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Abstract

Cellobiose-induced B-galactosidase and B-glucosidase activities of *Neurospora crassa.*

Russell, P. J. and C. B. Perry.

Cellobiosr-induced β-galactosidase and β-glucosidase activities of *Neurospora crassa*.

Three β-galactosidase (β-D-galactoside galactohydrolase: EC 3.2.1.23) activities have been shown in wild type Neurospora. One of them is induced by D-cellobiose, has an optimum activity at pH 6 and is precipitated at 50-to-75% saturation with ammonium sulfate (AS) (Perry and Lester 1973 Biochem. Biophys. Res. Commun. 54: 1476). Since D-cellobiose is an effective inducer for β-glucosidase (β-D-glucoside glucohydrolase: EC 3.2.1.21) activity in Neurospora, and since one of the known β-glucosidases, "cellobiase," has optimum activity at pH 6 (Eberhart and Beck 1970 J. Bacterial. 101: 408), we investigated whether or not the pH 6 form of β-galactosidase has any β-glucosidase activity.

Beta-galactosidase and β-glucosidase activities were determined by use of the chromogenic substrates, o-nitrophenyl-β-D-galactopyranoside (ONPG) and p-nitrophenyl-β-D-glucopyranoside (PNPG). These and the other procedures used in this study (e.g. growth conditions, medium, cellobiose concentration, etc.), were described by Perry and Lester (1973 Biochem. Biophys. Res. Commun. 54: 1476). A unit of enzyme activity is that amount which releases 1.0 μmole of ONP or PNP per hour at 37°C under the assay conditions. For ion exchange chromatography, diethylaminoethyl (DEAE) cellulose (Cellex D), was equilibrated with 0.01 M potassium phosphate, pH 6.8 containing 0.001 M EDTA, and poured into a 2.2 x 15-cm column (bed volume, 50 ml). Ammonium sulfate (AS) fractions were applied in volume of less than 5 ml and elution was carried out with a linear NaCl gradient (0.025 to 0.25 M) made in 0.01 M potassium phosphate buffer.

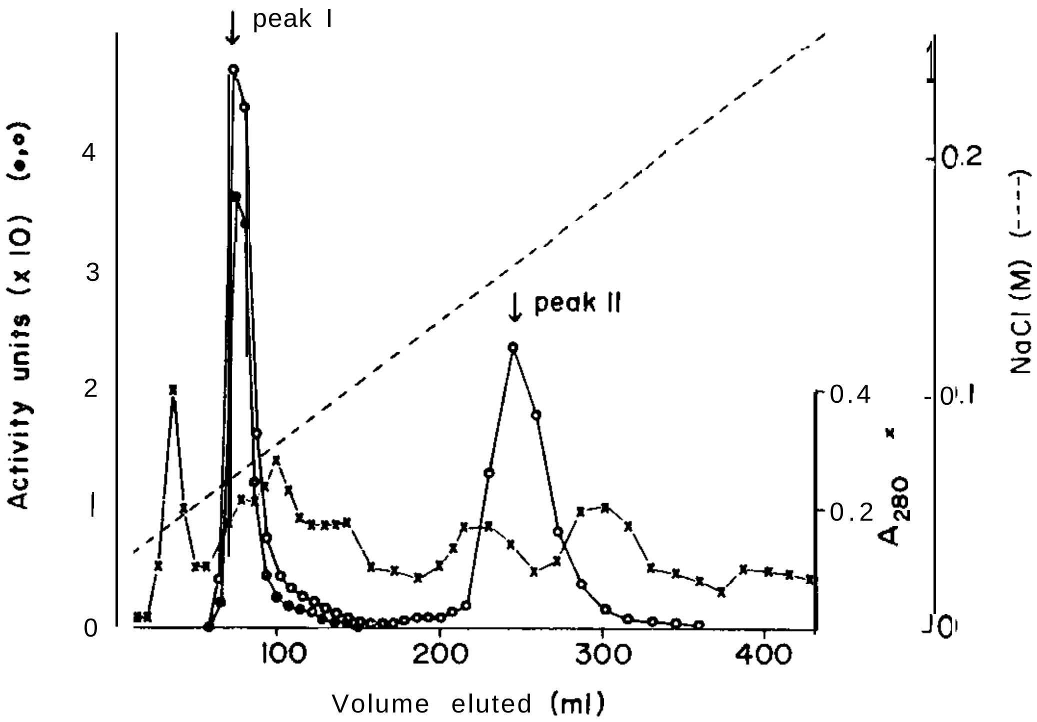

Figure 1. DEAE-cellulose chromatography of a resuspended 50-to-75% AS fraction from cellobiose-grown mycelia. The sample applied contained 101 units of β-galactosidase activity and 135 units of β-glucosidase activity. (•) β-galactosidase activity; (o) β-glucosidase activity; (x) absorbance at 280 nm; (---) NaCl concentration.

When extracts of cellobiose-grown mycelia were assayed at pH 6, both β-galactosidase and β-glucosidase activities were detected. The extracts were then subjected to AS fractionation. Very little of the enzyme activities (1% of the β-galactosidase and 4% of the β-glucosidase present in the crude extract) was precipitated by 0.50% AS; whereas, much more (75% of the β-galactosidase and 40% of the β-glucosidase) was precipitated by 50-75% AS. The 50.75% AS-fraction was then separated by chromatography on DEAE-cellulose. The fractions obtained were assayed for both activities (see Fig. 1). β-glucosidase activity appeared in two well-defined peaks (I and II), while β-galactosidase activity appeared in only one peak, which closely coincided with peak I of the β-glucosidase activity. There was, esentially, total recovery of both activities applied to the column. When activities were normalized to the peak maxima, the β-galactosidase peak and the β-glucosidase peak I were superimposable.

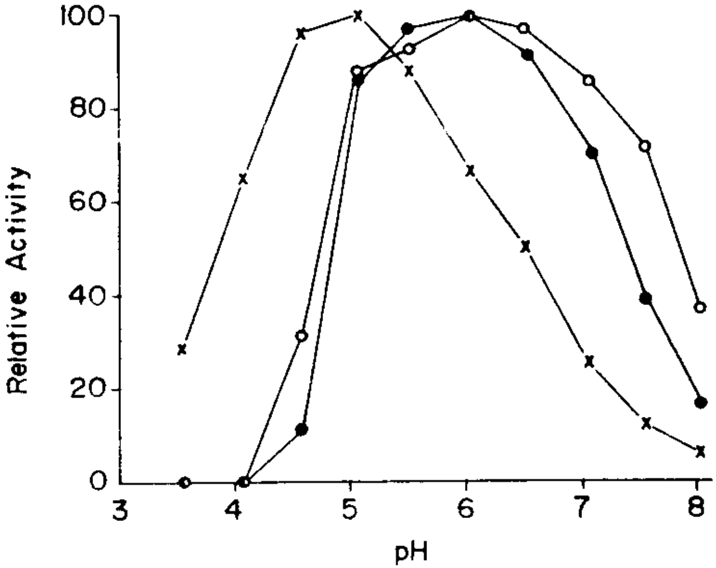

Figure 2. Effect of pH on DEAE-cellulose peak-! β-galactosidase activity (●), peak-1 β-glucosidase activity (o), and peak-II β-glucosidase activity (x). The number of units used were: peak-1 β-galactosidase, 1.30; peak-1 β-glucosidase, 1.30; and peak-11 β-glucosidase, 1.84. Activities were measured at pH optima.

Fractions corresponding to the two peaks were used to characterize further the β-galactosidase and β-glucosidase enzyme activities. Effects of pH on these activities are shown in Fig. 2. The pH optima of 5 and 6 for peak-II and peak-1 β-glucosidase activities are similar to the optima reported for aryl-β-glucosidase (Mahadevan and Eberhart 1964 Arch. Biochem. Biophys. 108: 22) and cellobiase (Eberhart and Beck 1970), respectively. The peak-1 β-glucosidase and β-galactosidase cannot be distinguished by the effect of pH upon their activities nor by the use of inhibitors. D-cellobiose or mercuric chloride. Finally, the peak-1 activities were tested for their thermal stabilities. At 50^{0}C, β-galactosidase and β-glucosidase activities showed very similar kinetics of inactivation, with half-lives of 3 to 4 min.

In summary, these data show that the cellobiose-induced, pH 6-β-galactosidase of Neurospora is associated with a β-glucosidase activity, possibly cellobiase. It is not known at this time whether this is because Neurospora possesses a single enzyme activity that has dual specificity for β-galactoside and β-glucoside substrates, or whether there are two separate enzymes that copurify in the procedures used.

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