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The Effect of Fructose, Galactose, and Glucose on the Induction of β-Galactosidase in *Escherichia coli*

CHARLENE CHU, CHRISTINA HAN, HIROMI SHIMIZU, AND BONNIE WONG

Department of Microbiology and Immunology, UBC

Carbon catabolite repression (CCR) refers to the ability of bacteria to preferentially utilize one carbon source over another. Phosphoenolpyruvate-dependent carbohydrate: phosphotransferase system (PTS) is the mechanism by which CCR is exerted. In the absence of preferred PTS-carbon sources, cAMP-CAP complexes are abundant and thus, the enzymes encoded in the *lac* operon can be expressed maximally. In our experiment, IPTG was used to induce expression of the *lac* operon in *E. coli* cells grown in 0.2% glycerol and then transferred to 0.1% of fructose, galactose, and glucose. β-galactosidase levels of each culture were measured by absorption spectroscopy of nitrophenol produced from the ONPG substrate. Results showed that the addition of galactose exhibited a decrease in β-galactosidase induction. However, both glucose and fructose displayed lower, but similar enzyme induction patterns to galactose. By the end of the experiment, enzyme induction in all three conditions (glucose, fructose, and galactose) was approximately 33% that of the control (glycerol). Initially, according to our "composition theory," we hypothesized that induction levels of galactose would follow that of lactose and induction levels with fructose would follow that of sucrose. However according to our results, galactose may exhibit feedback inhibition when there are higher levels, while there seems to be no preferential usage between glucose and fructose; thus, monosaccharides derived from dissacchrides are not necessarily utilized by bacteria in the same way.

The ability of bacteria to preferentially utilize one carbon source over another is termed carbon catabolite repression (CCR). It is also known as the "glucose effect", but can be exhibited by other carbon sources as well. Carbon sources are grouped into two classes: Class A and Class B. When grown in a mixture of carbon sources, bacteria preferentially utilize Class A substrates over Class B substrates. The system by which Class A substrates exert repression is called the phosphoenolpyruvate (PEP)-dependent carbohydrate:phosphotransferase system (PTS) (4).

The PTS transports and phosphorylates a large number of carbohydrates. PTS carbohydrates, especially glucose, result in a decrease in the phosphorylated form of Enzyme II, $EIIA^{Glc}$~P. $EIIA^{Glc}$~P activates adenylate cyclase which makes cAMP from ATP. A decrease in $EIIA^{Glc}$~P results in a decrease cAMP, which represses CAP-dependent operons – genes whose transcription is regulated by the cAMP-CAP complex (7). One example of a CAP-dependent operon is the *lac* operon in *Escherichia coli*.

When E. coli are grown in rich media, the cAMP concentration is low and there are few cAMP-CAP complexes to activate the *lac* operon. Thus, even in the presence of an inducer, such as lactose or its analog, enzyme induction remains low. However, when preferred carbon sources are absent, cAMP-CAP complexes are abundant and the capacity to synthesize *lac* operon encoded enzymes is maximal upon addition of an inducer.

Experiment A4 (6) utilizes glycerol, glucose and the two disaccharides lactose and sucrose to study β-galactosidase induction levels in *E. coli*. It showed strong reduction of activity due to lactose treatment but slower, more limited reduction of activity due to sucrose treatment. Lactose is composed of glucose and galactose, whereas sucrose is composed of glucose and fructose. Therefore we initially hypothesized that induction levels of galactose would follow that of lactose and induction levels of fructose would follow that of sucrose. However, upon further research we found that our "composition theory" does not make sense when the PTS system is taken into account. This is because the disaccharides are not split into the components until after transport so composition would have no bearing on the levels of induction. Even so, we went ahead with our initial experiment and investigated CCR by growing *E. coli* in glucose, fructose, galactose and glycerol. β-galactosidase activity was measured as an indication of cAMP levels and CCR relationship with respect to the different carbon sources.

MATERIALS AND METHODS

All materials and methods used in this experiment can be found listed in the "Materials and Equipment" section of Experiment A4 (6). *E. coli* K-12 wild-type strain B23 in M9 media containing 0.2% glycerol was incubated overnight in a 37°C shaking waterbath. The overnight culture's OD_{460} was then adjusted in the morning so that the OD_{460} reading at the start of the experiment was 0.15.

The protocol for this experiment followed that of Experiment A4 (6) with a few modifications. This experiment was carried out utilizing the following carbon sources: 10% glycerol, glucose, galactose and fructose. In general, the protocol involved growing *E. coli* in M9-0.2% glycerol (the control) and adding the lactose analogue isopropyl thiogalactoside (IPTG) to induce β-galactosidase formation. The various sugars listed above were then added to investigate their affect on bacterial growth and β-galactosidase activity. Bacterial growth was then monitored by taking *E. coli* samples at various time points and measuring the turbidity with a Spectrophotometer (OD_{460}). O-nitrophenyl galactoside (ONPG) was then added to those samples, and the amount of nitrophenol was then analyzed at A_{420} to measure β-galactosidase activity.

RESULTS

Growth of *E. coli* diverged shortly after transferring the culture into the three different conditions (glucose, fructose, and galactose), as indicated in Figure 1. Growth in each condition extensively overlaps with one another such that it is difficult to tell clearly which condition exhibited the highest or the lowest rate. Trends were not straight and there were several points that went off the line. Glucose exhibited a biphasic growth: growth slowed from around 42 minutes (~0.045 OD) to 48 minutes (~0.043 OD) after induction, and resumed at a rate similar to the first phase. However, no sample was taken in the 12 to 25 minutes after induction. Thus it is difficult to tell when the first phase began, although growth seemed to increase abruptly from 25 minutes to 30 minutes after induction. Glycerol exhibited a relatively lower average growth rate as compared to the other three conditions. Samples gave more erratic results; the measurement at 43 minutes after induction seemed to be an experimental error. Growth remained lower until it increased from around 0.042 OD at 48 minutes to 0.060 OD at 55 minutes, an almost 50% increase. Growth then seemed to stop near the end of the experiment around 68 minutes. The trends of fructose and galactose were similar; the trends were straighter and more reproducible. Both exhibited a steady increase in growth.

Figure 1: Effect of various sugars on *E. coli* B23 growth in M9 Media

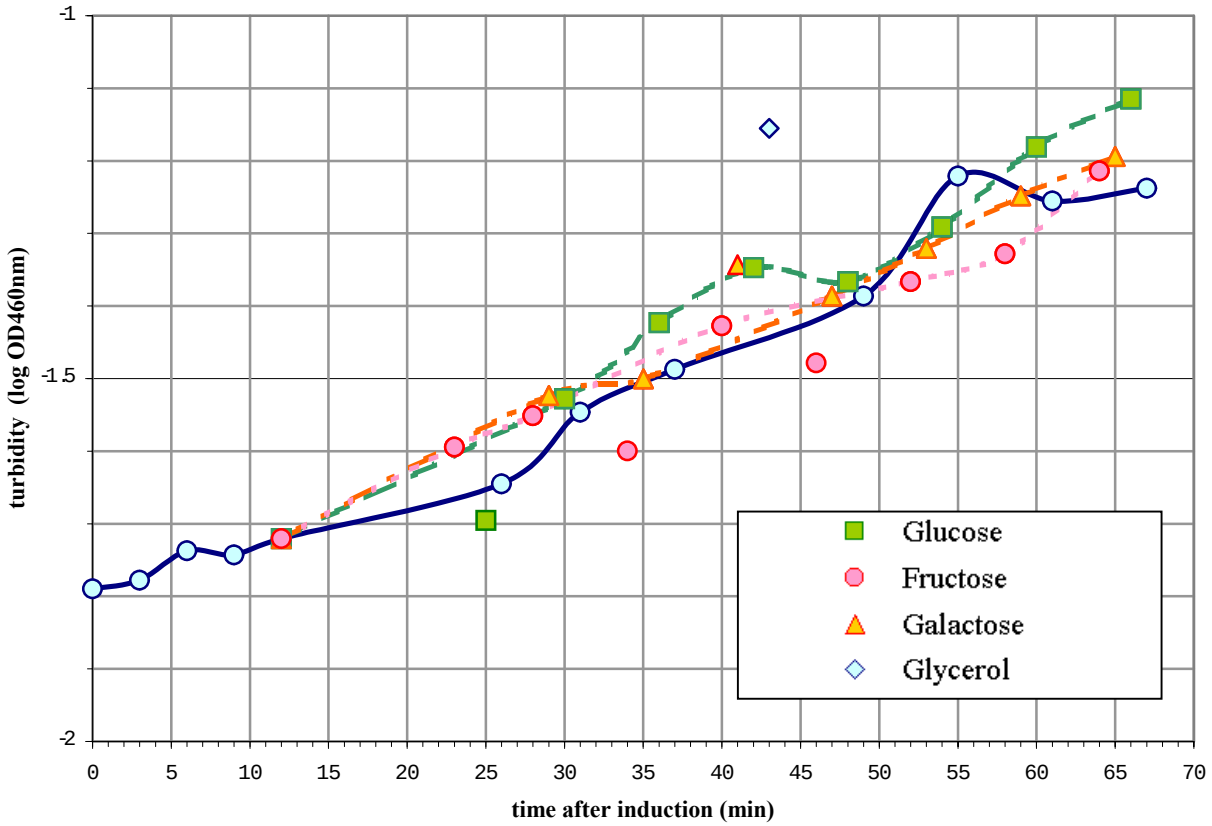

Figure 2: Effect of various sugars on enzyme activity of B-galactosidase from *E. coli* B23 growing in M9 Media

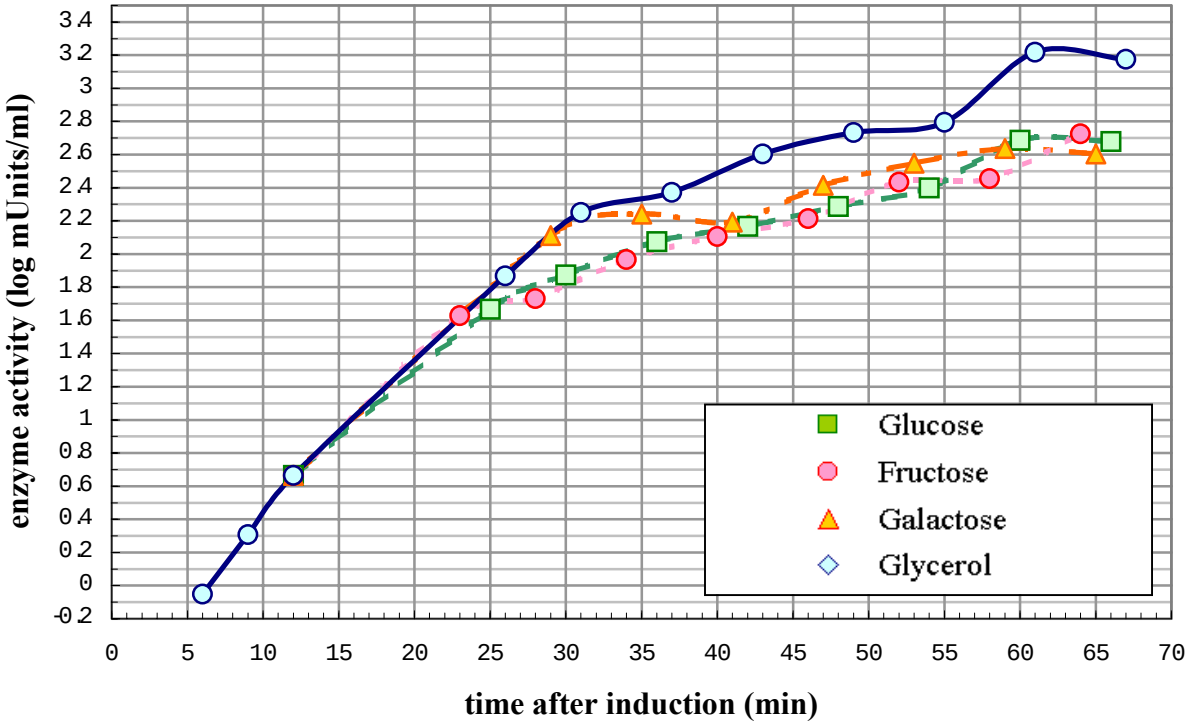

Figure 2 shows the enzyme activity of β-galactosidase in *E. coli* grown in the different conditions. Glycerol, the control, showed the highest enzyme activity level as expected. The trend was straight up till around 30 minutes when it shifted to another lower level. The addition of galactose caused a decrease in enzyme induction and induction remained low throughout the course of the experiment; enzyme induction tripled at the end of the experiment since the first time-point. However, decrease in induction occurred around 28-29 minutes (at least 10 minutes after the start of treatment). Samples had not been taken during the first 10 minutes, so the time and strength of the decrease at the start cannot be discerned. Glucose and fructose exhibited similar enzyme induction patterns, both causing a reduction in β-galactosidase activity that remained low throughout the course of the experiment. Enzyme induction increased 10-fold by the end of the experiment. In all three conditions (glucose, fructose, and galactose), enzyme induction by the end of the experiment was approximately 33% that of the control.

DISCUSSION

As shown in Figure 1, the culture grown in glycerol alone stopped growing after 55 minutes and resulted in the lowest turbidity reading at the end of the experiment. This indicates that glycerol alone is not sufficient to support the growth of *E. coli* after a certain point (0.06 OD at 55 minutes). It also implies that other nutrients may be needed to support the continuation of cell growth after the cell number of a culture has reached to a particular level. Under three other conditions (glucose, galactose and fructose), cells kept growing throughout the course of the experiment indicating that the three sugars can be utilized as sole carbon sources by *E. coli* and are sufficient to support its growth. Among them, glucose is the most preferential sugar to be utilized by *E. coli* as the average reading of turbidity of the particular culture is the highest. Since glucose and fructose exhibit a similar trend that is found in between that of galactose and glycerol, they may be assumed as intermediately preferential sugars utilized by *E. coli* in the experiment.

Figure 2 shows that glycerol did not repress nor slightly repress β-galactosidase induction. Glycerol is transported into *E. coli* through facilitated diffusion. Since it does not use the PTS, Enzyme II would be primarily phosphorylated and cAMP levels would increase. Upon addition of IPTG, a lactose analog, transcription of the *lac*

operon occurred utilizing available cAMP-CAP complexes, resulting in high levels of the enzyme (8). On the other hand, the addition of either of the three other sugars (glucose, galactose or fructose) all caused a decrease in the enzyme induction. Among glucose, galactose, and fructose, galactose exhibited the least decrease in β-galactosidase induction until around 57 minutes. A slightly higher repression in β-galactosidase induction by galactose after 57 minutes indicates that galactose represses β-galactosidase activity in a non-steady pattern. An explanation to this observation could be due to galactose's ability to induce β-galactosidase and also its ability to inhibit hydrolysis of lactose by β-galactosidase. When the concentration of galactose inside *E. coli* is low, β-galactosidase will be induced. However, when there is too much galactose around and being transported (by the CAP-dependent Gal P permease instead of PTS (2)) into the cell, the cell does not need to produce β-galactosidase to hydrolyze so much lactose—feedback inhibition (5).

Fructose and glucose exhibit a very similar trend in repressing β-galactosidase activity. According to our proposed "composition theory", fructose should exhibit a lesser ability to repress β-galactosidase activity than glucose. However, in Figure 2, their trends are too similar to be distinguished from each other. As a result, this Figure indicates that β-galactosidase activity does not follow the "composition theory" but does not necessarily contradict it either. The similarity of the fructose and glucose induction patterns might be due to the fact that fructose and glucose both utilize that PTS, leading to low levels of cAMP and low β-galactosidase induction (3). Further, it has also been shown that fructose and glucose are equally capable of exerting CCR over sucrose in *Saccharomyces cerevisiae*, both suppress sucrose metabolic enzyme expression at 5g/L (1). Thus, there seems to be no hierarchy of preferred usage between fructose and glucose.

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APPENDIX

Sample calculation for Enzyme Activity (glycerol time point: 60)

$$\frac{\text{Enzyme Activity}}{\text{ml of enzyme}} = A \times \frac{1}{t} \times \frac{10^6}{15{,}000} \times Nv \times \frac{1}{Ev}$$

$$= 0.090 \times \frac{1}{60} \times \frac{10^6}{15{,}000} \times 3.8 \times \frac{1}{0.4}$$

$$= 0.95\text{mUnits}$$