Structure-activity relationship of G protein-coupled receptor kinase 5 (GRK5) and its mutants

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G protein-coupled receptors (GPCRs) are the largest class of membrane receptors in eukaryotes. They regulate diverse functions like cardiac output, vision, and migration. GPCR kinases (GRKs) function as 'brakes' for GPCR signaling and thereby allow cells to adapt to changes in extracellular cues. Altered GRK activity is however implicated in cardiovascular diseases, diabetes, and cancer. In this study, we sought to define how GRK5 interacts with rhodopsin, a canonical model GPCR. After failing to capture the complex via chemical crosslinking, we introduced alterations into GRK5 to increase its affinity for rhodopsin such as swapping the N-terminus of GRK5 with that of GRK1, the native kinase for rhodopsin, and installing a K194R mutation to remove a crosslinking site that might compete for complex formation. We tested the mutants for kinase activity using a radiometric assay and for structural stability using a Thermofluor assay. We observed minimal changes in the kinase activity of these mutants, but deletion of the C-terminus decreased the structural stability of GRK5. We also studied the activity of GRK5 in the presence of c8-PIP_2, a soluble version of the common phospholipid PIP_2 known to activate GRKs and demonstrated inhibition. This inhibition is likely due to nonspecific binding of the compound to the catalytic domain of GRK5. Our findings confirmed that the mutants retain kinase activity and minimally altered structural stability, setting the stage for us to obtain a stable GRK5-rhodopsin complex for structural analysis.