Effect of Isopropylthiogalactoside on Induction of the Galactose Operon by D-Fucose in a Lactose Deletion Mutant of Escherichia coli

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G. Buttin (J. Mol. Biol. 7:164, 1963) demonstrated that D-fucose (6-deoxy-D-galactose) is an effective gratuitous inducer of the galactose operon in Escherichia coli. However, in the presence of an equimolar concentration of isopropyl-β-D-thiogalactoside (IPTG), which is a gratuitous inducer of the lactose operon, the induction of galactokinase in mutant 3300 of the bacterium by 4 × 10⁻³ M fucose was inhibited by 70%. B. Williams and K. Paigen (Federation Proc. 24:417, 1965) showed that this repression can be overcome by increasing the concentration of the inducer, induction and repression behaving as competitive phenomena. Moreover, these authors showed a similar interaction of fucose and IPTG in the formation of β-galactosidase in a strain of E. coli constitutive for the enzyme. In view of the possibility of an interaction during the simultaneous induction of the two operons, we considered that it would be interesting to investigate the effect of IPTG on the induction of the galactose operon by fucose in a strain of E. coli deleted for the lactose operon; in such a strain, an interaction could not occur.

E. coli 200 X74, a strain carrying a complete deletion of the lactose region (S. Naono, J. Rouvier, and F. Gros, Biochem. Biophys. Res. Commun. 18:664, 1965), was grown on glycerol-minimal medium supplemented with arginine, histidine, and thiamine. Four parallel cultures were obtained while the cells were growing in exponential phase at 37 C; IPTG and fucose were added, as indicated in Table 1. Samples were withdrawn from the cultures, and were treated with chloramphenicol (100 µg/ml) at regular intervals while the cells continued to grow logarithmically as determined by turbidity measurements at 650 mµ. The samples were subsequently assayed for galactokinase activity, by the method of Buttin, except that the reaction products were separated by one-dimensional paper chromatography with butan-1-ol-propionic acid-water (40:18:22; v/v) as solvent.

Each 150-µliter sample of bacterial suspension was incubated at 37 C for 10 min with 20 µliters of toluene in the presence of magnesium chloride (10⁻³ M), ethylenediaminetetraacetic acid (10⁻⁴ M), and β-mercaptoethanol (5 × 10⁻³ M). To each sample was added 100 µliters of a solution containing 0.25 µmole of C⁴-galactose (2 µc/µmole), 0.4 µmole of adenosine triphosphate, 0.33 µmole of magnesium chloride, 0.8 µmole of sodium fluoride, and 8 µmoles of glycylglycine-sodium hydroxide buffer (pH 7.5). The mixture was incubated for 15 min at 37 C; then 50-µliter samples were withdrawn into 400 µliters of 95% ethyl alcohol, and were spotted onto Whatman no. 4 chromatography paper. After development for 20 hr in butan-1-ol-propionic acid-water (40:18:22; v/v), spots were located by radioautography, excised, and counted on the paper by use of two closely opposed end-window Geiger-Muller tubes with a combined efficiency of approximately 15%.

The observed incorporation of labeled galactose into galactose-1-phosphate is shown in Table 1. The rates of synthesis of galactokinase in cells treated with fucose and with fucose and 5 × 10⁻⁴ M IPTG were almost identical; with cells treated with fucose and 5 × 10⁻³ M IPTG, the rate of synthesis of the enzyme in excess of that in the uninduced control was consistently inhibited by approximately 43%.

In a second experiment, the effect of D-fucose on the induction of the lactose operon by IPTG (5 × 10⁻⁴ M) was investigated in two different strains of E. coli growing exponentially in glycerol-minimal medium. After the addition of IPTG to the cultures, samples were withdrawn at regular intervals and treated with chloramphenicol for enzyme assay; 21 min after contact with the inducer, D-fucose was added to each culture in the amounts shown in Table 2. Sampling was continued for a further 30 min. The activity of β-galactosidase was assayed by the method of A. Kepes (Biochim. Biophys. Acta 76: 293, 1963). The differential rates of enzyme synthesis before and after the addition of D-fucose are shown in Table 2.

In the Cavalli strain of the bacterium, which is
inducible for both the lactose and the galactose operons, neither concentration of fucose caused a reduction in the differential rate of enzyme synthesis. In strain AB 1105, which is inducible for β-galactosidase but is galactose-negative, there was a stimulatory effect which was apparently due to the addition of fucose. These results are not in accord with those of Williams and Paigen, but no attempt was made to investigate the effect of fucose on the induction of the lactose operon by less than optimal concentrations of IPTG.

Our experiments eliminate the possibility that the interaction of IPTG and D-fucose in the induction of the galactose operon is caused (i) by the competition of two protein-synthesizing systems, operating simultaneously, for a limited supply of precursors or cellular apparatus, or (ii) by an effect of the induction of the lactose operon or a product thereof acting in an unspecified way as an inhibitor in the induction of the galactose operon. The results of the present experiments are satisfactorily accounted for in terms of IPTG acting as a competitive inhibitor in the reaction of fucose with its acceptor site in the cell (presumably the repressor). Such an explanation is in accord with the view of Buttin that the intervention of IPTG in the regulation of the biosynthesis of β-galactosidase and galactokinase takes place at two distinct cellular sites.

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