



Biochemistry: Amino Acid Effects on Amylase Production

Paper citation: Liu KS and Franckowiak B (2016). The Effects of L-glutamate, L-glutamine, and L-aspartic Acid on the Amylase Production of *E. coli* Transformed with pAmylase. J Emerging Investigators 73: 1-6

Paper questions

In reading through the assigned papers, please answer the following questions:

1. What's the central question being investigated by the researchers?

Amylase, the enzyme being studied, is used by human in the digestion of food. There are also human diseases involved in amylase deficiency or malfunction. Therefore, studying this enzyme is of broad relevance.

2. What does the introduction tell you about important concepts needed to understanding the experiment? In particular, what did you learn about amino acid synthesis and amylase enzymes?

The introduction provides all of the relevant background information needed to understand the paper. In doing so, it also provides a range of references one can look up to learn more about a particular topic. Finally, the introduction states what question is being answered by the paper.

In this paper, the introduction introduces the amylase enzyme and the basics of amino acid synthesis. We learn that amylases break down sugar and are used in a range of industrial applications. We also learn that L-glutamine and L-glutamate have previously shown effects on amylase production. Finally, we learn that these two amino acids are precursors for many other amino acids.



3. Which of the 4 major macromolecules of life were mentioned in this paper? List their monomers and polymers as they apply here.

This paper talks about sugars (monosaccharide monomers and polysaccharide polymers) and amino acids (the monomer to proteins).

4. Explain the principles behind these critical experiments used in this paper:

-Transforming the amylase into *E. coli*

Through a process called 'heat shocking', the DNA for the amylase is put into *E. coli* cells. This way, the bacteria cells will express the protein for amylase activity studies.

-Amylase activity assays

These two assays test for the overall activity of amylase in the collection of bacteria. Increased amylase activity is linked to increased amylase production. The assays look at the amount of substrate and products for the amylase-catalyzed breakdown of amylose and maltose to sugar.

-Measuring the ratio of amylase and CFU

By looking at this ration, the authors can differentiate between an increase in amylase production and a larger number of cells. One could fathom that L-glutamine and L-glutamate lead to more cells, which in turn contain more amylase. However, taking the ratio shows that there are indeed more amylase enzymes per colony.

5. What are the controls for the experiment? Did they use enough controls?

The control group in this experiment did not receive any additional amino acids while the three experimental groups received either L-glutamine, L-glutamate, or L-aspartic acid. This is a good negative control, but one could make an argument for trying more than just 3 out of 20 amino acids.



6. Why do the authors think that L-glutamate and L-glutamine can lead to increased amylase production?

L-glutamine and L-glutamate are good nitrogen sources, which cells need to grow well. This would help cells grow more quickly and more cells grow. They are also versatile precursors for other amino acids. Therefore, more amino acids might be readily available to make more protein.

7. What other experiments can you propose to strengthen the results of this paper? Try to think of one that could be done in your classroom and one that could be done at a university lab with access to any equipment you might deem necessary.

A follow up experiment would be to run an SDS-PAGE gel for the control and experimental groups to see if a band where amylase should be changes in intensity. At a university, one could perform mass spec, Western blots, or immuno blots. Additionally, one could use a better apparatus to measure color.

8. What is one potential pitfall of studying the effects of L-glutamate, L-glutamine, and L-aspartic acid on human protein production in a bacterial system?

The three added amino acids could have an effect that is specific to bacteria, for example through translation regulation. It is not necessarily true that the same effect would persist in a human system.

9. What are some shortcomings of this paper? Are the semi-quantitative experiments they used enough to support their results?

One could look at more amino acids and their effects on amylase production. One could also come up with a more quantitative assay that is more consistent among trials.



10. What is the big conclusion, or take-away message, the authors want the reader to remember?

L-glutamine and L-glutamate have a positive effect on amylase production in *E. coli*.