Research and Creative Experience for Undergraduates (RCEU) Summer 2017

COLLEGE OF SCIENCE
THE UNIVERSITY OF ALABAMA IN HUNTSVILLE

Purification of Human Hematopoietic Prostaglandin D-Synthase

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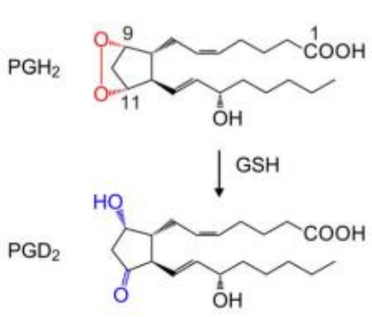
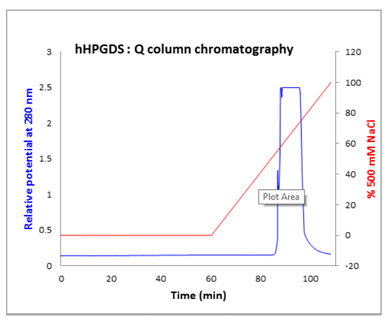
Introduction

Human Hematopoietic Prostaglandin D2-Synthase (h-HPGDS) **EC.5.3.99.2** is an isomerase that converts PGH_2 to PGD_2. In patients with muscular dystrophy, muscle cells where H-HPGDS is localized undergo necrosis [1]. For the purpose of neutron crystallization, purity of h-HPGDS is critical for the formation of a large crystal. To improve crystal quality, the purification protocol needed to be optimized. Anion-exchange chromatography was focused on specifically in the purification process due to lack of effective separation of the desired protein. A pH and salt gradient was established for more effective separation.



Reaction catalyzed by PGD-synthase [2]



First Q Column Chromatography attempt

Materials and Methods

I. Expression

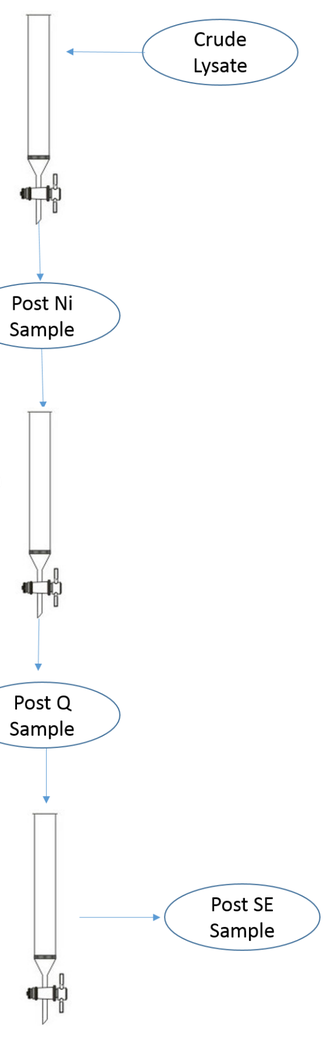
Recombinant *E. coli* with h-HPGDS expression vector in 10 L large growth batch

II. Purification

1. Nickel Column Chromatography
Start buffer: 50 mM sodium phosphate buffer, pH 8.0
300 mM NaCl
Elution buffer: SPB Start Buffer
500 mM imidazole
Wash buffer: SPB Start Buffer
10 mM imidazole

2. Quaternary Ammonium (Q) Column Chromatography
Protein bound at pH 7.5 but did not bind at pH 6.8, while unnecessary proteins eluted at salt concentrations of 300 mM or higher. To mitigate this issue, a pH and salt gradient was established.
Start buffer : 20 mM sodium phosphate buffer, pH 7.5
0.5 mM EDTA
0.1 mM DTT
1 mM PMSF
Elution buffer: Same as Start Buffer, pH 6.8
200 mM NaCl

3. Size Exclusion Chromatography
Sephracyl S100 was used for high resolution
Buffer: 20 mM HEPES-NaOH, pH 7.5
0.1 mM DTT
0.1 mM PMSF
100 mM NaCl



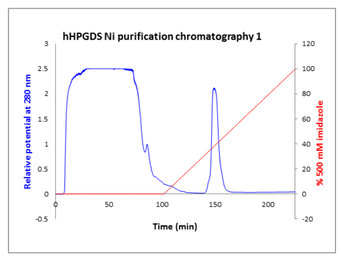
III. Activity Assay

- Glutathione S-Transferase (GST) Activity Assay
- Assay performed on samples from each post column fraction
- Readings were taken every 30 sec for 30 min at 340 nm

Data/Results

Post Column Fraction	Volume (mL)	Concentration (mg /mL)	Estimated amount (mg)	A_{280}/A_{260}	Specific Activity (μm/ml/min)
Crude lysate	200	20.20	4039.48	0.61	10.2
Post Ni	500	0.41	207.03	1.16	20.6
Post Q column	35	3.59	125.51	1.64	38.9
Post Size Exclusion	12	6.32	75.85	1.73	44.12

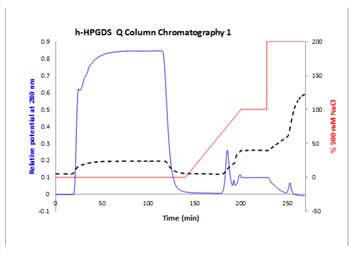
I. Nickel Column



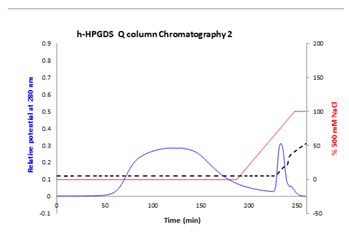
Nickel column purification chart

Purification was performed using a series of three columns. The first was a Nickel Column where the protein was eluted using an imidazole concentration gradient. The second column was a Q-column, where the optimized method to remove unnecessary proteins was a combined pH and salt gradient.

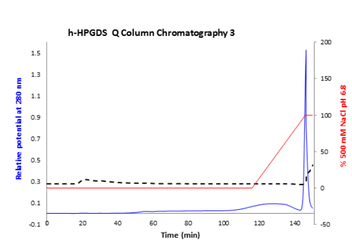
I. Q-Column



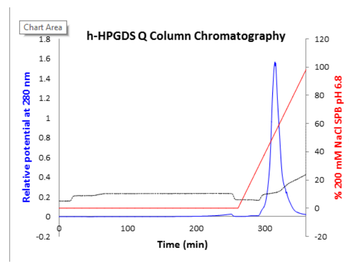
SPB buffer ph 6.8 with high NaCl in charged sample



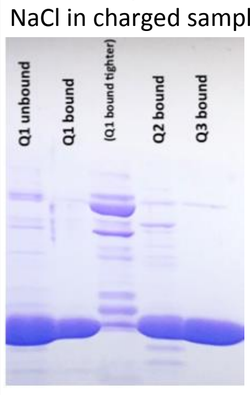
SPB buffer ph 6.8 with low NaCl in charged sample



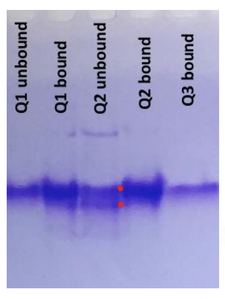
SPB Buffer pH 7.5 with low NaCl in charged sample



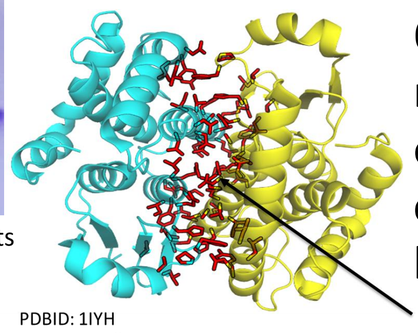
Optimized Q-Column Chromatography



SDS PAGE Results



Native PAGE results

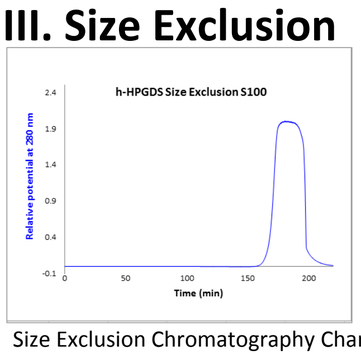


PDBID: 1IYH
X-ray Crystallography Structure

Interfacing residues shown in red

The hypothetical pI was originally determined to be 6.1. Accounting for interfacing residues that would have little effect on the overall surface charge changed, the hypothetical pH to 6.8.

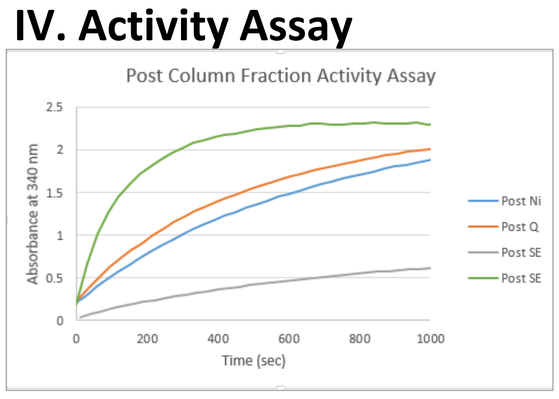
III. Size Exclusion



Size Exclusion Chromatography Chart

The final purification step was size exclusion chromatography. S100 was used for a higher resolution. The quality and quantity of the sample was determined after completion of each consecutive purification column. A Glutathione S-transferase (GST) activity assay was performed in order to determine the activity of purified h-HPGDS after each column chromotography. High activity indicates presence of h-HPGDS in the sample as well as purity of the sample.

IV. Activity Assay



GST Activity Assay for Post Column Fractions

Impact/Conclusions

Optimizing the purification protocol removes impurities that may hinder the quality of h-HPGDS crystal for neutron crystallography purposes. Neutron crystallography allows for a higher resolution, and therefore better structure determination, which is critical for determining a more specific inhibitor for h-HPGDS.

References

[1] Urade et al. (2012). International Journal of Microgravity Science and Applications. 29 (3) : 125-131
[2] Irikura et al. (2009). Journal of Biological Chemistry. 284 (12) : 7623-7630.

Acknowledgements

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