Expression and purification of F_{420} dependent glucose-6-phosphate dehydrogenase from *Nocardioidaceae bacterium*

Md Sabid Ahamed and Dr. Kayunta Johnson-Winters

University of Texas at Arlington, Department of Chemistry and Biochemistry, Arlington, TX-76019

Abstract

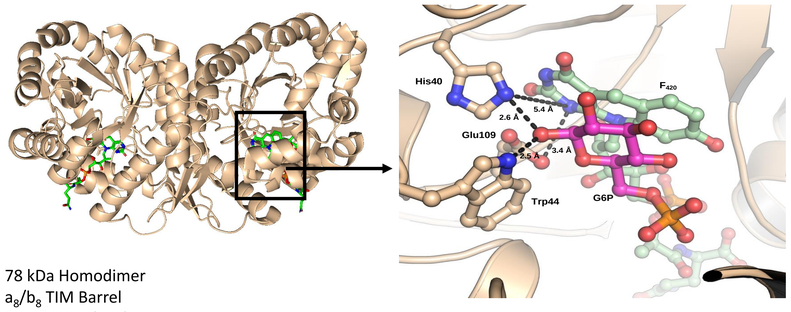
F_{420} dependent glucose-6-phosphate dehydrogenase (FGD), although originally discovered in *Nocardia*, has been extensively studied in *Mycobacteria* due to the health relevance for tuberculosis (TB) treatment. FGD catalyzes the conversion of glucose-6-phosphate (G6P) to 6-phosphogluconolactone, using oxidized F_{420} cofactor, which becomes reduced during catalysis. The focus of this project is on FGD from *Nocardioidaceae bacterium* (FGD-Noca), which shows activity towards additional sugar-6-phosphates, such as glucose-6-phosphate (G6P), D-mannose-6-phosphate (M6P), and D-fructose-6-phosphate (F6P), unlike the mycobacterial enzyme which only displays specificity towards G6P. The major goal of our research is to characterize the FSDs using steady-state and pre steady-state kinetic methods to provide detailed mechanistic insights for the FSDs. Our initial goal of this project is to express and purify FGD-Noca in order to kinetically characterize the hydride transfer reaction mechanism of *wt*FGD-Noca. The proposed experiments and preliminary results will be described here.

Background

Tuberculosis (TB) is a devastating and infectious disease. According to WHO nearly 7.5 million new cases of TB have been reported in 2022 which is 2.5% more than the previous year and hotspot regions are located in southeast Asia and African continent. The total number of global deaths caused by TB (including those among people with HIV) was 1.30 million in 2022. Understanding the biochemistry and physiology of active and persistent TB will help to reveal the basis of pathogenesis, making it possible to combat the disease more effectively. Kinetic Characterization on this F_{420}-dependent enzyme has led to the development of Nitroimidazole prodrugs.

https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2021

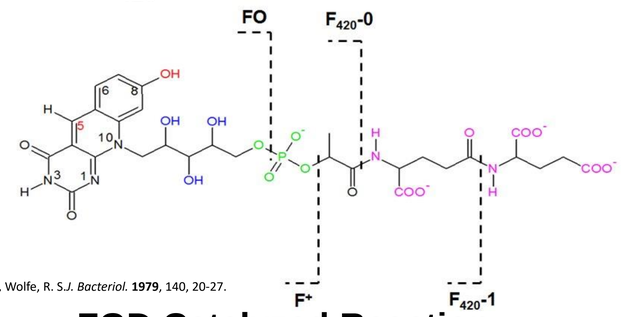
FGD Crystal Structure



78 kDa Homodimer
a_8/b_8 TIM Barrel
One F_{420} molecule per monomer

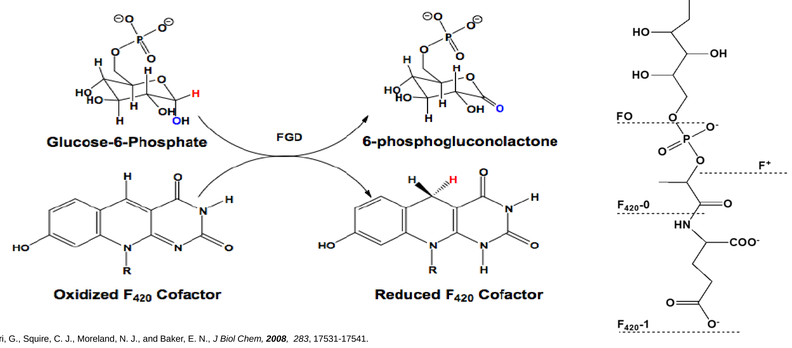
Bashiri, G., Squire, C. J., Moreland, N. J., and Baker, E. N., *J Biol Chem*, ***2008***, *283*, 17531-17541.

F_{420} Cofactor Structure



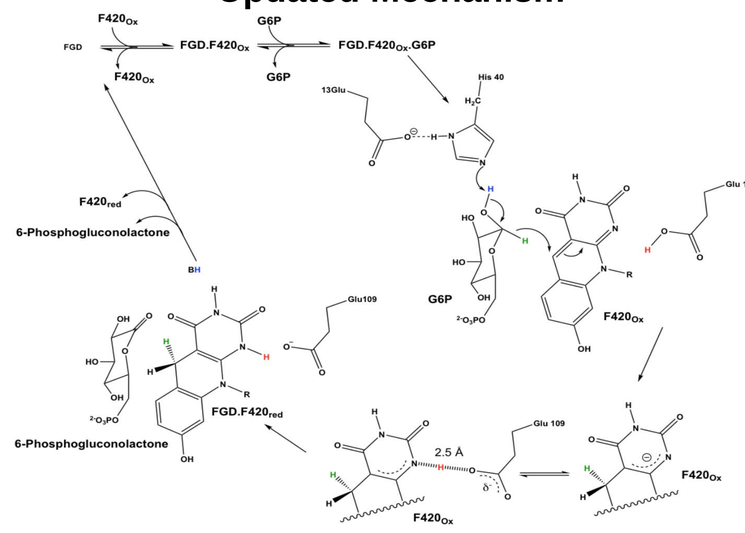
Eirich, L. D., Vogels, G. D., Wolfe, R. S.*J. Bacteriol.* **1979**, 140, 20-27.

FGD Catalyzed Reaction



Bashiri, G., Squire, C. J., Moreland, N. J., and Baker, E. N., *J Biol Chem*, ***2008***, *283*, 17531-17541.

Updated Mechanism

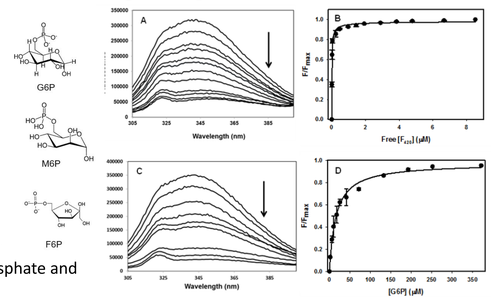


Oyugi, M. A.; Bashiri, G.; Baker, E. N.; Johnson-Winters, K. Mechanistic Insights into F_{420}-Dependent Glucose-6-Phosphate Dehydrogenase using Isotope Effects and Substrate Inhibition Studies. *Biochem. Biophys. Rep.* **2018**, 1866: 387-395.

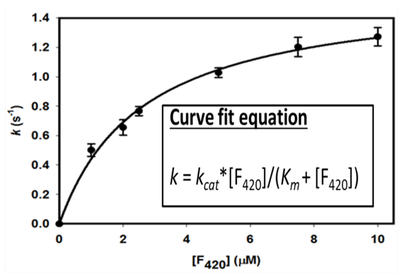
Proposed Binding Experiments

F420 binding:
[F420] = 0 µM to 5.5 µM
[FGD] = 350 nM
50 mM Tris, pH 7.0

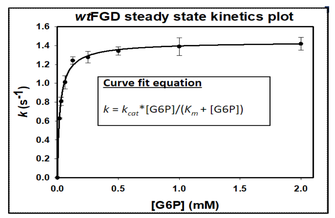
G6P binding:
[G6P] = 0 mM to 0.73 mM
[FGD] = 350 nM
50 mM Tris, pH 7.0
λex = 290 nm; λem = 340 nm
We will conduct the binding experiment for D-fructose-6-phosphate and D-mannose-6-sphate and Glucose-6-phosphate.



Proposed Steady-State Experiments

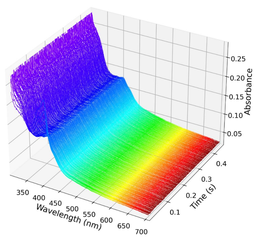


UV-Vis spectrophotometry to monitor the reaction at 420 nm
[G6P] = 2 mM; [F420] = 1-10 µM; [FGD] = 25 nM;
Buffer = 50 mM Tris, pH 7.0



UV-Vis spectrophotometry to monitor the reaction at 420 nm
[G6P] = 0 mM - 2 mM; [F420] = 10 µM;
[FGD] = 25 nM; Buffer = 50 mM Tris, pH 7.0

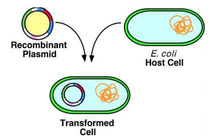
Proposed Pre Steady-State Experiments

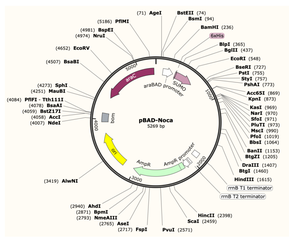


Rapid mixing methods using stopped-flow spectrophotometry
[G6P] = 2 mM; [F420] = 10 µM; [FGD] = 6 µM;
Buffer = 50 mM Tris, pH 7.0

Cloning

Gene created by Vector Builder

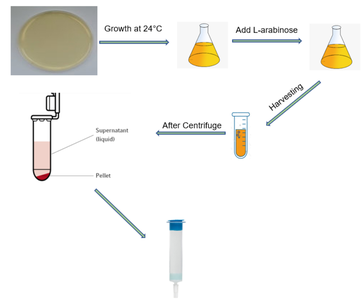




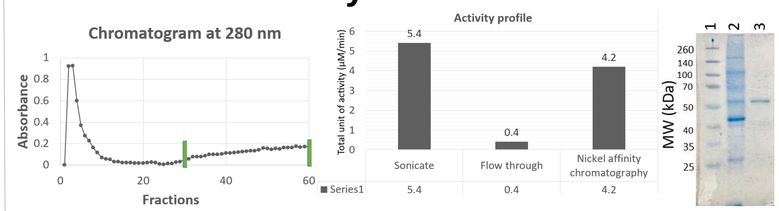
Transformations were done using NEB-10 beta competent cells

Expression and Purification

Expression
- Agar plate with 50 µg/mL ampicillin
- Growth in Terrific broth at 37°C for 12h
- OD_{600} (1.8-2.0)
- 50 mM L-arabinose
- Expressed for 36 hours at 24°C

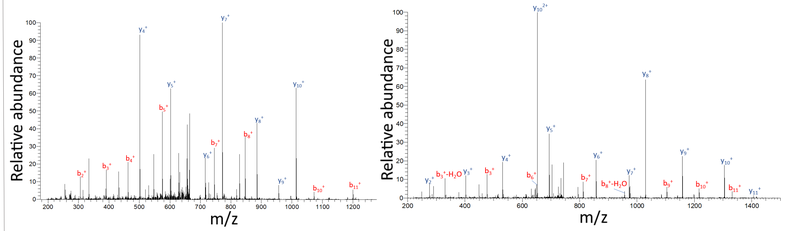


Preliminary Purification Data



1 = Ladder
2 = Sonicate
3 = Nickel affinity chromatography

LC-MS/MS Spectra



The protein was identified with 55% confidence

Conclusions

- The protein was identified with 55% confidence by In-Gel digestion experiment.
- **FWGALGLTPE** and **VTFEGDYYQL** are the two most confident peptides found.

Acknowledgements

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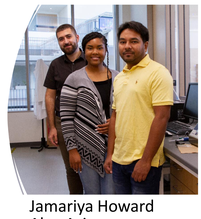
Dr. Kayunta Johnson-Winters



Jamariya Howard
Alaa Aziz