Structural Aspects of GPCR-G Protein Coupling

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G protein-coupled receptors (GPCRs) are membrane receptors; approximately 40% of drugs on the market target GPCRs. A precise understanding of the activation mechanism of GPCRs would facilitate the development of more effective and less toxic drugs. Heterotrimeric G proteins are important molecular switches in GPCR-mediated signal transduction. An agonist-activated receptor interacts with specific sites on G proteins and promotes the release of GDP from the G\(\alpha\) subunit. Because of the important biological role of the GPCR-G protein coupling, conformational changes in the G protein upon receptor coupling have been of great interest. One of the most important questions was the interface between the GPCR and G proteins and the structural mechanism of GPCR-induced G protein activation. A number of biochemical and biophysical studies have been performed since the late 80s to address these questions; there was a significant breakthrough in 2011 when the crystal structure of a GPCR-G protein complex was solved. This review discusses the structural aspects of GPCR-G protein coupling by comparing the results of previous biochemical and biophysical studies to the GPCR-G protein crystal structure.

Key words: GPCR, G protein, Structure

INTRODUCTION

In 1994, Alfred G. Gilman and Martin Rodbell were awarded the Nobel Prize in Physiology or Medicine for “their discovery of G-proteins and the role of these proteins in signal transduction in cells.” Eighteen years later, Brian K Koblika and Robert J Lefkowitz won the Nobel Prize in Chemistry for “studies of G-protein-coupled receptors (GPCRs)”

GPCRs are plasma membrane receptors that perform vital signaling functions in vision, olfactory perception, metabolism, endocrine system, neuromuscular regulation and CNS system (1).

Characterization of the structure and the dynamics of proteins are critical for a better understanding of the molecular basis of normal and abnormal physiological processes and drug development. Structural studies of GPCRs and their interaction with corresponding G proteins would provide...
important information on the biochemistry, biophysics and medicinal chemistry of these important therapeutic targets (6). Therefore, precise understanding of the structural mechanisms of GPCR-G protein coupling will leads to develop more effective and less toxic drugs with fewer side effects. Consequently, enormous effort has been put into the characterization of structures of GPCRs and G proteins. The crystal structures of various G proteins including Gαt, Gαi, Gβγ dimer and Gαβγ heterotrimer have been successfully obtained mostly in 90’s (7-16). Due to the technical difficulties of obtaining crystals of membrane proteins, however, few mammalian GPCR structures have been obtained mostly during past 6 years (17), and there is only one crystal structure of the receptor-G protein complex (18). This review will summarize the recent advance in the understanding of the structural aspect of G protein activation by GPCRs.

**GPCR-G PROTEIN INTERFACE**

High resolution crystal structures of various G proteins have been determined in their inactive (GDP-bound), transition (GDP·AlF-bound) and active (GTPγS-bound) states (7-16). These structural studies and other biochemical studies revealed the location of the nucleotide-binding pocket and the interface between Gα and Gβγ subunits (Fig. 1). The nucleotide-binding pocket is located between Ras-like domain and α-helical domain of Gα subunit surrounded by four flexible regions (p-loop, switch I, switch II, and switch III) (Fig. 1B). Ras-like domain hydrolyze GTP and provide binding sites for Gβ subunit (Fig. 1A). The N-terminus of Gα subunit is reported to be critical for the structure and function of Gα subunit (19-21) and is myristoylated or palmitoylated suggesting the role of this region in the attachment to the plasma membrane.

In 1988, Hamm et al. first identified that the C-terminus of Gα subunit is the critical binding site to the GPCR (22). This result, with the finding that the N-terminus of Gα subunit is myristoylated, allowed scientists to orient G proteins relative to plasma membrane and GPCRs (Fig. 2A). Since late 80’s, numerous efforts have been put to find the G protein-GPCR interface. The involvement of C-terminus of Gα subunit has been extensively investigated by various biochemical and biophysical studies including peptide competition assays (22,23), mutation studies (24-26), and crosslinking experiments (3,27,28). The crosslinking experiments provided that TM6 or intracellular loop (ICL) 3 of a GPCR is the major contact sites for the C-terminus of Gα subunit (27,28). Two different crystal structures of a GPCR with a Gα subunit C-terminal peptide confirmed the interaction of C-terminus of G protein with a GPCR (29,30). These two crystal structures agreed that C-terminus of Gα subunit is inserted into the cytoplasmic pocket surrounded by TM domains of a GPCR (29,30).

Besides the C-terminus, there are other regions that have been reported to interact with GPCRs. Crosslinking experiments suggested that αN of Gα subunit contacts with GPCRs (27,31,32). However, these studies are conflicting each other in terms of which regions of GPCR interact with αN of Gα subunit. α2-adrenergic receptor interacts with αN of Gα subunit with its ICL3 (31); Rhodopsin with its ICL1 (32); M3 muscarinic acetylcholine receptor with its ICL2 (27). α4/β6 loop of Gα subunit is also numerous reported to interact with GPCRs by mutation studies (33-35) and crosslinking experiments (27). A crystal structure of Gα subunit with ICL3 peptide showed that ICL3 interacts with α4/β6 loop (35), whereas the crosslinking experiment suggested that helix 8 of GPCR interacts with α4/β6...
GPCR-mediated G Protein Activation

loop (27). Few studies suggested that α3/β5 loop and β2/β3 loop also interact with GPCRs (5,27,36,37). In spite of these extensive efforts to identify the GPCR-G protein interface, it has been debating, until recently, what are the exact interface and the orientation of GPCR-G protein coupling.

The breakthrough in understanding the nature of GPCR-G protein interaction was made by the first crystal structure of the β2 adrenergic receptor (β2AR)-Gsβγ complex (Fig. 2) (18). In the structure, Gα subunit is in its nucleotide-free empty intermediate state. The C-terminus of Gα subunit is inserted into the cytoplasmic pocket of β2AR, which is consistent with previous biochemical studies and GPCR-Gα C-terminal peptide crystal structures. The crystal structure shows two other major contact sites; αN-β1 hinge and α4/β6 regions of Gα subunit, which are consistent with previous biochemical and biophysical studies. However, α3/β5 loop and β2/β3 loop of Gα subunit, which were reported to contact with a GPCR, did not interact with GPCR in the crystal structure. This inconsistency implies that there might be more than one conformation of the GPCR-G protein complex or different types of G protein interact with GPCR at different orientations. More studies are needed to determine the interface at different conformational stages or in different GPCR-G protein pairs.

**CONFORMATIONAL CHANGES OF G PROTEIN BY GPCR**

The long un-resolving question was how a GPCR activates G proteins and release GDP from a Gα subunit. Since the nucleotide-binding pocket is about 30 Å away from GPCR-interfacing sites of Gα subunit, the possible conformational shifts of Gα subunit during its activation by GPCR has been suggested. The recent crystal structure of β2AR-Gosβγ complex gave profound information on how a GPCR activates G proteins (Fig. 2B) (18).

The most striking feature of the crystal structure is the large movement of the α-helical domain of Gα subunit (Fig 2B). When compared to the GDP-bound inactive state of Gα subunit, the α-helical domain in receptor-bound nucleotide-free state is moved away from its original position, which opens up the nucleotide-binding pocket like a clamshell shape. Electron microscopy experiments also visualized the β2AR-Gosβγ complex, and this approach suggested that helical domain occupies various positions relative to the Ras-like domain, receptor and Gβγ (38). Earlier study with NMR showed that the receptor-bound empty pocket state of the heteromeric Gα subunit is conformationally dynamic (39). This feature is also predicted by Van Eps et al. shortly before the crystal structure came out (40). Van
Eps et al. measured the distance between chemical probes positioned on the Ras-like and α-helical domains of Gα subunit using double electron-electron resonance spectroscopy, and they observed a large increase in the distance between the domains upon receptor binding. Despite of this big movement, the crystal structure suggested that core of α-helical domain did not undergo conformational changes.

Other major sites of conformational changes are C-terminus and its adjunct α5 helix of Gα subunit (Fig. 3). In crystal structures of Gα subunit, the C-terminus is disordered and consequently not present in the deposited crystal structures (7-16). Many biophysical studies including NMR (41-44) or X-ray crystallography (29,30) suggested that the C-terminus forms helix upon coupling to the receptor. The crystal structure of β2AR-Gαsβγ complex also showed the C-terminus of Gα subunit as an α-helix confirming the previous findings (Fig. 3) (18). What is interesting about the C-terminus is that it is connected to α5 helix (Fig. 1B and Fig. 3). The α5 helix links the C-terminus to the β6-α5 loop that contains the conserved nucleotide-binding TCAT motif (Fig. 1B). In the crystal structure of β2AR-Gαsβγ, α5 helix is pull-up towards to the receptor resulting in about 6 Å-movement away from the nucleotide-binding pocket (Fig. 3) (18). Previously, Oldham et al. proposed the receptor-induced rigid-body movement of α5 helix upon by using electron paramagnetic resonance spectroscopy (45). Recently two hydrogen-deuterium exchange mass spectrometry (HDX-MS) studies showed conformational changes in the N-terminus of α5 helix near the β6-α5 loop (46,47). By changing the solvent from H2O to D2O, hydrogens in the amide bonds are switched to deuterons. The pattern of deuterium labeling provides information about the solvent accessibility and thus protein conformation. With this method, it was established that receptor binding increases the solvent accessibility of the N-terminus of α5 helix, which implies that this region becomes more flexible or dynamic. Increased flexibility and movement of α5 helix would affect the stability of nearby nucleotide-binding pocket and promote the release of GDP. The crucial role of α5 helix in transferring the structural changes of receptor-binding region to the nucleotide-binding pocket has also been well-described in previous mutation studies (48-51) in which the insertion of poly glycine (48) or proline (50) inhibited the signal transduction of activated receptors.

Similar to α5 helix, the strand of αN helix-β1 sheet region of Gα subunit may translate receptor-induced conformational changes to the nucleotide-binding pocket. αN/β1 hinge region is another major GPCR-G protein contact site in the crystal structure, and β1 sheet is connected to the p-loop that interacts with the phosphate group of GDP (Fig. 3) (18). The crystal structure does not show significant conformational changes in either αN helix or β1 sheet. However, fluorescence labeling study detected conformational changes of αN helix (52), and HDX-MS study detected increased solvent accessibility of β1 sheet suggesting the increased flexibility or dynamics in this region (47). This discrepancy

Table 1. X-ray crystal structures used in the Figures

| Structure                     | PDB number | State                  | Reference |
|-------------------------------|------------|------------------------|-----------|
| Gαβγ heterotrimer with GDP     | 1GOT       | Inactive               | 8         |
| Gαα subunit with GTPγS        | ITND       | Active                 | 13        |
| β2AR-Gs                       | 3SN6       | Receptor-coupled intermediate | 18          |
| Gαβγ heterotrimer with GDP     | 1GP2       | Inactive               | 10        |
may be due to the nature of crystallization of a protein which leads the protein in the energetically most stable state. In the solution environment, and αN helix-β1 sheet may be flexible or dynamic.

In the crystal structure and HDX-MS experiment, Gβγ subunits do not contact with β2AR and do not undergo conformational changes upon receptor binding (18,47). However, Gβγ subunits have been implicated to interact with the receptor or modulate the activation of G proteins. Several studies suggested that GPCR interacts with C-terminus of Gγ subunit (53), and GPCR uses Gβγ subunits to distort the Gα subunit nucleotide-binding pocket resulting in the release of GDP (54,55). The crystal structure is a snapshot of stable nucleotide-free β2AR-GαzGβγ complex, and it does not show the initial coupling modes. The involvement of Gβγ subunits in G protein activation might be the early stage of receptor coupling and needs more investigation.

**PERSPECTIVES**

Last twenty five years, the continuous efforts in biochemistry, biophysics, molecular and cell biology provided the basic information of structural mechanism of GPCR-mediated G protein activation. Numerous studies support the model that C-terminus and αN-β1 hinge region of Gz subunit are the major contact sites, and interaction of the receptor to these sites transmit conformational changes to nucleotide-binding pocket of Gz subunit. However, there are still more needed to be investigated. One of the great interests in this field is how GPCRs recognize specific G proteins. Biochemical, biophysical and mutational studies suggested several regions of Gz subunit or GPCR as factors that determines the selectivity, but without solid conclusion. More crystal structures with various GPCR-G protein pairs will help to understand the specificity of GPCR-G protein coupling. Second, GPCRs do not only act as a monomer but also as oligomers (56-58). What would be the coupling mode between G proteins and oligomerized GPCRs? Would GPCR oligomerization affect the G protein selectivity? Third, recent studies suggested for the preassembled complex (27,59-62). The preassembly of GPCR-G protein can speed-up the signal transduction and provide G protein selectivity of GPCRs (63). Therefore, understanding the conformation of preassembled GPCR-G protein complex will provide important aspect of GPCR-G protein coupling. Finally, although a number of studies suggested models for the conformational shift from receptor binding site to the nucleotide-binding pocket to explain the structural mechanism of GPCR-mediated G protein activation, we do not have a solid answer yet. A crystal structure is a snap shot of one conformation of the molecules. Therefore, more biochemical or biophysical studies are needed to understand this fundamental event of G protein activation.

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