Possible target for G protein antagonist: Identification of specific amino acid residue responsible for the molecular interaction of Gα 16 with chemoattractant C5a receptor.

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Abstract

Heterotrimeric G Proteins transduce ligand binding to a wide variety of seven transmembrane cell surface receptors into intracellular signals. The currently accepted model for the activation of G protein suggests that ligand-activated receptor accelerates GDP-GTP exchange reactions on the α subunit of the heterotrimeric G protein. At least seventeen distinct isoforms of the Gα subunit protein have been identified in mammalian organisms. Among them, the Gαq family consists of five members whose α subunits show different expression patterns. Gαq and Gα11 seem to be almost ubiquitously expressed, whereas Gα14 is predominantly expressed in spleen, lung, kidney and testis. Gα16 and its murine counterpart Gα15 are expressed in hematopoietic cells and has been shown to couple a wide variety of receptors to phosphoinositide-specific phospholipase C activity. Beta-isoforms of phospholipase C were shown to be activated by all members of Gαq family, i.e., Gαq, Gα11, Gα14 and Gα16 subunits either in reconstitution system or in experiments using cDNA transfection with intact Cos-7 cells.

There have been reports showing that a specific G protein-coupled receptor can interact with only a limited subset of the Gα subunits. Furthermore, the receptor may recognize the G protein as a heterotrimer, and the $\beta\gamma$ subunits can contribute to receptor specificity. Molecular and biochemical studies have identified specific regions or amino acids residue on the G protein-coupled receptor proteins that is essential in determining the receptor-G protein interaction specificity. It has been proposed that various structural determinants on the G protein were also involved in dictating G protein-receptor coupling selectivity. One of the clearly defined regions of interaction is in the extreme carboxyl terminal amino acid sequence of the Gα subunit. Several lines of evidence

support this assignment. The *unc* mutation of Gαs corresponding to a change in an amino acid residue adjacent to the carboxyl terminus can not be activated by β-adrenergic receptor stimulation. Functional analysis of chimeric Gαi/Gαs proteins showed that the carboxyl-terminal domain of Gαs specifies coupling of β-adrenergic receptors to the stimulation of adenylyl cyclase. Substitution of three amino acids at the extreme carboxyl terminus of the Gα subunit is sufficient to switch receptor specificity from that of the Gαq class to that of Gαi. Studies on the interaction between rhodopsin and transducin have demonstrated that the activated form of rhodopsin, i.e., metarhodopsin II, induces a conformational change in the a-transducin carboxyl-terminal decapeptide, further supporting the argument for involvement of the extreme carboxyl terminus of the Gα subunit in interaction with the receptor.

However, several studies suggest that the carboxyl terminus of the Gα subunit is clearly not the only structural determinant on the G proteins that is critical for dictating receptor-G protein coupling selectivity. The prevention of the interaction of transducin with rhodopsin by synthetic carboxyl-terminal peptides and monoclonal antobodies specific for the amino and carboxyl termini of a-transducin suggests that the amino terminus of the Gα subunit, as well as its carboxyl terminus, may constitute structural elements required for interaction with specific receptors. More recently, it was reported that deletion of six-amino acid extension at the extreme amino terminus of Gαq/11 subunits is sufficient to lose their constraining the receptor coupling selectivity, suggesting this amino terminus sequence element is critical for proper receptor recognition.

Other structural elements were proposed to be responsible for interacting with receptors. Experiment with an antibody directed against residues 110-119 within the α-helical domain of Gαs suggested that the epitope, the distal end of helix A and the beginning of the following loop, recognized by the antibody acts as one of the multiple contact sites for β-adrenergic receptor. Our previous study, in which numerous chimeras between Gα11 and Gα16 were examined for their ability to interact with the C5a receptor which couples to Gα16, but not to Gα11, suggested that the carboxyl-terminal 133 amino acids of Gα16 do not

alone account for its ability to interact with receptor, and that multiple regions of Gα16 are responsible for the functional difference between Gα11 and Gα16, including a segment encompassing residues 220-240 of Gα16. When aligned sequences of segment encompassing residues 220-240 are compared, there is a unique eight-amino acid extension (227-Ile-Ala-Leu-Tyr-Leu-Ala-Ser-Leu-234) in Gα16 subunit and its murine counter part, Gα15 that is distinct from Gα11 and Gαq subunits. We assume that this sequence variation may account for receptor specificity to a large extent.

With regard to the extension of this finding, we further characterize this unique eight-amino acid extension in Gα16 subunit to find critical amino acid residue which may play a key role in maintaining the specific coupling of Gα16 with C5a receptor.

For this purpose, Cos-7 cells are transiently transfected with specific cDNA clones encoding C5a receptors and various Gα11/Gα16 chimeric G proteins, and ligand-specific activation was determined by measuring changes in the levels of inositol phosphates that is the products of phosphatidylinositol hydrolysis.

In summary, we have defined single amino acid, Ala228 that confers upon Gα16 much of the C5a receptor specificity and it should contribute to a better understanding of the molecular basis of receptor-G protein interactions.