EXTRACELLULAR β-TRANSGLUCOSIDASE ACTIVITY FROM CONIDIA OF NEUROSPORA CRASSA

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Detailed study of carbohydrases has shown that many possess transglycosidase activity. Preparations of β-glucosidase from Aspergillus niger have been studied extensively (Crook and Stone, 1957; Barker, et al., 1955) and it has been found that during the hydrolysis of cellobiose to glucose, several additional oligosaccharides are produced. An explanation (Jermyn, 1957; Gottschalk, 1958) of these two apparently different activities of a single enzyme has the following mechanism:

1. GLUCOSYL OR + ENZ-H ——— ENZ - GLUCOSYL + R-OH
2. ENZ-GLUCOSYL + X-OH ——— ENZ-H + GLUCOSYL - OX

When X is hydrogen the reaction is a normal hydrolysis. But if X is a glycoside containing OH groups transglycosidation results and a new glycoside is synthesized.

This report describes additional properties of an extracellular enzyme preparation of Neurospora conidia already shown to contain cellobiase and p-nitro-phenyl β-D. glucosidase (Eberhart, 1961). These preparations contain β-transglucosidase activity that is responsible for the production of new oligosaccharides — principally with the formation of β-1,6 linkages.

METHODS AND MATERIALS

Cultures of Neurospora crassa strain SY7A were grown on 300 ml of a complete agar medium in 2,000 ml Fernbach flasks (Horowitz, 1947). After inoculation, the flasks were placed in the dark at 30°C for 4 days and then

3 days at 25°C. Cultures were fully conidiated by this time. The exogenous nature of the transglucosidase was the basis for its removal from the conidia by water (Eberhart, 1961).

For harvesting 200 ml of water was added to a Fernbach along with 3 glass beads (diameter 15 mm). Each flask was agitated and a conidial suspension was formed. Mycelia and bits of culture medium were removed from the mixture by filtration through several layers of cheesecloth. The mixture was then centrifuged in a Servall angle head centrifuge at 3,000 XG for 15 minutes. The supernatant solution was collected and filtered by suction through Whatman No. 2 paper, yielding a clear filtrated.

The above filtrate was dialized for 30 hours against distilled water at 5°C. The water was changed at 15 hours. This enzyme preparation was used in all the experiments here reported. When this preparation was kept frozen at -10°C, the β -glucosidase activity showed no change in 3 months.

Paper chromatography both ascending and descending was used to determine the carbohydrates in the reaction mixtures. The principal solvent was n-butanol:pyridine:water, 6:4:3 (by volume). Ascending runs were carried out for 24 hours. The chromatogram was removed from the chamber at 12 hours, dried and placed in the chamber for the remaining 12 hours. Descending runs took 14 hours. The other solvent employed was n-propanol:ethyl acetate:water 6:1:3 (by volume) Whatman No. 1 chromatography paper sheets were used throughout this investigation. The carbohydrate spots were detected by the action of alkaline silver nitrate, the general method of (Trevelyan, et al.,1950) which has been modified in our experiments. Spots were better defined and more even in texture if, instead of spraying, the chromatogram is dipped into 0.025 N NaOH dissolved in a 50-50 ethanol-acetone mixture. Using this method spots with 10^{-5} g. glucose or cellobiose could easily be detected on the paper.

Solutions of substrate, 10% (w/v) were prepared in .001 M citrate, .002 M phosphate buffer, pH 5.0. The usual procedure was to add 0.5 ml enzyme preparation to the test tube containing 1 ml of 10% buffered substrate and in-

cubated at 37°C for 24 hours before spotting chromatograms. Usually 10 λ of reaction mixture was placed on the paper.

All experimental results obtained by the above techniques were confirmed under sterile conditions, where the reaction mixture was filtered through a millipore filter — Swinney Adapter (Millipor Filter Corporation). The filter was type HA pore size 0.45.

Samples of the enzyme preparation were chromatographed both before and after incubation at 37°C for 24 hours. In no case were carbohydrate contaminant detected. The reactions described here were terminated by quickly drying the reaction mixture on the chromatographic paper.

Cellobiose (O-4-β-D-glucopyranosyl-D-glucose) was obtained from the National Biochemicals Corporation, and glucose from Merck & Company. Gentiobiose was purchased from the California Corporation for Biochemical Research. These compounds contained traces of glucose. Cellotriose was a gift of Dr. E. T. Reese.

Identification of transglycosidation products. Preliminary experiments using 0.1% buffered cellobiose showed that the glucosidase preparation had a high cellobiase activity. After 15 minutes cellobiose disappeared and only glucose was present.

When the cellobiose concentration was increased to 10%, three new spots were observed on the chromatograms after 24 hours incubation at 37°C. These spots were characterized by their R_G and are listed in Table 1.

The component R_G 0.54 had a mobility identical to a commercial sample of gentiobiose, while component R_G 0.21 had a mobility identical to that of cellotriose. These similarities between the transglycosidation products of R_G 0.54 and 0.21 and the known samples held true in both solvent systems used.

Component R_G 0.39 presented a more difficult problem for identification. A pure sample of this compound was chromatographically isolated from a reaction mixture using Whatman 3 MM paper. The R_G 0.39 substance was eluted with boiling water, and the eluate was filtered through Whatman No. 1 paper to remove remaining bits of chromatographic paper. The filtrate was subjected

Table 1

Properties of sugars produced by transglucosidation[≠].

R_G	Intensity of spot	Time of appearance	Tentative identification
1.0	+++	before 15 min.	Glucose
0.64	+++	substrate	Cellobiose
0.54*	++	4-6 hr.	Gentiobiose
0.39*	++	15 min.	0-6-β-D-gluco pyranosyl cellobiose
0.21*	+	10-11 hr.	Cellotriose

[≠]Neurospora extract + 10% cellobiose was incubated 24 hrs. and separated by ascending chromatography.

*Transglycosidation products.

to partial acid hydrolysis in a solution of 1 N H_2SO_4 at 100°C for 15 minutes. The hydrolyzate was neutralized with NaOH and evaporated to 1-2 ml. Chromatography showed that glucose, gentiobiose and cellobiose were present. The component R_G 0.39 (whose mobility indicates that it is a trisaccharide) probably possess a β 1,4 and a β 1,6 linkage. This compound is likely 0-6-β-D pyranosyl cellobiose which is also a major transglycosidation product of the β-glucosidase of Asperigillus niger (Crook and Stone, 1957; Barker, et al., 1955).

The appearance of 0-6-β-D-glucopyranosyl cellobiose in the reaction mixture after 15 minutes (Table 1) as compared to the appearance of cellotriose after 10-11 hours indicates that the β 1,6 linkage is synthesized preferentially over the β 1,4 linkage in this transglycosidation to cellobiose.

The synthesis of gentiobiose by our preparations demonstrates a parallel synthesis of 1,6 linkage by transglycosidation. The synthesis of gentiobiose is greatly increased by the addition of glucose in addition to the cellobiose in the standard reaction mixture.

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