The Purification and Characterization of *P. falciparum* Hypoxanthine Guanine Xanthine Phosphoribosyltransferase From Recombinant *E. coli*

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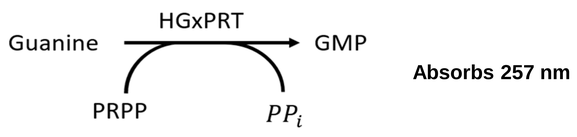
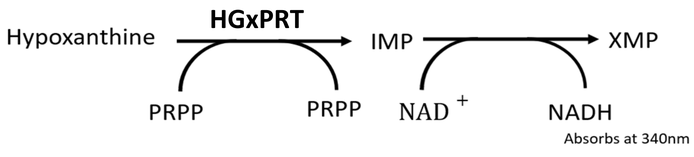
The purification and Isolation of IMPDH

The current protocol for the activity assay for HGXPRT is problematic and risky as it relies on the absorption of GMP. The absorption of GMP is around 257 nm, and many other molecules have a similar UV absorption so that could cause error in our studies. We are currently working on a coupled enzyme assay that would rely on the absorbance of NADH, which is around 340 nm and thus is would give us more accurate results.

We have acquired an E. Coli vector that expresses human IMPDH, which could be used in the coupled assay reaction. We plan to purify and isolate the IMPDH cell extract using an Affigel Blue column, Q-sepharose column, and a FPLC machine. The IMPDH binds to the beads in the columns while everything else gets filtered. A buffer is then used to wash the column which causes the enzyme to unbind from the beads, and the flow through is collected and used in the coupled assay reaction.

Enzyme Pathway





Introduction

The human malaria parasite, *Plasmodium falciparum*, is responsible for most of the million annual deaths from malaria. The underlying causes of this disease can be better researched by isolating the proteins and enzymes that it uses for synthesis of purine nucleotides. This parasite is developing a drug resistance to medicines commonly used for treatment, and scientists believe that the enzyme (HGXPRT) can be used to develop new treatments.

Our research goal is to sustainably produce and purify HGXPRT and to express it and assess its kinetics in the presence of substrates.

Our objective for this semester was to complete a coupled enzyme assay to test the activity and presence of HGXPRT, by measuring the absorbance of the byproducts.

Additionally, a new vector for human LDH5 is being developed for us to study its impact on the aerobic glycolysis and the growth of cancer cells.

Our research is focused on the study of the molecular mechanisms and the biochemistry underlying diseases .

Methods

- Overnight culture
- DNA and protein extraction
- Protein purification via cobalt chromatography and FPLC system
- Enzyme Assay
- Kinetic analysis
- Gel electrophoresis

Conclusion

Our work this semester mainly consisted of growing our E. Coli vector, extracting the DNA, and extracting the enzyme from the cell. Our first run through with the FPLC machine in order to purify the IMPDH enzyme was not a success since we did not get the results, we were expecting from the coupled assay reaction. To deal with this, we remade our buffers and had a second run with the purification protocol using the FPLC machine.

We were not able to run through a coupled assay reaction for a second time as our work was interrupted by the COVID-19 outbreak. We hope to continue where we left off with our research in the Fall of 2020.

For future work, we will express and the human HGPRT enzyme and we plan to work with a new vector LDH, and study its underlying impact on the spread and development of cancer.

Refrences

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