

PERSPECTIVE

Can a GDP-Liganded G-Protein Be Active?

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ABSTRACT

The replacement of GDP bound to the α -subunit of a G-protein by GTP is generally considered a crucial step in the activation of effectors by a G-protein. New data by Uğur et al. (2005) (p. 720) raise the possibility that for the heterotrimeric G-protein G_s , GDP-liganded G_s is able to activate the effector adenylyl

cyclase as potently and effectively as when G_s is in its GTP bound form. We summarize here the evidence that GTP is necessary for effector activation by G-proteins and discuss potential implications and limitations of data to the contrary.

Heterotrimeric G-proteins collect input from G-protein-coupled receptors (GPCR), including those for many neurotransmitters and hormones, tastes, odorants and light, and amplify such input by coupling to cellular effectors, primarily enzymes and ion channels (Taylor, 1990). The stoichiometry and spatial organization of receptors, G-proteins, and effectors play an essential role in this coordination and amplification (Ostrom and Insel, 2004).

Various lines of evidence, including detailed real-time analyses with biophysical approaches in the rhodopsin-transducin (G_t) system (Kahlert et al., 1990; Herrmann et al., 2004), have led to the following general scheme of G-protein function (Fig. 1; key elements of this model are also applicable to the "small" monomeric GTP-binding proteins of the ras superfamily). In their inactive state, G-proteins exist in a heterotrimeric, GDP-bound form. The interaction of the G-protein with an agonist or constitutively active receptor promotes GDP release; therefore, GPCRs can be considered guanine nucleotide exchange factors (GEFs). In the rhodopsin- G_t system, the activated receptor (i.e., *meta*-rhodopsin II) can be "frozen" in a complex with the nucleotide-free state of heterotrimeric G_t in the absence of guanine nucleotides. Addition of an excess of GDP attenuates the interaction of the heterotrimer with the active receptors, implying that GDP

release is an important part of the receptor/G-protein interaction. The release of GDP allows the subsequent binding of GTP, which physiologically is at a much higher cellular concentration than is that of GDP. The presence and binding of GTP induces the dissociation of the G-protein α -subunit (G_α) and the $\beta\gamma$ -dimer ($G_{\beta\gamma}$) from the receptor. Indeed, the separated subunits can be purified from illuminated retinal rod outer segment membranes by elution with GTP and its analogs (but not with GDP) (Kühn, 1980). Both G_α and $G_{\beta\gamma}$ can activate or inhibit effector mechanisms such as adenylyl cyclase, phospholipase C_β , or various ion channels. The interaction of the activated, GTP-liganded G_α with an effector (e.g., retinal phosphodiesterase γ) can be assessed biochemically (Deterre et al., 1986) and has been structurally resolved (Slep et al., 2001).

Besides the GPCR-induced GDP/GTP exchange, G-proteins of the G_i and G_s families can apparently be activated by the transfer of high energy phosphate onto GDP via nucleoside diphosphate kinase/ $G_{\beta\gamma}$ complexes. Recent data suggest that this reaction might be involved in the GPCR-independent, basal activity of G-proteins but does not contribute to GPCR-induced G-protein activation (Cuello et al., 2003; Hippe et al., 2003).

Whether GTP within the binding pocket of G_α originates from replacement of released GDP or from phosphate transfer onto local GDP, the γ -phosphate group seems to be essential for G-protein activation. Comparison of the crystal structures of heterotrimeric (inactive) G_{i1} (Wall et al., 1995) and

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Please see the related article on page 720.

ABBREVIATIONS: GPCR, G-protein-coupled receptor; G_t , rhodopsin-transducin; GEF, guanine nucleotide exchange factor; GAP, GTPase-activating protein; RGS, regulator of G-protein signaling; $GDP\beta S$, guanosine 5'-O-(2-thio)diphosphate.

G_t (Lambright et al., 1996) with those of G_{α_1} and G_{α_t} in the GDP- (Lambright et al., 1994; Mixon et al., 1995), GDP·AlF₄⁻ (transition) (Coleman et al., 1994; Sondek et al., 1994) and guanosine 5'-O-(3-thio)triphosphate-bound (active) states (Noel et al., 1993; Coleman et al., 1994) has provided a clear picture of the structural changes occurring during G-protein activation. The structure and orientation of all three so-called "switch elements" in the active conformation are essentially identical in G_{α_t} , $G_{\alpha_{i1}}$, and G_{α_s} (Sunahara et al., 1997). The interaction of activated G_{α_s} with its effector adenylyl cyclase has also been defined crystallographically (Tesmer et al., 1997).

The intrinsic GTPase activity of the G-proteins, possibly enhanced by GTPase-activating proteins (GAPs), hydrolyzes the bound GTP to GDP. Indeed, GAPs, regulators of G-protein signaling (RGS) or RGS-like proteins, have been identified for all four G_{α} subfamilies (Wieland and Chen, 1999; Wieland and Mittmann, 2003). Thus, the GTPase activity of the G-protein is considered the biochemical timer that terminates effector activation and induces the reassociation of the heterotrimer.

The essence of the above findings and ideas are that the GDP-bound form of a G-protein is inactive, whereas only the GTP-bound form is active and interacts with effectors. This well accepted view is challenged by a communication in this issue of *Molecular Pharmacology* (Uğur et al., 2005). The authors report that GDP and GTP seem equipotent and equieffective in enhancing adenylyl cyclase activity in membrane preparations from human embryonic kidney 293 cells engineered to overexpress a β_2 -adrenergic receptor/ G_{α_s} fusion protein, from *cyc*⁻ S49 cells (which lack G_{α_s}) that express β_2 -adrenergic receptors and that were transfected with G_{α_s} , and from S49 cells that express both β_2 -adrenergic receptors and G_{α_s} . Moreover, Uğur et al. (2005) report that neither GDP nor its stable analog GDP β S inhibit adenylyl cyclase activity that is stimulated by a combination of GTP and the receptor agonist isoproterenol. The authors also present data that argue against a conversion of GDP to GTP as possible explanation for the stimulatory effect of GDP. They report that an inhibition of the GTPase activity of G_{α_s} by treatment with cholera toxin enhances receptor-indepen-

dent adenylyl cyclase stimulation by GTP, but not GDP, and that an agonist-induced GDP release attenuates AlF₄⁻-promoted stimulation of adenylyl cyclase activity. Therefore, all the data presented are consistent with the authors' interpretation that GDP-liganded, receptor-activated G_{α_s} might be active and thus that binding of GTP would not be necessary to activate effector enzymes, at least for the β_2 -adrenergic receptor/ G_{α_s} -induced stimulation of adenylyl cyclase.

If the authors' findings and interpretation are correct, they would have important implications for our understanding of G-protein-mediated signal transduction, especially if applicable to other receptor/G-protein/effector combinations. Indeed, the findings imply that current ideas regarding GPCR-induced effector regulation would have to be reconsidered and revised. However, aspects of the present findings are in opposition to certain previous data, as well as to the well accepted model of G-protein activation (see above); thus, one must be cautious both in the interpretation of the data and in considering the implications, in terms of a revised model for G-protein signaling. Following are some of the possible implications and limitations of the authors' study:

1. A similar potency and effectiveness of GDP and GTP in the activation of G-proteins would lead one to question the physiological relevance of the intrinsic GTPase activity of the proteins as well as of activities identified for various regulatory proteins, such as GAPs (RGS) and GEFs (GPCR). It seems unlikely (and wasteful) that intrinsic GTPase activity, GAPs, and GEFs would have been evolutionarily retained if they served no biological purpose. On the other hand, it is interesting to note that although GAPs were described for most heterotrimeric G-proteins, a GAP for G_s remained elusive. A single study on a potential G_s -GAP has appeared (Zheng et al., 2001), but no follow-up studies regarding this GAP activity have been reported. This raises the possibility that G_s may differ from other G-proteins with regard to its regulation by GAPs. On the other hand, in the S49 *cyc*⁻ system, which was used by Uğur et al. (2005), a G_{α_s} mutant with enhanced intrinsic GTPase activity exhibits impaired β -adrenergic receptor-induced adenylyl cyclase stimulation

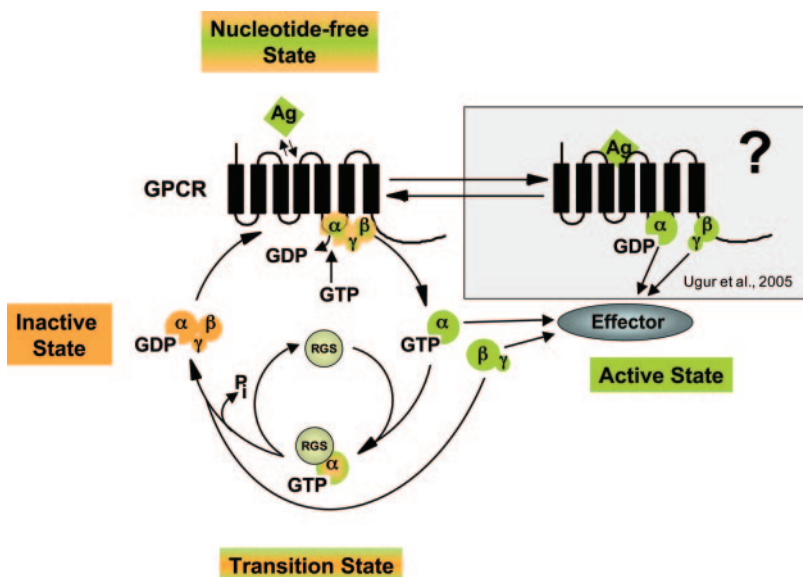


Fig. 1. Models of GPCR-induced activation of heterotrimeric G-proteins. In the classic four-state model of the G-protein activation cycle, the agonist (Ag)-activated GPCR serves as a GEF and catalyzes the GDP/GTP exchange. The dissociated G-protein, consisting of the GTP-liganded G_{α} subunit and the free $G_{\beta\gamma}$ dimer, represents the active state in which the G-protein interacts with effectors. RGS proteins, serving as GTPase-activating proteins, stabilize the transition state and thereby accelerate GTP hydrolysis by G_{α} . The inactive trimeric state of G-protein is recovered by the re-association of the GDP-liganded G_{α} with a $G_{\beta\gamma}$ dimer. This model allows a catalytic activation of multiple G-proteins by one GPCR. On the right side, the essential feature of the hypothesis proposed by Uğur et al., (2005) is shown. The authors propose that the agonist-activated GPCR (at least the β_2 AR) induce a conformation of the GDP-liganded G-protein in which (at least) G_{α_s} -GDP is able to activate an effector (i.e., adenylyl cyclase) and speculate that the association and dissociation of the agonist to the GPCR is the biological timer for effector activation.

- (Warner and Weinstein, 1999), pointing to a role for GTP binding and hydrolysis in that system.
- If the interaction with activated receptor rather than GTP binding were to induce an active conformation of the G-protein, a physical association between receptor and G-proteins needs to be maintained during effector activation to keep the G-protein in its active confirmation. It is thus interesting that the strongest GDP effects reported by Uğur et al. (2005) occur upon transfection of a β_2 -adrenergic receptor/ G_{α_s} fusion protein that secures such a physical association. Nevertheless, Uğur et al. (2005) also report GDP-induced activation without such fusion proteins. If receptor/G-protein association were to be maintained to keep the G-protein activated, a receptor/G-protein/effector stoichiometry of 1:1:1 has to be expected, whereas the measured relative abundances are clearly different (Ostrom et al., 2000).
 - The GDP/GTP exchange is most likely to be important for the dissociation of the heterotrimer and hence for effectors stimulated by $G_{\beta\gamma}$. It is well known, for example, that the stimulation of adenylyl cyclase type II by $G_{\beta\gamma}$ dimers requires 10- to 20-fold larger amounts of $G_{\beta\gamma}$ than the stimulation by activated G_{α_s} (Tang and Gilman, 1991). Thus, multiple G_i proteins, as the source of $G_{\beta\gamma}$ dimers, have to be activated simultaneously with G_{α_s} to generate the amounts of free $G_{\beta\gamma}$ dimers necessary for adenylyl cyclase regulation. This requires that G_i -coupled GPCR (among others) activate multiple G_i proteins upon agonist stimulation and is in accordance with stoichiometric studies (Ostrom et al., 2000) that suggest a single, agonist-activated receptor molecule can activate multiple G-protein molecules.
 - Although stimulation of adenylyl cyclase is the prototypical response of G_s activation (Simonds, 1999), other effectors exist (e.g., BK_{Ca} channels) that may even be more important than cAMP formation for some physiological responses of G_s -activating GPCR, such as smooth muscle relaxation (Horinouchi et al., 2003; Peters and Michel, 2003; Tanaka et al., 2003). Thus, even if GDP/GTP exchange would not be required for the stimulation of adenylyl cyclase, it could still be relevant for other effects of G_s stimulation.
 - Apart from these conceptual issues, it should be kept in mind that the data by Uğur et al. (2005) are based upon the combination of one receptor (the β_2 -adrenergic receptor) and one G-protein subunit (G_{α_s}) in membrane preparations and the measurement of only one effector activity (i.e., cAMP formation by adenylyl cyclase). Thus, unidentified peculiarities of the experimental conditions may have affected the results. For example, Uğur et al. (2005) report that GDP or GDP β S enhanced agonist-stimulated adenylyl cyclase activity. A stimulatory effect of GDP and GDP β S on adenylyl cyclase activity has been reported before, but this was interpreted as an inactivation of nucleotide free G_{α_i} from an active conformation that inhibits adenylyl cyclase (Piacentini et al., 1996; Lutz et al., 2002). Although the findings of Uğur et al. (2005) cannot be fully explained by a GDP-induced disinhibition of G_i , the data do not unequivocally rule out that possibility. It is noteworthy, therefore, that the authors observe a higher potency of GTP (compared with GDP) in the inhibition of agonist-stimulated adenylyl cyclase activity (compare Fig. 2, A–C, 10^{-4} to 10^{-5} M range in Uğur et al., 2005), an effect most likely to be mediated by basal GTP-induced G_i activation.
 - Certain other work disagrees with some of the observations of Uğur et al. (2005) Using the same G-protein/effector combination with a different receptor and cell type (i.e., prostaglandin E_1 -stimulation in human platelet membranes), others have observed a strong inhibition of cAMP formation by GDP β S in the presence of GTP (K. H. Jakobs, personal communication). In addition, in a different receptor/G-protein/effector combination but the same cell line as that used by Uğur et al. (2005), photoresponses mediated by melanopsin heterologously expressed in human embryonic kidney 293 cells are also blocked by GDP β S (Qiu et al., 2005). Other experimental considerations relate to the use of ATP-regenerating systems, which are designed to help maintain GTP concentrations in most adenylyl cyclase activity assays. No such system was present in the assays by Uğur et al. (2005). Moreover, the authors measured enzyme activity at 37°C and high Mg^{2+} concentrations, conditions that favor GTPases, nucleotidases, and other GTP-degradative enzymes. Hence, the possibility exists that Uğur et al. (2005) observed a similar action of GDP and GTP because of substantial hydrolysis of GTP, especially at low GTP concentrations. In this context, it is noteworthy that previous data with a fusion protein of the human β_2 -adrenergic receptor and rat $G_{\alpha_{sL}}$ (the same combination used by Uğur et al., 2005) indicate a clear difference in the potency of binding of GTP and GDP to that protein when the receptor is activated by an agonist (Seifert et al., 1998). GTP was found to be approximately 3 orders of magnitude more potent than GDP in inducing agonist displacement than GDP under conditions in which hydrolysis of GTP to GDP or transphosphorylation of GDP to GTP is less likely to occur than under the conditions used by Uğur et al. (2005). Nevertheless, these experimental conditions also apply to the studies with cholera toxin-treated membranes; thus, this possibility does not seem to explain the differences between GDP and GTP observed in the latter experiments.
- In conclusion, the data presented by Uğur et al. (2005) do not prove that the β_2 -adrenergic receptor induces an active conformation of G_{α_s} with GDP as the bound nucleotide. Nevertheless, the data raise the possibility that the binding of GTP is not an absolute requirement for the activation of a heterotrimeric G-protein, at least under the “artificial” experimental conditions that were used. However, additional experimental work with more strictly defined conditions (e.g., reconstitution of purified β_2 -adrenergic receptor/ G_{α_s} fusion proteins with purified adenylyl cyclase in vitro) has to be done to provide more rigorous proof of these new ideas. Until such additional evidence is available, the classic concept of GTP-liganded G_{α} subunit and/or a free $G_{\beta\gamma}$ as the active parts that interact with effectors remains the most accurate and well documented model to describe the activation of effectors by heterotrimeric G-proteins.

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