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Structural and Functional Characterization of Hyper-Phosphorylated GRK5 Protein Expressed From E. coli

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ABSTRACT

G protein-coupled receptor (GPCR) kinases (GRKs) are proteins in the cell responsible for regulating GPCRs located on the cell membrane. GRKs regulate active GPCRs by phosphorylating them at certain sites which causes them to stop normal signaling on the membrane. This ultimately affects how the cell responds to its environment. GRK5 is a kinase of particular interest due to its involvement in the pathology of diseases such as cardiac failure, cancers, and diabetes. Understanding the structure and function of GRK5 is essential for discovering ways to manipulate its behavior with these diseases, but not much is known about how GRK5 interacts with GPCRs. Although past studies used mammalian and insect cells to produce GRK5, this study aims to use E. coli cells to discover more about GRK5's structure and function. Previous studies revealed E. coli produce a hyper-phosphorylated version of the GRK5 protein. We attempted to crystalize this GRK5 produced from E. coli to reveal its conformation in a phosphorylated state that we hypothesize to be similar to its form when bound to GPCRs. We also tested the functionality of this GRK5 to reveal the effects of phosphorylation. We genetically edited the GRK5 gene in multiple E. coli samples to create GRK5 with less phosphorylation sites and tested activity levels by measuring the phosphorylation of GPCRs mediated by each GRK5 variant. Successfully creating an E. coli system for structural and functional analysis of GRK5 would help reduce time and costs for GRK5 research, and it could speed up the full understanding of the interactions between GRK5 and GPCRs.

KEYWORDS

G protein-coupled receptor kinase, GRK5, crystallography, phosphorylation, membrane binding