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Effects of the Biological Reagent IPTG on Expression of the Protein AFUDG

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The Effects of the Biological Reagent IPTG on AFUDG

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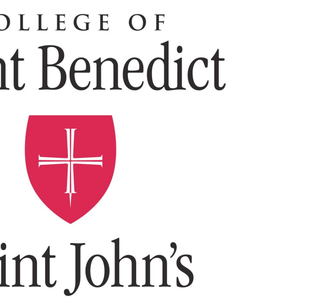
Introduction

The protein AFUDG is a Uracil DNA Glycosylase from Archeoglobus fulgidus, a eukaryote of the Archaea domain. This protein is a base repair enzyme and metalloprotein with a 4Fe-4S cluster cofactor located on it. We are studying this protein to find the most efficient and cost effective way to purify it for research and teaching labs. Studying AFUDG will also allow us ways to research folding activity of the protein, which is difficult because of the His(6) tag on the N-terminal. E Coli cells containing a plasmid with the gene for AFUDG and a gene for kanamycin resistance were streaked with cells to grow in colonies. Colonies were inoculated with IPTG, a biological reagent used to induce transcription and consequently translation of the protein AFUDG. Once an efficient and cost effective way to overexpress AFUDG is found, the methods and techniques can be used to aid in the affinity gel lab in Chem 202, as well as further explore the folding and activity of the protein. This can be done by later conducting thermal tests, which allows us to determine at which temperature the protein unfolds.



http://www.rcsb.org/pdb/explore/explore.do?structureId=2DDG
Figure 1. UDG (uracil-DNA glycosylase) protein structure. This protein is a homologue of AFUDG, which does not have a known crystal structure.

Discussion

- Throughout this research, an appropriate concentration of IPTG (0.66 mM) has been determined to use to induce growth of AFUDG
- Figure 3 shows unexpected data, as no bands can be seen in any of the 4 elutant fratctions. This could be a result of low protein concentration, as well as the protein not reacting with the buffer solutions as expected
- Final PAGE gel indicates that a large amount of protein came off the Affinity column in the Elutant 42 fraction (Figure 4)
- Based on Rf value of the protein and the equation obtained from Figure 2, the MW of the protein is 22.03 kD
- The large protein band size in E42 and MW of 22.03 kD shows that there are some impurities present as the completely pure protein has a MW of 23 kD (Figure 4)

Results

- The purpose of this research is to overexpress and purify AFUDG in *E. Coli*.
- Our goal was to grow AFUDG on large scale in *E. Coli* cells and then induce them with IPTG to express large amounts of the protein
- The protein was ran using concentrations of 0 mM, 0.33 mM, 0.66 mM, and 1 mM IPTG through a PAGE gel
- 0.66 mM IPTG mixed with AFUDG was chosen to mass produce the protein based on analysis and cost efficiency
- After determining 0.66 mM concentration IPTG would be used, the original tests were scaled up, to use on 2 L solution LB broth
- AFUDG was then separated from E. Coli by running it through a centrifuge
- Cells were lysed and centrifuged to separate AFUDG from E. Coli
- Lysed protein cells were run through an Affinity column using a High Affinity Ni-Charged Resin to bind and purify the protein.
- 4 fall through fractions and 4 elutant fractions were collected and run through a PAGE gel
- Procedure was scaled up and a sample of 30 mL of the protein was be run through 2 mL of the Ni-Charged resin to determine if the pure protein elutes when expected
- 30 fall through, 11 wash, and 8 elutant fractions were collected
- Fractions were run through PAGE gel and analyzed

Conclusion

- 0.66 mM IPTG is the most efficient/effective concentration when overexpressing AFUDG
- A high concentration of protein was found in elutant fractions, supporting expected results (Figure 4)
- Future experiments could be conducted to find a more accurate concentration of IPTG with the most positive effect on AFUDG expression
- Future temperature tests on denaturation of AFUDG

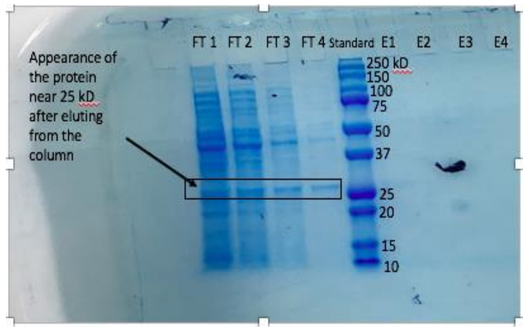


Figure 3. PAGE gel taken after running the protein through a 2 mL High Affinity Ni-Charged Resin column. 4 fall through and 4 elutant fractions are present as well as a standard. The box shows the band were the protein has appeared.

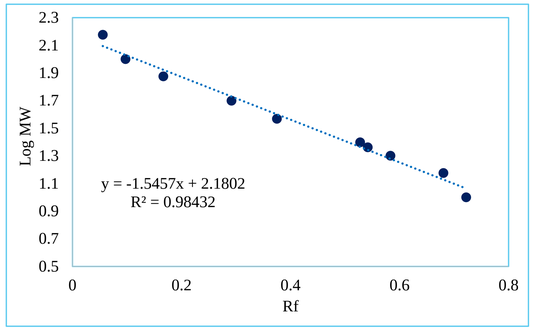


Figure 2. Plot of Rf values vs Log MW of protein standard and AFUDG on PAGE Gel after being run through an Affinity Gel column.

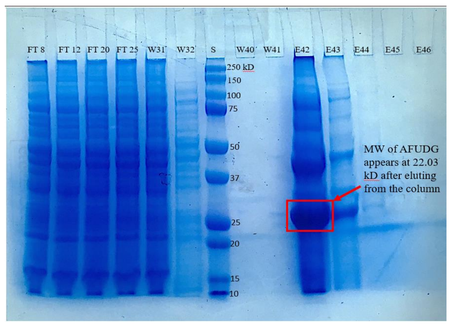


Figure 4. PAGE gel after running the protein through an 8 mL High Affinity Ni-Charged Resin column. 30 fall through (FT), 11 wash (W), and 6 elutant (E) fractions were collected. Fractions were compared to a protein standard (S) with certain molecular weights to determine Rf values and overall MW of AFUDG. The red box shows where the final protein appears.

Materials and Methods

Cell growth preparation
- 1 L solution of 25 g LB broth was made and added into 16 test tubes, 5 mL each and 4 Erlenmeyer flasks, 200 mL in each.
- A solution of 34 mg/mL of kanamycin was made. 200 µL of the solution was added to a beaker of 200 mL LB broth and 2 g agar
- 25 mL of solution was poured into 8 petri dish

Growing cells
- A petri dish was streaked with stock cells and placed in a shaking incubator for 16 hours
- 4 starter tubes were prepared with 10 mL LB broth, 10 µL kanamycin and 250 µL starter culture were added to each tube
- Place back in the shaking incubator.

Inducing gene expression
- 0 mM, .33 mM, .66 mM, and 1 mM concentrations of IPTG solution were added to different starter cultures.

Lysing cells
- Centrifuged cells were mixed with 30 mL of lysis buffer to break open *E. Coli* cells

Purifying protein
- The 4 concentrations of IPTG were run in an Affinity Gel Column
- 30 mL of AFUDG solution was run through column to purify the final protein

Literature Cited

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