REVIEW

A STRUCTURAL APPROACH TO G-PROTEIN SIGNALING MECHANISMS: α-SUBUNITS

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ABSTRACT

Guanine nucleotide binding proteins regulate a variety of physiological processes, including sensual perception, protein synthesis, hormonal regulation, vesicular and nuclear transport, cell growth and differentiation. They act as molecular mediators, cycling between inactive guanosine diphosphate (GDP)-bound and active guanosine triphosphate (GTP)-bound states. G-proteins are composed of three subunits: α, β, γ, where specificity mainly determined by α.The α-subunit consists of two domains: GTPase domain and α-helical domain. Activation results in conformational changes around so called switch I, II and III regions in GTPase- domain. Interaction of the receptor with the carboxyl terminus of α is clearly important. Carboxy terminus is also shown to be important in effector interaction.

Keywords: Heterotrimeric G-proteins, 3 D structure

G-PROTEİN SİNYAL İLETİ MEKANİZMALARINA YAPISAL BİR YAKLAŞIM:ALFA ALTBİRİMİ

ÖZET

Guanin nükletit bağlayıcı proteinler duyusal algılama, protein sentezi, hormonal düzenleme, salgı kesecikleri ve çekirdek taşınımı, hücre büyümesi ve farklılaşmasını da içine alan birçok fizyolojik sürecin düzenlenmesinde rol oynarlar. Bu proteinler (GDP)-bağlı dinlenim durumuyla (GTP)-bağlı aktif durumları arasında bir döngü geçirerek moleküler aracılık görevlerini yerine getirirler. G-proteinleri 3 altbirimden oluşmaktadır: α, β, γ. G-proteininin özgünlüğü α altbirimi tarafından belirlenir. α-altbirimi de iki bölgeden oluşur: GTPaz bölgesi ve α-heliks bölgesi. Aktivasyon GTPaz bölgesindeki anahtar I, II ve III olarak adlandırılan bölgelerde yapısal değişikliklere yol açar. Hem reseptör hem de efektörlerle etkileşimde karboksil ucunun önemli olduğu gösterilmiştir.

Anahtar Kelimeler: Heterotrimerik G-proteinleri, 3 boyutlu yapı

INTRODUCTION

Guanine nucleotide binding proteins regulate a variety of physiological processes, including sensual perception, protein synthesis, hormonal regulation, vesicular and nuclear transport, cell growth and differentiation. This superfamily includes members of small monomeric Ras-related proteins, the heterotrimeric G-proteins and the factors involved in protein synthesis. They act as molecular mediators, cycling between inactive guanosine diphosphate (GDP)-bound and active guanosine triphosphate (GTP)-bound states[1]. Signals carried by the majority of polypeptide hormones, all monoamine neurotransmitters, prostaglandins and ions such as Ca^{2+} and K^{+} are transmitted to their target cells through membrane receptors belonging to the GPCR (G-protein coupled receptors) superfamily that share a common structural and functional motif (seven transmembrane helices) and a common transduction mechanism (coupling to G-proteins). Therefore, G proteins play a key role in relaying signals from the plasma membrane to intracellular effectors, thereby triggering different cellular responses[2].

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A common structural feature of the heterotrimeric G proteins is their three subunits: a large a-subunit of 39-46 kDa, a β-subunit of 37 kDa and a γ-subunit of 8 kDa. The α-subunit has a binding site for GTP and GDP and an intrinsic GTPase activity. The β- and γ- subunits exist as a covalently bound complex and are only active in this form. All three subunits show great diversity: currently at least 20 different genes for α-subunits, 5 for β-subunits and 12 for γ-subunits are known in mammals. Distribution of G-proteins is heterogenous : some G-proteins are ubiquitous, whereas others only occur in specialized tissue.

Specificity of G protein function is mostly determined by the α-subunit: the α-subunit carries out the specific interaction with receptors preceding in the signal chain and with the subsequent effector molecules [3]. Recent studies have also shown that G β γ-subunits also interact with their specific effectors such as PLCa isoforms and type I adenylyl cyclase[4].

Overall Structure:

The α-subunit consists of two domains:

1) A domain similar to ras p21, a monomeric GTP-binding protein responsible from the signalling processes leading to cell multiplication and differentiation upon activation by receptor tyrosine kinases. This domain is called the "GTPase" domain since it is common in all GTPases.
2) An additional a-helical domain that is unique to heterotrimeric G-proteins.
The GTPase domain consists of six-stranded β-sheets surrounded by five a-helices (Fig. 1). This domain contains common regions involved in GTP-binding of all GTPases, the phosphate binding loop (P-loop) , the Mg^{2+}-binding residues and two guanine ring binding sites[4].

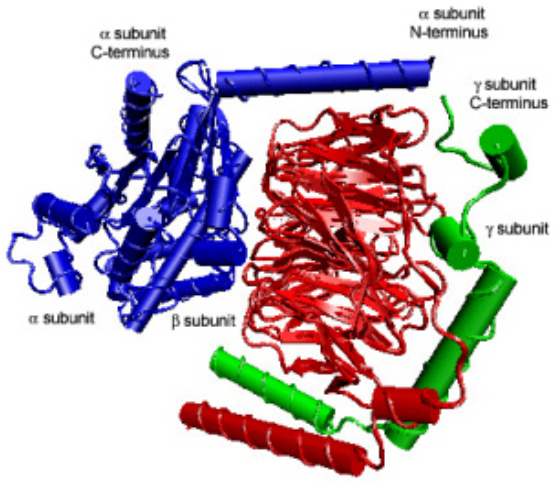

Fig. 1: Overall structure of heterotrimeric G-proteins (1GOT from Protein Data Bank)

The α-helical domain which can be regarded as a large insertion to the GTPase domain is connected to the GTPase domain by two linker regions. Secondary structure of the unit is completely helical and a long central α-helix (αA) is surrounded by 5 other short α-helices (αB- αF).

Structural Basis of the Activation of the α-Subunit:

The main conformational change that takes place during the transition form the inactive (GDP-bound) to the active (GTP-bound) form is a change in the positions of phosphates and Mg^{2+}.

All guanine-nucleotide binding proteins have five consensus sequences around the nucleotide binding pocket: the GXGCCGKS motif (where X is any amino acid) in the β, γ phosphate binding loop (P-loop); the N/TKXD motif responsible for the interaction with the nucleotide base; the Mg^{2+} - binding sequence DXXG ; a second guanine ring binding site , TCAT, that is homologous to the ras p21 sequence TSAK.

In both active and inactive conformations, the guanine nucleotide ring and the ribose unit stay deeply buried between the GTPase and α-helical domains, while Mg^{2+} and b-phosphates of GDP move from a surface-exposed position inward to a region protected between the domains.

This movement is a result of conformational changes around the γ-phosphate of GTP , localized to three regions called switch I, II and III. Switch 1 spans through Ser 173-Thr183, including Arg 174 and Thr 177. Arg 174 is the possible site for ADP-ribosylation and plays a key role in stabilizing the transition state and hydrolysis of GTP. The region referred to as switch 2 includes Gly 199 and α-helix 2. Switch 3 is a loop segment spanning residues between Asp 227 - Arg 238[4].

These residues have been derived from X-ray structural analyses of transducin (G αt) in complex with GTP γS, GDP and GDP.AlF_4- [5,6,7]. After activation, switch I moves toward the g phosphate, bringing the side chain of Thr 177 (in Gat) close to the γ-phosphate and Mg^{2+}. Mg^{2+} is required both to locate γ-phosphate properly and to stabilise guanine nucleotide complex [8]. The hydrogen bond established between the main chain NH of Gly 199 and γ-phosphate of GTP strecthes and rotates the switch II, which includes both the loop containing Gly 199 and helix α2.The switch III region has no direct interactions with γ-phosphate, but by interaction with switch II helps to fix the new conformation (Fig. 2)[8].

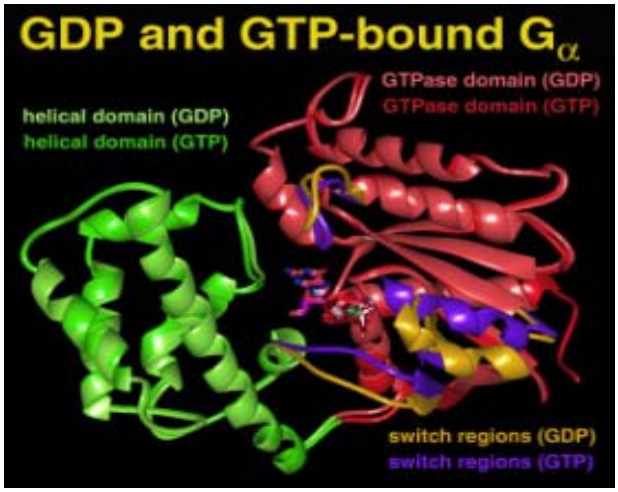

Fig. 2: Conformational changes around switch I and II during transition from inactive GDP- to active GTP-bound forms (http://www.bmb.psu.edu/faculty/tan/lab/gallery-proteins.html)

Interaction with β γ-subunits:

The β-subunit shows a propeller structure composed of seven motifs, each comprising four-stranded antiparallel sheets. Each of the seven motifs is called a β-blade. Contact region of α-subunit is opposite to where G γ subunit binds; so there is no contact between α- and γ-subunits.

There are two major contact sites between G α and G β. The first is the myristoylated or palmitoylated amino terminus of G α. The larger and more important site is the interface between the G α switch regions I and II and one of the G β's electronegative faces[9]. The N-terminal of isolated G α is disordered, but it becomes stabilized when it interacts with β-subunit. The overall structures of both α- and β γ-subunits have been preserved, except for the N-terminal region of the α-subunit and changes around switch regions[10].

The role of the switch II region is especially important in interaction with β γ. Conserved residues like Gly 199 and Gln 200 stabilize the transition state for GTP hydrolysis. In the β γ-bound form those switch regions lose their flexibility and firmly bind GDP. The replacement of GDP for GTP causes local but crucial conformational changes around switch I and II, thereby providing the appropriate conditions for release of G α from G β γ[8].

Interaction with Receptors:

How receptors interact and catalyse activation of G proteins is still unclear, however it is known that sequences in the second and third intracellular loops of receptors are important for selectivity and affinity for G proteins[11].

It is likely that direct interactions of receptor with the G protein α subunit are necessary to facilitate dissociation of GDP. The receptor possibly interacts with amino terminus of G α, thereby distorting the α-/ β γ- surface[11].

Interaction of the receptor with the carboxyl terminus of α is clearly important. The extreme C-terminal end of G α, the last five residues in particular, has been implicated as an important mediator of receptor-G protein interaction. Antibody studies targeting G α C-terminal sequences effectively block receptor-G protein interaction (Fig. 3). However, some reports indicate that G-proteins with identical residues at C-terminus sometimes couple to different receptors.

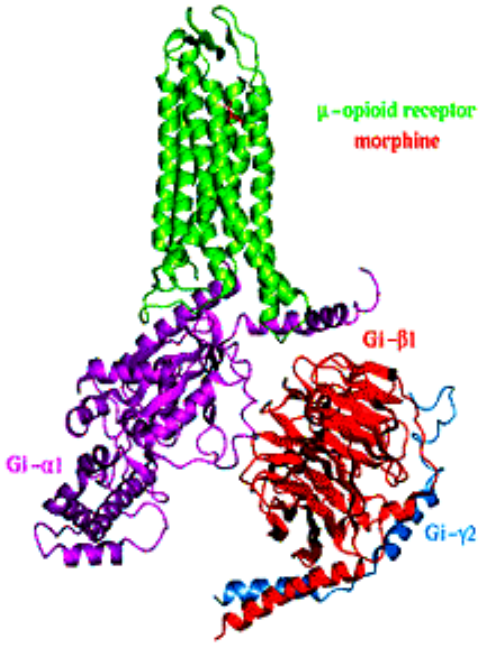

Fig. 3: A model of the complex of the a2a-adrenergic receptor with Gai using the rhodopsin crystal structure (1f88), the crystal structure of a G-protein trimer(1gp2) and the NMR structure of the C-terminal fragment of transducin in complex with photoactivated rhodopsin (1aqg) as the structural templates (http://mosberglab.phar.umich.edu/ projects/proj7.php).

Key residues in α2 and a4 helices have been found to be involved in G-protein coupling to its receptors. Mutations in residues in tha α4/ α5 loop, especially residues at the C-terminal end of the α4 helix, have suggested a role for these residues in receptor-mediated activation of G α subunits. On the other hand, a number of biochemical studies have also implicated the N-

terminus of G α subunits in receptor interactions.[12]. Segments of G β- and G γ-subunits may also contribute to the receptor interacting surface of heterotrimers[13].

Interaction with Effectors:

Each G α-family activates its specific effectors. Cocrystallization studies of G αs and the catalytic domains of adenylyl cyclase (AC) have identified specific contact regions within G αs at the α2-helix and the α3- β5 loop[14]. In addition, the α4- β6 loop of G αs also plays a role in adenylyl cyclase activation[15].

Using G αi/G αs chimeras, it was demonstrated that the carboxyl terminus of G αs, specifically the region G αs 235-356, contained the adenylyl cyclase activating region and mutation of residues within this region caused a decrease in the ability to stimulate adenylyl cyclase[16].

Studies using G αi2/G αs chimeras indicated to three distinctive regions involved in effector activation: residues 236-240 (corresponding to G αt α2/ β4); residues 276-285 (corresponding to G αt α3/ β5) and residues 349-356 (corresponding to G αt α4/ β6)[15].

Structural determinants for regulation of cGMP phosphodiesterase (PDE) by a G αt/i chimera and RGS9 were reported in an X-ray crystallographic study at 2.0 Å resolution by Slep et al. [17]. This study further confirmed that switch II and α3 domains are major sites of interaction between P γ (γ subunit of phosphodiesterase) and G α and that P γ also interacts with the G α α4/ β6 loop[18].

Future Prospects for G-protein Signaling:

The determination of crystal structures of G-proteins in various activation states have revealed the activation mechanism of G-proteins in detail. Comparison of G αt-GDP and G αt-GTP γS crystal structures has indicated to the presence of three flexible regions, which become more rigid and ordered upon GTP activation. The structures of extreme N- and C-terminals are not well known, since they are generally disordered or removed to prevent posttranslational modifications [13]. On the other hand, functional studies suggest that those regions play a key role in the activation process, especially on interaction with receptors and effectors.

The most important questions that remain to be answered in this respect are the structures of the extended complex of receptor/G α.GDP/G β γ, G α.GTP effector and G β γ/effector [9]. Difficulties in crystallization of membrane proteins have hampered these studies. The structure of bovine rhodopsin, a G protein coupled receptor was determined by Palczewski et al. only four years ago [19]. Solution-based techniques such as electron paramagnetic resonance, small angle X-ray scattering analysis, circular dichroism or high-resolution nuclear magnetic resonance are additional structural tools that can be employed to analyse protein-protein interactions when crystallisation is difficult for the protein of interest. These tools can be used in combination with biochemical and mutational approaches to provide deeper understanding of those mechanisms.

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