

**Celllobiosr-induced  $\beta$ -galactosidase and  
 $\beta$ -glucosidase activities of *Neurospora crassa*.**

$\beta$ -glucosidases, "cellobiase," has 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-celllobiose is an effective inducer for  $\beta$ -glucosidase ( $\beta$ -D-glucoside glucohydrolase: EC 3.2.1.21) activity in *Neurospora*, and since one of the known

Three  $\beta$ -galactosidase ( $\beta$ -D-galactoside galactohydrolase: EC 3.2.1.23) activities have been shown in wild type *Neurospora*. One of them is induced by D-celllobiose, 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-celllobiose is an effective inducer for  $\beta$ -glucosidase ( $\beta$ -D-glucoside glucohydrolase: EC 3.2.1.21) activity in *Neurospora*, and since one of the known

$\beta$ -galactosidase and  $\beta$ -glucosidase activities were determined by use of the chromogenic substrates,  $\alpha$ -nitrophenyl- $\beta$ -D-galactopyranoside (ONPG) and  $\alpha$ -nitrophenyl- $\beta$ -D-glucopyranoside (PNPG). These and the other procedures used in this study (e.g. growth conditions, medium, celllobiose 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  $\mu$  mole of ONP or PNP per hour at 37°C under the assay conditions. For ion exchange chromatography, diethylaminoethyl (DEAE) cellulose (Celltex 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.

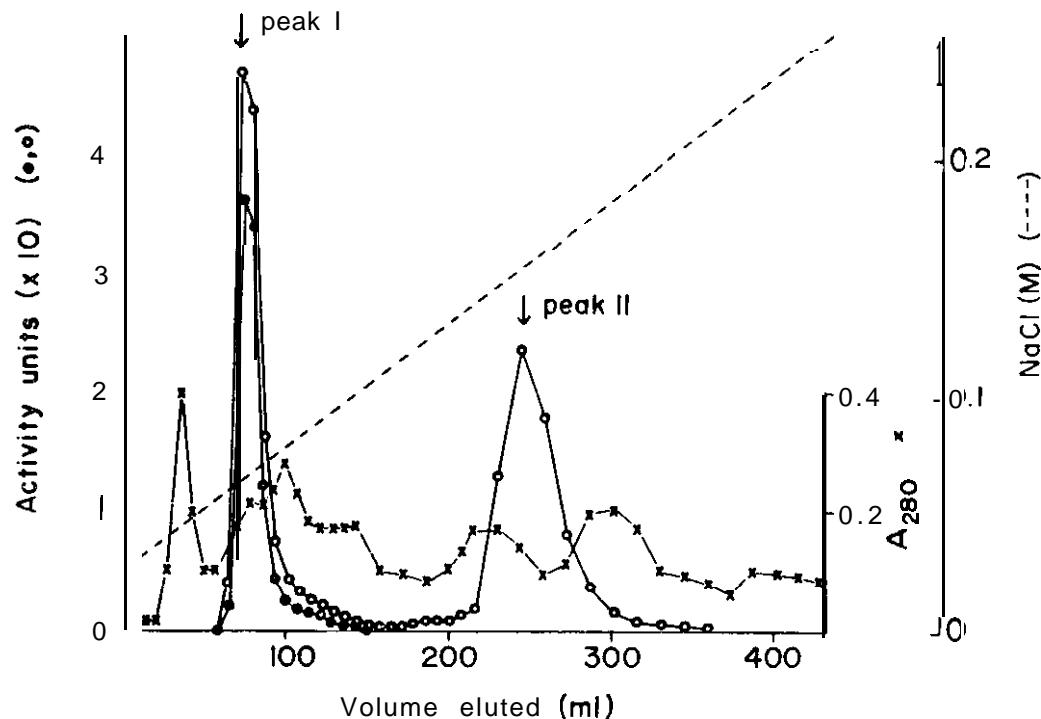


Figure 1. DEAE-cellulose chromatography of a resuspended 50-to-75% AS fraction from celllobiose-grown mycelia. The sample applied contained 101 units of  $\beta$ -galactosidase activity and 135 units of  $\beta$ -glucosidase activity. (●)  $\beta$ -galactosidase activity; (○)  $\beta$ -glucosidase activity;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

When extracts of cellobiose-grown mycelia were assayed at pH 6, both  $\beta$ -galactosidase and  $\beta$ -glucosidase activities were detected. The extracts were then subjected to AS fractionation. Very little of the enzyme activities (1% of the  $\beta$ -galactosidase and 4% of the  $\beta$ -glucosidase present in the crude extract) was precipitated by 0.50% AS; whereas, much more (75% of the  $\beta$ -galactosidase and 40% of the  $\beta$ -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).  $\beta$ -glucosidase activity appeared in two well-defined peaks (I and II), while  $\beta$ -galactosidase activity appeared in only one peak, which closely coincided with peak I of the  $\beta$ -glucosidase activity. There was, essentially, total recovery of both activities applied to the column. When activities were normalized to the peak maxima, the  $\beta$ -galactosidase peak and the  $\beta$ -glucosidase peak I were superimposable.

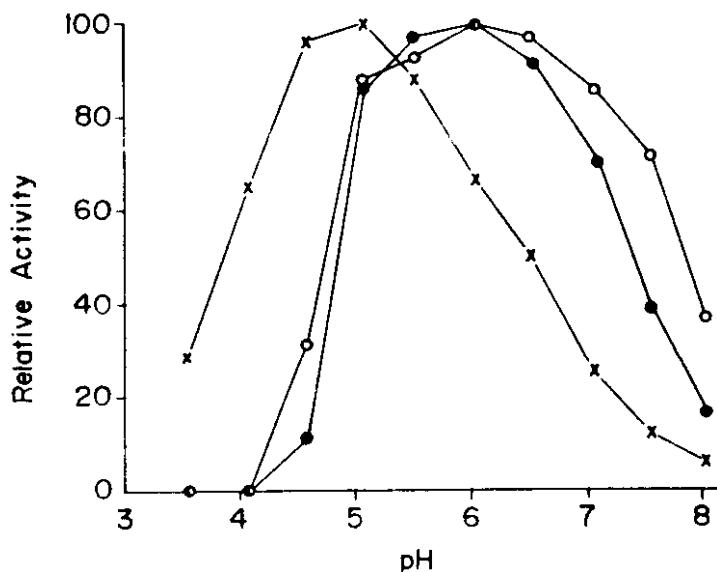


Figure 2. Effect of pH on DEAE-cellulose peak-1  $\beta$ -galactosidase activity (●), peak-1  $\beta$ -glucosidase activity (○), and peak-II  $\beta$ -glucosidase activity (×). The number of units used were: peak-1  $\beta$ -galactosidase, 1.30; peak-1  $\beta$ -glucosidase, 1.30; and peak-II  $\beta$ -glucosidase, 1.84. Activities were measured at pH optim.

Fractions corresponding to the two peaks were used to characterize further the  $\beta$ -galactosidase and  $\beta$ -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  $\beta$ -glucosidase activities are similar to the optima reported for aryl- $\beta$ -glucosidase (Mahadevan and Eberhart 1964 Arch. Biochem. Biophys. 108: 22) and cellobiase (Eberhart and Beck 1970), respectively. The peak-1  $\beta$ -glucosidase and  $\beta$ -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°C,  $\beta$ -galactosidase and  $\beta$ -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- $\beta$ -galactosidase of *Neurospora* is associated with a  $\beta$ -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  $\beta$ -galactoside and  $\beta$ -glucoside substrates, or whether there are two separate enzymes that copurify in the procedures used.