Induction of β-D-Glucosidase in *Streptomyces granaticolor*

M. Jirešová, Z. Dobrová, J. Náprstek, P. Ryšavý and J. Janeček

Department of Molecular Biology and Genetics, Institute of Microbiology, Czechoslovak Academy of Sciences, 142 20 Prague 4

Received January 10, 1983

**ABSTRACT.** β-D-Glucosidase in *Streptomyces granaticolor* is an inducible enzyme. Methyl-β-D-glucoside or cellobiose, added to a glycerol-containing medium, are most suitable inducers. The activity of β-D-glucosidase in a culture fully induced by cellobiose is 50 times higher than the basal level of the enzyme. β-D-Glucosidase is an intracellular enzyme, whose inducibility differs with culture age and reaches its maximum in a 10-h-old mycelium. The enzyme synthesis begins 2 h after the addition of the induced and reaches its maximum after a 10-h-induction.

The fact that streptomycetes represent a higher degree of organization among bacteria makes them an interesting model for studying regulatory systems as compared with other prokaryotes where the regulation of protein synthesis has been studied in more detail. Inducible enzyme synthesis is one of the best-known types of the regulation of protein synthesis. In streptomycetes most attention was devoted to the inducible synthesis of β-D-galactosidase (EC 3.2.1.23) (Dan and Szabó 1973; Vitalis and Szabó 1978, Sanchez and Hardisson 1979; Chatterjee and Vinig 1982). Chatterjee and Vinig (1981, 1982) studied also the induction of α- and β-D-glucosidase (EC 3.2.1.20,21) in *S. venezuelae*. The mechanism of induction of β-D-galactosidase in streptomycetes differs from the analogous regulation in *E. coli*. The differences include the chemical structure of the inducer, rate of enzyme synthesis and degree of induction. So far, only in the case of fructokinase (EC 2.7.1.4) has it been demonstrated that the induction is regulated at the transcription level even in streptomycetes (Martin and Sabater 1981). A detailed mechanism of induction has not yet been demonstrated for any other enzyme. The results obtained when studying the induction of β-D-glucosidase in *S. granaticolor* are presented in this communication. This enzyme is well suited for the study of protein synthesis regulation because it is easy to assay and exhibits a high degree of induction. Moreover, β-D-glucosidase is important in the bioconversion of cellulose to glucose (Ishaque and Kluepfel 1980).

**MATERIAL AND METHODS**

Organism and cultivation. *Streptomyces granaticolor* ETH 7347 (Říčicová and Řeháček 1968) was used. The minimal cultivation medium M 56 (Cohn and Monod 1951) containing 1 % Casamino acids (Difco) was supplemented with
a 1 % energy source. The microorganism was cultivated in 500-mL flasks containing maximally 70 mL of the medium. Cultivation was done on a reciprocal shaker at 27 °C. Vegetative inoculum was prepared by inoculating 50 mL of the medium with a loopful of spores and cultivating for 2 d. One mL of this culture was used to inoculate 50 mL of the fresh medium and cultivated 1 d. The obtained mycelium was diluted in such a way that the culture was in the exponential growth phase even further. Synthesis of β-D-glucosidase was induced by adding cellobiose or methyl-β-D-glucoside.

Assay of β-D-glucosidase. The enzyme activity was assayed using 4-nitrophenyl-β-D-glucopyranoside (PNPG), a chromogenic substrate, according to the method of Berghem and Petersson (1974); the method was partially modified. Cells or a cell-free extract (0.1—0.6 mL) were added to 0.3 mL of PNPG (5 mM) in 100 mM sodium phosphate buffer pH 7.0 (Fig. 1). The reaction mixture was incubated at 40 °C. The reaction was stopped by adding 0.6 mL 1 m Na2CO3. The amount of released 4-nitrophenol was determined colorimetrically at 425 nm. The enzyme activity is expressed in nkat. Specific enzyme activity is expressed in nkat per mg protein.

Preparation of cell-free extracts. The mycelium was centrifuged for 15 min at 15 000 g in the cold. The mycelium was centrifuged for 15 min at 15 000 g in the cold. The sediment was washed and resuspended in 100 mM sodium phosphate buffer (pH 7). The cells were disintegrated either by a 4-min sonication in a Raytheon sonicator at 9 kHz, or by vortexing the cell suspension with an equal volume of wetted ballotini beads No. 14 (3 × 1 min). The enzyme activity was assayed in the supernatant after a 15-min centrifugation at 20 000 g and 4 °C.

Preparation of toluenized suspension. 0.1 mL of toluene was added to 3 mL of the cell suspension and the mixture was shaken in closed test-tubes for 30 min at 25 °C. The enzyme activity was assayed directly in the toluenized suspension.

Cell proteins were determined according to Lowry et al. (1951).