# CATABOLITE REPRESSION IN ESCHERICHIA COLI: THE ROLE OF GLUCOSE 6-PHOSPHATE

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The formation of a number of bacterial catabolic enzymes is repressed by glucose and certain other readily metabolizable sources of carbon. The terms "glucose effect" (Epps and Gale, 1942; Monod, 1947) and "catabolite repression" (Magasanik, 1961) have been introduced to describe this phenomenon. Neither the chemical identity of the catabolite repressor nor its precise mode of action are known.

In the course of a study of the physiological role of phosphoenolpyruvate carboxykinase (PEPCK)* in Escherichia coli (Hsie and Rickenberg, 1966), a mutant was isolated which, unlike the parent strain, formed high levels of PEPCK even when grown in a medium containing glucose as sole source of carbon. Subsequent studies showed that in the mutant the syntheses of β-galactosidase, amylomaltase, isocitratase, tryptophanase, and acid phosphatase were also resistant to catabolite repression by glucose. The formation of these enzymes was inhibited by glucose in the parent strain.

Results reported in this communication show that whereas in the mutant neither glucose nor gluconate, when added singly to the medium, inhibited the formation of β-galactosidase or tryptophanase, a mixture of glucose and gluconate or glucose 6-phosphate (G-6-P) alone severely repressed the formation of the two

*Abbreviations used: PEPCK = phosphoenolpyruvate carboxykinase; G-6-P = glucose 6-phosphate; TMG = methyl-1-thio-β-galactoside; OAA = oxaloacetic acid.

enzymes. It is tentatively concluded that either G-6-P or a close metabolic derivative caused the observed catabolite repression.

## MATERIALS AND METHODS

E. coli K 12 strains AB 257, AB $257^{pc-1}$, AB 257-1, W 112-12-A-84, and 2000 $o^c$ were employed. AB $257^{pc-1}$, a spontaneous mutant derived from AB 257, was isolated on the basis of its ability to utilize succinate without lag after prior growth on glucose. The other strains are characterized in Table III. Bacteria were grown with aeration at 37° on the mineral salts medium "56" (Monod et al., 1951) containing the source of carbon specified and supplemented with the amino acids or vitamins required. Bacterial growth was measured turbidimetrically. Extracts were prepared by treatment of the washed bacteria, resuspended in the appropriate buffer, in a Raytheon 10 kc magnetostrictive oscillator. They were centrifuged for 30 minutes at 39,000 x g and supernates assayed for enzymic activities. Bacterial protein was determined by the method of Lowry et al. (1951). Phosphoenolpyruvate carboxykinase (PEPCK) was assayed by the isotope exchange method of Utter and Kurahashi (1955) modified by us (Hsie and Rickenberg, 1966). β-Galactosidase and β-galactoside permease activities were determined by measuring the rate of formation of o-nitrophenol from o-nitrophenyl-β-D-galactoside and the concentrative uptake of radioactive methylthiogalactoside (TMG) as described by Müller-Hill, Rickenberg, and Wallenfels (1964). Acid phosphatase was assayed by measuring the rate of formation of p-nitrophenol from p-nitrophenylphosphate in acetate buffer, 0.1 M, pH 5.5 (v. Hofsten, 1961). Tryptophanase activity was determined by measuring the rate of indole formation (Boezi and DeMoss, 1960). Amylomaltase activity was measured by following the rate of liberation of glucose from maltose (Loomis and Magasanik, 1966) using the glucostat reagent (Worthington Biochemical Corp., Freehold, N. J.). Isocitratase activity was determined by measuring the rate of glyoxylic acid phenylhydrazone formation (Dixon and Kornberg, 1959). The conditions of the enzyme assays were standardized so that the rates of enzymic activities were rectilinear functions of both the duration

of the assay and the concentration of bacterial protein used. The specific activities of PEPCK are expressed as mμM of $^{14}C$-labeled OAA formed per mg of bacterial protein per minute at 36°; of acid phosphatase as mμ M p-nitrophenol formed per mg of bacterial protein per minute at 37°; of β-galactosidase as mμ M o-nitrophenol formed per mg of bacterial protein per minute at 37°; of β-galactoside permease as the ratio of intracellular/extracellular TMG concentrations at 37°; of tryptophanase as μM of indole formed per mg of bacterial protein per minute at 37°; of amylomaltase as μM D-glucose formed per mg of bacterial protein per minute at 37°; of isocitratase as mμ M of glyoxylic acid phenylhydrazone formed per mg of bacterial protein per minute at 37°.

## RESULTS AND DISCUSSION

The growth of strains AB 257 and AB 257$^{pc-1}$ on various sources of carbon: Table I shows the generation times of the two strains utilizing different sources of carbon. The significantly more rapid growth of mutant AB 257$^{pc-1}$ on the

Table I

The generation time in minutes of AB 257 and AB 257$^{pc-1}$ on various sources of carbon

| | Maltose ($0.5 \times 10^{-2}$M) | Lactose ($0.5 \times 10^{-2}$M) | Glucose ($1.0 \times 10^{-2}$M) | Gluconate ($1.0 \times 10^{-2}$M) |
|---|---|---|---|---|
| AB 257 | 72 | 54 | 49 | 52 |
| AB 257$^{pc-1}$ | 60 | 54 | 48 | 51 |
| | Glucose 6-phosphate ($1.0 \times 10^{-2}$M) | Glucose ($1.0 \times 10^{-2}$M)+ Gluconate ($1.0 \times 10^{-2}$M) | Succinate ($1.5 \times 10^{-2}$M) | |
| AB 257 | 44 | 44 | 79 | |
| AB 257$^{pc-1}$ | 45 | 42 | 57 | |
| | Glycerol ($2.0 \times 10^{-2}$M) | Lactate ($2.0 \times 10^{-2}$M) | Acetate ($3.0 \times 10^{-2}$M) | |
| AB 257 | 73 | 93 | 224 | |
| AB 257$^{pc-1}$ | 54 | 52 | 156 | |

glycogenic compounds glycerol, lactate, acetate, and succinate is worthy of note. Unlike the parent strain, the mutant also showed no adaptive lag between growth on glucose and growth on lactose, maltose, acetate, or succinate. The lack of a glucose-lactose diauxie indicated that the expression of the lac operon was resistant to catabolite repression by glucose.

The effects of different sources of carbon on the formation of enzymes normally subject to catabolite repression: Mutant AB 257$^{pc-1}$ differed from the parent strain AB 257 in its insensitivity to catabolite repression by glucose with respect to the formation of a number of enzymes. Table II shows that the formation of PEPCK, isocitratase, acid phosphatase, amylomaltase, and β-galactoside permease was more resistant to the "glucose effect" in the mutant than in the parent strain. The experiments described in Table III were designed to compare the effect of growth on a number of different sources of carbon on the formation of β-galactosidase in strains in which the formation of β-galactosidase was either inducible or constitutive. It can be seen that in every case G-6-P or the mixture of glucose and gluconate were significantly more effective in repressing β-galactosidase formation than was glucose. Furthermore G-6-P and the mixture of glucose and gluconate repressed β-galactosidase synthesis in the mutant resistant to repression by glucose or gluconate. It can also be seen that constitutive and basal level β-galactosidase synthesis were repressed more effectively by G-6-P and the mixture of glucose and gluconate than by glucose. Neither G-6-P nor the mixture of glucose and gluconate inhibited the activity of crystalline β-galactosidase when tested at the concentrations employed in the preceding experiments.

Table IV shows that the formation of tryptophanase in both parent and mutant strains was more sensitive to G-6-P and the mixture of glucose and gluconate than to either glucose or gluconate alone.

Paigen (1966), on the basis of his study of transient catabolite repression, suggested that the compound responsible for catabolite repression was metabolically close to glucose. Prevost and Moses (1967), also studying transient repression,

Table II

The effect of growth on different sources of carbon on the formation of PEPCK, isocitratase, acid phosphatase, amylomaltase and β-galactoside permease

| Carbon source during growth | Experiment No. | PEPCK AB 257 | PEPCK pc-1 | Isocitratase AB 257 | Isocitratase pc-1 | Acid phosphatase AB 257 | Acid phosphatase pc-1 | Amylomaltase AB 257 | Amylomaltase pc-1 | β-galactoside permease AB 257 -TMG | β-galactoside permease AB 257 +TMG | β-galactoside permease pc-1 -TMG | β-galactoside permease pc-1 +TMG |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Glucose ($1.0 \times 10^{-2}$ M) | (1) | 0.8 | 5.6 | 0.10 | 0.26 | 0.4 | 1.8 | 7 | 24 | 4 | 8 | 3 | 92 |
| | (2) | 1.2 | 4.4 | 0.15 | 0.51 | 0.6 | 3.4 | | | | | | |
| | (3) | - | - | 0.19 | 0.42 | - | - | | | | | | |
| Succinate ($1.5 \times 10^{-2}$ M) | (1) | 14.3 | 9.2 | 1.4 | 0.7 | 3.5 | 4.3 | - | - | - | - | - | - |
| | (2) | - | - | - | - | 4.7 | 4.1 | | | | | | |
| Glycerol ($2.0 \times 10^{-2}$ M) | (1) | 10.4 | 9.6 | 1.4 | 0.9 | 2.1 | 2.3 | 104.3 | 144.3 | - | 109 | - | 111 |
| | (2) | - | - | - | - | 2.2 | 2.4 | | | | | | |
| Acetate ($3.0 \times 10^{-2}$ M) | (1) | 15.0 | 13.1 | 10.7 | 15.2 | 4.0 | 4.5 | - | - | - | - | - | - |
| | (2) | - | - | 8.8 | 9.5 | 5.0 | 5.1 | | | | | | |
| | (3) | - | - | 11.3 | 14.4 | - | - | | | | | | |

The cultures were grown on the medium specified for at least 10 generations to a density of approximately $5 \times 10^8$ bacteria per ml. For the induction of amylomaltase $5 \times 10^{-3}$ M of maltose was added and for the induction of β-galactoside permease $5 \times 10^{-4}$ M TMG was added to the growth medium containing the specified source of carbon.

Table III
The effects of growth on various sources of carbon on β-galactosidase formation

| | AB 257 ($i^+o^+z^+$) | | AB 257$^{pc-1}$ ($i^+o^+z^+$) | | AB 257-1 ($i^+o^cz^+$) | | W 112-12-A-84* ($i^-o^+z^+$) | | 2000$o^c$* ($i^+o^cz^+$) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | -TMG | +TMG | -TMG | +TMG | -TMG | +TMG | -TMG | +TMG | -TMG | +TMG |
| Maltose ($0.5 \times 10^{-2}$M) | 24 | 12,500 | 13 | 8,800 | - | - | - | - | - | - |
| Glucose ($1.0 \times 10^{-2}$M) | 8 | 200 | 9 | 9,400 | 1,700 | 7,300 | 4,500 | 5,100 | 970 | 15,250 |
| Gluconate ($1.0 \times 10^{-2}$M) | 8 | 4,400 | 13 | 9,800 | 2,400 | 11,800 | 9,800 | 10,200 | 480 | 9,150 |
| Glucose 6-phosphate ($1.0 \times 10^{-2}$M) | 3 | 21 | 2 | 230 | 190 | 2,600 | 930 | 1,500 | 230 | 2,200 |
| Glucose ($1.0 \times 10^{-2}$M) + Gluconate ($1.0 \times 10^{-2}$M) | 4 | 18 | 5 | 500 | 450 | 4,800 | 2,000 | 2,200 | 330 | 5,200 |
| Glucose ($1.0 \times 10^{-2}$M) + Glucose 6-phosphate ($1.0 \times 10^{-2}$M) | 3 | 5 | 2 | 13 | 150 | 1,600 | 540 | 760 | 200 | 1,670 |
| Succinate ($1.5 \times 10^{-2}$M) | 16 | 14,500 | 14 | 11,600 | - | - | - | - | - | - |
| Glycerol ($2.0 \times 10^{-2}$M) | 22 | 17,000 | 16 | 9,700 | 5,200 | 18,700 | 16,700 | 17,000 | 6,400 | 26,000 |
| Lactate ($2.0 \times 10^{-2}$M) | 19 | 14,500 | 12 | 11,300 | - | - | - | - | - | - |

The cultures were grown on the specified medium for more than 10 generations. TMG when present was employed at a concentration of $5 \times 10^{-4}$M. Values represent specific β-galactosidase activity.

*W 112-12-A-84 requires thiamine, histidine and cysteine, and 2000 $o^c$ thiamine for growth. The $i^-$ character of W 112-12-A-84 is suppressible (Müller-Hill, 1966).

Table IV

The effect of growth on different sources of carbon on the formation of tryptophanase in AB 257 and AB 257$^{pc-1}$

| Carbon source during growth | AB 257 | | AB 257$^{pc-1}$ | |
|---|---|---|---|---|
| | sp. act. | % rel. act. | sp. act. | % rel. act. |
| Glycerol ($2 \times 10^{-2}$ M) | 9,000 | 100 | 2,400 | 100 |
| Glucose ($1.0 \times 10^{-2}$ M) | 215 | 2.4 | 1,100 | 44 |
| G-6-P ($1.0 \times 10^{-2}$ M) | 74 | 0.8 | 13 | 0.6 |
| Gluconate ($1.0 \times 10^{-2}$ M) | 140 | 1.6 | 580 | 24 |
| Glucose ($1.0 \times 10^{-2}$ M) + G-6-P ($1.0 \times 10^{-2}$ M) | 6 | 0.1 | 4 | 0.2 |
| Glucose ($1.0 \times 10^{-2}$ M) + Gluconate ($1.0 \times 10^{-2}$ M) | 14 | 0.2 | 21 | 0.9 |

The cultures were pregrown on glycerol, washed once with sterile medium "56" and resuspended in the medium specified containing $5 \times 10^{-3}$ M L-tryptophan. The cultures were grown for 4 to 4.5 generations to a cell density of approximately $5 \times 10^{8}$ bacteria per ml. The bacteria were washed twice with sodium phosphate buffer 0.05 M, pH 7.8, treated with toluene for 10 minutes and the specific tryptophanase activity determined.

considered G-6-P, 6-phosphogluconate, fructose-1,6-diphosphate and NADPH likely candidates. The finding of Loomis and Magasanik (1966) that glucose still caused catabolite repression in a strain lacking hexose phosphate isomerase activity would appear to exclude fructose-1,6-diphosphate as the compound directly responsible for catabolite repression. Our finding that gluconate was ineffective in mutant AB 257$^{pc-1}$ and less effective than G-6-P in the parent strain makes it unlikely that 6-phosphogluconate or NADPH are closer to the compound responsible for catabolite repression than G-6-P itself. (6-Phosphogluconate added to the medium was not utilized as a source of carbon by the bacteria). The effectiveness of the mixture of glucose and gluconate in causing catabolite repression might be explained on the basis of a sparing effect of the 6-phosphogluconate formed by the phosphorylation of gluconate on the G-6-P derived from glucose.

It should also be pointed out that the intensity of the permanent repression observed in our experiments when G-6-P served as source of carbon was comparable in

degree to the transient repression in Paigen's and Moses and Prevost's experiments. Experiments designed to determine the steady-state concentrations of G-6-P in different strains grown under a variety of conditions are in progress; results will be published later.

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