Characterization of Guanylate Binding Protein 2

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Introduction

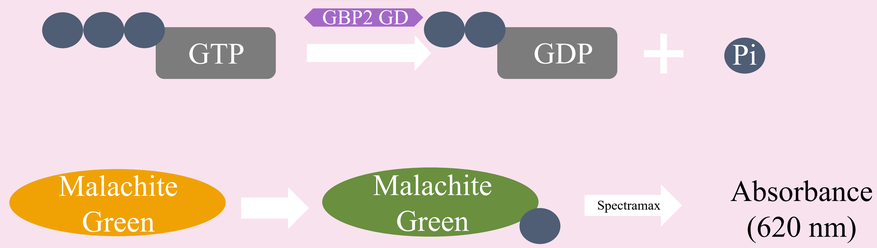
The innate immune response is the first line of defense to protect the body from pathogens and other foreign molecules. Protein signaling cascades are among the mechanisms used to properly respond to the pathogen, as well as activate other defense mechanisms. Guanylate Binding Protein 2 (GBP2) is a GTPase believed to be an important intermediate in such cascades, however the importance of specific structural elements and amino acid residues have yet to be determined.

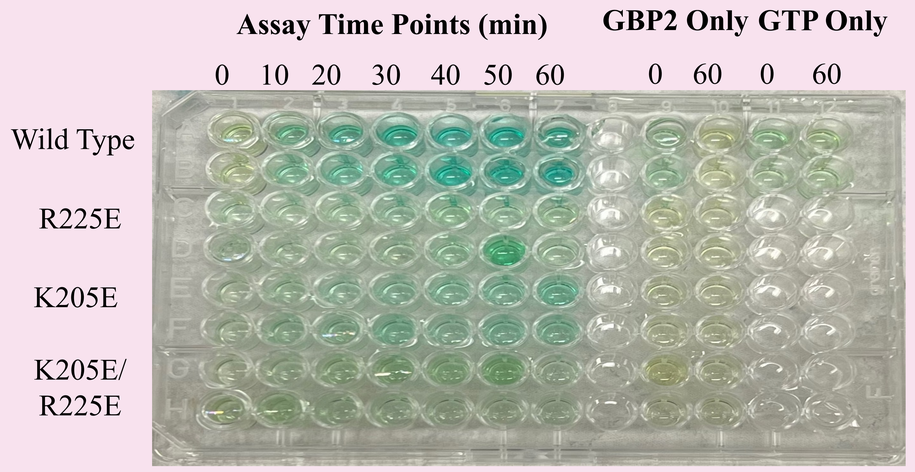
GBP2 General Structure

Methods

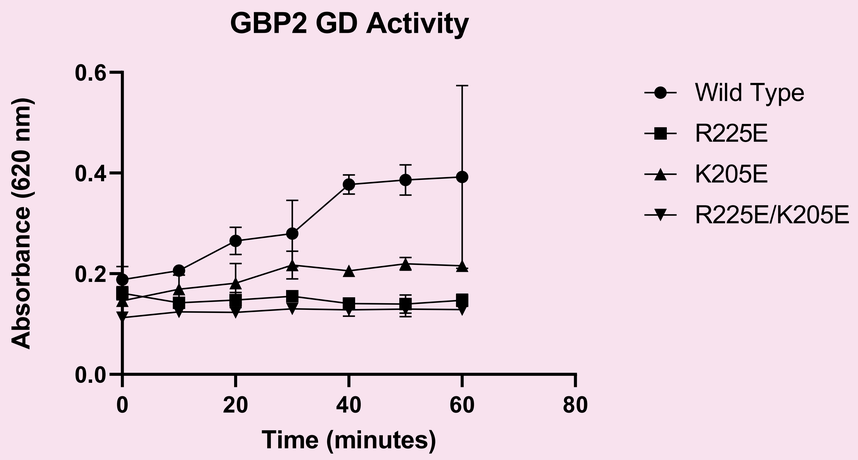
In order to study the activity of GBP2 and its mutants, the purified proteins must be obtained. This involves transforming competent E. coli cells, inducing their expression of GBP2 (which has been inserted into the lac operon), and then separating the pure protein from other cell debris. For this experiment, the wild type G domain of GBP2 was compared to mutants with differing amino acid residues at the 205th and/or 225th position in the primary structure. Each protein sample was combined with guanosine triphosphate (GTP), a molecule that is targeted by the GTPase activity of the GBP2 G domain. At each time point, a portion of the solution was added to Ethylenediaminetetraacetic acid (EDTA) in order to stop this GTPase activity. After 60 minutes, each mixture was combined with Malachite Green assay solution. After the 30 minute incubation period, the amount of absorbance of each sample at 620 nm was recorded.

Malachite Green Assay Analysis of GBP2 G Domain Activity





GBP2 G Domain Activity Assay Results



Discussion

These results show that upon mutation of the 205th and 225th amino acids in the primary structure of the GBP2 G domain, enzymatic activity is reduced. This implies that these residues play an important role in the overall activity and function of GBP2 within the cell. These residues are not located near the nucleotide binding site. Therefore, their ability to affect enzymatic function shows that there is a reciprocity in the long-range interactions that take place in facilitating protein function, rather than the active site solely acting on these areas. The fact that the activity of the K205E mutant was reduced, but not eliminated, indicates that the 225th residue may play a more important role in this interaction than the 205th residue.

Conclusion

The 205th and 225th amino acid residues in the GBP2 G domain demonstrate the ability to affect overall enzymatic function despite their lack of proximity to the protein's active site. Future research examining the impact of different mutations at the same residues might provide better insight into the two-way communication that occurs between them and the protein active binding site.

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