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G protein activation by G protein coupled receptors: ternary complex formation or catalyzed reaction?

David J. Roberts, Magali Waelbroeck*

Department of Biological Chemistry and Nutrition, School of Medicine, Université Libre de Bruxelles, Bat G/E, CP 611, 808 Route de Lennik, B-1070 Bruxelles, Belgium

Abstract

G protein coupled receptors catalyze the GDP/GTP exchange on G proteins, thereby activating them. The ternary complex model, designed to describe agonist binding in the absence of GTP, is often extended to G protein activation. This is logically unsatisfactory as the ternary complex does not accumulate when G proteins are activated by GTP. Extended models taking into account nucleotide binding exist, but fail to explain catalytic G protein activation.

This review puts forward an enzymatic model of G protein activation and compares its predictions with the ternary complex model and with observed receptor phenomenon. This alternative model does not merely provide a new set of formulae but leads to a new philosophical outlook and more readily accommodates experimental observations.

The ternary complex model implies that, HRG being responsible for efficient G protein activation, it should be as stable as possible. In contrast, the enzyme model suggests that although a limited stabilization of HRG facilitates GDP release, HRG should not be ''too stable'' as this might trap the G protein in an inactive state and actually hinder G protein activation. The two models also differ completely in the definition of the receptor ''active state'': the ternary complex model implies that the active state corresponds to a single active receptor conformation (HRG); in contrast, the catalytic model predicts that the active receptor state is mobile, switching smoothly through various conformations with high and low affinities for agonists (HR, HRG, HRG_{GDP}, HRG_{GTP}, etc.).

Keywords: Receptors; G protein coupled; Signal transduction; Heterotrimeric GTP-binding proteins; Models; Theoretical

1. Introduction: the ingredients

GPCRs represent the largest receptor family and are involved in the control of every aspect of our physiology and behavior [1,2]. They recognize extracellular signals including hormones and neurotransmitters, ions, light (photons), etc. and transmit this information to intracellular trimeric GTP-binding proteins, known as ''G proteins'', which in turn alter the activity of enzymes or proteins that are involved in second messenger generation.

Trimeric G proteins [3] are composed of two subunits. The Gα subunit recognizes GTP, hydrolyses it to GDP, and then associates with the Gβγ subunit. An activated receptor is necessary to facilitate the GDP release and GTP recognition (see below). The term ''G protein'' is, unfortunately, used rather loosely to designate either the guanyl nucleotide-free G protein, the isolated (GTP-bound) Gα subunit, or the trimeric (GDP-bound) $G\alpha_{GDP}$–Gβγ complex.

The Gα subunit three-dimensional structure (Fig. 1) can be divided into two domains: a ''Ras-like'' domain with clear structural homology to small G proteins and a helical domain [4–6]. The Gβ chain assumes a barrel-shaped β-propeller structure preceded by an α-helix [5]. It is intimately associated with the Gγ chain.

Abbreviations: GPCR, G protein coupled receptor; H, agonist; R, receptor; R*, activated (as opposed to resting) receptor state; G, G protein; K_s, specificity constant (in enzymology: V_{max}/K_m); S_{50}, agonist concentration necessary for half maximal stimulus induction; K_H, dissociation constant of the high agonist affinity ternary complex; EC_{50}, agonist concentration necessary for half maximal G protein activation; *v*, G protein activation rate

* Corresponding author. Tel.: +32 2 555 6215; fax: +32 2 555 6230.
E-mail address: mawaelbr@ulb.ac.be (M. Waelbroeck).

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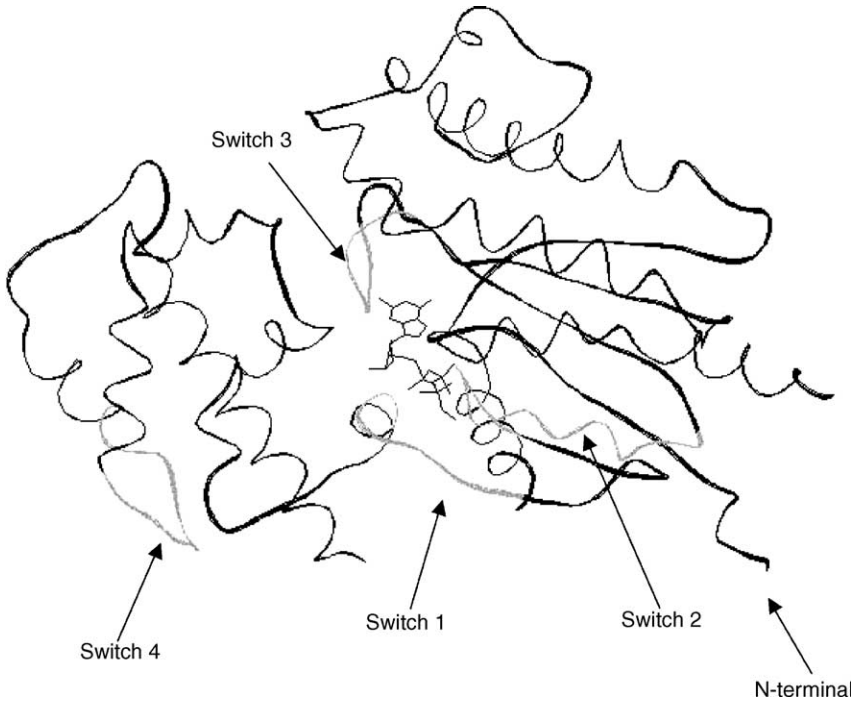

Fig. 1. Structure of the $G\alpha_{GTP\gamma S}$ complex. The four ''switch regions'' are shown in grey (Protein Data Bank structure: 1AZT). GTPγS lies between the Ras-like and helical domains.

The conformation of four Gα regions, known as ''switch 1'' to ''switch 4'' (Fig. 1), depend on the nature of the bound guanyl nucleotide (GDP or GTP) [4–6]. In the presence of GDP, switches 1 and 2 make extensive contacts with the Gβγ propeller base and the N-terminal Gα subunit sequence lies over the barrel side. The resulting complex is very stable, does not readily allow GDP dissociation and is inactive as far as signaling is concerned. GTP binding induces marked conformational changes in the four switch regions, and thereby induces the dissociation of Gα from Gβγ. This allows both subunits to interact with effectors or regulators, as outlined below.

Both G protein subunits are necessary to reconstitute the stable (high affinity) ''ternary complex'', HRG, in the absence of nucleotides [7]. GPCRs are unlikely to contact the ''switch regions'', which are at least 30 Å from the membrane surface [1]. They might use the Gβγ subunit as a lever to open a ''hinge'' between the Ras-like and helical domains in the Gα subunit and allow nucleotide release and recognition [1,8].

The interaction surface between G protein subunits and their regulators or effectors has been elucidated by X-ray diffraction and mutagenesis studies [9–13]. Like Gβγ, adenyl cyclase contacts switch 1, switch 2 and the α3-β5 loop of $G\alpha_S$. Similarly, the GTPase regulator protein RGS4 contacts switch 1, switch 2 and switch 3 of $G\alpha_i$. The interaction of Gβγ with phospholipase C (an effector) as well as with phosducin (a regulator protein) implicates the same propeller base that contacts the Gα subunit. All these results indicate that the dissociation of Gα from Gβγ and from the receptor, induced by GTP, is essential for effector activation [3,14,15].

2. Binding studies: the ternary complex model

Agonists and inverse agonists discriminate high and low affinity receptor states in the absence of guanyl nucleotides. In the presence of high GDP or GTP concentrations, most if not all receptors are found in a state with low affinity for agonists [14,15] and high affinity for inverse agonists [16]. Neutral antagonists, in contrast, recognize all receptor states with the same affinity.

The high agonist-affinity state can be reconstituted in the absence of nucleotides from purified receptors, Gα and Gβγ subunits [7]: this supports the ''ternary complex'' model of De Lean et al. [15]. This model is extraordinarily successful in describing the GPCR agonists' binding properties under the assumption that agonists and (empty) G proteins favour each other's interaction with the receptor (Fig. 2a). It has been extended to account for G protein interaction with the resting receptor state [17] and explain the effect of mutations that increase the agonists' affinity together with the receptor's spontaneous signaling ability [18].

3. The ternary complex model describes adequately agonist binding. Why should dose–effect curve predictions be out of bounds?

Agonist dose–effect curves can usually be described by two parameters: the agonist potency (EC_{50}) and efficacy [19]. De Lean et al. pointed out in 1980 [15] (pg 7108) that ''the formation of the high affinity state of the agonist–receptor complex is a prerequisite for its action''. This led

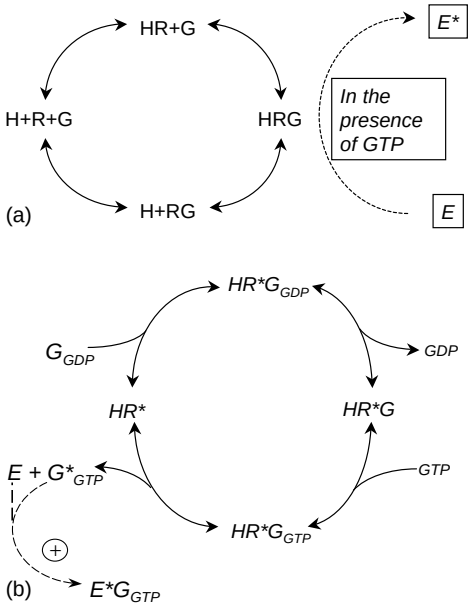

Fig. 2. The ternary complex and "enzyme" models of G protein activation. (a) The original ternary complex model described by De Lean et al. [15]: the receptor (R) recognizes the agonist (H) and G protein (G) in random order. Agonist binding facilitates G protein recognition and vice versa. The ternary complex is necessary for effector activation in the presence of GTP. (b) The "enzyme" model of G protein activation [31]. The active receptor state, R^*, stabilized by agonists, H, catalyzes G protein activation more efficiently than R. Recognition of an inactive G protein (G_{GDP}) is followed by GDP release and GTP binding. This induces the dissociation of the activated G protein subunits ($G\alpha_{GTP}$ and $G\beta\gamma$) from the receptor, which is now free to act on another G protein.

several authors to use the ternary complex as a measure of the stimulus leading to G protein activation [17,20,21]. Two fundamental properties of GPCR signaling cannot be explained by the ternary complex model, however:

1. The ternary complex does not accumulate in the presence of GTP [15], but G protein activation does not occur in the absence of GTP.
2. Agonists form ternary complexes with high affinities: they should, therefore, activate effectors at very low concentrations [20]. (The agonist intrinsic potency, S_{50}, is the agonist concentration necessary for half maximal stimulus induction. It must be equal to K_H if the ternary complex serves as stimulus for G protein activation. The agonist concentration necessary for half maximal effect, EC_{50} is by definition $\leq S_{50}$). Agonist EC_{50} values in functional assays are, in fact, significantly greater than K_H, and intermediate between the high and low affinity constants (see for instance [22–25]).

4. Is it sufficient to take into account GTP binding to the ternary complex?

The failure of the ternary complex model to explain G protein activation is most likely due to the fact that it was never meant to account for GTP binding to the G protein. It has been extended to a quaternary [23] complex (H.R.G.GTP) model that describes GTP binding to uncoupled and receptor-bound G proteins. High agonist EC_{50} values (compared to K_H) can be explained by this model [23]. It is, however, necessary to accept the unlikely hypothesis that GTP-bound G proteins are active only if they interact with an agonist-bound receptor (HRG^*_{GTP}), implying that they are inactive when uncoupled (G_{GTP}) or coupled to empty receptors (RG_{GTP}) [23].

To get around this problem, Onaran et al. analyzed a quinternary complex (H.R.Gα.GTP.G$\beta\gamma$) model, assuming that GTP is not sufficient per se to separate the two G protein subunits and that the receptor's cooperation is necessary to fully dissociate $G\alpha_{GTP}$ from $G\beta\gamma$ and achieve G protein activation [26]. According to this model, agonist-bound receptors activate G proteins by stabilizing $HRG\beta\gamma$ and $G^*\alpha_{GTP}$.

The quaternary and quinternary models of G protein activation predict—in contrast to experimental observations [1,27–29]—that the number of activated G proteins must always be much smaller than the number of agonist-bound receptors as several agonist-bound receptors are involved in non-signaling complexes (HR, HRG, HRG_{GDP}).

5. What about kinetic models?

A number of researchers have simulated G protein activation kinetics (reviewed in [30]; see also [31]), and predicted catalytic G protein activation with EC_{50} and efficacy values in good agreement with experimental observations. We went a step further by taking advantage of the similarities between the G protein activation cycle and enzyme-catalyzed reactions to develop the kinetic equations.

Enzymes are biological catalysts: at low concentration they accelerate chemical reactions without affecting the equilibrium constant. As pointed out by Purich [32], the underlying principles of biological catalysis apply equally well to the breaking and making of covalent, coordinate and non-covalent bonds. The G protein activation cycle, shown in Fig. 2b, is equivalent to a "ping-pong" enzyme-catalyzed reaction mechanism, taking the receptor as enzyme and the agonist as allosteric activator. The "enzyme" (activated receptor, HR^*) interacts with the first reactant (G_{GDP}) and facilitates the release of the first product (GDP); the second reactant (GTP) then binds to the "substituted enzyme" (ternary complex, HR^*G) and the second product (activated G protein) is released, allowing the freed "enzyme" (active receptor) to activate another G protein [1].

The ternary complex model is "embedded" inside the ping-pong enzyme model of G protein activation (Fig. 2b): in the absence of GDP or GTP the intermediate complex HRG accumulates. This explains why, as outlined below,

the predictions of the two models are very similar. The enzyme model, however, describes more accurately the G protein activation process.

6. Potency, efficacy, G protein specificity and desensitization

At saturating GTP concentrations, agonists induce G protein activation with a low potency, reflecting their low affinity for the (more numerous) uncoupled GPCRs (see [23,26,33], but also [28,30,31]). If the GTP concentration is decreased progressively, the proportion of ternary complexes (and the agonist potency) increases but the maximal rate of G protein activation decreases [33,34].

Most receptors are able to signal weakly, even in the absence of agonists [18]. Their constitutive activity is not only affected by mutations, but also by the incubation conditions: G proteins, nucleotides, sodium or magnesium ions, etc. [18,24,35,36]. This suggests that all receptors can be found in two states at equilibrium ($R \rightleftarrows R^*$). Agonist efficacy has been shown to be correlated with its ability to stabilize the receptor-empty G protein interaction [15,33]. If agonists stabilize the ternary complex by affecting the proportion of active receptors, R^*/R rather than the active receptor's affinity for G proteins, a single allosteric parameter ($\beta = \{[HR^*]/[HR]\}/\{[R^*]/[R]\}$) is sufficient to describe the effect of agonists on receptor activation [18]. Agonist efficacy is defined experimentally as its ability to induce G protein activation, compared to the maximal effect of the most efficient agonist. The ternary complex model, like the enzyme model of G protein activation, predicts that the ligand efficacy increases hyperbolically with β and with the receptor's constitutive activity, R^*/R [18,34].

Several receptors activate different G protein subtypes in response to agonists [2]. The experimental results can be grouped into two ''activation patterns'': agonists may induce the activation of different G proteins with the same EC_{50} value but different maximal effects (Fig. 3a), or activate the receptor's cognate G protein at low concentrations and alternative G proteins at much higher concentrations (Fig. 3b).

The formation of ternary complexes at equilibrium reflects the relative concentration and affinity of the G proteins for agonist-bound receptors: $[HRG^1]/[HRG^2] = K^1[G^1]/K^2[G^2]$ [20,37], and the relative activation rate (v^1/v^2) of the two G proteins, their concentrations and specificity constants: $v^1/v^2 = [G^1]K_s^1/[G^2]K_s^2$ [38]. Both equations have the same mathematical form and differ only in the meaning of the parameters: K measures the empty G protein's affinity for agonist-bound receptors at equilibrium while K_s corresponds to the rate of the HRG complex formation. Both G protein activation patterns (Fig. 3a and b) can therefore be explained similarly by equilibrium and enzymatic G protein activation models.

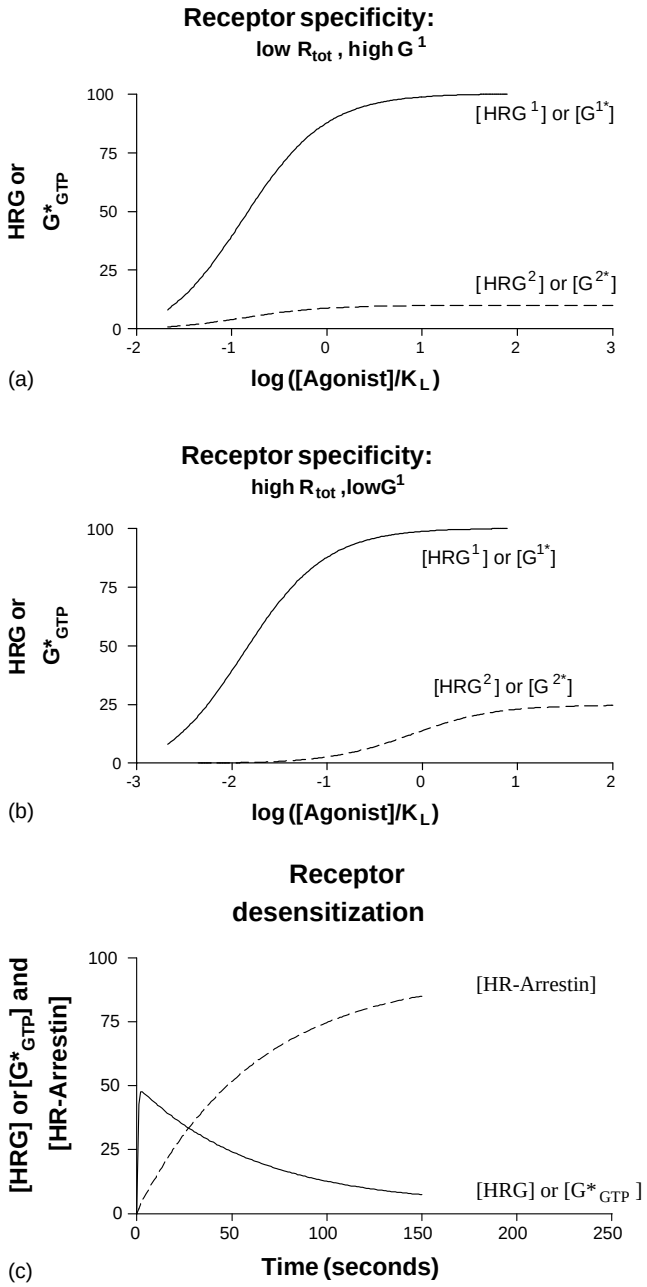

Fig. 3. G protein activation by ''promiscuous'' receptors and desensitization. Hypothesis: the receptor is capable of recognizing and activating two G proteins (with a preference for G^1 over G^2) or its cognate G protein and arrestin. (a) The receptor is not too selective and the concentration of G^2, high enough to allow receptor recognition despite G^1 competition. The agonist recognizes principally RG^1 complexes (for which it has a high affinity): low agonist concentrations are therefore sufficient to occupy all G protein-bound receptors [20,37]. (b) The receptor is highly selective for G^1 but this G protein is present in low concentrations. At low agonist concentrations, inactive or free G^1 proteins competitively inhibit receptor recognition by G^2. At high agonist concentrations, in contrast, G^1 proteins are depleted by binding to excess receptors or full activation, and do not interfere with the (low affinity) G^2 recognition. (c) The receptor's cognate G protein and arrestin compete for receptor recognition, but the G protein dissociates much faster from the receptor than arrestin. After short incubation periods, the receptor will recognize (and activate) preferentially G proteins; after long incubations, in contrast, the receptor interacts preferentially with the (high affinity) arrestin and is desensitized.

If both G proteins are in large excess compared to the receptor concentration, they compete for recognition of the same agonist–receptor complex. As a result, both ternary complexes will accumulate at the same low agonist concentrations, sufficient to form the most stable ternary complex (Fig. 3a) [20,37], and agonists will activate both G proteins at the same, low concentrations.

If the receptor is highly selective, the cognate G protein will compete efficiently with the second, less ''attractive'' G protein, and prevent its recognition by the receptor. If the receptor concentration is high enough, however, it might be possible at high agonist concentrations to deplete its cognate G protein (by binding or activating it completely). Under these conditions, the excess (spare) receptors will be able to recognize [39,40] and activate, with a low affinity, the ''less attractive'' G proteins (Fig. 3b).

As shown by Motulsky and Mahan [41]: if two ligands compete for the same binding site, the ligand with the highest affinity (usually associated with the slowest dissociation rate) binds preferentially at equilibrium. In contrast, if the incubation is very short, the ligand with the fastest dissociation rate binds first (Fig. 3c). The same reasoning can be applied to competition between G proteins and arrestins: if the G proteins have been fine-tuned for rapid dissociation and arrestins for slow dissociation from activated receptors, agonist-bound receptors will recognize and activate the G proteins first, followed by arrestin. Similar results can be simulated with the ''G protein binding'' and ''enzymatic G protein activation'' models.

7. Supramolecular receptor-G protein-effector complexes have been observed: doesn't that argue against the enzyme model of G protein activation?

Various findings indicate that receptor-G protein-effector complexes occur [42–44] and might be useful in facilitating effector activation by the G proteins. These observations might appear to support an extended ternary complex model, with agonists stabilizing receptor-activated G protein-effector interactions, as opposed to the ''hit and run'' interactions between the receptor and G protein that underlie the enzymatic model. The enzymes of several metabolic pathways are, however, known to associate in (sometimes gigantic) complexes. The catalytic sites of the various enzymes in these complexes are arranged so that the metabolites can be shuttled from one enzyme to the next, in a phenomenon known as ''enzyme channeling''. Its putative impact on metabolism is controversial [38,45], but some authors suggest that enzyme channeling does improve the flow through the whole reaction pathway [45]. Likewise, the close proximity or association of receptors and effectors might facilitate the interaction of G proteins with their effectors once they are activated by their receptors.

8. The free energy profile of G protein activation

Heck and Hofmann [27] recently analyzed the kinetics of transducin activation using the enzyme model of G protein activation. They estimated that the physiological transducin (G_{GDP}) and GTP concentrations are close to their respective K_m values [27]. This allows us [46] to write:

$$\frac{K_m^{G_{GDP}}}{[G_{GDP}]} = \frac{[R^*]}{[R^*G_{GDP}] + [R^*G_{GTP}]} \approx 1$$

$$\text{so that } [R^*] \approx [R^*G_{GDP}] + [R^*G_{GTP}]$$

and

$$\frac{K_m^{GTP}}{[GTP]} = \frac{[R^*G]}{[R^*G_{GDP}] + [R^*G_{GTP}]} \approx 1$$

$$\text{so that } [R^*G] \approx [R^*G_{GDP}] + [R^*G_{GTP}]$$

Under physiological conditions, the concentrations of uncoupled, empty- and occupied-G protein coupled rhodopsin molecules are very similar. Each light-activated rhodopsin molecule (R^*) is able to activate up to 1300 transducin molecules per second at 34 °C [27]: rhodopsin switches smoothly, more than 1000 times per second, from one conformation to the next, indicating that the free energy barriers that separate the different intermediates are small. This can be schematized by the free energy diagram shown in Fig. 4a (full line).

Agonists stabilize the ternary complex [15] that accelerates GDP release from inactive G proteins [47]. As shown in Fig. 4a, the two effects are correlated: if the activated ternary complex (R^*G) is more stable than the ''resting'' ternary complex (RG), the neighboring energy barriers will be lower, and the two reactions that lead to this complex (GDP but also GTP release) will be easier [46]. This explains why agonist efficacies are usually correlated with their high/low affinity ratios (see for instance [15,33]).

It has been suggested that agonists not only accelerate GDP release but also accelerate G protein activation per se [28,47], and that some activated receptors have a significant affinity for one of the two activated G protein subunits [21,26,46]. Receptor–G$\beta\gamma$ interactions have been demonstrated directly [48–50]. Agonists that stabilize $R^*G\beta\gamma$ accelerate the reactions that lead to this receptor state, including the release of $G\alpha_{GTP}$ and thereby accelerate G protein activation (Fig. 4b).

9. Why should we think in terms of "enzymatic" rather than "allosteric" G protein activation?

We all agree that the ternary complex HRG [15] and HRG$\beta\gamma$ [48–50] are important for G protein activation. Most of the predictions of the ternary [15] or quinternary complex models [26] and of the enzyme model of G protein activation [31] are very similar. It seems so much

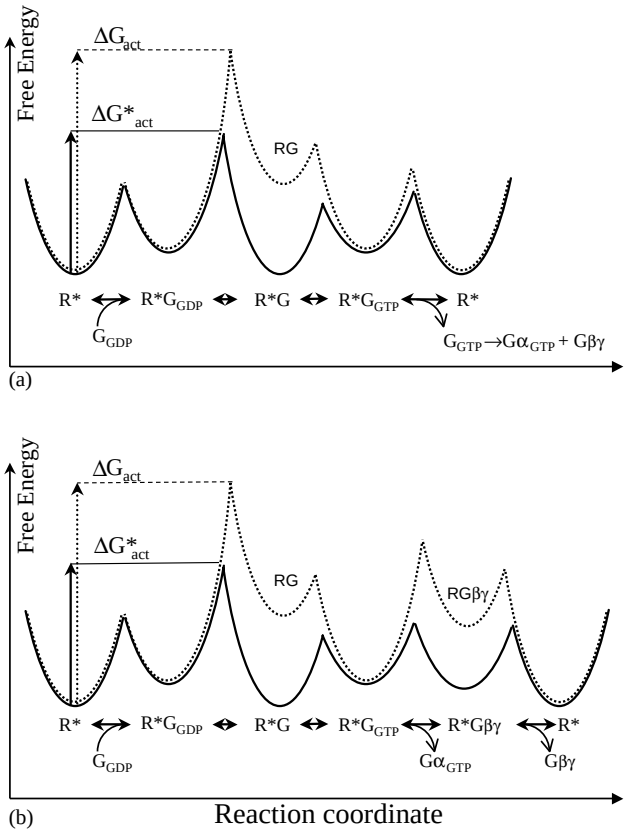

Fig. 4. Schematic thermodynamic profile of transducin activation by rhodopsin. The free energy profile of a reaction can be represented as a succession of parabolas which represent the energy necessary for stretching, bending and twisting each reaction intermediate. The probability of finding rhodopsin in a given conformation ($[R^*] \approx [R^*G] \approx \{[R^*G_{GDP}] + [R^*G_{GTP}]\}$) was estimated as explained in the text (8: The free energy profile of G protein activation). The more populated rhodopsin conformations have lower energies, and the free energy barrier that separates two reaction intermediates is inversely related to that step's rate constant. If activation by a photon stabilizes the rhodopsin-transducin (R^*G) (panel a) complex, this will facilitate the reactions that lead to these states: GDP but also GTP release. If it also stabilizes the rhodopsin-G$\beta\gamma$ ($R^*G\beta\gamma$) complex, the release of $G\alpha_{GTP}$ will also be accelerated (panel b).

easier to understand equilibrium reactions rather than kinetics: should we really bother with kinetic models of G protein activation?

As explained above: equilibrium models of G protein activation cannot explain catalytic G protein activation. The enzyme model of G protein activation describes more accurately our present understanding of the reaction: it is therefore beneficial to draw on the concepts that were developed by enzymologists to enhance our understanding of G protein activation.

The main difference between the two models lies in our understanding of the function of the ternary complex. The ternary complex model suggests that the HRG complex is essential per se for biological activity, and therefore implies that it should be as stable as possible. Binding sites are optimized by increasing their affinity for the ligand, that is, by *slowing* G protein dissociation. In contrast, the enzyme model of G protein activation suggests that the ternary complex is merely the means of accelerating G protein activation by facilitating GDP release. It should not be "too stable": otherwise the receptor and G protein might be trapped in this (inactive) state. Enzymes are in fact optimized by *accelerating* their products' (GDP and activated G protein) dissociation rates: the shorter the product's time of residence on the enzyme, the more substrate the enzyme can convert in a given time period.

As explained above, it would be difficult to simultaneously explain high selectivity (associated with slow cognate G protein dissociation) and rapid desensitization (that necessitates fast G protein and slow arrestin dissociation) within the ternary complex model. In contrast, rapid product dissociation rates have little effect on ping-pong enzyme selectivity: high "enzyme" specificity is fully compatible with rapid desensitization.

Another fundamental difference between our understanding of the ternary complex model and enzyme models of G protein activation lies in our understanding of the "active state" concept. Taken at face value, the ternary [15,17,18] and quinternary [26] complex models suggest that it is possible to define a single active receptor conformation: HRG or HRG$\beta\gamma$, respectively. In contrast, the enzyme model of G protein activation [31] suggests that active receptors are extremely mobile, easily switching from one conformation to the next: $HR^* \rightarrow HR^*G_{GDP} \rightarrow HR^*G \rightarrow HR^*G_{GTP} \rightarrow HR^* + G\alpha_{GTP} + G\beta\gamma$ (Fig. 2), and that the only role of agonists is to facilitate these conformational changes. If this is true, several distinct X-ray structures will be needed in order to have a complete picture of G protein activation.

10. Conclusion

The ternary complex model is a wonderful tool to use for the analysis of GPCR binding, but we have put forward a number of points that this model is ill equipped to accommodate when considering G protein activation. In order to understand how the receptors actually activate G proteins, it would be much better to envisage GPCRs as "enzymes" catalyzing this process. Considering the system in this manner does not merely provide a new set of numbers or formulae, but, more importantly, a different philosophical outlook which more easily accommodates common observations.

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