Repression of $\beta$-Galactosidase Synthesis by Glucose in Phosphotransferase Mutants of Escherichia coli

REPRESSION IN THE ABSENCE OF GLUCOSE PHOSPHORYLATION

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SUMMARY

Glucose and $\alpha$-methyl glucoside repress $\beta$-galactosidase synthesis in wild type Escherichia coli and in mutant strains deficient in Enzyme I or in the heat-stable protein of the phosphoenolpyruvate-phosphotransferase system. Although the mutants do not grow on glucose and accumulate only a small amount of $\alpha$-methyl glucoside, they are more sensitive to repression by these compounds than are their parent strains. This repression is presumably due to the lowering of the intracellular concentration of cyclic 3',5'-AMP by glucose and $\alpha$-methyl glucoside, since it can be prevented by addition of the nucleotide. In contrast, a mutant deficient in glucose Enzyme II activity was resistant to repression by glucose and $\alpha$-methyl glucoside. Evidently, the repression of $\beta$-galactosidase synthesis by these sugars does not require phosphorylation by the P-enolpyruvate-phosphotransferase system. It does, however, require the presence of Enzyme II activity for the sugars.

An Enzyme I mutant and a heat-stable protein mutant which did not grow on lactose would grow on lactose in the presence of cyclic 3',5'-AMP or of isopropylthio-$\beta$-galactoside. Therefore, an intact P-enolpyruvate-phosphotransferase system is not required for lactose utilization by E. coli.

Cyclic adenosine 3',5'-monophosphate stimulates the synthesis of $\beta$-galactosidase and many other inducible enzymes in Escherichia coli (1, 2). Cyclic AMP stimulates $\beta$-galactosidase synthesis by acting, directly or indirectly, through the lac promoter locus, to increase the transcription of lac messenger RNA (3). Glucose represses $\beta$-galactosidase synthesis both by lowering the intracellular concentration of cyclic AMP (4) and by inhibiting the uptake of inducers (5). We have been studying the cyclic AMP-dependent repression of $\beta$-galactosidase synthesis produced by glucose. In the absence of direct measurements of the intracellular concentration of cyclic AMP in our experiments, we have used an indirect assay. We have assumed that any repression which can be overcome by the addition of cyclic AMP is due to the lowering of cyclic AMP levels by the repressor. Since $\alpha$-methyl glucoside produces a cyclic AMP-dependent repression of $\beta$-galactosidase synthesis similar to that produced by glucose, it is clear that this repression does not require extensive metabolism of the repressing sugar, but at most requires its phosphorylation (2).

We have now studied the repression of $\beta$-galactosidase synthesis by glucose, $\alpha$-methyl glucoside, and mannitol in a number of mutant strains of E. coli deficient in various components of the phosphoenolpyruvate-phosphotransferase system, in order to determine whether this repression is in fact dependent upon the phosphorylation of the added sugar, or whether the free sugar itself is an active repressor. We find that the cyclic AMP-dependent repression of $\beta$-galactosidase synthesis by glucose and other sugars does not require phosphorylation of the sugar by the P-enolpyruvate-phosphotransferase system. All that is required for this repression is the presence of phosphotransferase Enzyme II activity with specificity for the added sugar. In contrast, the inhibition of inducer uptake by glucose appears to require phosphorylation of the glucose.

The phosphotransferase system, recently described by Kundig, Ghosh, and Roseman (6), participates in the accumulation and phosphorylation of a number of sugars in bacterial cells (7). This system can be depicted schematically as follows:

Phosphoenolpyruvate + HPr $\xrightarrow{\text{Enzyme I}}$ phospho-HPr + pyruvate

Phospho-HPr + sugar $\xrightarrow{\text{Enzyme II}}$ sugar phosphate + HPr

Enzyme I catalyzes the transfer of phosphate from PEP to a small, histidine-containing protein, HPr. Enzyme II catalyzes the subsequent transfer of phosphate from phospho-HPr to the receptor sugar. There is a family of Enzyme II species, some constitutive and some inducible, and each specific for an individual sugar or group of sugars (8).
All chemicals were obtained commercially. \( ^{14} \)C-\( \alpha \)-Methyl glucoside, 3.2 mCi per mmole, was purchased from Amersham-Searle, and \( ^{14} \)C-TMG, 8.1 mCi per mmole, from New England Nuclear.

The E. coli strains used in these experiments are described in Table I. The phosphotransferase components in strains MO and MO-X19 were assayed by Dr. C. F. Fox (personal communication); assays of these components in the other strains were reported by Fox and Wilson (9). Cells were grown aerobically at 37\(^\circ\) in Medium A, a minimal medium (4) supplemented with thiamine (5 \( \mu \)g per ml). In all experiments with the W strains, this medium was also supplemented with methionine (50 \( \mu \)g per ml). Sucinate (0.5%) was the carbon source. Three kinds of agar plates were used to study the growth and fermentation patterns of these strains. Minimal agar contained Medium A and 2\% agar. MacConkey agar contained the standard MacConkey ingredients, except that the lactose was omitted. Tetrazolium agar contained 25.5 g of Antibiotic Medium 2 (Difco) and 50 mg of 2,3,5-triphenyl-tetrazolium chloride per liter. The tetrazolium was autoclaved before use, so that positive colonies appear white, and negative colonies are red. All types of agar were supplemented with various carbon sources at 1\%.

Cyclic AMP and IPTG were added to a final concentration of \( 10^{-3} \) M, when indicated. At the end of each experiment, cells were streaked on glucose tetrazolium agar (W1805 and W1805 D1) or mannitol MacConkey agar (all other strains); in every case, the cells had the appropriate fermentation characteristics.

Uptake of \( ^{14} \)C-\( \alpha \)-methyl glucoside was measured by a modification of the method of Hagihara, Wilson, and Lin (10). Cells were grown to a density of about 1 mg per ml, wet weight. Chloramphenicol (50 \( \mu \)g per ml) and sodium azide (0.01 \( \text{M} \)) were added, and incubation was continued at 37\(^\circ\) for 30 min. Finally, \( ^{14} \)C-\( \alpha \)-methyl glucoside (4 \( \times \) \( 10^{-4} \) M, 0.1 mCi per mmole) was added, and, after incubation for 10 min at 37\(^\circ\), duplicate 1-ml samples were collected on Millipore filters (0.45 \( \mu \)m pore size), washed with 10 ml of Medium A at room temperature, air dried, and counted in a scintillation fluid containing toluene (750 ml), Triton X-100 (250 ml), and Omnifluor (10 g). Uptake of \( ^{14} \)C-TMG was measured in a similar manner. TMG permease I (the lac permease) was induced by growth of the cells for many generations in the presence of \( 10^{-3} \) M IPTG. Following induction, the cells were centrifuged, washed once, and re suspended at a density of about 1 mg per ml, wet weight, in Medium A containing 50 \( \mu \)g per ml of chloramphenicol. The cultures were divided, and glucose was added to one aliquot to \( 10^{-3} \) M. \( ^{14} \)C-TMG was added (\( 10^{-4} \) M, 0.02 mCi per mmole), and, after incubation for 15 min at 37\(^\circ\), samples were collected and counted as described above. Uptake of both compounds is expressed as nanomoles per mg, wet weight, after correction for the apparent uptake by cells heated at 100\(^\circ\) for 5 min.

\( \beta \)-Galactosidase was induced by incubation with \( 10^{-3} \) M IPTG and assayed in toluene-treated cells according to the method of Pardee, Jacob, and Monod (11). One unit of enzyme activity is the amount which hydrolyzes 1 mmole of o-nitrophenyl-\( \beta \)-D-galactoside per min.

**RESULTS**

Initially, we compared the repression of \( \beta \)-galactosidase synthesis in mutant strains deficient in Enzyme I (strain 1109) or in HPr (1101) and their parent strain (1100). In the experiment shown in Fig. 1, we measured the amount of \( \beta \)-galactosidase made during a 20-min induction period in the presence of increasing amounts of glucose, in the presence and absence of \( 10^{-3} \) M cyclic AMP. We used a high concentration of inducer (\( 10^{-3} \) M IPTG), in order to minimize repression due to interference with inducer uptake. In the absence of glucose, all three strains made approximately the same amount of \( \beta \)-galactosidase (about 10 units per 10\(^{8} \) cells). This finding is in agreement with that of Fox and Wilson (9), who reported that both \( \beta \)-galactosidase and the lactose permease in strain 1103 and could be induced to wild-type levels by methyl-\( \beta \)-thiogalactoside. In the parent strain, \( \beta \)-galactosidase synthesis is repressed by about 80\% at high concentrations of glucose; 50\% repression occurs at about \( 7 \times 10^{-4} \) M. In both mutant strains, the glucose repression of \( \beta \)-galactosidase synthesis is more severe, and the repression is produced by lower concentrations of glucose. In the mutants high concentrations of glucose repress by 90\% and 50\% repression is produced by 2 to 3 \( \times \) \( 10^{-4} \) M glucose. In all three strains, cyclic AMP at \( 10^{-4} \) M restores \( \beta \)-galactosidase synthesis in the presence of 0.01 M glucose to about 50\% of the unpressed level, and at 3 to 5 \( \times \) \( 10^{-4} \) M restores enzyme synthesis to more than 80\% of the unpressed value (not shown). \( \beta \)-Galactosidase synthesis is independent of IPTG concentration above 3 \( \times \) \( 10^{-4} \) M, in the presence or absence of glucose.

**Table I**

Growth and fermentation properties of strains used

<table>
<thead>
<tr>
<th>Strain</th>
<th>Glucose minimal agar growth*</th>
<th>Glucose MacConkey agar fermentation</th>
<th>Mannitol MacConkey agar fermentation</th>
<th>Glucose tetrazolium agar fermentation</th>
<th>( \alpha )-Methyl glucoside uptake</th>
<th>Phosphotransferase system</th>
<th>Source and reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1100</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>13.1</td>
<td>Intact</td>
<td>Reference 9</td>
</tr>
<tr>
<td>1101</td>
<td>±</td>
<td>+</td>
<td></td>
<td>-</td>
<td>1.2</td>
<td>HPr*</td>
<td>Reference 9</td>
</tr>
<tr>
<td>1103</td>
<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
<td>0.7</td>
<td>Enzyme I*</td>
<td>Reference 9</td>
</tr>
<tr>
<td>MO</td>
<td>+</td>
<td>+</td>
<td></td>
<td>-</td>
<td>7.8</td>
<td>Intact</td>
<td>C. F. Fox*</td>
</tr>
<tr>
<td>MO-X19</td>
<td>-</td>
<td></td>
<td></td>
<td>-</td>
<td>0.3</td>
<td>Enzyme I*</td>
<td>C. F. Fox*</td>
</tr>
<tr>
<td>W1895</td>
<td>1</td>
<td>1</td>
<td></td>
<td>1</td>
<td>10.6</td>
<td>Intact</td>
<td>Reference 13</td>
</tr>
<tr>
<td>W1895 D1</td>
<td>+</td>
<td>+</td>
<td></td>
<td>-</td>
<td>0.6</td>
<td>Enzyme I*</td>
<td>Glucose*</td>
</tr>
</tbody>
</table>

* Growth was scored as follows: +, growth at 24 hours; ±, no growth at 24 hours, small colonies at 48 hours; −, no growth at 48 hours.

* Personal communication.
Strain 1100 metabolizes glucose. At the lower glucose concentrations, this strain probably metabolized all of the available glucose within the 20-min induction period. It is difficult to compare the glucose repression of β-galactosidase synthesis in this strain with repression in strains 1101 and 1103, which do not metabolize glucose. Accordingly, we studied the repression produced by the nonmetabolized glucose analogue, α-methyl glucoside. Fig. 2 presents the results of such an experiment.

In strain 1100, high concentrations of α-methyl glucoside repress β-galactosidase synthesis by about 50%. Again, α-methyl glucoside is a much more powerful repressor of β-galactosidase synthesis in the mutant strains than in their parent. In the mutants, α-methyl glucoside represses β-galactosidase synthesis more completely, and is active at concentrations several orders of magnitude lower than is required to repress enzyme synthesis in the parent strain. In all the strains, cyclic AMP at $10^{-3}$ M overcomes this repression significantly, and at $5 \times 10^{-8}$ M completely (not shown).

The repression of β-galactosidase synthesis by α-methyl glucoside in the HPr mutant, strain 1101, was studied in more detail in the kinetic experiment shown in Fig. 3. Here, α-methyl glucoside at $10^{-4}$ M, with and without $10^{-3}$ M cyclic AMP, was added to cells which had been induced for 10 min with $10^{-3}$ M IPTG, and the appearance of β-galactosidase was followed. This concentration of α-methyl glucoside has no effect on β-galactosidase synthesis in the parent strain. In the mutant, however, it produces a typical transient repression, lasting about 20 min. This transient repression is abolished by cyclic AMP.

Because we were surprised that glucose and α-methyl glucoside repressed β-galactosidase synthesis in strains 1101 and 1103, we thought it was important to confirm some of the physiological properties of these strains. Table I presents data on the growth and fermentation characteristics of these strains, as well as on their ability to accumulate α-methyl glucoside. Strain 1100 grows on glucose minimal agar, and gives positive fermentation reactions on both glucose and mannitol MacConkey agar and on glucose tetrazolium agar. Strain 1103 does not grow on glucose and is negative on all of these indicator plates. This is the phenotype expected of a mutant strain devoid of Enzyme I activity. Strain 1101 is slightly leaky for HPr, and has prop...
properties intermediate between the other two strains. It grows slowly on glucose minimal agar (no growth is apparent at 24 hours of incubation, but small colonies appear by 48 hours) and is positive on glucose MacConkey agar, but negative on mannitol MacConkey agar and glucose tetrazolium agar. The inability of the mutants to grow on glucose was confirmed in studies using liquid media. Strain 1100 had a doubling time of 60 min in Medium A supplemented with 0.5% glucose. In contrast, the growth of strain 1103 in this medium was unmeasurable (doubling time greater than 12 hours). The mutants are clearly defective in their ability to metabolize glucose. They are also defective in their ability to accumulate α-methyl glucoside. As shown in Table I, the mutant strains accumulate only 5 to 10% as much α-methyl glucoside as does the parent strain; as expected, strain 1101 accumulates somewhat more α-methyl glucoside than does 1103. The intracellular concentration of α-methyl glucoside in the mutant strains is only slightly greater than the extracellular concentration. Between 50 and 90% of the α-methyl glucoside accumulated by wild-type E. coli is present as α-methyl glucoside 6-phosphate (10, 12). Therefore, a gross defect in α-methyl glucoside uptake, such as we observed in the mutants, must represent a marked decrease in α-methyl glucoside phosphorylation. Cyclic AMP at 10^-3 M has no effect on the growth and fermentation characteristics of these strains, but does cause a small (about 15%) decrease in α-methyl glucoside uptake.

Obviously, glucose and α-methyl glucoside can both produce a cyclic AMP-dependent repression of β-galactosidase synthesis under conditions in which their phosphorylation and metabolism is negligible. Repression of β-galactosidase synthesis in these strains is not restricted to glucose and its analogues. Mannitol, which is not accumulated by these strains, also represses enzyme synthesis in them (Fig. 4). This mannitol repression of β-galactosidase synthesis is also abolished by cyclic AMP (not shown).

Experiments with another Enzyme I mutant, strain MO-X19, and its parent strain, MO, provide further evidence that the repression of β-galactosidase synthesis by glucose does not require glucose phosphorylation. For some unknown reason, β-galactosidase synthesis in strain MO is relatively resistant to glucose repression. High concentrations of glucose repress β-galactosidase synthesis in strain MO by only about 30%. Although the Enzyme I mutant is unable to grow on glucose or to accumulate α-methyl glucoside (Table I), it is more sensitive to glucose repression than is its parent strain (Fig. 5).

Clearly, repression of β-galactosidase synthesis does not depend on an intact P-enolpyruvate-phosphotransferase system. We next wanted to determine whether this repression required Enzyme II activity towards the repressing sugar, or whether it was completely independent of the P-enolpyruvate-phosphotransferase system. To investigate this, we studied the repression of β-galactosidase synthesis in strain W1895 and its “glucose permease-negative mutant,” W1895 D1. This mutant is deficient in Enzyme II activity towards glucose and α-methyl glucoside (Table I). Although it will grow on a high concentration of glucose, it is unable to phosphorylate α-methyl glucoside. Growth of the mutant on glucose is probably due to phosphorylation of the glucose by glucokinase and ATP. Fig. 6 compares the glucose repression of β-galactosidase synthesis in strains W1895 and W1895 D1. Glucose represses β-galactosidase synthesis in the parent strain to about the same extent and at about the same concentrations as it does in strain 1100. In the mutant, however, glucose is a very weak repressor of β-galactosidase synthesis. Glucose at 10^-3 M represses β-galactosidase synthesis by 85% in the parent strain, and by only 25% in the mutant. The glucose repression of β-galactosidase synthesis in strain W1895 is overcome almost completely by 10^-3 M cyclic AMP.

The altered regulation of β-galactosidase synthesis in strain W1895 D1 was demonstrated even more dramatically in an experiment in which α-methyl glucoside was used as the repressor (Fig. 7). α-Methyl glucoside at 10^-2 M represses β-galactosidase synthesis in the parent strain by about 50%. In contrast, this concentration of α-methyl glucoside produces a negligible (about 10%) decrease in β-galactosidase synthesis in strain W1895 D1.

![Fig. 4. Effect of mannitol on β-galactosidase synthesis by strains 1101 (○) and 1103 (■)]. Experimental design and symbols are the same as in Fig. 1, except that mannitol was used in place of glucose.

![Fig. 5. Effect of glucose on β-galactosidase synthesis by strains MO (○) and MO X19]. Experimental design and symbols are the same as in Fig. 1.
Sugars other than glucose can repress β-galactosidase synthesis (14). Glucose 6-phosphate and mannitol both repressed β-galactosidase synthesis in strain W1895 D1 to the same extent as in the parent strain, and this repression was overcome by cyclic AMP. Thus, the resistance of strain W1895 D1 to glucose repression is due to its defective glucose transport system, rather than to a generalized abnormality in its repression mechanism. Apparently, Enzyme II activity toward glucose is required for the repression of enzyme synthesis by glucose.

Because we were interested in the relationship between the phosphotransferase system and lactose metabolism, we investigated the ability of some of these strains to grow on lactose and accumulate 14C-TMG (Table II). Strain 1100 grows on lactose minimal agar, and is positive on lactose MacConkey and lactose tetrazolium agar. Strains 1101 and 1103 do not grow on lactose (9). Both strains are negative on lactose tetrazolium agar; 1103 is also negative on lactose MacConkey agar, whereas 1101 is positive. The failure of strains 1101 and 1103 to utilize lactose was puzzling, since lactose is not thought to be a substrate of the phosphotransferase system in E. coli (15). We found that both strains, 1101 and 1103, would grow on minimal lactose agar, if this agar was supplemented either with cyclic AMP or with IPTG (Table II). These cells did not grow on cyclic AMP alone; rather, the addition of cyclic AMP allowed them to utilize and grow on lactose. In the presence of cyclic AMP, strain 1103 became positive on lactose MacConkey agar, and both became positive on lactose tetrazolium agar.

All of these strains accumulate 14C-TMG (Table II). In some experiments, the mutant strains accumulated TMG to the same extent as did the parent strain (Table II); in others, the parent accumulated somewhat more than the mutants. Glucose significantly inhibits the accumulation of TMG by strain 1100. In contrast, glucose has only a negligible effect on TMG uptake by strains 1101 and 1103 (Table II). Apparently glucose must be phosphorylated (or further metabolized) in order to inhibit TMG uptake. Cyclic AMP has no effect on the inhibition of TMG uptake by glucose.

**DISCUSSION**

Glucose or α-methyl glucoside can repress β-galactosidase synthesis by two mechanisms. First, they lower the intracellular concentration of cyclic AMP (4), and second, they decrease the uptake and intracellular concentration of inducer (5, 6).

G. Aurbach, R. L. Perlman, and I. Pastan, unpublished observations.

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**Table II**

Lactose-metabolizing properties of phosphotransferase mutants

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth* on lactose minimal agar</th>
<th>Fermentation on lactose MacConkey agar</th>
<th>Fermentation on lactose tetrazolium agar</th>
<th>14C-TMG uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>14C-TMG uptake</td>
</tr>
<tr>
<td>1100</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>9.5 (0.01 mM)</td>
</tr>
<tr>
<td>1101</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>8.4 (0.01 mM)</td>
</tr>
<tr>
<td>1103</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>11.3 (0.01 mM)</td>
</tr>
</tbody>
</table>

* Growth was determined after 48 hours of incubation at 37°C.

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This small amount of repression may be due to α-methyl glucoside itself; on the other hand, it could be accounted for by contamination of the α-methyl glucoside with as little as 0.1% glucose. The observation that α-methyl glucoside does not repress β-galactosidase in strain W1895 D1 was first made by Dr. D. Kessler and has been reported by Schaeffer (13).
In short term experiments, these two processes are relatively distinct. However, in longer experiments these processes are related, since these compounds also repress the synthesis of the lac permease. Cyclic AMP abolishes the glucose repression of permease synthesis but has no effect on the inhibition of permease activity (17). We have used a high concentration of inducer in order to avoid repression due to decreased inducer uptake. The repression of β-galactosidase synthesis that we observe can be almost completely abolished by the addition of cyclic AMP, and so this repression is presumably due entirely to the lowering of cyclic AMP levels by the repressor. We have measured β-galactosidase synthesis during the 20-min period following the addition of glucose to organisms growing on succinate. Thus, we have measured primarily what other workers have called “transient repression” (18). However, we think that both are probably due to lowered cyclic AMP levels (17). The differences between transient and catabolite repression probably reflect the fact that glucose or its metabolites or both can lower cyclic AMP levels by several different mechanisms. We have shown that transient repression does not appear to require any glucose metabolism. Any free sugar for which the cells have Enzyme II activity—glucose, α-methyl glucoside, or mannitol—can cause this repression. The permanent form of catabolite repression apparently requires glucose metabolism. Many glucose metabolites, including lactate and acetate, can lower cyclic AMP levels in E. coli (4). In addition, the repression of β-galactosidase synthesis produced by glucose 6-phosphate and by pyruvate can be prevented by cyclic AMP, and so is presumably due to the lowering of cyclic AMP levels by these repressors. The permanent form of catabolite repression may depend on the lowering of cyclic AMP levels by these or other glucose metabolites. Diaminonic growth is probably due both to a decreased cyclic AMP concentration and to inhibition of inducer uptake. Diaminonic growth can be overcome either by cyclic AMP (19) or by increasing the inducer concentration (20).

Since α-methyl glucoside represses β-galactosidase synthesis and lowers cyclic AMP levels, it was clear before this study was initiated that either free α-methyl glucoside or α-methyl glucoside 6-phosphate was the effective agent. In the phosphotransferase mutants, the rate of phosphorylation of glucose and α-methyl glucoside is much less than that of the parent strain, but the mutants are very much more sensitive to repression by the sugars. Therefore, it appears that the free sugars are responsible for the repression. What is required for repression is phosphotransferase Enzyme II activity for the repressing sugar. The increased sensitivity of the Enzyme I mutants to glucose repression may be due to the fact that these mutants have increased Enzyme II activity (21). The function of Enzyme II in this process is not known. The sugar-Enzyme II complex itself may be directly involved in the lowering of cyclic AMP levels, possibly by promoting the release of cyclic AMP from the cell into the medium (4). Alternatively, if Enzyme II catalyzes the facilitated diffusion of sugars across the cell membrane (21), the lowering of cyclic AMP levels may not depend on Enzyme II itself, but on the subsequent interaction of the sugars with some other receptor in the cell or cell membrane. In addition, Enzyme II activity is dependent upon a number of separable, enzymatically inactive fractions (22). Perhaps only one of these components is involved in the repression of enzyme synthesis. Acetate and lactate, which lower cyclic AMP levels in E. coli (4), are not substrates or the phosphotransferase system. The mechanisms by which these compounds and other glucose metabolites lower cyclic AMP levels are not known.

The finding that strains 1101 and 1103 would not grow on lactose was unexpected, since the phosphotransferase system is not thought to be involved in lactose transport in E. coli (15), although it is involved in lactose transport in Staphylococcus aureus (23). Our finding that these strains would grow on lactose in the presence of cyclic AMP or IPTG provides further evidence that an intact phosphotransferase system is not required for lactose utilization by E. coli. It is not clear how IPTG or cyclic AMP allows the cells to grow on lactose. Lactose is a poor inducer of the lac operon. In order to induce synthesis of the lac enzymes, lactose must first be transported into the cell by the lac permease and must then be converted into an active inducer by β-galactosidase (24). In addition, glucose forms from the hydrolysis of lactose by β-galactosidase can repress lac enzyme synthesis. Because of the nature of lactose induction, glucagon repression of lac enzyme synthesis will have a cumulative effect on induction of the lac operon by lactose. Since strains 1101 and 1103 are very sensitive to glucose repression, it is possible that they are never able to accumulate enough active inducer from lactose to induce their lac enzymes efficiently. In contrast to lactose, IPTG can enter the cell by some pathway other than the lac permease, and it is itself a potent inducer, having a high affinity for the lac repressor (25). Thus, IPTG induction of the lac operon circumvents the cumulative effect of glucose repression. Cyclic AMP is presumably effective because it relieves the glucose repression of lac operon expression.

Since glucose does not inhibit TMG uptake in strains 1101 and 1103, we conclude that phosphorylation (and perhaps subsequent metabolism) is required to produce inhibition of TMG uptake. Our results are consistent with the recent findings of Kaback (20) that glucose 6-phosphate and glucose 1-phosphate are involved in the regulation of sugar transport in membrane preparations from E. coli. Our results are not consistent with the hypothesis that glucose interferes with TMG uptake by a “common carrier” mechanism (5).

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