G-Protein Subunit Dissociation Is not an Integral Part of G-Protein Action

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G Proteins as Signal Transducers

The concept that a guanosine triphosphate (GTP) binding protein (or G protein) is a transducer of receptor-to-effector signal transduction was formulated in the 1970s for hormone-dependent adenylyl cyclase.[1] It has been shown that binding of the hormone to the receptor triggers exchange of guanosine diphosphate (GDP) for GTP on the G protein, thereby converting the G protein from the inactive conformation to the activated form. In its GTP-bound state, the G protein activates adenylyl cyclase to produce cyclic adenosine monophosphate (cAMP). Hydrolysis of the GTP terminates the signal. Since the rate of GTP hydrolysis is approximately 100 times slower than the rate of production of cAMP by the catalytic unit, one hormone–receptor complex is able to generate many cAMP molecules per minute. Another amplification step is between the receptor and the G protein, since the hormone–receptor complex interacts transiently with the G protein and dissociates from it, once the G protein has been loaded with GTP. The receptor then interacts with other G protein molecules. The amplification factor of this step has been estimated to be about 10 in the β-adrenergic-dependent adenylyl cyclase system.[2] For the light-dependent activation of cyclic guanosine monophosphate (cGMP) dependent phosphodiesterase by rhodopsin, it was found that each activated molecule of rhodopsin activates approximately 300 phosphodiesterase molecules.[3] This mechanism of activation is known as "collision coupling".[2] Many other G-protein-coupled receptor systems have since been discovered, but the main features of the signaling pathway remain essentially similar to those initially described for hormone-dependent adenylyl cyclases. The prevailing dogma for the action of G-protein-coupled receptors is still based on the detailed biochemical studies performed on hormone-dependent adenylyl cyclase.

The Dogma

The action of heterotrimeric G proteins is generally discussed in terms of Gα from Gβγ subunit dissociation (Figure 1 A). Let us review the evidence for the prevailing dogma, which depicts G-protein activation by dissociation.

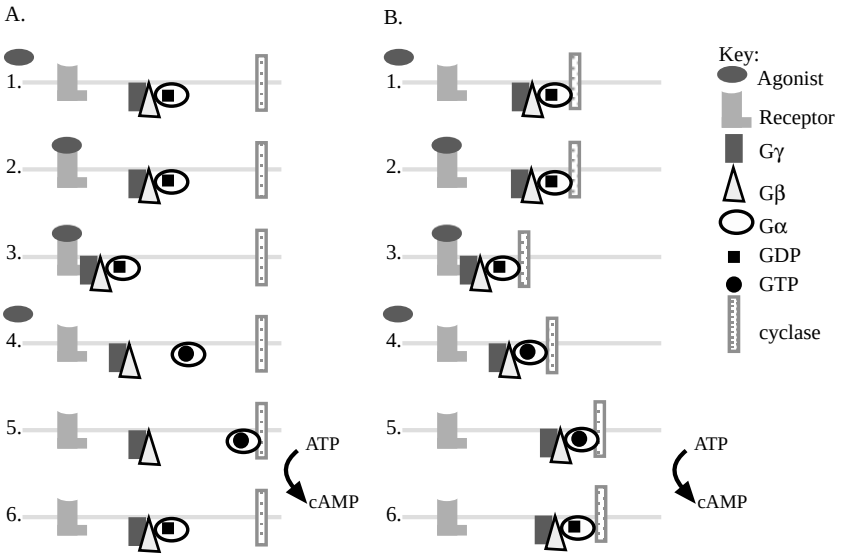

Figure 1. *Models of adenylyl cyclase activation. A) The G-protein-dissociation model. 1. The receptor, the G protein, and the catalytic unit are all separate entities. 2. The hormone binds to its receptor. 3. Subsequent to hormone binding, the affinity of the receptor for the G protein is increased and the G protein loaded with GDP forms a complex with the receptor. 4. Complex formation is followed by a conformational change which opens up the nucleotide binding site and allows the exchange of the bound GDP for cytoplasmic GTP, which is in excess. The GTP-bound G protein dissociates from the receptor and separates into the Gsα(GTP) and Gβγ subunits. 5. The Gsα(GTP) seeks and binds to the catalytic unit of adenylyl cyclase, thereby activating it. 6. Upon GTP hydrolysis, the Gsα(GDP) dissociates from the catalytic unit and reassociates with Gβγ. B) The G-protein-associated model. There are two differences between this model and the one depicted in (A): The G protein remains associated with the adenylyl cyclase throughout the activation cycle and it does not dissociate into Gsα(GTP) and Gβγ. In both models the receptor acts catalytically.*

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1) The nonhydrolyzable GTP-analogue GTPγS induces the dissociation of G proteins to give Gα(GTPγS) and Gβγ.[1, 4]
2) The addition to isolated membrane preparations of purified Gα subunits bound to the nonhydrolyzable GTP-analogues GTPγS or GPPNHP induces full activation of downstream effector enzymes such as adenylyl cyclase.[1, 4]
3) The over-expression of Gβγ subunits or their addition to the experimental system opposes the activation caused by Gα subunits in a number of experimental systems.[4, 5] It should be noted that the β and γ subunits can only be separated from each other at high concentrations of urea or guanidine hydrochloride. Thus one can treat the β and γ subunits as a single entity.
4) The subunits of the retinal G-protein transducin dissociate from the membrane and from each other when activated by rhodopsin.[6]

The studies with transducin were the only direct biochemical experiments showing subunit dissociation which utilized GTP. Since transducin was the first G-protein system to be analyzed, it was tacitly assumed that all G proteins dissociate upon the binding of GTP. Regarding other G proteins, subunit dissociation has not been demonstrated to occur upon GTP binding, and the evidence for it is mainly indirect, as summarized above. Although it is clear that G-protein subunits *can* dissociate under extreme experimental conditions promoting activation, it is far from clear that this is what actually happens in cell membranes under physiological conditions. It seems to us that the question of whether a G protein actually dissociates upon GTP binding needs to be addressed in each specific case. Since both Gα and Gβγ subunits possess effector functions, it is important to know in each case whether these two entities separate and act at distant targets or whether they remain attached and act simultaneously on the same target or targets in close proximity. We believe that for many G proteins the data is more consistent with the model that we proposed in 1988[2] and that the changes between the inactive GDP-bound and active GTP-bound states can be explained by changes in the conformation of the complex (Figure 1 B).

Kinetic Evidence against G-Protein Dissociation

In 1984, the key experiments which led to the dogma presented in Figure 1 A were published.[2, 4] These studies concerned Gs, which activates adenylyl cyclase, and Gi, which inhibits adenylyl cyclase. It was shown that GTPγS leads to the dissociation of Gs, in the presence of 50 mM Mg^{2+} ions, 150 mM NaCl, and the detergent Lubrol PX. It was also shown that Gsα bound to GTPγS activated the adenylyl cyclase moiety fully, in the complete absence of the Gβγ subunits. Furthermore, it was shown that the addition of Gβγ subunits to adenylyl cyclase inhibited the enzyme. These findings were taken to mean that GTP must also induce Gs to dissociate into Gsα(GTP) and Gsβγ. The actual experiment to verify this assumption, utilizing GTP, was not performed until the late 1990s (see below). Nevertheless, the assertion was accepted and over time became dogma for all G proteins. The model presented (Figure 1 A) also offered a molecular explanation for the inhibition of adenylyl cyclase by the inhibitory G protein, Gi. It was suggested that Gi dissociates into GTP-bound Giα and Gβγ. Gβγ then competes with Gsα(GTP) for the catalytic unit and so causes inhibition of the enzyme.[4] This dissociation model therefore offers not only a molecular explanation for the activation of adenylyl cyclase but also for its inhibition.

The subunit-dissociation model for adenylyl cyclase activation is based on two main assumptions: 1) The G protein, the activating receptor, and the adenylyl cyclase are separate physical entities; 2) upon hormone binding, the hormone-bound receptor interacts with the GDP-bound G protein and induces the exchange of GTP for GDP. The GTP-bound G protein dissociates from the receptor and splits into Gsα(GTP) and Gsβγ (Figure 1 A).

Kinetic studies have indeed demonstrated that the receptor acts catalytically. The evidence for the catalytic role of the receptor comes from two experimental approaches. Both measure the rate of β-agonist-dependent adenylyl cyclase activation in the presence of the nonhydrolyzable GTP-analogue GPPNHP. This strategy allows one to measure the "on" activation rate without the "off" GTPase step. In native membranes, a gradual reduction in the number of β-adrenergic receptor molecules, by means of an affinity label directed against the receptor, did not diminish the maximal level of activation of Gs or cyclase attained, but did proportionately reduce the rate of adenylyl cyclase activation. The rate of cyclase activation correlated linearly with receptor concentration.[7] When the β-adrenergic-dependent adenylyl cyclase system was reconstituted from purified Gs, purified β-adrenergic receptor, and purified adenylyl cyclase, similar results were obtained: The total pool of enzyme was activated in the presence of agonist and GPPNHP, and the rate of activation correlated linearly with the concentration of receptor in the reconstituted vesicles.[8, 9] It should be noted that the kinetic experiments were conducted under a very wide range of concentrations of β-adrenergic receptor, Gs protein, and adenylyl cyclase. Thus, the rate-limiting step indeed seems to be the interaction between the agonist-bound receptor and the G protein.

The catalytic nature of the receptor is the only aspect of G-protein activation which is shared by both the subunit-dissociation model (Figure 1 A) and our original model. Our model, however, differs from the subunit-dissociation model in two important aspects: Firstly, the Gs protein is not a separate entity from the catalytic moiety but is tightly associated with it and, secondly, Gs does not dissociate into the Gαs and Gβγ subunits upon activation (Figure 1 B).[2]

The subunit-dissociation model does not take account of the experimentally observed kinetics of adenylyl cyclase activation. These kinetics were found to be first order, where the pseudo-first-order constant was directly proportional to receptor concentration.[7–9] A comprehensive kinetic analysis of the different models for receptor→Gs→adenylyl cyclase activation[10] revealed that the only model which is compatible with the observed first-order kinetics of cyclase activation is the one which assumes that Gs is always coupled to the catalytic unit.[10] All other mechanisms would yield a complex kinetic pattern of adenylyl cyclase activation.[10] Thus the assumption that Gs is

separate from the catalytic unit of adenylyl cyclase must be incorrect. Indeed, Gs is firmly attached to the catalytic unit (see below). The separation of Gαs from Gβγ would also result in complex kinetics of enzyme activation,[10] which are not experimentally observed.

Thus, both of the assumptions on which the subunit-dissociation model rests (namely, that the receptor, G protein, and effector are separate physical entities and that the G protein itself dissociates into its α and βγ components) are incompatible with the observed experimental data.

Biochemical Evidence against G-Protein Dissociation

Biochemical evidence against G-protein dissociation has come from a number of experimental approaches. Purification of adenylyl cyclase in its inactive GDP-bound form and its GPPNHP-activated form yielded complexes of the same molecular weights.[12] In both cases, the G protein copurified with the catalytic unit on both anion-exchange and molecular-sieve columns. These results show clearly that Gs is tightly coupled to the catalytic unit, both in the inactive GDP-bound state and in the GPPNHP-activated state. Further analysis of GPPNHP-activated adenylyl cyclase in its highly purified form demonstrated that the Gs heterotrimer remained intact, with a stoichiometry of 1:1 between the α and the β subunits.[13] Furthermore, the stoichiometry between Gs and the catalytic unit was 1:1; this confirmed earlier reports.[13]

As stated above, G-protein dissociation has been demonstrated only for G proteins complexed with GPPNHP or GTPγS under extreme conditions (in the presence of detergent or high concentrations of Mg^{2+} ions or salt). Direct biochemical experiments actually show that GTP does not induce Gs subunit dissociation. Rebois and co-workers[14] measured the effects of $MgCl_2$ and various purine nucleotides on Gs subunit dissociation and activation. Subunit dissociation was assayed by measuring the amount of Gβ subunit that was coprecipitated by Gsα-specific antiserum. Gs activation was determined by the ability of the protein to reconstitute adenylyl cyclase activity in rat lymphoma membranes lacking Gsα (S49cyc$^-$). High concentrations of $MgCl_2$ caused bound GDP to dissociate from Gs and inactivated the protein, unless high concentrations of GDP or GTP were present in solution. $MgCl_2$ also caused a concentration-dependent dissociation of Gs subunits. GTPγS shifted the $MgCl_2$ concentration response curve for subunit dissociation to much lower concentrations of $MgCl_2$; this suggests that GTPγS promotes subunit dissociation. On the other hand, GDP and GTP were equally effective in shifting the curve to higher concentrations of $MgCl_2$. These results suggest that the compound that activates Gs in vivo, GTP, is no more effective at promoting Gs subunit dissociation than is GDP.[14] These results strongly suggest that GTP does not induce Gs protein dissociation as a necessary step for adenylyl cyclase activation.

Recently another direct biochemical experiment was performed to examine whether the Gs protein functions as a heterotrimer in intact membranes.[15] When Gsα was completely and irreversibly activated with GTPγS and incorporated into stripped S49cyc$^-$ cells, it was a poor substrate for cholera toxin and a weak stimulator of adenylyl cyclase unless Gβγ was also incorporated. Furthermore, the level of adenylyl cyclase stimulation corresponded to the amount of Gs heterotrimer that was formed in the membranes from GTPγS-activated Gsα and Gβγ. These data suggest that adenylyl cyclase is stimulated by an activated Gs heterotrimer in cell membranes.

Pheromone-Induced Mating Response in Yeast

An excellent system in which to test ideas concerning the dissociation of G-protein subunits and their relationship to receptors is provided by the mating response of the yeast, *Saccharomyces cerevisiae*. Haploid *S. cerevisiae* cells have **a** or α mating type and respond to pheromone mating factors secreted by cells of the opposite mating type. In response to the mating factors, **a** and α haploid cells undergo cell cycle arrest in the late G1 phase, fuse to each other, and eventually give rise to diploid **a**/α cells.[16] The secreted pheromones bind to the **a**/α-factor receptors, products of the genes *STE2* and *STE3*. The mating signals are transduced by a G protein, the product of the genes *GPA1* (which encodes Gα), *STE4* (Gβ), and *STE18* (Gγ). Deletion of *GPA1* is lethal, because the free Gβγ subunits activate the signal transduction pathway, leading to growth arrest. Successful mating requires the presence of all of the genes coding for G-protein subunits.[16]

These features of the pheromone mating system show that the G protein acts on the effector system through the Gβγ subunits. These findings have also been taken as a molecular genetic proof of the G-protein-dissociation model. The published three-dimensional structure of the G protein[17] offered a unique opportunity to test whether or not the mating G protein must dissociate. From the three-dimensional structure it can easily be seen that the C-terminal end of the Gα subunit is in close proximity to the N terminus of the Gβ subunit. We constructed a *STE4–GPA1* gene fusion, which led to the production of a protein in which the C terminus of Ste4 was coupled to the N terminus of Gpa1. The fusion was then introduced into yeast cells from which *STE4* or both *STE4* and *GPA1* had been deleted. The chimeric protein was fully functional in the transduction of pheromone signals, and in promoting growth arrest and mating.[18] It is therefore likely that the receptor, upon activation, induces conformational changes in the G protein that expose binding interfaces which allow communication with downfield effectors. The chimeric protein cannot undergo dissociation, but it can undergo the changes in conformation induced by the activated receptor. The finding that the fusion construct is fully competent to convey the signals ascribed to both the Gα and the Gβγ subunits implies that wild-type proteins, albeit demonstrably capable of dissociation, may not actually dissociate. Even if they do dissociate, it is likely that they remain in very close proximity to each other throughout the GTPase cycle. These results fit the currently emerging picture of signal transmission by multiprotein complexes, which exist as large assemblies of receptors – G proteins – effectors throughout the activation cycle.

Structural Considerations

It has been argued (for example, in ref. [17]) that the interfaces for contact between Gα and Gβγ and for contact with the effector overlap. Therefore, in order for an effector to interact with the Gα subunit, the Gβγ would have to dissociate away. A close examination of the contact sites shows that the residues involved in contact between Gα and Gβγ are distinct from the residues involved in contact with the effector, and therefore both interactions may be able to occur simultaneously (see Figure 1 in ref. [18]).

Conclusion

Analysis of the current status of our knowledge of the mode of signal transduction of G-protein-coupled receptors suggests that the dissociation of G proteins into their subunits is not essential for their mechanism of action. Although the rhodopsin-dependent activation of transducin involves its dissociation, G-protein subunit dissociation is not part of the activation of adenylyl cyclases by hormones or pheromones. Detailed studies are now needed to examine the behavior of other G proteins involved in the numerous signaling pathways discovered in the last decade.

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