were suspended in 11 ml of sterile water, using glass beads if necessary, and 1.0 ml of the suspension (about 4 mg of spores) was used to inoculate each 250-ml culture flask containing 50 ml of *Trichoderma* salts solution in 0.05 M potassium citrate buffer (pH 4.4) with 0.3% glucose. Cultures were incubated for 25 h at 28°C with reciprocal shaking (100 strokes per min), centrifuged, and washed twice with 2 volumes of water. The washed mycelium was suspended in 0.1 M potassium phosphate buffer of appropriate pH and modified *Trichoderma* salts solution (0.5 the normal concentration but omitting urea and KH_2PO_4). The mycelium grown by this technique had very short hyphal filaments. Small samples could be pipetted fairly uniformly, although brief blending (about 10 s in a Virtis homogenizer) improved the homogeneity of the suspension without affecting its response, i.e., respiration. At the time of harvest, glucose was completely depleted from the medium and the pH was unchanged.

Induction. For induction studies, the suspension was diluted 1:1 with the inducer solution, giving a final mycelial dry weight of 1.5 to 2.0 mg/ml. Induction usually was carried out in 50-ml flasks containing 8 ml of reaction mixture (0.25 strength *Trichoderma* salts lacking urea and KH_2PO_4 , in 0.05 M potassium phosphate buffer, unless specified to the contrary) and incubated on a reciprocal shaker (100 strokes per min) at 28°C, except where noted. The entire contents of the flask were harvested and centrifuged for each sample. The incubation period usually did not exceed

24 h. Precautions were taken to maintain asepsis only for longer incubation periods. For sugar absorption studies, the same procedure was used, except that the suspensions were filtered through glass fiber filters on Buchner funnels for rapid removal of mycelium.

Respirometric methods. Typical methods were used, employing a Gilson differential respirometer at 30°C; 2 ml of mycelial suspension was added to the vessel, and 0.5 ml of substrate was added to the side arm. Results are expressed as Q_{O_2} , i.e., microliters of O_2 per hour per milligram (dry weight) of mycelium.

Assays. Carboxymethyl cellulase (CM cellulase; EC 3.2.1.4.) and filter paper cellulase (FP cellulase) were measured by following the increase in reducing power of an 0.5% solution of CM cellulose or of a 50-mg strip of Whatman no. 1 filter paper per 1.5 ml, respectively, by the dinitrosalicylic acid procedure (16). β -Glucosidase (EC 3.2.1.21) activity was assayed by the hydrolysis of 7.5 mM cellobiose (Eastman Kodak Co., Rochester, N.Y.) as previously described (26), with the resulting glucose measured by a glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, Ohio). Mycelial-associated enzyme activity was assayed by using suspensions of washed mycelium which had been blended in a Virtis-45 homogenizer (Virtis Research Equipment, Gardiner, N.Y.) for 30 s at moderate speed. Enzymes were assayed at 50°C in sodium citrate buffer (0.05 M at pH 4.8) containing 0.001% merthiolate (30 min of incubation for CM cellulase and β -glucosidase; 60 min for FP cellulase). A unit of enzyme activity represents 1 μ mol of glucose (or glucose equivalent) released per min. Protein was measured on trichloroacetic acid (5%) precipitated material by the procedure of Lowry et al. (13). Residual sugars (in uptake studies) were measured by the phenol-sulphuric acid method (7).

Electrophoresis. Extracellular proteins were separated by electrophoresis on an Ortec unit (Ortec Inc., Oak Ridge, Tenn.) for 1.5 h at the suggested power settings, using a gel slab with a 4.5 to 8% polyacrylamide gradient and a buffer system as described in the Ortec operation manual. After removal, the gel was fixed in 10% trichloroacetic acid at room temperature for 30 min, rinsed, and placed in Coomassie brilliant blue stain (1.25 g of Coomassie blue-G, 46 ml of acetic acid, 454 ml of 5% methanol) overnight; the gel was destained with an Ortec destainer in 10% acetic acid.

RESULTS

Factors influencing induction. Of various carbohydrates tested (at 0.3 to 0.5 mg/ml), only sophorose and cellulose induced significant amounts of CM cellulase. Cellobiose, lactulose, and methyl- β -glucoside did not induce CM cellulase, but the latter did induce β -glucosidase, which remained associated with the mycelium. Lactose was a weak inducer, giving about 1/10 the amount of CM cellulase given by an equal amount of sophorose, and at a much slower rate. Low concentrations of lactose, cellobiose, or glucose (down to 1 μ g/ml) did not induce cellulase. Induction by cellulose was slow, the enzyme not appearing until after 2 days and slowly increas-

ing for an additional 4 days. Therefore, sophorose was used as an inducer in the subsequent studies. No induction of CM cellulase occurred in the absence of oxygen (flasks flushed with N_2). The presence of nutrient salts increased the yield slightly over buffer only. Thus, an exogenous supply of nitrogen was not necessary for induction. Sophorose concentrations from 1.5 to 1,500 $\mu\text{g}/\text{ml}$ did not significantly increase either mycelial or cell-free β -glucosidase over constitutive levels.

Induction of CM cellulase by sophorose was markedly affected by pH, with the optimum at about 2.8 (Fig. 4) (23). Above pH 3.0, the rate of formation and yield of cell-free enzyme de-

creases sharply, and no CM cellulase activity could be detected in the mycelium. The optimum temperature for production of enzyme was 28°C (Fig. 1), and there was a lag of about 1.5 h before cell-free cellulase could be detected. Whereas temperature had a negligible effect on lag, effects on rate of formation (optimum at ca. 34°C) and yield were markedly temperature dependent. The low yield at 39°C was not due to lethal effects, since both rate of absorption of sugar and respiration were more rapid at 39 than at 30°C. A substantial amount of sophorose was taken up before CM cellulase activity could be detected (Fig. 1B); generally, 70 to 80% of the CM cellulase was produced after 70 to 80% of

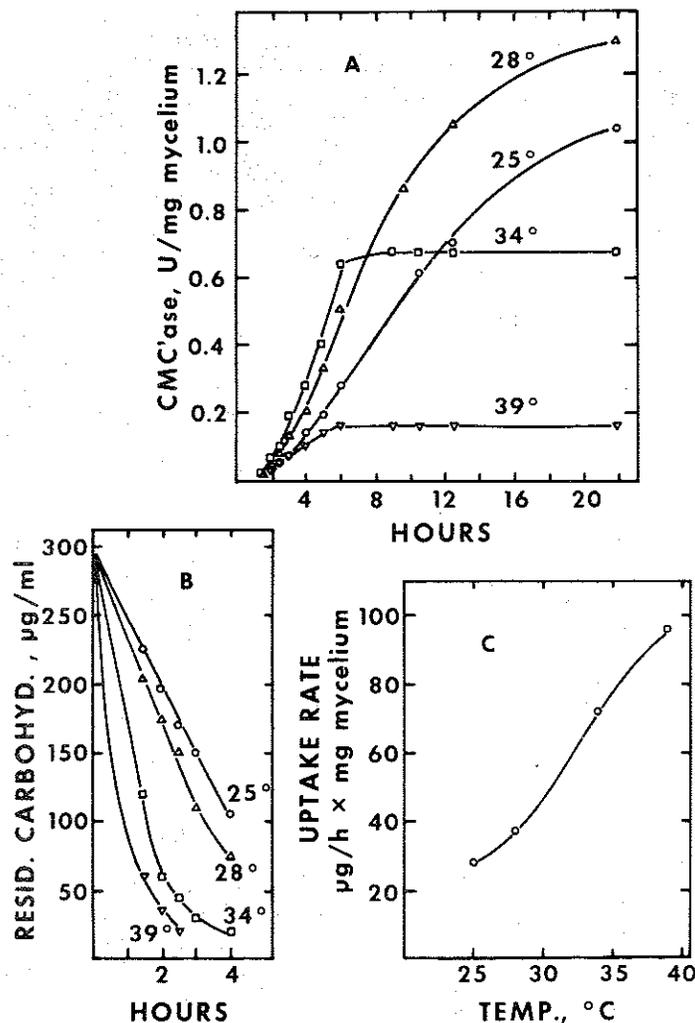


FIG. 1. Effect of temperature on induction and uptake of sophorose. Mycelia were incubated at the designated temperature at pH 2.8 with 300 μg of sophorose per ml. (A) Time course for extracellular CM cellulase production; (B) residual sugar remaining in the medium; (C) rate of sugar absorption at the various temperatures as $\mu\text{g h}^{-1} \text{mg}^{-1}$ of mycelium. Symbols: \circ , 25°C; Δ , 28°C; \square , 34°C; ∇ , 39°C.

the sophorose was absorbed by the fungus.

The response of CM cellulase production to increasing concentrations of sophorose (1.5 to 1,500 $\mu\text{g/ml}$) was hyperbolic and followed the Michaelis-Menten equation. The maximum CM cellulase produced was 1.8 U of mycelium per mg, and 0.15 mM gave half the maximal response.

During induction, no CM cellulase could be detected in the mycelium before its appearance in the medium. The amount of activity associated with the mycelium was about 30% of the total activity at the onset of induction and declined to less than 10% during the rapid phase of cellulase production.

Rates of sugar uptake. In comparison with other sugars tested, sophorose uptake under standard induction conditions was quite slow, approximating a linear response to initial concentration (Fig. 2A). Uptake rates were influ-

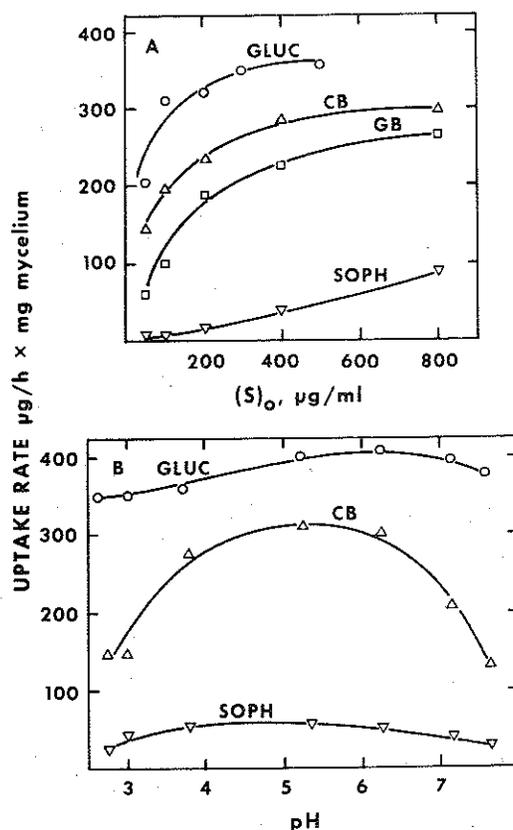


FIG. 2. Effect of substrate concentration (A) and pH (B) on rate of sugar uptake in micrograms per hour per milligram of mycelium. For the various substrate concentrations, optimal conditions for CM cellulase induction were used; i.e., pH 2.8 at 28°C. For effect of pH, the initial sugar concentrations were 300 $\mu\text{g/ml}$, and the incubation temperature was 28°C.

enced by pH (Fig. 2B), with sophorose and cellobiose showing similar relative patterns, the optimum rates (at pH 5) being about twice those at pH 2.8, whereas the best CM cellulase yields were found with sophorose at pH 2.8. The yield at pH 5 was reduced by 80% (Fig. 4).

Hydrolysis of sophorose and cellobiose. Generally, glucosidases have a broad specificity for glucosidic bonds; e.g., *Aspergillus* β -glucosidase and α -glucosidase hydrolyze all β - or α -linked diglucosides, respectively, except for α - α -trehalose (24, 26). It was assumed that *Trichoderma* β -glucosidase would follow this pattern and hydrolyze sophorose as well as cellobiose. Because the β -glucosidase activity in these induction assays was associated with the mycelium, whole cell suspensions were used to measure the relative rates of cellobiose and sophorose hydrolysis. From Lineweaver-Burk plots the K_m of mycelial β -glucosidase was somewhat higher for sophorose (1.4 mM) than for cellobiose (1.2 mM), and no substrate inhibition as occurred with cellobiose above 6.0 mM was observed for sophorose up to 9.5 mM. Maximum velocities for cellobiose and sophorose hydrolyses were 0.07 and 0.05 $\mu\text{mol of glucose mg}^{-1} \text{min}^{-1}$, respectively. These were constitutive activities within the mycelium. Nojirimycin, a potent inhibitor of β -glucosidase (25), inhibited both cellobiose and sophorose hydrolysis, with a ratio of nojirimycin to substrate of about 6×10^{-4} (wt/wt) giving 50 and 60% inhibition of cellobiose and sophorose hydrolysis, respectively. Thus, it appeared that if sophorose entered the cell it would be cleaved to glucose somewhat slower than cellobiose.

Sophorose metabolism. Several types of experiments were carried out comparing the metabolism of sophorose with cellobiose and glucose for clues as to inducer specificity and the mechanism of induction. Glucose and cellobiose supported a rapid increase in respiration rate which fell as the sugars were depleted, whereas respiration on sophorose was considerably slower and extended for a longer time (Fig. 3). Measurements at various pH's show that exogenous respiration on sugars (sophorose, glucose, or cellobiose) falls off abruptly below ca. pH 3.5, decreasing to zero (i.e., the endogenous level) around pH 2.5 (Fig. 4). This cannot be ascribed to retarded sugar uptake, particularly with glucose or cellobiose (Fig. 2; Table 1). The rate of sophorose absorption is much lower than that of the other sugars but appears more than adequate to account for respiratory activity, assuming 50% assimilation and 50% oxidation to CO_2 and water and that endogenous respiration continues at about the same rate in the presence of exogenous sugars.

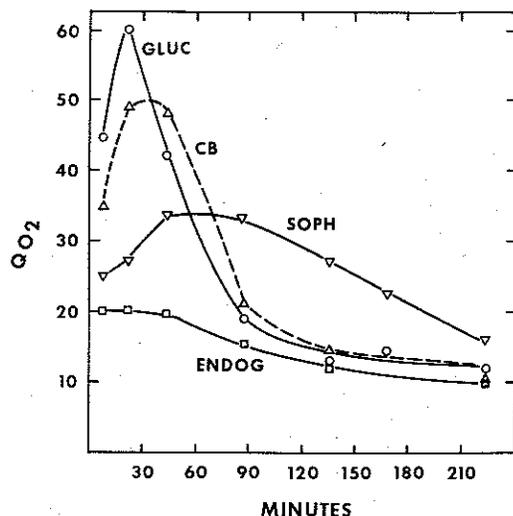


FIG. 3. Influence of the type of sugar on the rate of respiration. Mycelia were incubated with 500 μg of sugar per ml at pH 5.0 (0.05 M potassium phosphate). Symbols: \circ , glucose; Δ , cellobiose; ∇ , sophorose; \square , endogenous (no carbon source added).

TABLE 1. Absorption and respiration of selected sugars^a

Sugar	Absorption ($\mu\text{g}/\text{mg}$ mycelium)	Respiration ($\mu\text{g}/\text{mg}$ mycelium) ^b
Glucose	178	58
Cellobiose	156	48
Sophorose	47	29

^a Experimental conditions were pH 5.0; substrate concentration was 500 $\mu\text{g}/\text{ml}$; and mycelial concentration was 1.93 mg/ml for absorption and 3.86 mg/ml for respiration. Data are from measurements at 30 min. —, No sugar added.

^b Values indicate micrograms of carbohydrate consumed, assuming complete oxidation to CO_2 and water.

Above ca. pH 5, marked variation in exogenous respiration of sugars was seen, although endogenous respiration was unaffected (Fig. 4). Rate of absorption of sophorose at these pH's could be limiting, but this did not appear likely for cellobiose and was even less likely for glucose, because in both cases the rate of absorption was greatly in excess of respiration. The respiration rate on sophorose in this experiment approached that on glucose because relatively high sugar concentrations (10 mg/ml) were used to better resolve the changes in respiratory rates at the various pH values.

Nojirimycin caused almost complete inhibition of respiration on either sophorose or cellobiose, when used at 1/100 the concentration of these sugars. The same concentration had no

effect on either glucose or endogenous respiration (Fig. 5).

Respiration on glucose was relatively unaffected by acid treatment of mycelium. Acid treatment blocked respiration initially on sophorose or cellobiose for over 1 h, and then recovery was observed. This indicates that a surface-located agent is responsible for the absorption of cellobiose and sophorose (14).

Deinduction studies. The induction phenomenon can be characterized by three parameters: length of lag, rate of formation, and yield of enzyme. Several studies were carried out to attempt experimental separation. Mycelium was incubated with 300 μg of sophorose per ml for 6 h, by which time 80% of the sugar had been taken up. One set was centrifuged, washed, and resuspended in the same 6-h medium from which it was removed, a second set was similarly washed and resuspended in fresh medium without sophorose, and the control was allowed to incubate without interruption. Hardly any CM cellulase was produced from cultures resuspended in fresh medium, whereas those cultures which were resuspended in their original medium continued to produce cellulase at a rate approaching the control. Continued cellulase production re-

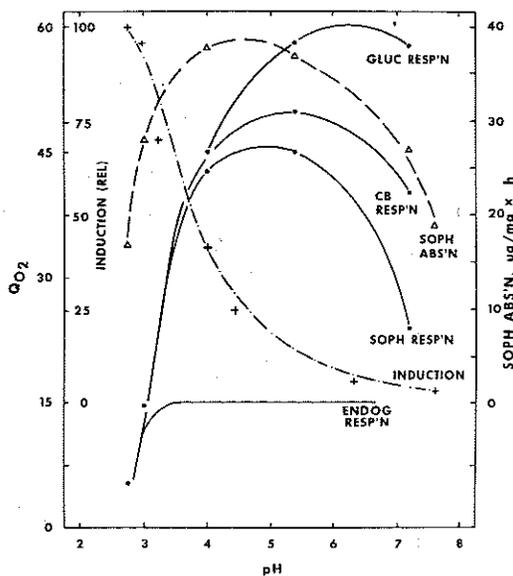


FIG. 4. Comparative effect of pH on respiration, CM cellulase induction, and sophorose absorption. For respiration, mycelium was grown for 24 h on glucose, washed, and incubated in water for 24 h. Respiration data from O_2 uptake of 2.1 mg of mycelium per vessel at 3 h and substrate concentration of 10 mg/ml. Absorption data from Fig. 2B. Symbols: \bullet , respiration on sugars as noted; Δ , absorption of sophorose; +, induction of CM cellulase.

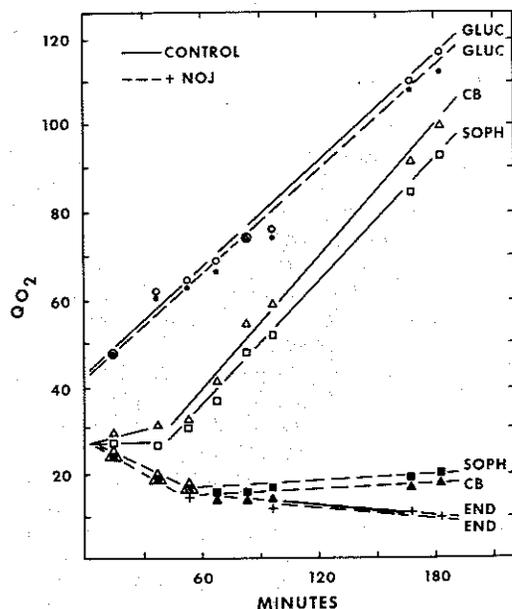


Fig. 5. Effect of nojirimycin on respiration on glucose, cellobiose, or sophorose. Substrate at 5 mg/ml; nojirimycin at 0.05 mg/ml; 0.04 M potassium phosphate buffer at pH 6.1, 3.9 mg of mycelium per vessel, prepared as described in legend to Fig. 4. Symbols: \circ , glucose; Δ , cellobiose; \square , sophorose; +, endogenous. Closed symbols indicate nojirimycin.

quired the presence of the inducing medium; as soon as the organism was separated from its medium, cellulase production ceased. Supernatant fluid taken from a culture incubated in the standard medium without sophorose had no inducing affect when combined with washed mycelium previously incubated with sophorose for 6 h. Furthermore, the amount of cellulase induced by supernatant fluids taken from the inducing mixture decreased with length of preincubation time. When the mycelium was incubated in sophorose medium, its sensitivity to sophorose and its ability to produce cellulase increased as the sophorose was consumed. At the same time, the potency of the supernatant to induce cellulase decreased, and when sophorose was totally consumed the supernatant induced no cellulase even with preinduced mycelium. Presumably, low levels of residual sophorose were responsible for continued induction, and preinduced mycelium was more sensitive to induction than was uninduced mycelium. To test this, mycelia were incubated with and without sophorose for 5 h, washed and suspended in the 5-h medium of the sophorose-containing mixture or in fresh media containing either 10 or 30 μ g of sophorose per ml, and incubated for an additional 20 h (Fig. 6). The enzyme production was

slower in the uninduced mycelium. In both cases, the response to the 5-h medium approximated that from the addition of 10 μ g of sophorose per ml. Attempts to characterize the inducing material in supernatants from sophorose culture were not inconsistent with the active material being traces of sophorose. For example, the inducing material was dialyzable, heat stable (withstood autoclaving for 5 min), was destroyed at pH 12 at 100°C, but was more stable at pH 1.

Nature of the cellulase enzymes induced by sophorose. The cellulase system of *Trichoderma* is a complex of endo- and exo-glucanases which can hydrolyze crystalline cellulose to soluble sugars (9, 28). In the experiments so far, CM cellulose was the substrate in the assay because of its reactivity; hence we measured only endo- β -glucanase activity. To investigate other cellulase components induced by sophorose, the fungus was cultured on sophorose or cellobiose at 250 μ g/ml for 24 h and the cell-free medium was concentrated 13-fold by ultrafiltration to permit examination for activity against substrates other than CM cellulose. For comparative purposes, a culture was incubated with 0.5% ball-milled cellulose in the standard salt solution for 9 days, and the filtrate was used without concentration or further treatment. No protein or cellulase activity could be detected in

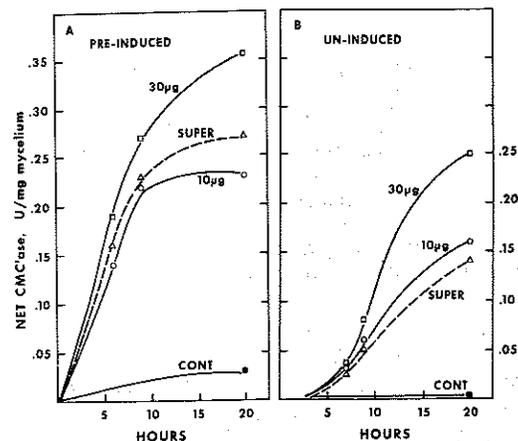


Fig. 6. Inducing potential of a 5-h supernatant fluid from a reaction mixture initially containing 100 μ g of sophorose per ml, compared with the inducing potential of 30 and 10 μ g of sophorose per ml. After 5 h of incubation with 100 μ g of sophorose per ml (A) or without sophorose (B), mycelia were centrifuged, washed, and suspended either in fresh media with 0, 10, or 30 μ g sophorose per ml or resuspended in the 5-h supernatant fluid. Symbols: \bullet , control, suspended in fresh medium without sophorose; \circ , suspended in fresh medium with 10 μ g of sophorose per ml; Δ , resuspended in the 5-h supernatant; \square , suspended in fresh medium with 30 μ g of sophorose per ml.

the concentrate from the cellobiose culture (Table 2). β -Glucosidase activity was lacking in all the preparations. The specific cellulase activities were lower in the sophorose culture concentrate than in the cellulose culture filtrate. Although the cellulase induced by sophorose had significant activity against filter paper, indicating that the enzyme complex was capable of degrading crystalline cellulose, its specific activity was about half that of the cellulose-induced system. The ratio of CM cellulase to FP cellulase was higher in the sophorose-induced system. These data indicated that some cellulase component(s) involved in the degradation of crystalline cellulose was absent or present in lower concentration in the system induced by sophorose. To visualize differences in the protein patterns, the three preparations were subjected to polyacrylamide gel electrophoresis (Fig. 7). A large protein band corresponding to purified cellobiohydrolase (EC 3.2.1.91) was present in both sophorose- and cellulose-induced preparations, as well as a series of bands corresponding to some partially purified endo-glucanases. Conspicuously absent from the sophorose-induced system were several of the more slowly migrating proteins in the region where other endo-glucanases and exo-glucanase migrated; these proteins were present in filtrates from cellulose cultures.

DISCUSSION

Cellulase can be induced in *Trichoderma* by very low levels of sophorose, with as little as 1.5 μ g/ml giving measurable induction. This quantity is in the range of 1/1,000 of the mycelial dry weight, and the effect on respiration and growth is negligible. When the sophorose concentration is less than 500 μ g/ml, the lag time for the appearance of cellulase is between 1.5 and 2.0 h. Lag times of 2 to 5 h have been reported previously for *T. reesei* (10, 12). Berg and Pettersson (2) reported that, when incubated on a suscep-

TABLE 2. Comparison of enzyme activities from cultures incubated on cellobiose, sophorose, or cellulose

Enzyme source ^a	Protein (mg/ml)	CM cellulase (U/mg of protein)	FP ^a ase (U/mg of protein)	CM/FP cellulase ratio
Cellobiose	0	0	0	
Sophorose	1.61	9.6	0.24	40
Cellulose	0.72	12.9	0.51	26

^a Filtrates of cultures incubated in cellobiose or sophorose medium, containing 250 μ g of the sugar per ml, for 24 h and concentrated 13-fold before assaying. Filtrate from a 9-day cellulose (0.5% ball-milled) culture was used without concentrating.

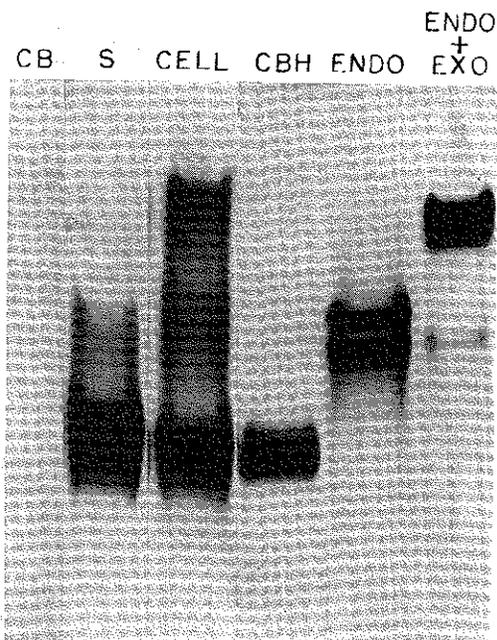


FIG. 7. Polyacrylamide gel electrophoresis; proteins stained with Coomassie brilliant blue. Cellobiose and sophorose culture filtrates were concentrated 13-fold in an Amicon stirred cell, using a PM 10 membrane (Amicon Corp., Lexington, Mass.); about 80 μ l of the concentrates (120 μ g of protein in the sophorose preparation) and 100 μ l (70 μ g of protein) of the cellulose culture filtrate were applied. About 10 μ g of the cellulase components was applied. CBH, Cellobiohydrolase; ENDO, 1,4- β -endo-glucanase; EXO, 1,4- β -exo-glucanase.

tible form of cellulose, much of the cellulase produced by *T. viride* remained bound to the mycelium and was released into the medium only after the cellulose was consumed and the organism was starving. Similarly, Eriksson and Hamp (10) working with *T. reesei* (QM6a), found that none of the cellulase induced by sophorose could be detected extracellularly. Contrary to these reports, we find that in our system there is no appreciable accumulation of cellulase associated with the mycelium before the appearance of the enzyme in the medium, indicating that synthesis and secretion of cellulase are closely associated events occurring only with intact mycelium under aerobic conditions of favorable temperature and pH.

The effects of pH and temperature on the induction by sophorose resemble those reported for cellulose fermentations; i.e., pH 3.0 and 28°C are optimal for cellulase production (1, 22). With sophorose, we found that the yield of cellulase increased even at pH 2.5. Such a low pH is suboptimal for a cellulose fermentation because

growth ceases (4), whereas in the sophorose induction system growth is not necessary for enzyme production.

Sophorose is taken up by the fungus at about 1/5 to 1/10 the rate of other sugars tested. The uptake rates agree relatively well with those found by Loewenberg and Chapman (12), who followed uptake with tritiated sophorose. The slowness in sophorose uptake may be related to its effectiveness as an inducer in that conditions which increase the uptake rate (higher pH and temperature) lead to lower cellulase yields. However, comparison of the effects of pH on sophorose absorption, respiration, and induction of CM cellulase (Fig. 4) shows that the suppression of induction at higher pH cannot be ascribed to limited absorption and metabolism. At lower pH's, where both rate and extent of induction are maximal, exogenous respiration is either completely suppressed or is below detection by our methods; absorption does occur but at a retarded rate—only 50% of maximal. Since pH within viable cells is relatively unaffected by that of the surrounding solution, the induction process, or key steps, occur at the cell surface. This supports the concept of coupled synthesis and secretion at the cell surface (15) and may help explain why only low levels of cell-bound or intracellular CM cellulase are found.

Loewenberg and Chapman (12) suggest that the more rapidly sophorose is catabolized by the organism, the smaller the induction response. One possible fate for sophorose, after it is absorbed by the mycelium, is its reaction with β -glucosidase; the glucose produced enters the respiratory system. Sophorose is respired by *Trichoderma*, although at a lower rate than either glucose or cellobiose. The observation that nojirimycin inhibits respiration on cellobiose as well as sophorose suggests that β -glucosidase is involved in the catabolism of the sugars.

Because sophorose is not a gratuitous inducer, the kinetics of its induction of cellulase are complicated by other metabolic reactions. However, the dose response curve does display saturation kinetics, indicative of a reversible reaction. Induction is reversible in that further cellulase formation ceases as soon as the inducer is removed from the medium. Although much of the cellulase is produced after most of the sophorose is consumed, a low, residual level of sophorose may be responsible for continued induction. The fact that no cellulase is produced after sophorose is removed discourages the hypothesis that cellulase is cryptically associated with the mycelium and released as the cells age.

Sophorose, at concentrations from 1 to 1,500 μ g/ml, does not induce β -glucosidase, but this

enzyme is present in the mycelium apparently as a constitutive enzyme; i.e., the activity is about the same when the mycelium is incubated with glucose as it is with sophorose. Although some cellulase components are induced by sophorose, it appears that a group of enzymes produced in cellulose cultures is lacking in the sophorose-induced system. The properties of the missing enzymes are not known, but they aid in the hydrolysis of crystalline cellulase as indicated by the higher CM cellulase to filter paper cellulase ratio found in sophorose-induced cellulase. It is interesting that cellobiohydrolase, which has been suggested as a key factor in the attack of crystalline cellulose, is abundantly induced by sophorose. The lack of a complete cellulase system induced by sophorose raises some interesting questions for further study. Is there more than one inducer for the complete cellulase system, or are the differences in components between sophorose- and cellulose-induced enzymes the result of fermentation differences such as the length of time or growth-associated events which occur in the cellulose culture but not with the sophorose incubation?

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