Effect of different substrates on the transcription level of the Lac operon in
*Escherichia coli*.

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Introduction

This experiment is designed to address the question of how much transcription occurs at the lac operon by observing the enzymatic activity when different substrates are added. Gene regulation is important on a cellular level as certain genes can be shut off when they are not being expressed to conserve energy (Freeman 2014). Regulation of transcription is essential for a cell to control its metabolic pathway. The lac operon has been studied extensively due to its ability to act like a switch to control and regulate cell transcription. Lac operons are comprised of DNA in multiple genes that encode for specific proteins. In the bacterium used in the lab *Escherichia coli*, a lac operon codes for three different genes that facilitate the breakdown of lactose to provide energy for the bacteria (Emerson & Li 2010). Jacob and Monod are recognized for their work on gene regulation and discovering the relationship between enzyme expression levels and cellular transcription (Jacob & Monod 1961). This research marked the beginning of understanding gene control and regulation (Beckwith 1967). They researched the *E. coli* lac operon and identified that the genes that are encoded in the lac operon are coordinated together.

The first gene of the lac operon for *E. coli*, lacZ, codes for β-galactosidase; β-galactosidase is an intracellular enzyme capable of breaking bonds. *LacY* codes for lactose permease; permease is responsible for transporting lactose in and out of the cell membrane into the cytoplasm (Mieschendahl, et al., 1981). *LacA* codes for galactoside O-acetyltransferase, an enzyme that can transfer acetyl groups to β-galactosides. This gene does not influence the role of the lac operon; only lacZ and lacY are necessary in the breakdown of lactose (Freeman 2014). For the lac operon to be activated two conditions
must be satisfied; lactose, an activator, must be present, and cyclic adenosine
monophosphate (cAMP) must be present (Santillan & Mackey 2004). The presence of
different enzymes will affect the transcription rates of the lac operon. The lac operon for
*E. coli* has both a negative and positive control function. The primary regulator for the lac
operon is a negative control, called the lac repressor coded for by the *LacI* gene. The
transcription of the genes is dependent on the presence of lactose. When lactose binds to
the repressor protein, it induces a conformational change in the repressor causing it to fall
off and DNA to be released. This then permits the RNA polymerase to travel to the
promoter and initiate transcription. If lactose is absent the repressor protein remains in the
conformation that prevents the polymerase from binding to the promoter and
transcription of *lacZ*, and *lacY* is prevented. The lac operon has a positive control
function with the catabolite activator protein (CAP) and cAMP, which regulates what
glucose can bind to. When lactose and glucose are absent, the CAP will attempt to bind to
the promoter to induce transcription at low levels. Both the negative control and positive
control help the cell conserve energy when lactose or glucose is detected in sufficient
quantity in the cell.

This lac operon can be thought of as a switch as it has repressors and operators
that can be turned on or off when a more favorable energy source is present. To
determine how transcriptionally active the lac operon is an assay has been created to
indirectly measure the amount of transcription occurring throughout the reaction. One of
the proteins coded for in the lac operon is β-galactosidase. β-galactosidase has three
enzymatic activities, the first being its ability to cleave the lactose bonds to form the
products of glucose and galactose. The second activity of β-galactosidase can catalyze the
hydrolysis of lactose to allolactose. Thirdly, β-galactosidase can cleave the bonds within allolactose (Juers et al., 2012). Orthonitrophenylgalactoside (ONPG) is commonly used as an analog of lactose. ONPG is used in this experiment because, when cleaved by β-galactosidase, the product is yellow in color. This yellow color serves as a measure of how transcriptionally active the lac operon is by measuring the enzymatic activity of β-galactosidase. The transcriptional activity can be quantified through changes in absorbance measured on the spectrophotometer. To stop the reactions in the tubes, Na₂CO₃ is added to alter the pH, thus, inactivating β-galactosidase and stopping the reaction.

The effect of different substrates is examined with: 1. isopropyl thiogalactopyranoside (IPTG, a synthetic analog of lactose), 2. lactose by itself, and 3. a lactose and glucose mix. This experiment was repeated a week later with substitutions for the substrates including glucose, IPTG, and a glucose and IPTG mix. In this study we show how transcriptionally active the lac operon for *E. coli* is by examining different substrates; we see preference to the IPTG synthetic analog compared to pure lactose, pure glucose, or a mix of the substrates. The results also showed that when two substrates are present in the same tube, the lac operon would show preference for the one that is more easily digestible.

**Materials and Methods**

*Escherichia coli*

*Escherichia coli* was grown in a bacterial cell suspension. To all of the tubes including the control, 1mL of the bacterial suspension was added at the beginning of the experiment.
Measurement of Substrates

Various substrates were added to the 10-labeled tubes in 1mL quantities at 15, 30, and 70 minutes during incubation. Each tube received 1mL of the bacterial suspension of *E. coli* before the incubation period began. A control tube was prepared with 1mL of the bacterial suspension and 1mL of distilled water. Three tubes received lactose at 15, 30, and 70 minute periods during incubation. Three tubes similarly received IPTG at 15, 30, and 70 minute periods, and three tubes received a mix of glucose and lactose at the 15, 30, and 70 minute incubation periods. The tubes were incubated in water at a temperature of 37°C. After 70 minutes of incubation, 1mL of lysis buffer and 1mL of 0.5% ONPG was added to the tubes, and then the tubes were returned to the incubator for an additional 15 minutes. After the 15 minutes, the tubes were removed and 1mL of Na₂CO₃ was added to each tube and inverted to mix and stop the reaction.

We repeated this experiment a week later with the following substitutions for the substrates, 0.1% glucose, 0.1% IPTG, and a mix of 0.1% glucose and 0.1% IPTG. Three tubes received 1mL of glucose, three tubes received 1mL of IPTG, and three tubes received a 1mL mix of IPTG and glucose, at 15, 30 and 70 minute time periods during incubation. See appendix for data table, and exact procedure followed.

β-galactosidase Assay

When ONPG is cleaved by β-galactosidase it yields galactose and o-nitrophenol, which is a molecule yellow in color. This compound serves as an assay to indirectly measure the transcriptional activity of the lac operon. The transcriptional activity can be quantified through changes in absorbance measured on the spectrophotometer.
Spectrophotometry

A Spectronic 20D spectrophotometer was calibrated with a control tube as a blank, and readings were taken at 420 nm. The absorbance was measured for all of the tubes with varying amounts of substrates, after the incubation period was complete.

Data analysis

The absorbance values from both experiments were subjected to analysis in Microsoft Excel. The effect of different substrates on the timing and quantity of the enzyme β-galactosidase, was examined with results.

Results

The purpose of this lab was to examine the effects of different substrates on the transcriptional level of the lac operon by quantifying the enzymatic activity of β-galactosidase. The bacterial suspension used was isolated from E. coli. At specific times during incubation substrates were added to the 10 reaction tubes. The first experiment looked at the effects of lactose, IPTG, and a lactose glucose mix on β-galactosidase and its transcriptional activity. The enzymatic effects of these substrates were quantified by spectrophotometry. Figure 1. shows that when lactose was present, small changes in absorbance occurred indicating that transcription was occurring to a small degree. The sample with IPTG showed a rapid increase in absorbance during incubation suggesting the lac operon was more transcriptionally active as more β-galactosidase bonds were being cleaved. The sample with a glucose lactose mix had no significant change in absorbance, suggesting transcription was occurring but only to a small degree. The Figure 1. confirms that the lac operon for E. coli will be transcriptionally active in the presence
of lactose. Biologically speaking, we would expect to see a disparity and change in absorbance illustrating that transcription is occurring when lactose is present because, lactose is an inducer of the lac operon for E. coli (Freeman 2014).

This experiment was repeated a week later with glucose, IPTG, and a mix of glucose and IPTG. Figure 2. shows that when glucose was added there was little to no change in absorbance, indicating minimal transcription. The tube with a mix of glucose and IPTG, showed an increase in absorbance when the substrate was first introduced (between 15 and 30 minutes) and then plateaued. There was more transcriptional activity within the mixed tube compared to the tube with pure glucose. The tube with only IPTG, displayed a greater increase in absorbance compared to the tube with a mix of glucose and IPTG and the pure glucose tube. The Figure 2. revealed that the lac operon was most transcriptionally active with the pure IPTG compared to pure glucose and the IPTG glucose mix.
Figure 1. The effect of different substrates on the transcriptional level of the lac operon through quantifying the enzymatic activity of β-galactosidase. The lac operon in *E. coli* controls gene regulation and synthesizes enzymes in the presence of lactose. Tubes were prepared with varying contents and different substrates were added at different intervals over a period of time. The tubes were incubated in a water bath throughout the experiment. The substrates used were lactose, IPTG, and a mix of glucose and lactose. All tubes received the same amount of bacterial suspension. A control was used to blank the spectrophotometer at 420nm. The absorbance values were recorded on a Spectronic 20D spectrophotometer. The lines were determined and graphed with Microsoft Excel.
Figure 2. The effect of different substrates on the transcriptional level of the lac operon through quantifying the enzymatic activity of β-galactosidase. The lac operon in *E. coli* controls gene regulation and synthesizes enzymes in the presence of lactose. Tubes were prepared with varying contents and different substrates were added over a period of time at different intervals. The tubes were incubated in a water bath throughout the experiment. The substrates used were glucose, IPTG, and a mix of glucose and IPTG. All tubes received the same amount of bacterial suspension. A control was used to blank the spectrophotometer at 420nm. The absorbance values were recorded on a Spectronic 20D spectrophotometer. The lines were determined and graphed with Microsoft Excel.
Discussion

The purpose of this lab is to examine the effects of different substrates on the transcriptional levels of the lac operon. The lac operon in *Escherichia coli*, codes for genes and proteins responsible for the catabolism of lactose. The enzymatic activity was quantified indirectly with absorbance, through an assay that can be measured with a spectrophotometer. This yellow color from ONPG serves as an indicator of how transcriptionally active the lac operon is.

The results showed lower levels of transcription at different times with the substrates tested. When lactose binds to the repressor it leads to transcription of the lac operon. Low intracellular levels of lactose will initiate the synthesis of the enzymes. This is evident in the results when looking at the tubes that received lactose; the trend showed a slight increase in absorbance suggesting low levels of transcription was occurring. *E. coli* metabolizes lactose and is used for energy, therefore depleting the concentration of lactose present overtime. We hypothesized the results would show the highest transcriptional activity with lactose because *E. coli* metabolizes lactose, however this was not reflected in our results. The literature validates our results as to why lactose presented lower levels of transcription compared to the IPTG tubes according to Kuo et al., (2003).

IPTG differs from lactose, as it is not hydrolyzed or used by the cell meaning its concentration will remain constant. When IPTG was added to the reaction, transcription was initiated as seen by the significant increase in absorbance values quantified by the change in color. IPTG is therefore a constant activator of the lac operon, thus why we see maximum transcriptional activity (Borralho et al., 2002).
The glucose lactose mix showed a minimal change in absorbance and was similar to the results of the pure lactose tube; suggesting limited transcriptional activity was occurring in both tubes. Only lactose has the ability to bind to the repressor protein and cause a conformational change. When glucose is present in the cell it inhibits the transport of the lactose, thus reducing transcriptional activity of the lac operon, however transcription will continue to occur through the metabolism of glucose. It is believed that glucose inhibits the transcription of enzymes by changing the transcription factors present; this is commonly referred to as the “glucose effect” (Kimata et al., 1997). The positive control element in the lac operon controls CAP. When glucose levels are sufficiently low and cAMP levels are high, CAP will be able to bind with the promoter directing the RNA polymerase to bind, initiating transcription of the lac operon. However, when the levels of glucose are high and the cAMP levels are low, the “glucose effect” inhibits CAP from binding to the promoter and transcription production will be significantly lower. Thus, while glucose does not have the ability to bind to the repressor protein like lactose does, the presence of glucose will influence the transcription levels and cause “Catabolite repression” (Ullmann 2009). If lactose and glucose are present simultaneously, *E. coli* will metabolically favor glucose relative to lactose. When glucose is present, the cell can conserve energy, as it does not need to breakdown lactose as an energy source. However, after the glucose is depleted, *E. coli* will then begin metabolizing lactose. These positive and negative control elements serve as a form of regulation to monitor the energy usage and metabolism of the cell; if there is a sufficient amount of lactose or glucose in the cell then no energy needs to be expended on the binding of cAMP (Kuo et al., 2003).
The following week, the experiment was carried out under the same conditions but with different substrates. The tube containing pure glucose showed relatively small changes in absorbance and very minimal transcription compared to the IPTG tube. The two tubes that contained the IPTG and a mix of IPTG and glucose exhibited comparable trends from the previous week. IPTG has the ability to remain at a constant concentration activating the lac operon, which induces higher levels of transcription. When glucose and IPTG were mixed, the overall change in absorbance was slightly lower compared to the pure IPTG tube. This could be attributed to the fact that the concentration of IPTG in the mixed tube was lower than the tube with pure IPTG. Overall, the highest transcriptional activity was seen when IPTG was present, compared to the other substrates tested. IPTG’s ability to serve as a constant activator of the lac operon makes it an optimal inducer of transcription.

For future experiments, varying the concentrations of these substrates to identify the thresholds of the lac operon could help expand on this research. This would also address the question of how concentration affects the transcription levels of the lac operon. To continue this research even farther, examining how different, more complicated substrates and energy sources affect this lac operon for *E. coli* could be interesting, for example looking at how sucrose influences transcription levels. Gene regulation could be studied in more complicated lac operons to determine how they function differently with more genes to coordinate (Franke et al., 2010). This would help contribute to the existing knowledge on gene expression and regulation. In this lab, the cell suspension that was looked at was from a bacterium, *E. coli*. It would be very fascinating to look at the implications of lac operons within eukaryotes to determine if
there are any connections between eukaryotes and prokaryotes with regards to gene expression and regulation. To expand this information further, we should examine the trends observed and connect them with clinical research. Any type of disease or condition that is caused from out of control energy expenditure is connected with gene regulation. For example, diabetes is a disease where one's body cannot regulate the amount of sugar present. Studying diabetes to potentially establish a medicine or method to help the body regulate sugar could improve many people's lives. By understanding gene regulation and expression of these diseases and conditions, we as a society could potentially save many lives and make a positive impact within the biomedical and molecular biology field.
### Appendix

Table 1. The addition and timing of substrates added during the experiment to measure transcription of the lac operon through a β-galactosidase assay.

<table>
<thead>
<tr>
<th>Time in Procedure (min)</th>
<th>0</th>
<th>40</th>
<th>55</th>
<th>70</th>
<th>85</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Add Water</td>
<td></td>
<td></td>
<td></td>
<td>For ALL tubes- Add</td>
</tr>
<tr>
<td>Lactose (70 min rxn)</td>
<td>Add Lactose</td>
<td></td>
<td></td>
<td></td>
<td>1 mL lysis buffer and 1 mL ONPG. Mix and return to water bath.</td>
</tr>
<tr>
<td>Lactose (30 min rxn)</td>
<td></td>
<td></td>
<td>Add Lactose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactose (15 min rxn)</td>
<td></td>
<td></td>
<td></td>
<td>Add Lactose</td>
<td></td>
</tr>
<tr>
<td>IPTG (70 min rxn)</td>
<td>Add IPTG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPTG (30 min rxn)</td>
<td></td>
<td></td>
<td>Add IPTG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPTG (15 min rxn)</td>
<td></td>
<td></td>
<td></td>
<td>Add IPTG</td>
<td></td>
</tr>
<tr>
<td>Glucose + Lactose (70 min rxn)</td>
<td>Add Glucose and Lactose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose + Lactose (30 min rxn)</td>
<td>Add Glucose and Lactose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose + Lactose (15 min rxn)</td>
<td>Add Glucose and Lactose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note**: The quantity and timing of adding the substrates is detailed in the table. The table was the same when the experiment was repeated a week later, however the only substitutions were with the substrates. The substrates used were, Glucose, IPTG glucose mix, and pure IPTG.
Reference List


Freeman, Scott. *Biological Science, 5*th ed: Pearson Education. 2014


