Glucose-Lactose Diauxie in Escherichia coli

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Growth of Escherichia coli in medium containing glucose, at a concentration insufficient to support full growth, and containing lactose, is diauxic. A mutation in the gene, CR, which determines catabolite repression specific to the lac operon, was found to relieve glucose-lactose but not glucose-maltose diauxie. Furthermore, a high concentration of lactose was shown to overcome diauxie in a CR+ strain. Studies on the induction of β-galactosidase by lactose suggested that glucose inhibits induction by 10⁻³ M lactose. Preinduction of the lac operon was found to overcome this effect. The ability of glucose to prevent expression of the lac operon by reducing the internal concentration of inducer as well as by catabolite repression is discussed.

Growth of Escherichia coli in media containing two carbon sources at concentrations insufficient to support full growth is often diphasic, depending on the specific carbon sources supplied; the phenomenon has been termed diauxie (11). When lactose is supplied, the presence of either glucose or gluconate gives rise to diauxie. It has been shown that, in a glucose-lactose medium, the bacteria utilize glucose exclusively during the initial growth phase, and, upon depletion of glucose, utilize lactose during the second growth phase (11). The enzyme β-galactosidase, which is required for growth on lactose, has been shown not to be synthesized during growth on glucose and to appear only upon depletion of glucose shortly before growth resumes (5). The glucose concentration in this experiment was initially 2 × 10⁻³ M and decreased to a negligible concentration by the end of the first growth phase. During the second growth phase, the enzyme was initially synthesized at the maximal differential rate. Therefore, the concentration of lactose present in these experiments (5 × 10⁻³ M) is sufficient to cause full induction of β-galactosidase in lac+ cells in the absence of glucose. Thus, it appears that glucose, even at very low concentrations, completely inhibits the induction of β-galactosidase by 5 × 10⁻³ M lactose.

It has been shown that glucose represses many different enzymes (12). However, it appears that the repression is elicited by catabolites of glucose rather than by glucose per se (9, 12). The phenomenon has been termed catabolite repression (10). It is thought that the degree of repression of a specific operon is determined by the concentration of one or more catabolites which can be derived from different carbon sources. We have recently been able to isolate a mutant strain of E. coli which is insensitive to catabolite repression specific to the lac operon (8). The strain carries a mutation in a gene, CR, which maps genetically distant to the lac operon and near the tryptophan genes (Loomis and Magasanik, J. Mol. Biol., in press).

We used this mutant to determine whether catabolite repression is primarily responsible for the diauxic growth of E. coli on a mixture of glucose and lactose. We found that the primary cause is not repression, but interference by glucose with the uptake of lactose in glucose-grown CR+ cells containing only a basal level of β-galactosidase permease. The ability of the catabolite-insensitive mutant to grow without interruption on a mixture of glucose and lactose may reflect the higher level of permease in the glucose-grown CR− cells.

**MATERIALS AND METHODS**

**Chemicals.** Isopropyl-thio-β-D-galactoside (IPTG) and o-nitrophenyl-β-D-galactoside (ONPG) were obtained from Mann Research Laboratories, New York, N.Y.

**Media.** Minimal medium was made by adding to 1 liter of water 10 g of K₂HPO₄, 0.2 g of MgCl₂, 0.2 g of Na₂SO₄, 5.0 g of NaCl, 0.2 g of sodium citrate, 0.46 mg of FeSO₄, 4.4 mg of CaCl₂, and 0.5 mg of thiamine. The pH was adjusted to 7.0 with concentrated HCl. Carbon sources were added as indicated. The nitrogen source was 0.4% (NH₄)₂SO₄.

**Bacterial strains.** E. coli strain 3000 is an HfrH strain which is lac− (i+z+y+) CR+ and requires thiamine. Strains LA-12 and LA-12G were isolated from strain 3000 and shown to be specifically insensitive to catabolite repression of the lac operon (CR−) (8).

β-Galactosidase assay. β-Galactosidase activity was...
measured by the method reported by Loomis and Magasanik (7). When cells were induced by 10^{-3} M lactose, the activity was corrected to account for the inhibition of ONPG hydrolysis by lactose which was found to be 16%.

**Diauxie experiments.** The bacteria were grown overnight at 37°C in minimal medium containing 10^{-3} M glucose. The cultures were diluted in fresh medium to 2 \times 10^8 bacteria per milliliter and were allowed to grow at 37°C for about two generations. The cells were then collected on membrane filters (Millipore Filter Corp., Bedford, Mass.), washed with warm, carbon-free medium, resuspended in minimal medium with various carbon sources, and incubated with shaking at 37°C.

**RESULTS**

**Diauxie in CR− strains.** Strain LA-12 was selected from strain 3.000 after treatment with ethylmethane sulfonate on a medium containing glucose, 10^{-3} M IPTG, and N-acetyl lactosamine as sole source of nitrogen (8). Strain LA-12G is a derivative of strain LA-12 selected for rapid growth on glucose. Both strains were shown to be insensitive to catabolite repression specific to β-galactosidase and β-galactoside permease (8). β-Galactosidase synthesis in strain LA-12G was shown to be resistant to catabolite repression elicited by a variety of carbon sources, even when anabolism was limited by nitrogen starvation (8). No other effects of the mutation were observed.

Strain 3.000 was found to grow in minimal medium containing 10^{-3} M glucose and 10^{-2} M lactose in a diauxic manner; strain LA-12G, however, grew in this medium without interruption (Fig. 1a,b). Likewise, minimal medium containing 5 \times 10^{-4} M glucose and 10^{-2} M lactose gave rise to a diauxie in the growth of strain 3.000 but not in the growth of strain LA-12 (Fig. 1c,d). Minimal medium containing 5 \times 10^{-4} M glucose and 10^{-2} M maltose gave rise to a diauxie in the growth of both strain 3.000 and strain LA-12 (Fig. 1e,f). It is apparent that a mutation in the CR gene relieves glucose-lactose diauxie but not glucose-maltose diauxie.

To define further the relation of the CR gene to diauxie, we determined the differential rate of β-galactosidase synthesis in CR+ and CR− cells suspended in media containing 10^{-2} M lactose and either 10^{-3} M glucose or 2 \times 10^{-4} M glycerol. As can be seen in Fig. 2, cells of both strain 3.000 and strain LA-12G formed β-galactosidase at a high rate in the media containing glycerol and lactose. The CR+ cells of strain 3.000 formed no appreciable enzyme in medium containing glucose and lactose. The CR− cells of strain LA-12G suspended in medium containing glucose and lactose synthesized β-galactosidase, but at a low

![Fig. 1. Glucose-lactose and glucose-maltose diauxie.](image-url)
initial rate which increased with growth in this medium. Thus, a mutation in the CR gene allows induction by 10^{-3} \text{M} \text{ lactose} in the presence of glucose. However, it is apparent that glucose has an effect on induction by 10^{-2} \text{M} \text{ lactose} in the CR^{-} cells as well as in the CR^{+} cells.

Induction by lactose. β-Galactosidase synthesis in lac^{+} CR^{+} cells is completely prevented by glucose when 5 \times 10^{-4} \text{M} \text{ TMG} is used as inducer (3). However, preinduction of β-galactosidase permease-positive cells reduces the degree of repression to about 50\% (3). Preinduction of permease-negative cells does not reduce the strong repression by glucose under these conditions (3). It appears that permease activity overcomes the strong repression by glucose. Therefore, preinduction of the lac^{+} CR^{+} strain 3.000 might allow induction of β-galactosidase by 10^{-2} \text{M} \text{ lactose} in the presence of glucose.

IPTG at a concentration of 10^{-3} \text{M} was added to a culture of strain 3.000 growing in glucose-containing medium. After various periods of time, the cells were collected on membrane filters (Millipore Filter Corp., Bedford, Mass.), washed, and suspended in a medium containing 10^{-2} \text{M} \text{ glucose} and 10^{-2} \text{M} \text{ lactose}. As can be seen in Fig. 3, β-galactosidase is rapidly induced by 10^{-3} \text{M} \text{ IPTG} under these conditions. Cells which were immediately removed from the medium containing IPTG failed to synthesize β-galactosidase in the glucose-lactose medium, whereas those which had been incubated in the presence of IPTG for either 5 or 15 min continued to synthesize β-galactosidase in the glucose-lactose medium. It appears that a short period of preinduction gives rise to partial protection against glucose inhibition of induction by 10^{-2} \text{M} \text{ lactose} and that further synthesis of the products of the lac operon gives greater protection.

![Fig. 2. Induction of β-galactosidase by lactose. The cells were cultivated with aeration at 37 C in minimal media containing 2 \times 10^{-4} \text{M} \text{ glycerol} or 10^{-2} \text{M} \text{ glucose} as source of carbon. During exponential growth, 10^{-2} \text{M} \text{ lactose} was added to the cultures. Growth was followed by measuring the optical density of the cultures at 530 \mu\text{m} in a Klett-Summerson photoelectric colorimeter (1 unit = 3 \times 10^{6} \text{ bacteria per milliliter}). Samples were withdrawn for the measurement of β-galactosidase. Strain LA-12G on glycerol plus lactose (○) and on glucose plus lactose (□). Strain 3.000 on glycerol plus lactose (●) and on glucose plus lactose (△).](http://jb.asm.org/)

![FIG. 3. Effect of preinduction on β-galactosidase formation in a medium containing 10^{-4} \text{M} \text{ glucose} and 10^{-2} \text{M} \text{ lactose}. A culture of strain 3.000 in the exponential phase of growth in minimal medium containing 10^{-2} \text{M} \text{ glucose} received 10^{-3} \text{M} \text{ IPTG}. The increase in β-galactosidase activity was determined (●). Samples of the culture were withdrawn immediately after the addition of IPTG (○), 5 min later (△), and 15 min later (□). The cells were collected by filtration on a membrane filter, washed with medium free of IPTG, and suspended in minimal medium containing 10^{-2} \text{M} \text{ glucose} and 10^{-2} \text{M} \text{ lactose}. The cultures were incubated with shaking at 37 C, and their increase in β-galactosidase and in optical density at 530 \mu\text{m} was measured. One unit of optical density, determined on a Klett-Summerson photoelectric colorimeter, corresponds to 3 \times 10^{6} \text{ bacteria per milliliter.}](http://jb.asm.org/)
Loss of diauxie. Cohn and Horibata (3) have also shown that the repression of β-galactosidase by glucose is reduced to about 50% when the concentration of TMG is increased to $2 \times 10^{-3}$ M. Therefore, a high concentration of lactose might also give rise to reduced repression of the lac operon by glucose and thus allow continuous growth in a glucose-lactose medium. A culture of strain 3,000 was suspended in a medium containing $10^{-4}$ M glucose and $8 \times 10^{-2}$ M lactose. The bacteria grew with no observable lag (Fig. 4). It appears that diauxie is overcome by a high concentration of lactose.

**DISCUSSION**

It is known that lactose enters the cell by means of the β-galactoside permease and is converted to the active inducer by β-galactosidase (1). Consequently, the induction of the lac operon by lactose is facilitated by these enzyme activities.

Cohn and Horibata (3) have shown that preinduction of β-galactosidase and β-galactoside permease, but not of β-galactosidase alone, overcomes the severe repression by glucose in cells suspended in medium containing low levels of TMG. Kepes (6) has shown that the presence of glucose in the medium greatly reduces the internal concentration of TMG in cells lacking β-galactoside permease activity. The presence of permease activity overcomes this effect of glucose. Thus, it is likely that the severe inhibition by glucose of β-galactosidase synthesis in uninduced cells incubated in the presence of $10^{-2}$ M lactose is the result of a reduction of the internal concentration of lactose by glucose to a level insufficient for induction.

We have shown that preinduction of the lac operon for 5 min allows induction by $10^{-4}$ M lactose in the presence of glucose (Fig. 3). Furthermore, a high concentration of lactose ($8 \times 10^{-2}$ M) was shown to overcome diauxie (Fig. 4), suggesting that the lac operon was induced by $8 \times 10^{-2}$ M lactose before depletion of glucose. Both of these observations may be explained as the result of an increased internal concentration of lactose in cells growing in the presence of glucose, resulting, in the first case, from increased permease activity, and, in the second case, from the higher external concentration of lactose.

Clark and Marr (2) have shown that the repression of β-galactosidase in permeate-negative cells by glucose is stronger when the cells are induced by concentrations of IPTG less than that which gives maximal induction. It is possible that the reduction in internal concentration of inducer by glucose accounts for this effect.

The glucose analogue, α-methyl-D-glucoside, repressed β-galactosidase in cells induced by $10^{-4}$ M TMG (4). However, no repression by $10^{-2}$ M α-methyl-D-glucoside is observed in cells incubated in the presence of $10^{-4}$ M IPTG (Loomis and Magasanik, unpublished data). It is possible that α-methyl-D-glucoside represses β-galactosidase by reducing the internal concentration of inducer leading to a lower rate of induction.

Diauxic growth in medium containing $10^{-2}$ M lactose and insufficient glucose to support full growth may result, therefore, from the complete repression of the lac operon by reduction of the internal inducer concentration by glucose.

The primary effect of the mutation which makes E. coli resistant to catabolite repression of the lac operon (CR-) does not appear to be an alteration of the mechanism of induction. This is shown by the observation that the rate of the uninduced synthesis of β-galactosidase, and of the induced synthesis of β-galactoside-permease, in inducible and in constitutive strains, is not reduced by glucose when these strains carry the
CR− gene (Loomis and Magasanik, J. Mol. Biol., in press). In view of the results discussed so far, it is therefore somewhat surprising to find that the mutation to CR− has specifically abolished the glucose-lactose diauxie (1). The CR− cells, in contrast to CR+ cells, can be induced by 10−3 M lactose in the presence of glucose, though initially the rate of enzyme synthesis is much lower in the presence of glucose than in its absence (Fig. 2). We suggest that this induction is a reflection of the higher basal level of β-galactosidase permease of glucose-grown CR− cells compared with glucose-grown CR+ cells. The limitations of the assay method make it difficult to determine the uninduced levels of permease with sufficient accuracy to prove this assumption; however, the basal level of β-galactosidase is three times higher in glucose-grown CR− cells than in CR+ cells, and the two activities show in all cases a coordinate response to induction and repression (Loomis, Ph.D. Thesis, Massachusetts Institute of Technology, Cambridge, 1965). It is therefore likely that the basal level of β-galactosidase permease of glucose-grown CR− cells is three times higher than that of CR+ cells. Uninduced CR− cells should consequently be able to take up lactose more rapidly in the presence of glucose than uninduced CR+ cells. The hydrolysis of this lactose, favored by the higher basal level of β-galactosidase, should provide the inducer for the lac operon (1). Furthermore, since CR− cells are able to synthesize the products of the lac operon in the presence of glucose twice as fast as CR+ cells (8), their susceptibility to induction by lactose rapidly increases in the glucose-lactose medium. In fact, the kinetics of β-galactosidase synthesis in CR− cells incubated in the presence of 10−3 M lactose and 10−3 M glucose (Fig. 2) are very similar to those of CR+ cells incubated in such a medium after a 5-min period of preinduction (Fig. 3).

In summary, the phenomenon of glucose-lactose diauxie, the preferential utilization of glucose before lactose, appears to result from catabolite repression specific to the lac operon. Catabolite repression reduces the basal level of permease in glucose grown CR+ cells so that insufficient activity is present to overcome the ability of glucose to reduce the internal concentration of inducer. It appears that glucose can affect the rate of expression of the lac operon by at least two distinct mechanisms: by reducing the internal concentration of inducers of the lac operon, as shown here, and by catabolite repression of the lac operon depending on a functional CR gene, as shown in a previous publication (8).

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LITERATURE CITED


