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Galactose Repression of β-Galactosidase Induction in *Escherichia coli*

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ABSTRACT

BEGGS, WILLIAM H. (University of Minnesota, Minneapolis), AND PALMER ROGERS. Galactose repression of β-galactosidase induction in *Escherichia coli*. J. Bacteriol. **91**:1869–1874. 1966.—Galactose repression of β-galactosidase induction in *Escherichia coli* was investigated to determine whether the galactose molecule itself is the catabolite repressor of this enzyme system. Without exception, β-galactosidase induction by cells grown in a synthetic salts medium with lactate or glycerol as the carbon source was more strongly repressed by glucose than by galactose. This relationship existed even when the organism was previously grown in the synthetic medium containing galactose as the source of carbon. Two observations suggested that the ability of galactose to repress β-galactosidase formation by *Escherichia coli* depends directly upon the cells' capacity to catabolize galactose. First, galactose repression of β-galactosidase synthesis was markedly enhanced in bacteria tested subsequent to gratuitous induction of the galactose-degrading enzymes with D-fucose. Second, galactose failed to exert a repressive effect on β-galactosidase in a galactose-negative mutant lacking the first two enzymes involved in galactose catabolism. Glucose completely repressed enzyme formation in this mutant. This same mutant, into which the genes for inducible galactose utilization had been introduced previously by transduction, again exhibited galactose repression. Pyruvate was found to be at least as effective as galactose in repressing β-galactosidase induction by cells grown in synthetic salts medium plus glycerol. It is concluded that the galactose molecule itself is not the catabolite repressor of β-galactosidase, but that repression is exerted through some intermediate in galactose catabolism.

Efforts to elucidate the mechanism by which glucose and certain related compounds are able to inhibit the biosynthesis of a variety of inducible degradative enzymes have led to the catabolite-repression hypothesis (10). Many subsequent reports have strengthened the original proposal (4, 5, 11–13).

One of the primary questions in this area, which to date remains unanswered, concerns the nature and identity of the catabolite repressor. Apparently, some degree of repressor specificity does exist; that is, it appears as though different catabolite-repressor molecules exist for different glucose-sensitive enzymes (9, 14). McFall and Mandelstam (9) concluded that the real repressor of inducible, degradative, glucose-sensitive enzymes is either the end product of the enzyme system in question or a very closely related molecule. In support of this, they reported that β-galactosidase synthesis in *Escherichia coli* was repressed by its two end products, glucose and galactose, and that galactose was about twice as effective as glucose. Pyruvate, which is an end product of tryptophanase activity (17), exerted only slight inhibition of β-galactosidase synthesis, but it was found to be a better repressor of tryptophanase induction than was glucose. Galactose, on the other hand, had little or no effect on tryptophanase. From these and other studies of galactose-negative mutants of *E. coli*, it was concluded by McFall and Mandelstam (9) that glucose exerts its repressive effect on β-galactosidase induction via conversion of glucose to galactose. Nakada and Magasanik (13) presented evidence which contradicts this hypothesis.

The results reported here indicate that galac-

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tose exerts its repressive effect on β-galactosidase induction through some metabolic intermediate in galactose catabolism, and that the galactose molecule itself is not the catabolite repressor.

MATERIALS AND METHODS

Organisms. Three strains of *E. coli* were employed in these studies. The K-12 wild type was obtained from G. D. Novelli of Oak Ridge National Laboratory, Oak Kidge, Tenn. W13, Lederberg's W 3350 strain, is a *gal*⁻ mutant of *E. coli* K-12 which lacks both galactokinase and galactose-1-phosphate uridyltransferase. W70/4 is a doubly lysogenic derivative of W13 which carries both λ and λdg, and is therefore *gal*⁺. The W13 and W70/4 strains were supplied by Kenneth Paigen of Roswell Park Memorial Institute, Buffalo, N.Y. Strains K-12 and W13 were maintained on nutrient agar slants; W70/4 was maintained on minimal salts-galactose-agar slants.

Medium. The basal medium consisted of sodium citrate, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.7 g; NaCl, 1.0 g; KH_2PO_4, 1.0 g; K_2HPO_4, 1.0 g; $(NH_4)_2SO_4$, 4.0 g; and deionized water, 1 liter (final *p*H, 6.8 to 7.0). Sterilization was accomplished by autoclaving for 15 min at 121 C and 15 psi. Additions of various carbon sources were made from sterile aqueous solutions to a final concentration of 5 mg/ml.

Chemicals. Reagent-grade chemicals were employed in all media and solutions. D-Glucose and glycerol were obtained from Mallinckrodt Chemical Works, St. Louis, Mo.; D-galactose (substantially glucose-free), from the Sigma Chemical Co., St. Louis, Mo.; D-fucose, from K and K Laboratories, Plainview, N.Y.; and lactic acid, from J. T. Baker Chemical Co., Phillipsburg, N.J. D-Galactose-*1*-C^{14} (5 mc per mmole) was obtained from the New England Nuclear Corp., Boston, Mass.

Inoculum. Inoculum cells were grown in 250-ml Erlenmeyer flasks containing 80 ml of basal medium plus glycerol (galactose in the case of strain W70/4). Incubation of *E. coli* K-12 was carried out for 15 to 18 hr with reciprocal shaking at 37 C. Strains W13 and W70/4 were incubated for 18 to 24 hr under the same conditions.

Experimental cultures. Cells used for the induction experiments were grown in 250-ml Erlenmeyer flasks containing 80 ml of the basal medium plus the appropriate carbon source. Inoculations were made to 3 to 5% from the appropriate inoculum cultures, and the flasks were incubated for 4 to 5 hr at 37 C with reciprocal shaking.

Induction experiments. β-Galactosidase-induction experiments were performed with either 25- or 50-ml Erlenmeyer flasks containing 10- or 20-ml samples of experimental culture, respectively. Appropriate carbon sources were added to a final concentration of 5 mg/ml, and the inducer, isopropyl-β-D-thiogalactopyranoside (Mann Research Laboratories, New York, N.Y.), was added to a final concentration of 1 μmole/ml. During induction the flasks were incubated at 37 C with reciprocal shaking.

Assay for β-galactosidase. At appropriate time intervals, 2.0-ml samples of culture were removed and added to tubes (18 by 150 mm) containing 0.1 ml of toluene. The tubes were placed in a water bath (37 C), and were shaken manually at frequent intervals for 20 min. β-Galactosidase was assayed by a modification of the method described by Lederberg (8). Into test tubes (13 by 100 mm) were placed 0.6 ml of sodium phosphate buffer (0.02 M, *p*H 7.5), 0.2 ml of toluene-treated cell suspension, and 0.2 ml of substrate (0.005 M *o*-nitrophenyl-β-D-galactopyranoside, Mann Research Laboratories). Reactions were run for 5, 10, or 20 min at 37 C, and were stopped by the addition of 2.0 ml of 1 M Na_2CO_3. To correct for turbidity contributed by the cells, blank tubes were employed which contained 0.8 ml of buffer, 0.2 ml of toluene-treated cells, 2.0 ml of Na_2CO_3, and no substrate. *o*-Nitrophenol formation was determined with a Klett-Summerson photoelectric colorimeter fitted with a blue (420 mμ) filter, and was quantitated by relating the readings to a standard curve. β-Galactosidase activity is expressed as micromoles of *o*-nitrophenol formed per hour per milliliter of culture. In all experiments, enzyme activity was plotted as a function of time. A comparison of the initial and final optical density readings of the several induction systems in any particular experiment reveals that the patterns obtained reflect quite accurately the differential rates of enzyme synthesis.

Assay for galactokinase. The technique of Sherman (16), employing radioactive D-galactose, ion-exchange paper, and liquid scintillation counting, was used with minor modifications for the assay of galactokinase. Galactokinase activity is expressed as millimicromoles of galactose-1-phosphate formed per hour per milliliter of culture.

Growth. Growth was estimated with a Klett-Summerson photoelectric colorimeter, with a blue (420 mμ) filter, and the readings were converted to optical density units. An optical density of 0.100 is equivalent to about 5×10^8 cells per milliliter.

RESULTS

A typical study comparing the effects of D-galactose and D-glucose on β-galactosidase induction by glycerol-grown and lactate-grown cells of *E. coli* K-12 is shown in Fig. 1. Both hexoses were found to repress, but it is apparent that glucose was more effective than was galactose.

Glucose utilization is essentially a constitutive system in *E. coli*, whereas galactose utilization is an induced property. These factors may account for the observations in Fig. 1, and, in reality, galactose may be the better repressor.

To investigate this possibility, galactose-grown cells of *E. coli* were permitted to induce β-galactosidase in the presence of glucose or galactose (Fig. 2). Glucose was shown to be the better repressor or source of repressor substance, even after previous growth of cells with galactose as the sole carbon source.

The experiment presented in Fig. 3 is similar to that of Fig. 2, except that the cells were grown

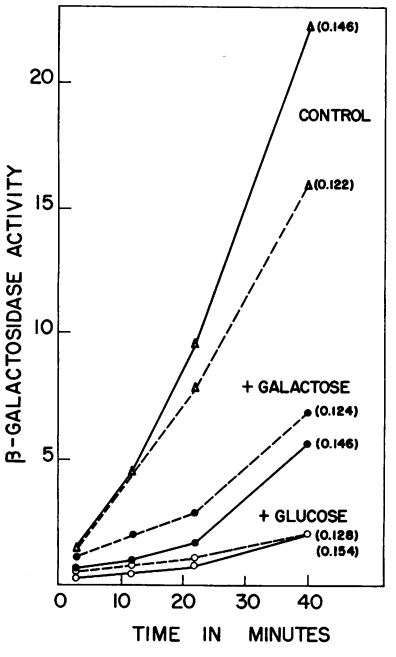

FIG. 1. *Effect of glucose and galactose on β-galactosidase induction by Escherichia coli K-12 grown on two different carbon sources. One culture was grown in glycerol medium to an optical density of 0.110, at which time three samples were removed and placed in separate flasks (solid line). A second culture was grown in lactate medium to an optical density of 0.102, and was divided in the same manner (broken line). Enzyme induction was followed in the presence of glucose (○), or galactose (●), or no additional carbon source (△) as indicated. Numbers in parentheses represent optical density readings of the cultures after 40 min of induction.*

in basal medium plus glucose. Under these conditions, galactose failed to exert any repressive effect on β-galactosidase induction.

It has been shown (5, 15) that the ability of a particular carbon and energy source to repress the formation of certain glucose-sensitive enzymes is directly related to the rate at which the bacteria in question are able to utilize or degrade that substrate for energy and biosynthetic intermediates. These data are consistent with the catabolite-repression hypothesis, and it would seem probable, therefore, that the ability of galactose to repress β-galactosidase is dependent upon the ability of the bacteria to catabolize galactose. To test this possibility, lactate-grown *E. coli* K-12 was incubated with D-fucose prior to induction of β-galactosidase. D-Fucose has been shown to be a gratuitous inducer of the galactose-degrading enzymes (1). The data in Fig. 4 show that galactose was much more effective as a repressor of β-galactosidase in the fucose-treated cells than in the untreated cells. Galactokinase is the first enzyme involved in galactose metabolism; it was found that, at time zero, the treated culture had a galactokinase activity of 242 units/ml, whereas the value for the control culture was 17 units/ml.

To establish further that catabolism of galactose is a prerequisite to its function as a repressor, mutant strains of *E. coli* were employed. The W13 strain lacks both the inducible galactokinase and transferase enzymes, and is therefore unable to grow on galactose as a sole source of carbon and energy. In such an organism, we would expect galactose to exert some repressive effect on β-galactosidase synthesis only if the galactose molecule itself is the repressor. However, galactose should exert no effect if catabolites produced from it are necessary for repression. In contrast, β-galactosidase induction in the W70/4 strain

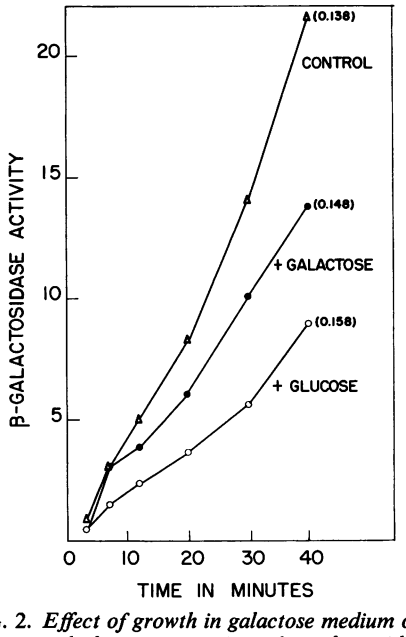

FIG. 2. *Effect of growth in galactose medium on the galactose and glucose repression of β-galactosidase in Escherichia coli K-12. E. coli K-12 was grown in the basal medium plus galactose. The bacteria were harvested by centrifugation, and were then suspended in fresh basal medium without a carbon source. Samples of this suspension were placed in each of three flasks; the cells were starved for 10 to 15 min, with shaking at 37 C, to deplete any catabolite pool. Glycerol was then added to each, followed by glucose and galactose to the appropriate flasks. Induction of β-galactosidase was followed as previously described. The optical density of the cells at zero-time was 0.104. Numbers in parentheses represent optical density readings of the cultures after 40 min of induction.*

(W13 carrying λ and λdg) would be expected to exhibit galactose sensitivity similar to that observed in the wild-type *E. coli* K-12.

Lactate-grown cultures of W13 and W70/4 were examined for their capacity to induce β-galactosidase in the presence and absence of D-galactose. Both strains were incubated with D-fucose prior to addition of inducer for β-galactosidase. It is apparent (Fig. 5) that galactose exerted a pronounced repression on β-galactosidase induction in the W70/4 strain; there was little or no effect on the W13 strain, in which synthesis of this enzyme was similar in either the presence or absence of galactose. Other experiments revealed that, even without prior incubation with D-fucose, galactose repressed β-galactosidase formation in the W70/4 strain, but galactose had no repressive effect in the W13 strain. Also, β-galactosidase induction in both mutant strains was repressed strongly by glucose.

It may be argued that the W13 strain does not show galactose repression by virtue of its impermeability to galactose. However, preliminary studies with 5×10^{-5} M galactose-*1*-C^{14} revealed that, within 8 min at 25 C, W13 cells were able to accumulate intracellular galactose to levels in excess of the extracellular concentration. The rate and level of accumulation were found to be slightly less when cells were pretreated for 90 min with D-fucose. This is not surprising in view of the report by Horecker, Thomas, and Monod (7) which indicated an inducible exit permease for galactose.

McFall and Mandelstam (9) reported that, although pyruvate is an even better repressor of tryptophanase than is glucose, pyruvate fails to repress β-galactosidase to any significant degree. With *E. coli* K-12, the pyruvate effect on tryptophanase was confirmed in this laboratory. However, the effect observed on β-galactosidase induction was contrary to the earlier work (Fig. 6).

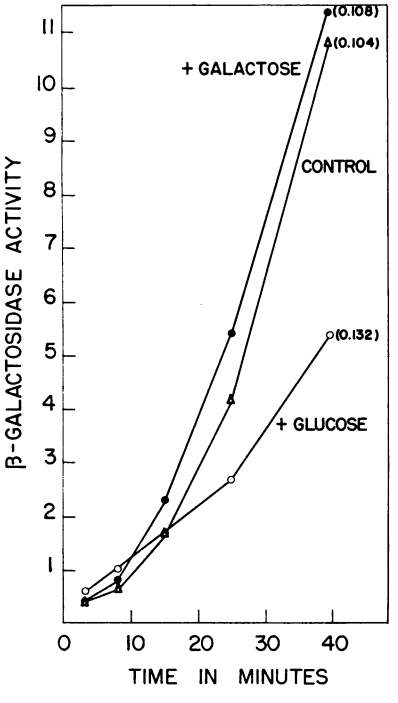

FIG. 3. *Effect of previous growth in glucose medium on the galactose and glucose repression of β-galactosidase in Escherichia coli K-12. Details of the experimental procedure were identical to those of Fig. 2. The optical density of the cells at zero-time was 0.084. Numbers in parentheses represent optical density readings of the cultures after 40 min of induction.*

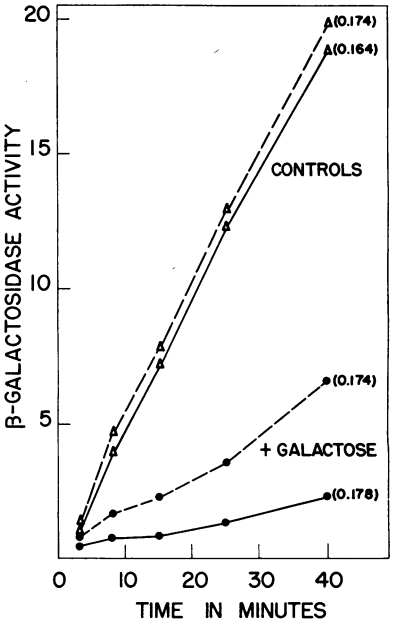

FIG. 4. *Effect of D-fucose induction of the galactose enzymes on the galactose repression of β-galactosidase in Escherichia coli K-12. Cells were grown in lactate medium. At 90 min prior to zero-time, the culture was split into two parts, one of which was made 10^{-3} M with respect to D-fucose. Just prior to zero-time, the treated and untreated cultures were each divided into two portions, and galactose was added to one of each pair. β-Galactosidase induction was followed in the presence and absence of galactose in both the fucose-treated (solid line) and untreated (broken line) cultures. The zero-time optical density readings were 0.138 in each case. Numbers in parentheses represent optical density readings of the cultures after 40 min of induction.*

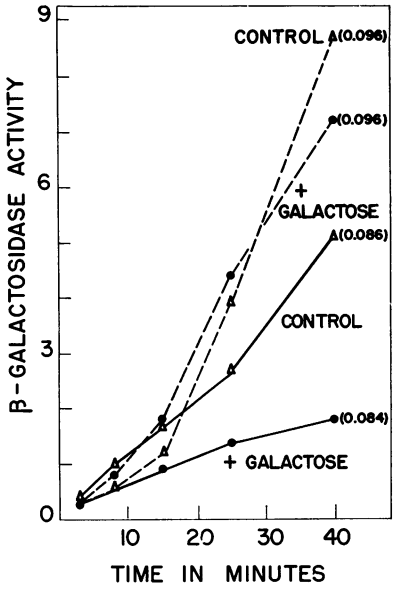

FIG. 5. *Galactose repression of β-galactosidase in Escherichia coli strains W13 and W70/4 after previous induction of the galactose enzymes with D-fucose. The two strains were cultured in basal medium plus lactate; 90 min prior to zero-time, D-fucose (10^{-3} M) was added to 40-ml samples of each. At zero-time, the cultures were divided into two flasks, and D-galactose was added to one of each pair of flasks. Strain W70/4 (solid line) and strain W13 (broken line) each had a zero-time optical density of 0.072. Numbers in parentheses represent optical density readings of the cultures after 40 min of induction.*

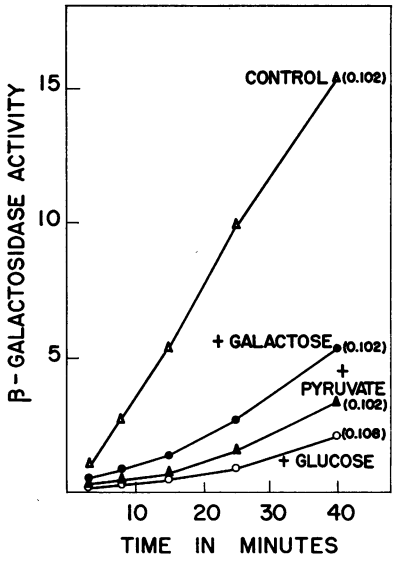

FIG. 6. *Comparison of the effects of pyruvate, galactose, and glucose on β-galactosidase induction by Escherichia coli K-12. Cells were grown in basal medium plus glycerol to an optical density of 0.076. The culture was then divided into four parts, and induction was followed in the presence of carbon sources as indicated. Numbers in parentheses represent optical density readings of the culture after 40 min of induction.*

From our data it seems clear that pyruvate was at least as effective a repressor of β-galactosidase induction as was galactose, if not better.

DISCUSSION

McFall and Mandelstam (9) suggested that glucose exerts its inhibitory effect on β-galactosidase via conversion to galactose. This presumably entails operation of the galactose-degrading enzymes in reverse. The enzymes involved are galactokinase, galactose-1-phosphate uridyltransferase, and uridine diphosphogalactose-4-epimerase. This system is inducible, and the induction process is coordinate rather than sequential (1). In addition, each of the enzymes is repressed by glucose (18). Strong repression of β-galactosidase can be demonstrated if cells are grown in a medium containing glucose and lacking an inducer of the galactose enzymes. Under such conditions, the levels and activities of galactokinase, transferase, and epimerase would be at an absolute minimum. On the basis of these considerations alone, it seems unlikely that the glucose effect on β-galactosidase involves conversion of glucose to galactose.

In the present study it was found that, without exception, β-galactosidase induction in bacteria grown on lactate or glycerol was more strongly repressed by glucose than by galactose. Even when *E. coli* K-12 was previously grown on galactose, glucose was still the better repressor of enzyme induction. The latter observation tends to minimize the possibility that a poorly developed galactose permeation mechanism is responsible for weaker galactose repression of cells grown on lactate or glycerol.

Our evidence strongly suggests that the effectiveness of galactose as a repressor of β-galactosidase depends directly upon the ability of the organism to catabolize the molecule. When the repressive effect of galactose on β-galactosidase was tested subsequent to gratuitous induction of the galactose-degrading enzymes in *E. coli* K-12, it was found that the repressive ability of the sugar was markedly enhanced.

Further evidence that catabolism is required for repression was obtained from studies em-

ploying galactose mutants. *E. coli* W13 lacks the first two enzymes for metabolic utilization of galactose, and yet, as indicated earlier, this organism is capable of galactose uptake (2, 3, 6). The fact that galactose exerted no significant repressive effect on β-galactosidase in the W13 strain, even when the cells were first treated with fucose, argues strongly against the idea that the galactose molecule is the catabolite repressor of β-galactosidase. Introduction of the genes for inducible galactose utilization into *E. coli* W13 yielded an organism (W70/4) in which β-galactosidase was again repressed by galactose; this effect was enhanced by prior treatment with D-fucose.

The *E. coli* K-12 wild-type strain employed in these studies demonstrated pyruvate-sensitive β-galactosidase induction when the organism was grown in basal medium plus glycerol. Pyruvate appeared to repress β-galactosidase more severely than did galactose, and it is possible that the pyruvate molecule is closer to the "real" catabolite repressor of β-galactosidase than is galactose.

The evidence presented herein is in agreement with the conclusion reached by Nakada and Magasanik (13), and suggests rather strongly that, although galactose is an excellent source of repressor for β-galactosidase induction in *E. coli*, the galactose molecule itself is not the catabolite repressor.

ACKNOWLEDGMENTS

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