DOES 5-FLUOROURACIL CAUSE CATABOLITE REPRESSION?

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1. Introduction

Induced β-galactosidase synthesis is severely inhibited by 5-fluorouracil in *Escherichia coli* cells. In their contribution concerning the mode of action of this base analogue Peck et al. [1] concluded, that this inhibitory effect was catabolite repression. This opinion is in contradiction to that of others [2, 3], who considered the inhibition as the incorporation of 5-fluorouracil into *lac* mRNA and the subsequent translation of this altered mRNA into enzymically inactive protein. The explanation of Peck et al. [1] contradicted also our previous, unpublished experiments, in which we failed to detect catabolite repression of induced β-galactosidase synthesis in wild type *E. coli* cells by 5-fluorouracil. In another yet unpublished experiment we have found that thymidine, the way it was used by Peck et al. [1], may in itself inhibit β-galactosidase synthesis. This possibility, i.e. that nucleosides are able to evoke catabolite repression, was also raised by Moses and Prevost [4].

In this paper we present evidence that thymidine evokes catabolite type repression in wild type and in a mutant *E. coli* strain.

2. Materials and methods

The *E. coli* K 12 wild type and CA 8050 bearing the UV5 mutation in the *lac* promoter gene were used. Induced tryptophanase synthesis showed typical catabolite repression in both strains when growing on glycerol, glucose was added later to the induced culture. The *lac* operon of the CA 8050 strain, however, was glucose-insensitive as a consequence of the promoter mutation. Medium and growth conditions were the same as has been described previously [5]. An overnight culture of the bacteria was diluted in fresh, prewarmed mineral salts + glycerol medium to a $A_{570} = 0.075$ and cultivated at 37°C in a reciprocal shaker (Gallenkamp IH-350). At a $A_{570} = 0.300$, i.e. in the log phase, at 0 min the cells were induced with 5×10^{-4} M TMG (methyl-β-D-thiogalactoside) and 10^{-3} M L-tryptophan simultaneously for β-galactosidase and tryptophanase production, respectively. The estimation of β-galactosidase activity was the same, as described previously [5]. For the determination of tryptophanase activity the cells after addition of 50 μg/ml chloramphenicol were washed with 0.05 M, pH 7.8 sodium phosphate buffer, resuspended in the same buffer to the original volume, and 0.5 ml of this suspension was incubated at 37°C for 60 min with 0.5 ml of 5×10^{-4} M pyridoxal phosphate and 0.2 ml 5×10^{-2} M L-tryptophane. This indole produced was detected by the addition of 3.6 ml Ehrlich reagent and the absorbance of the samples was read at 570 nm in a Unicam SP1800 spectrophotometer. The enzyme activity is expressed as 1 unit = 1 μmole L-tryptophane hydrolyzed/hr/1 ml cell suspension.

The chemicals applied were of analytical grade from commercial sources.

3. Results

Fig. 1 demonstrates the effect of 10^{-3} M glucose and 10^{-3} M thymidine on induced β-galactosidase and tryptophanase synthesis in *E. coli* K 12 wild type strain. Glucose causes severe transient repression of β-galactosidase synthesis for about 15 min which the cells then escape, and it is followed by a less severe catabolite repression. The tryptophanase synthesis

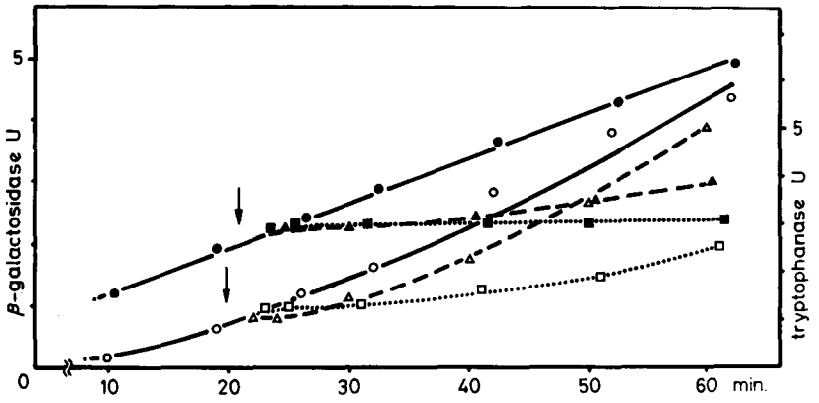

Fig. 1. The effect of 10^{-3} M glucose and 10^{-3} M thymidine (added at the 20th and 21st min, respectively, as indicated by the arrows) on induced β-galactosidase and tryptophanase synthesis, in *E. coli* K 12 wild type strain.

	β-Galactosidase	Tryptophanase
Control	o—o	●—●
10^{-3} M glucose	□·····□	■·····■
10^{-3} M thymidine	△—△	▲—▲.

shows severe inhibition nearly throughout the experiment. The repressive effect of thymidine is less pronounced: it is shorter and the degree of inhibition is less.

From fig. 2 it can be seen that in *E. coli* CA 8050 both 10^{-3} M glucose and 10^{-3} M thymidine fail to repress induced β-galactosidase synthesis. This is in good agreement with the fact that the UV5 mutation of the *lac* promoter region renders it insensitive to catabolite repression [1, 6]. On the other hand the induced tryptophanase synthesis is still sensitive to both glucose and thymidine. The somewhat enhanced β-galactosidase synthesis in the presence of glucose in this strain is explained by enhanced growth caused by

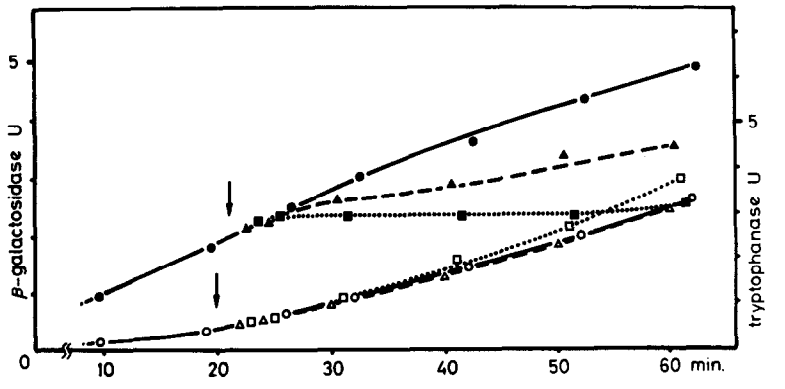

Fig. 2. The effect of 10^{-3} M glucose and 10^{-3} M thymidine (added at the 20th and 21st min, respectively, as indicated by the arrows) on induced β-galactosidase and tryptophanase synthesis, in *E. coli* CA 8050, *lac* promoter mutant strain.

	β-Galactosidase	Tryptophanase
Control	o—o	●—●
10^{-3} M glucose	□·····o	■·····■
10^{-3} M thymidine	△—△	△—△.

this sugar as indicated by the increased optical density of the cultures at 570 nm. In the presence of 10^{-3} M thymidine we could not detect any difference to control cells between the growth rates.

We could also demonstrate that the repression caused by glucose and that by thymidine are both partly inhibited by adding 10^{-3} M cyclic adenosine-3′,5′-monophosphate (cAMP) both in the case of β-galactosidase and tryptophanase in *E. coli* K 12 and of tryptophanase in *E. coli* CA 8050 cells.

4. Discussion

In their MS UV5 strain – carrying the same mutation in the *lac* promoter gene as does the CA 8050 we used – Peck et al. [1] have found in the presence of glucose 90% β-galactosidase synthesis of that in glycerol medium, whereas the corresponding figure for tryptophanase has been 5–10%. By contrast, in the parental MS 1054 p^+ strain, both enzymes have been synthesized in glucose medium at 5–15% of the rate in glycerol medium. They have expected in MS UV5 the tryptophanase synthesis to be more sensitive to inhibition by 5-fluorouracil than the synthesis of β-galactosidase. They have succeeded in obtaining experimental evidence to this hypothesis. Thus they concluded that 5-fluorouracil caused catabolite repression.

Here we wish to call attention to the fact that Peck et al. [1] have always added about 3.5×10^{-4} M thymidine together with 5-fluorouracil, probably to protect DNA synthesis from possible effect of this base analogue. (It may behave as a thymine analogue too.)

It is well known that catabolite repression is mediated through the cAMP-system in *E. coli* [7] and can be reversed in part by adding cAMP to the repressed culture [8, 9].

Our above results, i.e. that: i) thymidine is able to repress induced β-galactosidase and tryptophanase synthesis in wild type *E. coli* cells; ii) it represses only tryptophanase production in a *lac* promoter mutant strain, where the β-galactosidase synthesis is also glucose resistant; iii) the repression is counteracted in part by cAMP; all this indicates that thymidine causes catabolite repression. On this basis we do not think there is evidence enough that 5-fluorouracil evokes catabolite repression in the presence of thymidine.

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