

The Effect of Cyclic 3', 5'-Adenosine Monophosphate on the Effect of Glucose, Fructose, and Sucrose Supplements on the Induction of *lacZ* in *Escherichia coli* B23 Grown in Minimal Medium with Glycerol

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Catabolite repression refers to the inhibition of genes whose products are involved in sugar metabolism within cells growing on a preferential carbon source. The transient repression of the *lac* operon observed when glucose is added to *Escherichia coli* growing on glycerol is associated with a decrease in the intracellular concentration of cyclic adenosine 3', 5'-monophosphate (cAMP). Previous studies have shown that sucrose and fructose affect the expression of β -galactosidase activity in induced cells. The basis for these observations was not established. In this study, *E. coli* B23 was shifted to growth on glucose, sucrose, and fructose in order to determine whether these effects were minimized by exogenous cAMP levels. Cells cultured on glycerol media were induced to express the *lac* operon for fifteen minutes and then supplemented with an alternative catabolite and cAMP. The results showed similar reduction in measured *lac* expression in cultures supplemented with glucose, fructose, and sucrose. Exogenous cAMP abolished the glucose- and fructose-mediated repression, but not sucrose. This supports that fructose catabolism is regulated by a mechanism similar to glucose mediated catabolite repression, but sucrose must be acting by some other mechanism.

Although expression of *Escherichia coli*'s *lac* operon involves a complex regulatory system that is still not completely elucidated, it serves as a model for prokaryote gene regulation. The inhibitory effect of glucose on the expression of the *lac* operon has been extensively studied, specifically in the synthesis of LacZ (β -galactosidase), an enzyme required for lactose catabolism. When grown in glucose-containing media, *E. coli* synthesizes less β -galactosidase, regardless of inducer presence, allowing for preferential metabolism of glucose. The current understanding of the glucose effect, also referred to as catabolite repression, involves the enzymes of the phosphoenolpyruvate:carbohydrate phosphotransferase system (PTS) (13). Activation of adenylate cyclase, the biosynthetic enzyme that produces cyclic 3', 5'-adenosine monophosphate (cAMP), is linked to the PTS system (17, 18). The role of cAMP in glucose-mediated repression is evidenced by a decrease in the intracellular cAMP concentration of glucose utilizing cells (7). The addition of cAMP to the growth medium is shown to antagonize the glucose effect with varying levels of relief, depending on the growth conditions (20, 15). Although it is not the sole mediator, the cAMP:cAMP-receptor protein (cAMP:CRP) complex acts as an activator in the transcription of the *lac* operon (11, 3).

A more severe transient repression occurs when glucose is added to cells growing in conditions favouring maximum β -galactosidase synthesis, such as glycerol media. Synthesis is abolished almost completely for up to one generation, then resumes at a

slower rate (12). Although the mechanism behind transient repression is not well studied, certain observations have been made. Resembling glucose mediated permanent catabolite repression, transient repression is linked to a decrease in intracellular cAMP (15, 21) and was proposed to be controlled by a similar mechanism (15). However, unlike the glucose effect, it is not due to a decrease in cAMP synthesis (6). Also, the addition of cAMP in sufficient quantity abolishes transient repression (15, 5).

Since most past research on the regulation of the *lac* operon has focused on the effect of glucose, the aim of our study was to investigate whether the effects of sucrose and fructose were caused by cAMP-dependent mechanisms similar to glucose. We confirmed previous findings that fructose and sucrose decrease the level of β -galactosidase synthesis (1, 2), albeit to different degrees. Exogenous cAMP was added simultaneously with the supplementation of the different carbon sources to the isopropyl- β -D-thiogalactopyranoside (IPTG) induced cultures. We discovered that the fructose effect, like the glucose effect, is dependent on cAMP as the repression of β -galactosidase synthesis was abolished. The effect of sucrose on the catabolic enzyme synthesis did not change with exogenous cAMP, suggesting a cAMP-independent system of repression.

MATERIALS AND METHODS

Materials. Isopropyl- β -D-thiogalactopyranoside (IPTG; Fermentas Life Sciences, Burlington, ON, Cat # R0392). o-Nitrophenyl- β -D-galactopyranoside (ONPG; Sigma Chemical Company, St. Louis, MO, Cat # N1627). Cyclic adenosine-3', 5'-monophosphate (cAMP; United States Biochemical Corporation, Cleveland, OH, Cat # 19085). Glycerol (Sigma Chemical Company, St. Louis, MO, Cat # G5516). Glucose (Sigma Chemical Company, St. Louis, MO, Cat # G5250). Sucrose (Sigma Chemical Company, St. Louis, MO, Cat # S9378). Fructose (Sigma Chemical Company, St. Louis, MO, Cat # F0127).

Bacterial Strains and culturing. *E. coli* B23 (MICB 421 teaching lab frozen stock, University of British Columbia) were grown overnight (12 hours) in M9 minimal media (16) supplemented with 0.2% w/v glycerol at 37°C under mild aeration (150 rpm) in a shaking water bath. A sufficient aliquot of the overnight culture was added to each of four flasks containing 102 ml M9 minimal media supplemented with glycerol (0.2% w/v) to obtain a final turbidity of 0.1 OD₄₆₀ units. The four cultures were then incubated for an additional 4 hours at 37°C under mild aeration (150 rpm) in a shaking water bath. Sampling and timing were started when cultures were simultaneously induced to express *lacZ* by addition of IPTG (final concentration 280 μ g/ml). Cultures were maintained at 37°C and aerated at 200 rpm during sampling. At 15 minutes post-induction, one of three catabolites (glucose, sucrose, or fructose) was added to different respective cultures at a final concentration of 0.2%. In the cAMP treatment, culturing techniques were performed as described above, except cAMP (final concentration 5 mM) was also added to each culture along with the catabolite.

Sampling and growth assay. A 3 ml aliquot was withdrawn from each culture flask immediately after induction. Cultures were then sampled simultaneously at 5 minute intervals from induction to 80 minutes post-induction. All culture aliquots were immediately placed on ice after sampling. Turbidity of each undiluted sample was measured at 460 nm with a Spectronic 20 spectrophotometer to assess growth over the time course. Cells were then permeabilized by a 400 μ l addition of toluene, followed by 15 seconds of vortexing. A 1 ml fraction of the aqueous (protein-containing) phase was collected and stored at 4°C.

Enzyme assay. A 400 μ l aliquot of the aqueous fraction of each sample was added to 1.2 ml of Tris and 0.2 ml of ONPG, pre-warmed to 30°C. Samples were maintained at 30°C in a water bath and monitored for development of yellow color, at which time 2 ml of 0.6 M Na₂CO₃ was added. The elapsed time from sample addition to Na₂CO₃ addition was recorded. Absorbance at 420 nm was measured using a Spectronic 20 spectrophotometer for each sample and converted to enzyme activity as previously described (1).

RESULTS

Growth was determined by measuring turbidity of cultures over time. All cultures showed similar patterns over the 80 minutes; the growth rates of cells varied little between different sugar supplements. However, it should be noted that growth on glucose, fructose, and sucrose, unlike glycerol (Fig.1), did not reach any peak within the 80 minutes. This suggests that the glycerol was limited in cultures without supplement. The addition of external cAMP showed an increase in the overall growth in all cultures. An example of *E. coli* growth is demonstrated in with glycerol (Fig.1).

The specific activity of β -galactosidase produced in each culture was quantified over time (Fig. 2). All sugars, when compared to the glycerol control, showed a reduction in β -galactosidase synthesis. Of the cAMP

treated cultures, the glucose- and fructose-supplemented cultures showed an increase in β -galactosidase levels; β -galactosidase levels did not increase in the sucrose-supplemented culture even though the cAMP-treated glycerol control also showed an increase in β -galactosidase production.

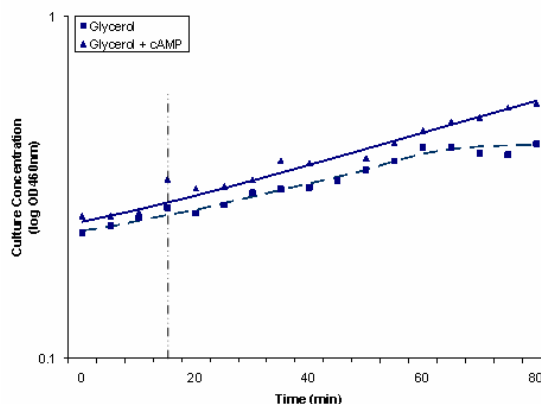


Fig. 1. Effect of exogenous cAMP on growth of *E. coli* in M9-minimal medium supplemented with glycerol. 5 mM cAMP was added at 15 minutes (indicated by the dashed line) to the cAMP-treated flask.

DISCUSSION

No difference in growth rates was observed (Fig. 1) or expected (1) between cells growing on the different catabolites. Additionally, growth of cells on the glycerol-supplemented media appeared to stop at a culture density corresponding to 0.42 OD₄₆₀ units (at around 70 minutes post-induction). The apparent cessation of growth suggests that *E. coli* B23 cells do not exceed a prescribed cell density when glycerol is available as the sole carbon source or that the cells consumed all of the glycerol. This growth limitation has been previously reported and may warrant further investigation (1). The cultures supplemented with the other 3 catabolites did not reach maximum turbidity indicating that the alternative catabolites support a higher density of cells. This higher density may be the result of the additional nutrients extending growth beyond the glycerol limit seen in the cultures growing only on glycerol.

Cells grown in glycerol media to which cAMP was added exhibited no limitation of growth. This implies that strictly attributing the upper cell density limit to nutrient limitation may be oversimplified. Our observations provide strong support for an interactive relationship between nutrient availability and extracellular cAMP concentration for cells grown on glycerol, whereby increasing concentrations of cAMP have an antagonistic effect on the slowed growth caused by nutrient limitations, such as growth on

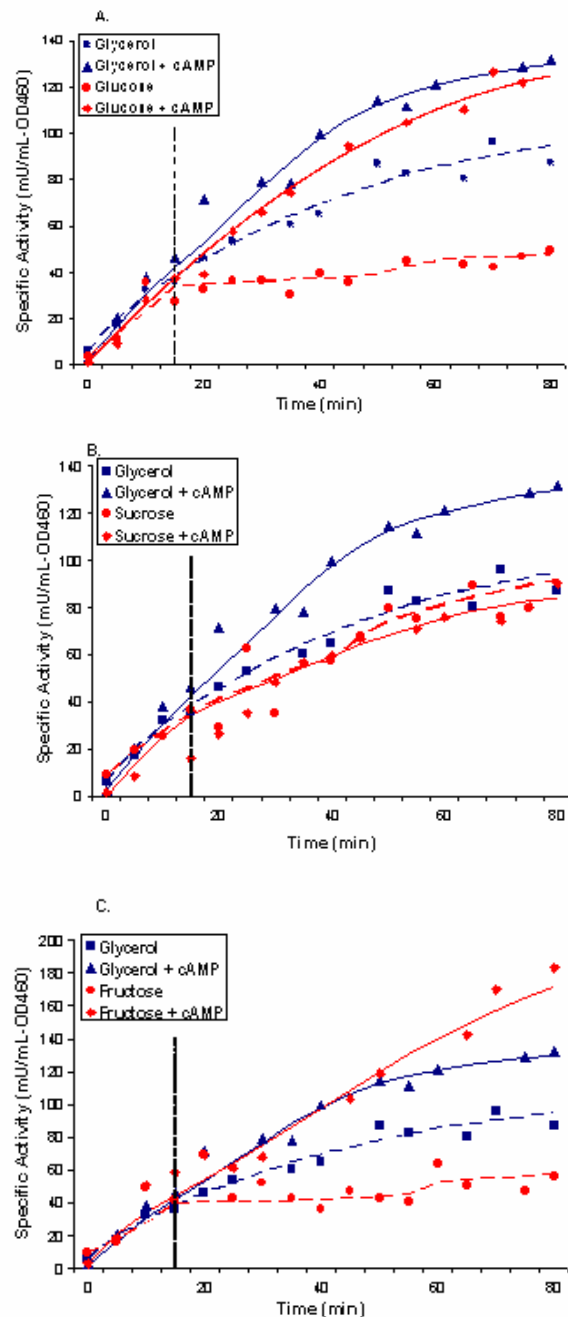


Fig. 2. Effect of exogenous cAMP on various sugar effects with respect to β -galactosidase synthesis. All cultures contained 0.2% w/v of their respective sugar. 5 mM cAMP was added at 15 minutes (indicated by the dashed line) to the appropriate flasks. Glycerol is used as a control. A) Glucose; B) Sucrose; C) Fructose.

minimal media. The growth curves (Fig. 1) indicate that the addition of cAMP 15 minutes after induction does not cause any major change on the growth of the cells growing on glycerol. Previous studies suggest that the addition of exogenous cAMP has been shown to decrease growth rate of cells grown in glucose media,

and that this growth inhibition is less pronounced in cells grown on glycerol (9). These reports disagree with our results (Fig. 1). However, because such findings are sparsely replicated in the literature, the results presented here should not be discarded. In our study, growth rates of cells grown on glucose, fructose, or sucrose in the cAMP treatment did not largely differ from those in cultures to which cAMP was not added.

Based on previous research, we expected to observe transient catabolite repression upon the addition of alternative catabolites to cultures growing on glycerol M9 media (1, 12). In the condition without cAMP addition, transient catabolite repression was observed for all sugars, but at varying degrees (Fig. 2). Glucose and fructose showed a distinct pattern of transient repression (Fig. 2a, Fig 2c), whereas sucrose showed the pattern with subtlety (Fig 2b). Cells grown on glycerol did not exhibit repression of *lacZ* expression (Fig 2). Similar results have been extensively reported in the literature (19). Even though glycerol uptake is mediated by a PTS-independent pathway, which allows intracellular cAMP levels to remain at a high basal level, the addition of cAMP enhanced the synthesis of β -galactosidase as if the levels were still slightly limited. Since sucrose is a putative PTS sugar, we expected cells grown on sucrose to also exhibit transient repression (20, 8). However, in the present study, only a subtle transient repression was observed (Fig 2). The absence of distinct transient catabolite repression in cells grown on sucrose may be indicative of a PTS-independent uptake mechanism for sucrose. Although such a mechanism for sucrose uptake has not yet been reported, previous studies have supported the existence of a PTS-independent system for uptake of the PTS sugar, fructose (19). Thus, a mechanistically analogous system for sucrose uptake, which has yet to be characterized, may account for our results. It has also been shown that sucrose enters cells at a slower rate than glucose due to a limited number of transport enzymes (2). Our methods may have precluded detection of sucrose-mediated transient catabolite repression due to the excessive latency between addition of sucrose to the medium and observable catabolite repression.

Cells growing on preferred carbon sources, such as the PTS sugars glucose, fructose, and sucrose, exhibit decreased intracellular cAMP. This low cAMP availability limits the level of the CRP-cAMP complexes involved in transcriptional activation of the *lac* operon (4). In agreement with previous findings (14, 20), addition of exogenous cAMP to cells growing on glycerol, glucose and fructose enhanced measured β -galactosidase synthesis. The corresponding increase in β -galactosidase was gradual and became noticeable 10 minutes after the addition of cAMP.

Although catabolite repression is not known to occur in cells growing on glycerol, we observed an increase in β -galactosidase synthesis after the addition of cAMP (Fig. 2). This result indicates that even for cells growing on glycerol, a basal level of repression may be occurring. Perlman *et al.* report a similar finding (15).

In the cultures to which cAMP was added, cells growing on sucrose did not exhibit increased production of β -galactosidase compared to similar cultures to which cAMP was not added (Fig 2B). We hypothesize that the reason why a similar increase in β -galactosidase activity was not observed is because β -galactosidase was not limited by the available cAMP level. This is shown by the high level of β -galactosidase present in cells growing on sucrose in the no-cAMP treatment, but this level is lower than the β -galactosidase level in the culture growing on glycerol. This suggests that induction in glycerol was enhanced. The lack of an effect on the cells growing in sucrose indicates that sucrose limits expression in some way that cAMP is no longer limiting.

β -galactosidase levels increased the most in cells growing on fructose after the addition of cAMP. This increase was higher than the increase seen for the cells growing in glucose, which in turn was higher than the increase seen for the cells growing on glycerol (Fig 2). This suggests that cells growing on sugars such as fructose have lower levels of intracellular cAMP which result in greater repression of the *lac* operon than in cells growing on sugars such as glucose and glycerol. Because our results indicated a cAMP-independent mechanism of the sucrose effect, we cannot elucidate the position of sucrose in relation to this hierarchy.

FUTURE EXPERIMENTS

The findings presented here not only elaborate on previous works (1, 10), but also primes new research directions by generating new testable hypotheses. The most significant hypothesis to come about from our research was the existence of a PTS-independent sucrose uptake system that precludes cAMP-mediated catabolite repression of the *lac* operon. A simple genetic test to evaluate this hypothesis would involve attempting to grow *E. coli* mutants with defective *cya* or *crp* genes. If sucrose is independent of cAMP or CRP, such strains will grow on sucrose, but not on glucose or fructose.

To determine whether or not a particular carbon source induces cAMP-mediated catabolite repression, a similar experiment, with a modified design that uses the *cya* or *crp* mutants described above, could be performed. By preventing the synthesis of cAMP and CRP, respectively, observed changes in β -galactosidase synthesis rates would indicate the existence of cAMP-

independent catabolite repression caused by the particular carbon source being examined.

Previously, we attributed the absence of catabolite repression in cells grown on sucrose to an absence of intracellular sucrose due to limitations in uptake of the sugar. This delayed uptake was observed as latency between addition of sucrose to the culture and a measurable increase in intracellular sucrose concentrations. Thus, a modified version of the procedure presented here, one that extends sampling time to include uptake and metabolism of sucrose, may be warranted. An evaluation of whether or not sucrose induces catabolite repression based on the data would be permissible since sucrose uptake is assumed to have occurred.

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