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The significance of the free energy of hydrolysis of GTP for signal-transducing and regulatory GTPases

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Abstract

A large number of GTP/GDP binding proteins, which in general have intrinsic and/or stimulatable GTPase activity, have been identified in recent years and are involved in a wide range of cellular regulatory and signal transducing processes. A common property of these proteins is that they exist in what is generally described as an active form when GTP is bound and an inactive (resting) form when GDP is present. Thus, the intrinsic or stimulated GTPase activity of these 'enzymes' serves to turn off a signal or to terminate a regulated process. It has been suggested that these proteins, together with ATPases whose prime function is to convert the free energy of ATP hydrolysis into another form of energy or into energy-requiring chemical reactions should be grouped together under the heading of 'energyases'. In this article, this suggestion is examined from the point of view of identifying the role of the free-energy of hydrolysis of GTP in the signal-transducing or regulatory process of the GTPases. It is concluded that there is a qualitative difference between ATPases and classical GTPases, in the sense that a quantitative relationship between the free-energy of GTP hydrolysis and the appearance of this energy in a different form cannot be directly defined. The significance of the high free energy of hydrolysis is that it allows efficient transition from the active to the inactive state of GTPases in spite of the tendency of the strong interaction of the GTP-bound form with a partner molecule ('effector'), an essential feature of their mode of action, to stabilize the GTP-bound form.

Keywords: GTP-bound; Intrinsic; ATPases; Hydrolysis

GTPases involved in signal transduction and in the regulation of various cellular processes have many similarities both with each other and with several classes of ATPases involved in energy transduction. This similarity is seen at the structural and mechanistic levels and suggests evolution from common ancestors. The similarity is particularly strong between GTPases and ATPases involved in chemomechanical energy transduction, where there is high similarity of (but also important differences between) active site structures and details of the interaction of the proteins with nucleotides. ATPases which couple the free-energy of hydrolysis of ATP to an energy-requiring chem-

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ical process are in many cases structurally less well related, but there appear to be common principles in all these proteins. It has recently been suggested that they should not be referred to as ATPases or GTPases, but as energyases [1], since their important function is not that they produce diphosphates from triphosphates, but that they use the free energy change associated with this reaction to drive a process such as movement or chemical synthesis. In many cases, it is clear what the role of the free-energy of hydrolysis is, at the level of thermodynamics or energetics, but in the case of signal-transducing GTPases this is less obvious. Nevertheless, statements to the effect that the free energy of hydrolysis of GTP is used in energy transduction, or more explicitly that the free energy is used to drive a conformational change involved in energy transduction, are often made. In this article, an attempt is made to investigate the exact relationship between the free energy of GTP hydrolysis and events involved in the transduction process.

GTPases involved in signaling and regulation exist typically in two states, or conformations, which are correlated with the state of the nucleotide at the active site. Thus, when GTP is bound, they are in the active state [2]. The essential property of the GTP-bound state appears to be that it has high affinity for a partner protein, which is often described as an effector molecule. This interaction is required, for example, for a signal to be passed to other molecules in a signal transduction cascade. At the simplest level, the role of GTP-hydrolysis is clear: it leads to (re)generation of the inactive GDP state. This state is inactive in the sense that it interacts weakly with the effector molecule and therefore does not transmit a signal to it. Thus, the hydrolysis reaction represents the process of switching off the signaling GTPase. The question to be asked here is whether the high free energy of hydrolysis of GTP plays a significant (i.e. essential) role in the signal transduction process. Expressed in another fashion, we can ask whether the process of switching off could function efficiently if the free energy of hydrolysis of GTP were significantly smaller than it is. To approach this question, a simplified system containing GTPase, GTP (initially) and effector is examined. The reactions and equilibria in this system are shown in Scheme 1.

The purpose of the following analysis is not to attempt to model the dynamics of such systems, since this would involve consideration of all the factors involved, including proteins with GTPase—stimulatory activity (in many cases GAPs, or GTPase activating proteins) and GEFs (GTP/GDP exchange factors). GAP activity will, in general, merely catalyse the establishment of the equilibrium situation described in Scheme 1, unless the GAP is present at concentrations which lead to saturating binding to G. In the latter case, its effect will be dependent on whether it binds preferentially to the GTP or GDP states, as analyzed below for effectors. In several cases where the GAP interaction has been characterized quantitatively, the interaction is stronger with the GTP state. It would then lead to effects on the equlibrium between GTP and GDP states similar to those described for the effector below. The effects of GEFs are not included in the analysis, since we are examining the tendency, or potential, of the system to 'turn off' as a result of the GTPase reaction, which is the opposite of the effect of active or activated GEFs. In other words, we are concerned at present with the deactivation phase of the activity cycle, not the activation phase. Clearly, under activating conditions, both phases are operative and will, at the simplest level, compete with each other.

The overall equilibrium constant for Scheme 1 corresponds to the equilibrium constant for GTP hydrolysis free in solution. It is referred to as K_{hyd} and is directly related to the standard free-energy of hydrolysis of GTP by the relationship

$$\Delta G^{\circ} = -\mathrm{RT}\ln K_{hyd}$$

It is related to the equilibrium constants of Scheme 1 by the relationship

$$K_{hyd} = K_1 \cdot K_2 \cdot K_3 \cdot K_4 \cdot K_5 \quad (1)$$

Rearranging this equation we obtain

$$K_3 = \frac{K_{hyd}}{(K_1 \cdot K_5) \cdot (K_2 \cdot K_4)} \quad (2)$$

equilibrium constant for GTP hydrolysis when effector is bound

relative affinity of GTP and GDP to G

relative affinity of effector to the GTP and GDP-bound forms of G

This form of the equation has been chosen since it illustrates some important requirements of the system. The following points can be made:

1. A fundamental property of the system is that $K_2 \cdot K_4$ (i.e. the ratio of effector affinity to the GTPase with GTP or GDP, respectively, bound to the active site) should be large, since this is the basic property leading to signal transmission.

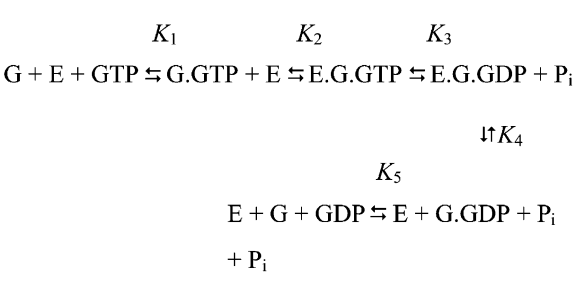
2. Despite the requirement that $K_2 \cdot K_4$ is large, inspection of equation 2 suggests that there is a limit to its magnitude. Thus, if $K_2 \cdot K_4$ were very large, K_3 would be very small. If K_3 were significantly smaller than 1, this would mean that the system would tend to remain in the E.G.GTP state as long as the effector is bound to the GTPase. Stated another way, even in the absence of activation of the GTPase by an exchange factor (which is the normal process leading to replacement of GDP by GTP, and which should occur in a regulated fashion), interaction of the effector with the GDP form of the GTPase would lead to generation of the E.G.GTP complex. Thus, the effector/GTPase system would tend to switch itself on, an unwanted property, since switching on should only occur as a result of regulated exchange of GTP for GDP. As will be seen later, whether this effect would be serious or not depends on whether the large value of $K_2 \cdot K_4$ is generated by a large value of K_2 or of K_4.

3. The magnitude of K_3, which is an expression of the distribution between the GTP- and GDP-bound states when effector is bound, is dependent on all the parameters on the right-hand side of the equation. The large value of K_{hyd} helps to keep K_3 large, which is a requirement for efficient switching to occur, even if $K_2 \cdot K_4$ is large, thus providing the thermodynamic driving force to generate the GDP state and overcome the tendency of preferential binding of E to G.GTP to stabilize the GTP-bound state.

Important factors for GTPases are their affinities for GTP and GDP (K_1 and $1/K_5$, respectively). Although they vary considerably, for a large group of them they are quite high. For the sake of discussion, they are taken as 10^{10} M^{-1}, commensurate with values measured for the Ras proteins and several other proteins of the Ras superfamily [3,4]. The exact magnitude of these constants is not of great significance for the arguments presented, but a high affinity is in fact necessary for the functioning of classical GTPase switches (this is to ensure that only regulated exchange of GTP for GDP occurs, which is achieved via low intrinsic dissociation rate constants that are accelerated by activated or recruited exchange factors). Of more significance for the present arguments are the relative affinities of GTP and GDP, as will be seen, but they are roughly equal for Ras. Thus, $K_1 \cdot K_5$ has a value of approximately 1. A large value of $K_1 \cdot K_5$ is not to be expected (i.e. considerably stronger binding of GTP than GDP to *G*), and is also not observed in practice, since this would erode the potential provided by the large value of K_{hyd}. For the interaction of Ras with the

Ras-binding domain (RBD) of its effector Raf-1, K_2 is approximately 10^8 M^{-1}, and $1/K_4$ is approximately 10^3 smaller (i.e. 10^5 M^{-1}) [5]. For the sake of illustration, we assume $K_2 \cdot K_4$ to be 10^3 (i.e. $10^8 \times 10^{-5}$), and using a value for K_{hyd} of 10^6 M, we can then calculate K_3 to be 10^3 M, showing that the equilibrium between GTP and GDP is very much in favor of GDP[1]. The factor of 10^3 which has been 'lost' in this constant in comparison to the situation free in solution, where the equilibrium constant for GTP hydrolysis is approximately 10^6 M, has been 'used' to generate the 10^3-fold difference in affinity between the GTP- and GDP-bound forms of Ras–Raf.

What would happen if the free energy of GTP hydrolysis were much lower? Going to the extreme case of $\Delta G^\circ = 0$, K_{hyd} would be 1 M, and from equation 2, K_3 would not be 10^3 M, but 10^{-3} M (assuming $K_1 \cdot K_5 = 1$ and $K_2 \cdot K_4 = 10^3$). Thus, the system would tend to be 'stuck' in the 'on' state (GTP state).[2] How could this situation be overcome? A small value of $K_1 \cdot K_5$ would help, i.e. GDP would have to be bound much more strongly than GTP. However, this would cause problems in another aspect of the mechanism of such systems, i.e. in the interaction with exchange factors. Thus, such a system (GTPase which binds GTP much more weakly than GDP) could function in the required fashion as far as GDP release under the influence of exchange factor is concerned, but the resulting stable complex with the exchange factor would not be easily dissociated by the weakly bound GTP [6]. A relatively modest effect of this type is in fact seen for EF–Tu, which has a approximately 100-fold higher affinity of GDP than GTP.



Scheme 1. G is the GTPase, E the effector and P_i is inorganic phosphate

The arguments presented so far are of a semi-quantitative nature, but they allow a first identification of the significance of the large equilibrium constant of GTP hydrolysis for the mode of action of signaling GTPases. Stated simply, it provides the driving force for the turned on system to turn off, although there is an opposite tendency (i.e. self on-switching) inherent in the manner in which the GTPases interact with their effectors (stabilization of the GTP state by interaction with effector). In the following, this is analyzed more quantitatively to illustrate the points, which have already been made qualitatively. One of the problems in this undertaking is lack of knowledge concerning relative concentrations of components of the systems under in vivo conditions. We have therefore examined extreme model situations and looked at the influence of effector concentration on the distribution between the GTP and GDP forms of the GTPase at equilibrium on the one hand, and of the effect of GTPase concentration on the amount of effector bound to which form of the GTPase on the other hand. Both of these approaches lead to similar conclusions, but we will only refer in detail to the second approach (i.e. we assume that the GTPase is present in excess over effector). Based on Scheme 1, the following relationship between the fraction of the effector bound to the GTPase as a function of the GTPase concentration can be derived.

[1] It should be noted that the distribution between GDP and GTP is not given directly by K_3. This is partly because K_3 is a composite constant comprising two steps (GTP hydrolysis and P_i release). Thus, its dimensions are M (i.e. molar), and the actual distribution of nucleotide between GDP and GTP is given by $K_3/[P_i]$. Taking the concentration of P_i in the cell to be approximately 1 mM, the ratio of GDP–GTP is then 10^6 if $K_3 = 10^3$. Note that this does not distinguish between the states E.G.GDP and E.G.GDP.P_i. The distribution between these two states will depend on the relative values of the equilibrium constant for GTP cleavage on the enzyme and for P_i release from E.G.GDP.P_i, for which there is no information at present. As shown below, the actual distribution is also influenced by further steps in the mechanism.

[2] Note that the actual distribution between GDP and GTP will be dependent on the prevailing concentration of P_i in the same manner as noted in the previous footnote.

$$\text{Fraction of effector bound to GTPase} = \frac{\sum[E.G]}{\sum[E]} = \frac{[E.GTP] + [E.GDP]}{[E] + [E.GTP] + [E.GDP]}$$

$$= \frac{1}{1 + \frac{K_{hyd}(1 + K_4/[G])}{K_1K_2K_4K_5[P_i]}} + \frac{1}{1 + K_4/[G] + [P_i]K_1K_2K_4K_5/K_{hyd}} \quad 3)$$

Fraction of E bound to the GTP state of the GTPase

Fraction of E bound to the GDP state of the GTPase

In principle, the species E.G should also be considered. However, prevailing nucleotide concentrations in the cell are so high that this can be ignored. It should also be pointed out that a further simplification is that the influence of the relative concentrations of free GTP and GDP is ignored, since in the absence of an exchange factor or activated receptor exchange of nucleotides will not be of significance. Of course, in reality, in some systems exchange factors are constitutively active, but the purpose of the present discussion is to examine the potential for on-switching of the system without the involvement of these factors. For the present discussion, we assume simply that we have allowed a certain undefined concentration of GTP to be hydrolyzed to GDP and P_i, but will consider a specified prevailing concentration of P_i (typically 1 mM).

In Fig. 1A,C,E, we have plotted the fraction of effector bound to the GTPase against the GTPase concentration for 3 different values of K_{hyd}. Using a value of 10^6 M, which applies to GTP under physiological conditions, and the values of the other constants which apply to the Ras–Raf system as described above, it can be seen that the fraction of effector bound remains low as long as the GTPase concentration is low compared to K_4, which is the dissociation constant of E from its complex with G.GDP (green curve in Fig. 1A). As this value is approached and passed, a progressively higher fraction of the effector becomes bound to the GTPase. According to our understanding of how such systems function, we must assume that the magnitude of K_4 is tuned appropriately to physiological concentrations so that in the absence of exchange factor catalyzed replacement of GDP by GTP, this fraction stays low.

Two more curves are shown in Fig. 1A. In black, the situation is shown for the hypothetical case that the free-energy of hydrolysis of GTP is 50% of the actual value, or expressed in terms of equilibrium constants, that K_{hyd} has a value of 10^3 M. Interestingly, with the set of equilibrium constants chosen for the Ras–Raf system, the resulting curve is indistinguishable from that calculated for $K_{hyd} = 10^6$ M. This tells us that, under these conditions for this system, the free energy of GTP hydrolysis could be significantly lower than it is and the 'efficiency' of the switching off process would still be sufficient to ensure that the process works. This is illustrated more directly in Fig. 1B, which shows that there is no tendency to form the effector-GTPase-GTP complex even at the highest concentration of GTPase used for the calculation. Reducing the free energy of hydrolysis even further (to zero, i.e. $K_{hyd} = 1$ M) leads to the red curves in both figures. Thus, as shown in Fig. 1A, association of the effector with the GTPase becomes significant at lower GTPase concentrations, and, as is evident from Fig. 1B, this is due to formation (or stabilization) of the complex between the effector and the GTP form of the GTPase. Despite this, it can be seen that effector binding and generation of the GTP form of the GTPase is still insignificant up to approximately 10^{-7} M GTPase concentration, underlining the fact that the switching off process is relatively insensitive to the equilibrium constant of the hydrolysis process.

The situation changes when certain adjustments are made to the constants describing the system.

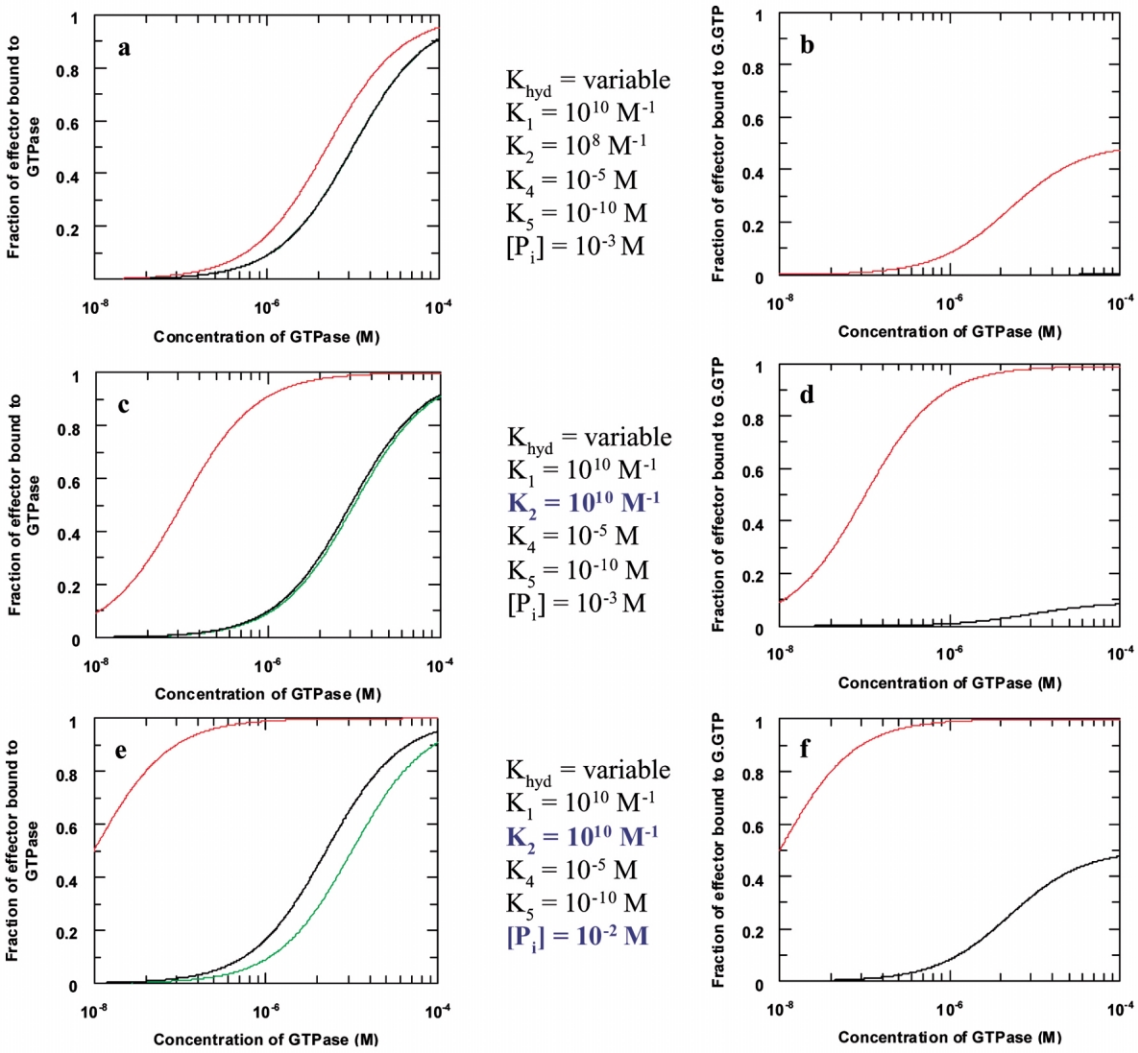


Fig. 1. Fraction of effector bound to a GTPase as a function of the GTPase concentration at constant low effector concentration according to Scheme 1 and equation 3. On the left, the sum of the bound forms (i.e. [E.G.GDP] + [E.G.GTP]) is plotted, on the right only [E.G.GTP]. The constants used for the simulations are shown in the boxes (constants changed between successive plots are highlighted in blue). K_{hyd} has values of 10^6 M (green curves), 10^3 M (black curves) or 1 M (red curves). In 1b, the green and black curves are not distinguishable, while in 1d,f, the green curve is not distinguishable from the *X*-axis.

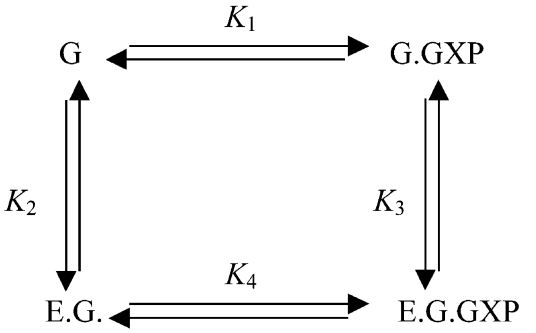
Increasing K_4 (i.e. weakening the affinity of effector for G.GDP) has very little effect on the curves (not shown in the figure), and this is understandable for the following reason. While an increase in K_4 leads to a corresponding decrease in K_3, according to equation 2, thus increasing the tendency for GTP formation when effector is bound, it also means that a higher concentration of effector is needed to achieve this effect. In contrast, increasing K_2 by a factor of 10 (i.e. increasing the affinity of the GTP form of the GTPase to the effector) has a marked effect, as shown in Fig. 1C,D. Thus, in the curve for $K_{hyd} = 1$ M, significant interaction between the GTPase and its effector occurs even at low GTPase concentration, and Fig. 1D shows this to be due to formation of the

complex between the effector and the GTP form of the GTPase. The influence on the curve for $K_{hyd}=10^6$ M is negligible, but noticeable for $K_{hyd}=10^3$ M. The effect of changing another parameter which tends to favor the formation of GTP, the free P_i concentration, has a further significant effect. Thus, increasing the P_i concentration from 1 to 10 mM (i.e. to a value which is not physiologically unrealistic) shifts the curves for $K_{hyd}=1$ M and $K_{hyd}=10^3$ M towards lower concentrations, this being due, as before, to production of the effector-GTPase-GTP complex. However, the curve corresponding to the real value of K_{hyd} (10^6 M) is still unaffected.

The simulations shown in Fig. 1 illustrate one main point, which is, in fact, the most important aspect of the high free energy of hydrolysis of GTP. This is that it ensures an efficient switching off process of the GTPase, and thus of its interaction with its effector, and that this process is robust, in the sense that changes in the constants defining the system do not have an effect on this 'efficiency'. These changes could be differences between different GTPase-effector systems or changes in conditions (e.g. P_i concentration).

The main conclusion so far is that the high free energy of hydrolysis of GTP allows differential binding of effector to the GTP and GDP forms of the GTPase while still retaining the potential to turn off the signal by hydrolysis of GTP. In this sense, the free energy of hydrolysis of GTP is 'used' to allow strong interaction between the GTP form of the GTPase and the effector while not preventing the switching off process from occurring. A question, that remains to be answered, is how this is achieved at the level of the interaction between nucleotide and effector binding sites on the GTPase. To investigate this question, we can examine a GTPase, nucleotide and effector system in terms of the equilibrium relationships describing the manner in which nucleotide and effector binding to the GTPase influence each other reciprocally (Scheme 2).

Since the overall equilibrium constant for generating the ternary complex E.G.GXP must be the same regardless of which of the two possible pathways is taken starting from the individual components, the following relationship must hold:



Scheme 2. Equilibrium relationships in the interaction of a GTPase (G) with an effector (E) and a guanine nucleotide (G×P).

$$K_1 \cdot K_3 = K_2 \cdot K_4 \tag{4}$$

There are two versions of this equation, one for GTP and one for GDP. From these two equations, and assuming that K_1^{GTP} is equal to K_1^{GDP}, it is easy to derive the following relationship:

$$\frac{K_3^{GTP}}{K_3^{GDP}} = \frac{K_4^{GTP}}{K_4^{GDP}} \tag{5}$$

We can now consider how the fundamentally required property of the system, i.e. the higher affinity of effector to the GTP form than to the GDP form of the GTPase (large value of the left hand side of Eq. (5)), could be achieved. One of the two principle possibilities is that the affinity of GDP in the ternary complex is smaller than in the binary complex, with the GTP affinity remaining unchanged, thus leading to a large value of the right hand side of Eq. (5) (and consequently of the left hand side). This will be referred to as Model 1. In the other extreme situation, the affinity of GTP in the ternary complex would be increased in the ternary complex compared with the binary complex, and the GDP affinity would remain unchanged, thus leading to a large value of both sides of Eq. (5) (Model 2). Of course, a combination of both effects could occur. From first principles, it seems very likely that the mechanism of Model 2 will dominate. The reason for this is that according to Model 1, the effector would have the properties of a GDP-specific exchange factor

towards the GTPase, weakening GDP binding and allowing it to be replaced by GTP. Thus, the effector would tend to switch the system on in the same manner as exchange factors. This is contrary to the basic principle of the mechanism of signal transduction in such systems. Thus, Model 2, i.e. strengthening of GTP binding in the ternary complex seems to be more reasonable, and this appears to supported by the finding that the rate constant for GTP release from its complex with Ras is dramatically reduced on interaction with Raf [5]. However, GDP release is also slowed down, which is exactly the opposite effect of that expected from Model 1. Unfortunately, it is extremely difficult to obtain quantitative data on the degree of retardation of GTP and GDP dissociation by interaction with Raf, since it becomes immeasurably slow, but we must assume that the retardation (and corresponding increase in affinity) is quantitatively more for GTP than for GDP, thus keeping GTP binding tighter than GDP binding and thus the affinity of effector to the GTP state higher than to the GDP state.

These arguments lead to the notion that binding energy of GTP is used to stabilize an otherwise unstable form of G, which has high affinity for E. Thus, the true (intrinsic) binding energy for GTP is expressed in the E.G.GTP complex. At the level of binding to G alone, the overall affinity of GTP, which is similar to that of GDP, is actually the product of an unfavorable isomerization reaction and extremely tight binding. We can envisage this thermodynamically (but probably not mechanistically) as:

$$\mathrm{G} \quad \overset{K_{\mathrm{iso}}}{\leftrightarrows} \quad \mathrm{G}^{*} \quad \overset{K_{\mathrm{bind}}}{\leftrightarrows} \quad \mathrm{G}^{*}.\mathrm{GTP}$$

Scheme 3. Two-step binding of GTP to a GTPase (G)

Here, G is a conformation of the GTPase which binds weakly to the effector, while G* is a conformation which binds strongly. The overall (measured) affinity for GTP is given by:

$$K_{\mathrm{GTP}} = \frac{K_{\mathrm{bind}}}{1 + 1/K_{\mathrm{iso}}} \tag{6}$$

K_{iso} must be $\ll 1$ (otherwise the GTPase would bind strongly to the effector even in the absence of GTP), so that this equation simplifies to

$$K_{\mathrm{GTP}} = K_{\mathrm{bind}} \cdot K_{\mathrm{iso}}$$

Binding of effector stabilizes the G* state, so that K_{bind}, which is much larger than the measured K_{GTP}, determines the affinity of GTP in the ternary complex.

What is the nature of the conformational change induced by GTP binding? In this state, the binding site for the effector must be correctly organized for interaction with the effector molecule. In the best characterized example of such an interaction, we can identify this process. In the case of the Ras protein, mainly two areas are structured differently in the GTP- and GDP-bound forms of the GTPase [7–9]. These are the so-called Switch I and Switch II regions. The second of these does not appear to be directly involved in effector binding, but the first, Switch 1, was identified in early studies as being involved in effector binding (hence the earlier name of effector loop). Although the structure of the Ras–Raf complex has not been determined, the structure of a highly analogous complexes are known. In the complex between the Ras-binding domain of Raf-1 (RBD) and the Ras-related protein Rap1A [10], it can be seen that the interaction of Rap1A with RBD involves the effector loop or Switch I region of the GTPase. The structural change in Switch I on GTP hydrolysis involves the transition from a well defined, highly ordered state when GTP is bound, to a disordered state, whose structure is not well defined by electron density in crystals of small GTPases in the GDP state [7–9]. The well-defined structure of Switch I appears to be mainly stabilized by the interaction of the main chain NH of the conserved Thr-35 with the γ-phosphate group of GTP, as well as by the interaction of the side chain hydroxyl group of the same residue with the Mg^{2+} ion which is coordinated to the β- and γ-phosphate groups. Loss of these interactions as a result of GTP hydrolysis results in loss of the ordered structure of Switch I. An interpretation of these structural changes in terms of Scheme 3 is that in state G of the GTPase, Switch I is highly disor-

dered, whereas it is ordered in state G*. Ordering of Switch I costs energy, for entropic reasons, and this is 'paid for' by the binding energy of GTP. This fits in well with the observation that although GTP makes more interactions with Ras than does GDP, the affinities are almost equal, suggesting that the additional binding energy provided by these interactions is used to stabilize an otherwise unstable state of Switch I. To complete the picture, we must postulate that the conformation of Switch I in the GTP bound state is appropriate for effector interaction. The experimental evidence suggests that these conformations (i.e. of Switch I in the GTP bound state with and without effector bound) are in fact not identical, as would be expected at the simplest level, but residues which appear to differ significantly between the two states are not directly involved in the interaction.

The arguments presented lead to the conclusion that the high free energy of hydrolysis of GTP is important for the switch function of GTPases, but in a different manner to that which can be derived for some of the best characterized systems which transduce the free energy of hydrolysis of ATP into another energy form or another chemical bond. Taking the example of cross-bridge cycling in muscle contraction, a detailed knowledge of the thermodynamics and structural dynamics of the ATP-myosin-actin interaction leads to a direct interpretation of the coupling of changes in free energy to production of mechanical work and this can be understood in the framework of a structural model [11]. For the GTPases, the relationship is more nebulous, but nevertheless tangible. It is perhaps best stated in the following manner: The function of the high free energy of hydrolysis of GTP by GTPases is to provide the driving force for switching off (generation of the GDP-form of the GTPase) to occur in spite of the opposing force inherent in the mechanism of signal transduction, i.e. the strong interaction of the effector with the GTPase.GTP complex, which tends to stabilize the switched on state. The large free energy of hydrolysis leads to a robust system, i.e. one with a strong tendency to turn off over a variety of conditions (e.g. different concentrations of the protein components and possibly of other interacting molecules, different concentrations of Pi and nucleotides, different combinations of equilibrium constants for different systems).

$$\mathrm{G} \overset{K_{\mathrm{iso}}}{\leftrightarrows} \mathrm{G}^{*} \overset{K_{\mathrm{bind}}}{\leftrightarrows} \underline{\mathrm{G}^{*}.\mathrm{GTP}}$$

$$\mathrm{AM} \overset{K_{\mathrm{amdiss}}}{\leftrightarrows} \mathrm{A}+\mathrm{M} \overset{K_{\mathrm{ATP}}}{\leftrightarrows} \underline{\mathrm{A}+\mathrm{M}.\mathrm{ATP}}$$

Scheme 4. Comparison of the utilization of nucleotide binding energy in a signal-transducing GTPase and in actomyosin (A = actin, M = myosin). It should be noted that the actual mechanistic pathway for producing A + M.ATP is known to proceed by formation of AM.ATP followed by dissociation of actin, but the theoretically possible pathway used in this scheme allows a better comparison with the formalism used in Scheme 3 and repeated here. The underlined species is, in both cases, intrinsically unstable due to the potential of GTP or ATP hydrolysis to GDP or ATP. In the case of actomyosin, experimental values for the constants are known (K_{amdiss} = approx. 10^{-9} M, K_{ATP} = approx. 10^{11} M^{-1}) [12], whereas for GTP we only have a value of the product of K_{iso} and K_{bind} (approx. 10^{11} M^{-1} for Ras) [3]. ADP interacts much less strongly than ATP with myosin and competes only weakly with actin binding.

Can we draw analogies to more classical energy-transducing systems in a manner, which helps to understand the role of GTP in such GTPase/effector systems? In the case of actomyosin ATPase, this analogy is quite illustrative. The fundamental energetic features of the latter system can be defined by stating that myosin can form very stable complexes with actin on the one hand and ATP on the other, and that these interactions compete with each other by an allosteric mechanism. This can be formulated in a similar manner to that of Scheme 3:

The significant feature of the reaction schemes shown is that an intrinsically unstable state of the proteins (the G* state of the GTPase or the dissociated state of actomyosin at the concentrations occurring in mucle tissue) is stabilized by very strong interaction with a nucleoside triphosphate. As discussed in the legend to Scheme 4, the individual equilibrium constants are known for actomyosin, but not for GTPases. However, if we assume that the nucleotide-free state of G resembles the GDP state in terms of the distribution

between the G and G* conformations, as implied by arguments presented above, K_{iso} can probably be related directly to the weakening of binding of effector on replacing GTP by GDP, which in the case of Ras is by a factor of approximately 10^3. Taking K_{iso} to be 10^{-3}, K_{bind} would then have a value of 10^{14} M^{-1} to explain the observed affinity of 10^{11} M^{-1}. Subsequent hydrolysis of GTP or ATP generates the respective GDP or ADP states, with an accompanying strong tendency to return to the stable starting state or conformation of the proteins (i.e. to G.GDP, which binds weakly to effector, or to AM.ADP, in which the protein–protein interaction is of similar stability to that in the AM complex). In the case of actomyosin, it is the reestablishment of the original strong protein–protein interaction which leads to liberation of the free energy of ATP hydrolysis as mechanical work. In the case of the GTPases, the highly irreversible hydrolysis of GTP to GDP allows reformation of the conformation of the GTPase which binds weakly to the effector. A lower free-energy of hydrolysis of ATP would, of course, mean that less work could be produced per cross-bridge cycle in actomyosin contractile systems, whereas in the GTPases, there would be a less pronounced tendency for the switch to turn off spontaneously, which would be exacerbated by interaction with the effector, which preferentially stabilizes the G*.GTP state.

Whether GTPases involved in signal transduction and regulation can be described as energy-transducing systems is, perhaps, merely a question of semantics. There is certainly a different quality concerning the direct coupling of chemical and a different form of energy in GTPase systems when compared with, for example, the actomyosin/ATP interaction of many contractile systems. As a compromise, it can be stated that the free energy of hydrolysis of GTP is used in such systems to render switching off of the active state essentially irreversible in the absence of events or factors which allow the reverse process (i.e. activation) to occur in a controlled fashion by regulated exchange of GTP for GDP.

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