Biochemical Characterization of Human Guanylate Binding Protein 2

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Abstract

Interferon-inducible guanylate binding proteins (GBPs) are contributors to our ability to fight infectious agents. GBP1 is the most well-characterized member of this family. However, recent studies are focusing on the roles of GBP2 and the other members because they also play an important role in immunity that is not well understood. Biochemical alterations of the environment and its effect on GBP2 activity can relate to how the protein functions in cell-autonomous immunity. We created constructs of the GTPase domain of GBP2 (GD) and studied its activity in different environments. This was accomplished through recombinant protein purification using affinity, ion-exchange, and size exclusion chromatography. We also generated a mutant of the GD using site-directed mutagenesis and finally purified it using the same purification strategy as the wild type (WT). We tested the activity of the protein in the presence of different cation co-factors and compared the activity of the WT and mutant at different pH levels. It was found that GD was only active with Mg^{2+} and Mn^{2+} cation co-factors.

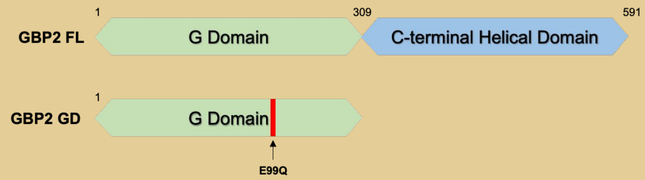
Introduction

Cell-autonomous immunity is very important for us to fight the infectious agents that enter our body. A family of interferon-inducible guanylate binding proteins (GBPs) play a central role in this immunity in humans. It is known that they provide defense to viral, bacterial, and protozoan pathogens. This occurs when interferons, signal proteins that are released by our cells in response to infectious agents, stimulate the effector proteins. GBP2, an efficient GTPase, is the main focus of this research project. The aim of this research was to compare the activity of GD compared to the active site mutant (E99Q) with different pH levels and cation co-factors.

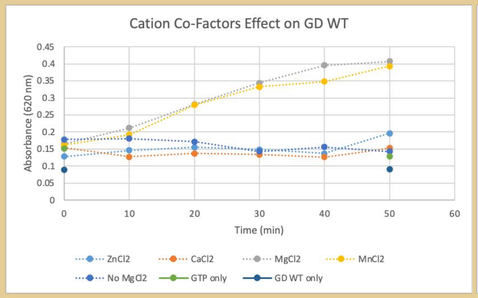
Methods

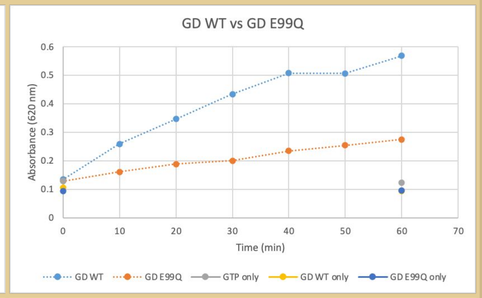
We made constructs of the GD of GBP2 (1-309) and generated the E99Q mutant by site directed mutagenesis. GBP2 GD and E99Q was overexpressed in E. coli cells. The cells were grown in LB broth containing 50 µg/mL kanamycin at 37°C at OD_{600} of 0.8, the cells were induced with 0.4 mM IPTG. Post induction, the cells were grown at 20°C for 16-20 hours. After incubation, the cells were harvested and resuspended in lysis buffer. The resuspended cell pellet was sonicated and centrifuged at 31,000 X g at 4° C for 45 min.The supernatant was poured into nickel column and recombinant protein was eluted with imidazole. After that, affinity, ion-exchange, and size exclusion chromatography were performed. We performed assays (malachite green) with different cation co-factors as well as different pH levels of GTPase assay buffers. We measured the phosphate production by reading the absorbances in a microplate reader spectrophotometer.

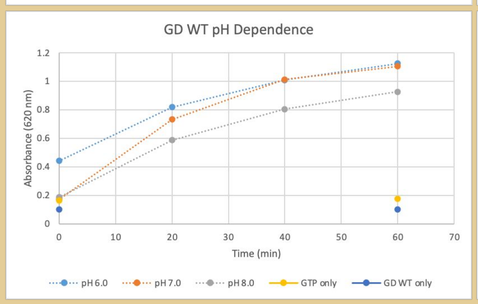
Site-Directed Mutagenesis

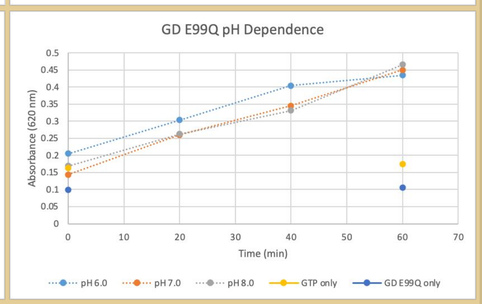


Results



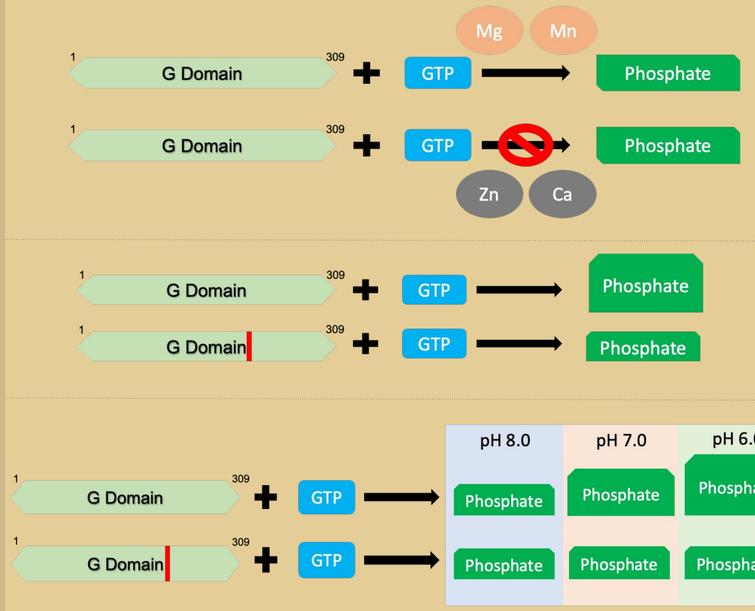






Conclusion

GD WT was only active in the presence of Mg^{2+} and Mn^{2+}. GD WT was more active than the GD E99Q. GD E99Q replaces the glutamate residue in GD WT with glutamine. The glutamate residue has a negative charge while the glutamine has a neutral charge. We believed that the GD WT and GD E99Q would show the same activity at acidic pH levels because the increase in positively charged ions will neutralize the negative charge on the glutamine residue. However, GD WT activity was fastest in pH 6.0 compared to pH 7.0 and 8.0. Therefore, GD WT showed pH dependency and GD E99Q did not. This shows that the negatively charged glutamate residue has a role in the activity of the protein, which future studies can investigate.



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References

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