Bella Cambra

Investigation to Determine the Location of Yeast Invertase

Introduction

This investigation focuses on the location of yeast invertase. Yeast (*Saccharomyces cerevisiae*) is a single-celled eukaryote and fungi that divides asexually by budding. Yeast uses the enzyme, invertase, to hydrolyze the breakdown of sucrose into glucose and fructose. In this laboratory the scientific question aims to determine precisely where the hydrolysis of sucrose takes place in yeast. My preliminary observations indicate that wheat, pollen, and red bread mold are also eukaryotic cells that use invertase to hydrolyze sucrose. Wheat (*Triticum aestivum L. cv Yecovo Rojo*) is a grain that uses invertase to produce simple sugars and hexose that are used for energy and carbon by the cell. Invertase also aids in cell development and growth. There are two types of invertase in wheat. One form of invertase is soluble and can be found intracellularly in the cytoplasmic regions of the cell. The other form of invertase is found extracellularly bound to the cell wall (Krishnan *et al*., 1985). Red bread mold (*Neurospora crassa*) uses invertase to hydrolyze sucrose into glucose and fructose which are required for cell growth and development and also help to provide carbon and energy to the cell. Invertase found in red bread mold is secreted to extracellular regions of the cell (Lee and Free, 1984). Pollen (*Lilium anratum*) is a pollen found in lillies that uses invertase to produce sugars that permit germinating pollen grains to grow and fulfil their high requirements for carbon. Carbon is crucial for respiration and the synthesis of the pollen tube wall. There are two forms of invertase found in lily pollen. One form is found intracellularly in the cytoplasmic regions of the cell. The second form is found bound extracellularly to the cell wall (Singh and Knox, 1984). Based on my observations in this experiment, my hypothesis states that invertase is located in both intracellular and extracellular regions of the call. This hypothesis will be experimentally addressed by separating yeast cells from their extracellular fluid by centrifugation and performing both supernatant and pellet tests to determine whether or not glucose and fructose are present. If glucose and fructose are present a benedict's test will be positive indicating that yeast invertase is present as well.

Discussion

In this experiment we used a Benedict's test to detect the presence of reducing sugars. A positive benedict's test will result in a color change and the forming of a precipitate. A negative benedict's test will not result in a change. Monosaccharides are all reducing sugars and will always result in a positive Benedict's test. Some disaccharides are reducing sugars and some are not. For example, although sucrose is not a reducing sugar and will result in a negative Benedict's test, lactose is a disaccharide that is a reducing sugar and will result in a positive Benedict's test. Polysaccharides are not reducing sugars and will always result in a negative Benedict's test. After performing the Benedict's test on various macromolecules, we found that only carbohydrates gave positive results. Since glucose and fructose are reducing sugars but sucrose is not, a positive benedict's test indicates that sucrose has been broken down into glucose and fructose. A negative benedict's test indicates that the Benedict's reagent did not react. Yeast alone did not react with Benedict's reagent because there were no reducing sugars present. Sucrose alone also did not react with Benedict's reagent because it cannot break down by itself into glucose and fructose. Glucose alone reacted with Benedict's reagent because glucose is a reducing sugar. The yeast that were fed sucrose did however react with Benedict's reagent. This result indicates that yeast has the ability to break down sucrose because the yeast and sucrose alone could not react with Benedict's reagent. This implies that the presence of invertase in yeast can hydrolyze sucrose into the monosaccharides, glucose and fructose. We separated the yeast into extracellular and intracellular fractions by centrifugation. Centrifugation resulted in a pellet containing intact yeast cells separated from the extracellular fluid. The pellet represented intracellular contents and the supernatant represented their extracellular fluid. The supernatant test showed a positive Benedict's test but the pellet test showed a negative result. The positive supernatant test indicates that the breakdown of sucrose occurs only in the extracellular fluid because glucose and fructose were present. Therefore, this result indicates that yeast invertase is also located only in the extracellular fluid.

Conclusion

My hypothesis for this experiment was that invertase would be located in both intracellular and extracellular regions of the cell. It was predicted that if yeast cells were separated from their extracellular fluid and tested for the presence of invertase both tests would show positive results indicating that invertase was present. It was also predicted that if the hypothesis were to be falsified by the data then none or only one of the tests would show positive results. Our experimental outcome showed that invertase is located only in the extracellular regions of yeast since the supernatant test showed positive results but the pellet test showed negative results. The positive supernatant test indicates that glucose and fructose were present in the solution and therefore yeast invertase was as well. The experimental outcome falsified my

hypothesis because only the supernatant test showed positive results and I hypothesized that the pellet test would as well. Based on the data, my revised hypothesis is that invertase is located only in the extracellular regions of the cell.

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References

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