EFFECT OF 5-FLUOROURACIL ON β-GALACTOSIDASE SYNTHESIS IN AN *ESCHERICHIA COLI* MUTANT RESISTANT TO CATABOLITE REPRESSION OF THE *LAC* OPERON

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1. Introduction

5-Fluorouracil severely inhibits the synthesis of β-galactosidase by wild-type cells of *Escherichia coli.* It has been suggested [1] that the mechanism of the inhibition involves incorporation of 5-fluorouracil into β-galactosidase mRNA and the subsequent translation of this altered mRNA into enzymically inactive protein. This explanation gained support from the finding of Nakada and Magasanik [2] that cultures of *E. coli* induced in the presence of 5-fluorouracil contain a protein that cross-reacts with anti-β-galactosidase antiserum. An alternative explanation [3] is that 5-fluorouracil causes a severe catabolite repression which prevents transcription of the *lac* operon [4].

We have been able to confirm that this latter explanation is correct by studying a mutant that is insensitive to catabolite repression of the *lac* operon.

2. Materials and methods

The bacterial strains we used were MS 1054 (F^- *thr leu arg str lac* $i^+ p^+ o^+ z^+ y^{del}$) [5]; UV5 (Hfr H str^+ *lac* $i^+ p^r o^+ z^+ y^+$) [6], and MS UV5 (*thr leu arg lac* $i^+ p^r o^+ z^+ y^+$) which was constructed by mating MS 1054 and UV5 and selecting recombinants on lactose-minimal-streptomycin agar supplemented with threonine, leucine and arginine. Media, maintenance and growth of the organisms, induction of β-galactosidase, sampling into chloramphenicol and estimation of β-galactosidase and of bacterial protein have been described previously [5, 7]. Tryptophanase was induced with 500 μg DL-tryptophan/ml; for its estimation, 1 ml samples of the chloramphenicol-treated culture were centrifuged, and the bacteria were washed once, treated with toluene and assayed [8]. 5-Fluorouracil (40 μg/ml) was added together with thymidine (80 μg/ml) 10 min before induction of the enzymes.

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3. Results and discussion

In strain MS UV5, the *lac* promoter carries the mutation UV5 [9] which renders transcription of the *lac* operon specifically resistant to catabolite repression [6]. (Contrast strain LA12-G [10], which shows general resistance to catabolite repression [11, 12]). Thus in MS UV5 we found that the differential rate of synthesis of β-galactosidase in glucose medium was about 90% of that in glycerol medium, whereas the corresponding figure for tryptophanase (another catabolite-sensitive enzyme) was 5–10%. By contrast, in the parental p^+ strain MS 1054, both enzymes were synthesized in glucose medium at 5–15% of the rate in glycerol medium.

If 5-fluorouracil exerted its inhibitory effect by catabolite repression, one would expect that

in MS UV5 the synthesis of tryptophanase would be far more sensitive to inhibition than the synthesis of β-galactosidase. This expectation was confirmed experimentally: in MS UV5 growing in glycerol medium the differential rate of tryptophanase synthesis in the presence of 5-fluorouracil was less than 5% of that in its absence, whereas the corresponding figure for β-galactosidase synthesis was at least 110% (values above 100% indicate that the synthesis of enzyme was inhibited less than that of bulk protein). In MS 1054, on the other hand, both enzymes were synthesized in the presence of 5-fluorouracil at less than 5% of the rate in its absence. All differential rates were constant for at least 2.5 hr.

Plainly, then, the UV5 mutation alleviates the inhibitory effect of 5-fluorouracil on β-galactosidase synthesis. We conclude that in the presence of 5-fluorouracil cultures of wild-type *E. coli* fail to form β-galactosidase because they are severely catabolite repressed [3]. We cannot account for the claim [2] that cross-reacting material is synthesized in substantial amounts by such cultures.

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