The Effects Of Increasing Glucose On The Human Metabolism

Abstract

Neutral red dye transports through a membrane of yeast cells using active transport.. We decided that changing glucose concentration could overcome inhibition of active transport channels in S. cerevisiae and lead to an increase of ATP made available to the cell; therefore an increase in dye absorption in the alternate hypothesis. Microspectrophotometric readings were taken after the neutral red dye and exposure to the sodium azide. Results showed significant increase in neutral red dye uptakes to the amount of glucose introduced but not results expected.

Introduction

Results of the experiment indicate that there is a higher absorption rate in yeast cells in when sodium azide is introduced. Sodium azide is a metabolic inhibitor, it was hypothesized that it would lead to a decrease in the dye uptake, and glucose was increased to overcome this. The results proved the hypothesis since there was an increase in dye absorption. The results showed that sodium azide did have an influence on the dye concentrations, but the uncertainty of how variant it was remained.

Cells have a plasma membrane in any living organism, which is a bilayer of lipids and regulates the flow of materials into and out of the cell. There are three variations of transport, Simple diffusion, facilitated diffusion and active transport. Active transport is used during this experiment that involves a chemical reaction of hydrolysis of ATP that is used for transport to take place. In the experiment, a pH of 6.8 is used to make the solution more basic, with a more acidic environment; the neutral red dye cannot be absorbed properly by the cell. A recent article by Japanese researchers discovered that sodium azide inhibited more than 90% of E. coli, the azide inhibited multi-site ATPase activity by lowering catalytic cooperatively (Futai 1987).

An experiment performed by researchers in 1979 showed when sodium azide was applied on Saccharomyces cerevisiae cells growing in a medium, the results showed that the frequency of mutations increased up to 26 times. These mutations resulted because sodium azide acted as a weak hazard at a low pH. The other experiment showed that no mutagenic effect was observed when azide was applied to resting cells in buffer at pH 4.2 (Silhánková L, Smiovská V, Velemínský J, 1979).

Methods

Similar methods derived the previous experiment (Rowan 2010) were used. There were four different suspensions of yeast. 20mM HEPES, 0.056mM, 0.112 mM, and 0.224mM. These were set aside and incubated for thirty minutes lightly vortexing in between to guarantee a thoroughly mixed solution. After thirty minutes, two hundred micro liters of both control and azide mixed solution were pipetted into microfuge tubes of the concentrations 0.00%, 0.25%,0.50%, 0.75%, and 1%, vortexed and set those aside for another thirty minutes. When finished, the sample were taken to a microcentrifuge apparatus and spun for two minutes at 5,000 rpm. When the samples were taken out, the liquid was extracted from the samples making sure not to disturb the newly constructed pellet, and suspended in 1ml of yeast growing medium. This process was repeated two more times until the dye was depleted. After the third suspension was completely, the samples were resuspended in four hundred micro liters of yeast growing medium and lightly vortexed. After this step, one hundred micro liters of solution were transferred into a microtitre plate using three rows for control and three rows of azide and read in the microspectrophotmeter to graph and interpret the results.

Results

The azide showed a different result than what was expected in the alternative hypothesis. The points observed (figure 1) at data points around 0.32 on 56mM and 0.35 on 224mM in azide, can be assumed that an experimental error happened during the transfer of the samples into the wells of the microtitre plate. As well as significant cross contamination between different concentrations of red dye in the Saccharomyces cerevisiae. Incubation of dye and yeast for only 30 minutes was not enough time for the active transport to occur. Relative standard deviation of the azide groups are between roughly 0.08 and 0.12 on all three 56mM, 112mM, and 224mM, suggesting that the results are not distributed evenly.

Similar findings were found in the control group, which have a relative standard deviation value between 0.00 and 0.01, also indicating that results are not normally distributed. The graph was increasing until 0.25% that had a dramatic drop in absorbance for the control group. This could have been caused by dye still contained in the sample or the loss of a pellet. Since, both the azide and control groups showed an abnormal distribution patterns, the alternate hypothesis was rejected.

For the control 56mM, the line ascends until 0.25 nm resulting in possible contamination. 56 mM azide shows too much absorbance of dye in glucose as it is above the control. 112mM azide is mostly consistent with control, showing a dip or a loss of a pellet. 224 mM absorbance level is consistent with hypothesis until 1% showing a dramatic increase over control.

Discussion:

The main purpose of this experiment was to determine the effects of higher glucose concentration on inhibition caused by sodium azide. If glucose concentration was increased, it

would show a final result of decrease in dye concentration. The results using 56mM of glucose showed the highest amount of dye absorbance, regardless if sodium azide is there or not. This suggests that both a lack of glucose concentration, as well as an excess, can cause a decrease in metabolic function and transport activity. A few studies found showed a correlation between metabolic activity and glucose concentration.

Results from a study done on glucose concentration in growth medium and the metabolic activities of L. mononcytogenes showed that an increase in glucose concentration led to a decrease in metabolic activity and a decrease in catalase functions. It can be assumed that acidic pH levels are also a result of excess glucose concentrations (Alm and Friedman, 1962). The pH in our experiment was adjusted at 6.8 once. A study that compared glucose and pH levels showed that metabolic functions are better when the pH is maintained at exactly 7 (Margaritis and Vogrinetz, 1983).The null hypothesis states that there is no effect on dye concentration with increased glucose concentration. When our results were compared with those from the credible sources, the results and correlations were repeated between glucose, sodium azide, and dye absorbance. It can be assumed acceptable to reject not only the alternate hypothesis, but the null hypothesis too.