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ARTICLE

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Exchange factors, effectors, GAPs and motor proteins: common thermodynamic and kinetic principles for different functions

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Abstract In this article, we review the properties of several classes of proteins that interact with ATPases and GTPases involved in energy and signal transduction. We show that certain common basic thermodynamic principles apply to the manner in which the nucleotide hydrolases interact with their partner molecules, and that the principles involved in signal transduction can be quantitatively modified to give systems with the known properties of energy transducing systems. As an example, actin can be described as an exchange factor for myosin, with its exchange activity being specific for ATP or inorganic phosphate in the myosin.ADP.P_i complex, in contrast to the unspecific exchange activity of guanosine nucleotide exchange factors operating on GTPases involved in signal transduction and regulatory processes. These common aspects are reflected in shared structural features, suggesting an evolutionary relationship between such systems.

Keywords Exchange factors · Motor proteins · GTPases · ATPases · Effectors

Introduction

Modern methods in molecular and cell biology have led to the identification of a large number of biological interactions between proteins and other protein molecules, smaller molecules or nucleic acids. A common recurring theme concerns the interaction of a protein with two or more different ligands, and the manner in which the interaction of a ligand at one site affects the properties of a second, possibly distal, site in its interaction with a different ligand. The modulation of thermodynamic and kinetic properties in ternary complexes with respect to binary complexes in such systems forms the basis for energy transduction, signal transduction and the regulation of such crucial processes as protein synthesis, protein trafficking and protein targeting, as well as of gene expression. A particularly important general class of systems is that in which protein-protein interactions are modulated by interaction with nucleotides, in particular with ATP or GTP. The underlying principle in all such systems is that spatially separate (or partially overlapping) binding sites on a protein molecule (motor protein, signal transducing protein or regulatory protein) communicate with each other via structural changes of the protein. We discuss here such proteins from the point of view of the basic thermodynamic and kinetic coupling between individual binding and dissociation events and apply the basic principles involved to several examples of characterized systems. The examples discussed all involve proteins that interact with nucleoside di- and triphosphates. While this is partly determined by our areas of specific interest, it also reflects the fact that a large number of energy- and signal-transduction processes involve such proteins.

Basic principles involved in coupling between sites

There is a large body of literature dealing with functional coupling between binding sites on proteins based on the principle of allostery. The basic concept here is that binding of a ligand at one site on a protein changes, or tends to change, the structure of the protein such that other binding sites, which may be remote from the occupied site, change their properties. In early work, there was emphasis on a connection between

Dedicated to Professor H. Gutfreund on the occasion of his 80th birthday

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allostery and cooperativity in oligomeric proteins. However, it is clear that the principles also apply to binding of non-identical ligands to non-identical sites on monomeric proteins, except for that of the postulated tendency to maintain symmetry in an oligomeric molecule. Rather than repeating the arguments from the literature (for example see Gutfreund 1995) or referring the reader to individual works, we present here a simple treatment specifically related to the systems under discussion.

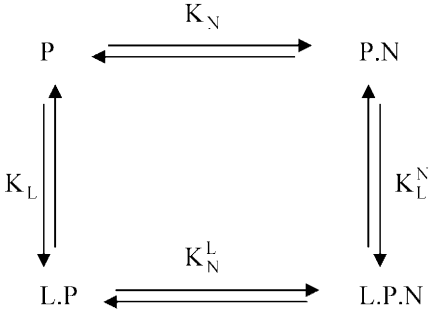
Scheme 1 shows the generalized model for interaction of two ligands (L for a protein ligand, N for a nucleotide) to a protein P.

Since the overall equilibrium constant for producing L.P.N from its components L, P, and N must be the same regardless of which of the two possible pathways are taken, the following relationship holds:

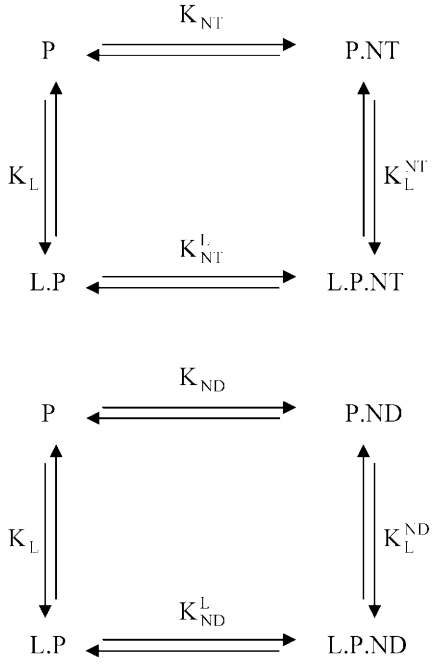
$$K_N K_L^N = K_L K_N^L \text{ or } \frac{K_N^L}{K_N} = \frac{K_L^N}{K_L} \quad (1)$$

Thus, there is the same change in affinity in going from the binary to the ternary complex for both types of ligand (nucleotide and second ligand). Stated another way, if the binding of N weakens (or strengthens) the binding of L to P by a certain factor, L also weakens (or strengthens) the binding of N by the same factor. There is no way in which this law can be violated. However, the reciprocal effects on the kinetics of the interactions could, in principle, be different. Thus, in a system showing "exchange" activity, the dissociation of one of the two ligands from the ternary complex could be accelerated more than the other. This would imply that, in order to retain thermodynamic balance, its association must also be affected more than that of the other ligand, in the sense that it must be increased more (or decreased less).

In systems in which nucleoside triphosphate hydrolysis occurs, we have two similar schemes (Scheme 2).



Scheme 1. Model for binding of two ligands to separate but interacting sites on a protein. P = protein (nucleotide binding), N = nucleotide, L = second ligand (protein, nucleic acid, peptide, etc.). The equilibrium constants are defined in the direction of binding (affinity constants)



Scheme 2. ND is a nucleotide diphosphate and NT a nucleoside triphosphate

From similar considerations to those applied above:

$$\frac{K_L^{NT}}{K_L} = \frac{K_{NT}^L}{K_{NT}} \quad \text{and} \quad \frac{K_L^{ND}}{K_L} = \frac{K_{ND}^L}{K_{ND}}$$

from which

$$\frac{K_L^{NT} K_{NT}}{K_{NT}^L} = \frac{K_L^{ND} K_{ND}}{K_{ND}^L} \quad (2)$$

and

$$\frac{K_L^{NT}}{K_L^{ND}} = \frac{K_{NT}^L K_{ND}}{K_{ND}^L K_{NT}}$$

Equations (1) and (2) form the basis for discussion of all types of systems we are considering.

Nucleotide exchange factors

Proteins which function as nucleotide exchange factors are encountered in a large number of systems. For example, a common property of many GTPases involved in signal transduction and regulation is that they have GDP bound in the inactive (resting) state, and that spontaneous dissociation of GDP is extremely slow, with typical half-lives of hours or days. The biological significance of this property is that spontaneous activation (replacement of GDP by GTP) is inhibited, and that this only occurs in a regulated manner. The protein agents involved in the last step of this activation mechanism can be described collectively as exchange proteins or exchange factors (exchangers). They may be cytosolic proteins acting in the cytosol, integral membrane

proteins, cytosolic proteins which are recruited to the membrane, or components of subcellular organelles. Before examining specific examples of this type of activity, we present some general arguments which must apply.

Basic mechanism

The simplest possible mechanism for displacing one ligand by another is classical competition for binding to one site. This is the end product of the action of an exchange factor working on, say, a GTPase, since GTP replaces GDP at the same binding site. However, this process cannot be accelerated by a putative exchanger which operates by the same mechanism, i.e. by also binding to the nucleotide binding site. This is because the rate of binding of the exchanger would be dependent on the spontaneous rate of GDP dissociation, so that even if the exchanger itself then dissociated more rapidly, there would be no acceleration of the overall rate. Although mechanisms can be envisaged in which one ligand displaces another at the same site after initial formation of a ternary complex in which partial dissociation of one ligand and partial association of the displacing ligand occur (this has been called facilitated dissociation; Prinz and Striessnig 1993), this is not formally different from a more general model in which binding of the exchanger occurs at a distinct site and exerts its effect via a conformational change. This is the situation whose linked equilibria are described in Scheme 1 and Eq. (1), and presently available kinetic and structural evidence strongly support such a mechanism.

It is possible to conceive of an exchange mechanism which does not involve changes of affinity of the displaced ligand in the binary and ternary complexes. Thus, the rate constant for dissociation of the ligand could be increased without changing its affinity to the protein if the association rate to the protein-exchanger complex is increased by the same factor. The degree of acceleration would be limited by the fact that the association rate constant can never be faster than the limit dictated by diffusion rates. However, since the effective association rate constants of many ATP- and GTP-binding proteins are 2–3 orders of magnitude lower than this, there is, in principle, room for acceleration. A consequence of this type of mechanism would be that there is not a cyclical change of affinity of the exchange factor and the protein, which is an essential feature of energy transducing systems and perhaps of others as well. While available evidence (as discussed below) suggests that mechanisms based purely on this effect do not occur (at least in systems examined so far), there is a suggestion of a contribution of an effect of this type in several systems. This leads to a larger increase in the dissociation rate of GDP than would be expected on the basis of the change in affinity of GDP on interaction with the exchanger, and in practical terms increases the "efficiency" of the exchanger. Expressing this in another manner, the kinetic efficiency (effectiveness is perhaps a better word) of the exchange factor (defined as the ratio of the dissociation rates of the same ligand from the ternary complex and from the binary complex) is higher than the thermodynamic efficiency (defined as the ratio of the affinities of the same ligand in the binary and ternary complexes).

Degree of competition and relative affinities of exchanged ligand and exchanger

Although the mechanism discussed is not a simple competitive binding mechanism, the basis is nevertheless competition between binding of the two ligands to different protein conformations (i.e. exchanged ligand and exchanger). This situation is in fact dealt with in classical enzymology (Segel 1975). In the present treatment, we express the degree of this competition quantitatively by the term $\frac{K_N^L}{K_N}$, and this represents the degree of weakening of binding of the exchanged ligand on interaction of the protein with the exchanger. In the extreme case, $\frac{K_N^L}{K_N} = 0$ (meaning that $K_N^L = 0$) and the situation is formally equivalent to classical competition involving one site. Cases in which this pertains are not to be expected, since this would also imply that K_L^N is also zero, so that infinite concentrations of L would be needed to generate the ternary complex, the formation of which is a requirement for exchange activity. We should thus expect $\frac{K_N^L}{K_N}$ to be small, so that significant exchange activity is developed, but not so small that unrealistically high concentrations of exchanger are needed.

Relative affinities of exchange factors and nucleotides

Related to this point are the affinities of ligands and exchangers. Systems which are tightly regulated will need to have very slow spontaneous dissociation rates of ligands from the binary complexes. This implies high affinities. As already pointed out, according to the model envisaged, exchange activity depends on a competition in the binding of ligand and exchanger, so we can expect intuitively that their respective affinities should not be too different. We can envisage two extreme cases. In one case, the exchanger affinity to the protein is much less than that of the ligand (e.g. GDP). Substantial weakening of GDP binding in the ternary complex would imply equal weakening of the exchanger, so that this would tend to generate significant concentrations of the crucial ternary complex only at very high concentrations of exchanger, which are unlikely to pertain. At the other extreme, the exchanger would have an affinity to the protein which is much higher than the already high affinity of the ligand. While this could lead to significant acceleration of dissociation of the ligand at realistic concentrations of the exchanger, it would result in formation of an extremely stable complex which could

only be dissociated with difficulty (i.e. slowly and inefficiently) by the displacing molecule (i.e. GTP), an undesirable feature in simple exchange systems. It therefore seems reasonable to conclude that the affinities of exchanger and exchanged ligand should not be too different, although it should be borne in mind that what will really be of importance is a factor which might be described as an effective affinity and which takes prevailing concentrations of the different species into consideration. Thus, GTP is likely to be present at much higher concentrations than an exchange factor (or GDP) in most systems, so that its affinity need not be as high as that of the other species.

Specificity of exchange reactions

Since the function of an exchange factor is to displace one ligand and allow a different ligand to bind, a naive expectation might be that the exchange activity is specific for the displaced ligand. In the context of an NTPase, in the extreme case this would mean that $\frac{K_{ND}^{L}}{K_{ND}}$ is large, but $\frac{K_{NT}^{L}}{K_{NT}} = 1$ (i.e. there is no change in affinity of NTP in the binary and ternary complexes). This also means that the affinity of P for the exchange factor (which must be high) is also unchanged by the presence of NTP at its binding site. For this reason, NTP would not be able to induce dissociation of the protein-exchange factor complex, and the system would effectively be arrested in this state, which in most cases would be disadvantageous. Thus, in general, exchange activity will apply to both types of ligand, at least in systems in which the exchange activity per se is the main purpose of the interaction. As discussed below, the situation is different for energy transducing systems involving motor proteins in which one of the partner proteins (the polymeric protein) has exchanger activity.

Example of exchange activity

One of the most carefully studied examples using isolated components is that of the interaction of the GTPase Ran with its exchange factor RCC1 (Klebe et al. 1995). These are proteins involved in the regulation of nuclear transport. Using purified proteins expressed in *Escherichia coli*, the equilibrium constants in Table 1 were obtained for fluorescent derivatives of GDP and GTP (mantdGDP and mantdGTP).

In terms of the equilibrium constants, it can be seen that the exchange reaction, as expressed by the relative affinities of the nucleotides in binary and tertiary complexes, is relatively unspecific $\left(\frac{K_{ND}}{K_{ND}^{L}} = 3 \times 10^5, \frac{K_{NT}}{K_{NT}^{L}} = 2 \times 10^5\right)$. However, since GTP is bound more than an order of magnitude less strongly than GDP to Ran, a similar effect is seen at the level of the ternary complex. In general, the expectation from first principles stated above holds, i.e. the exchange activity is not specific.

A deeper understanding of the exchange mechanism can be obtained from examination of the individual rate constants of partial reactions in the scheme. These are shown in Table 2. It can be seen that the rate constants for dissociation of the ternary complexes (both dissociation of RCC1 and of GDP or GTP) are essentially similar, regardless of the phosphorylation state of the nucleotide. An interesting observation here is that the weaker binding of GTP than GDP in the binary complex with Ran arises from the faster dissociation rate, whereas in the ternary complex it is the association rate which is responsible for the lower affinity. In the cell, rebinding of GDP is presumably prevented by the much higher concentrations of GTP than of GDP. Another point which emerges is that there is a larger effect of RCC1 on the rate of GDP release (factor of 1.4×10^6) than there is on the affinity of GDP to Ran (factor of 3×10^5), a possibility which was alluded to on theoretical grounds. To maintain thermodynamic balance, this is compensated for by a higher association rate constant of GDP to the Ran.RCC1 complex than to Ran alone.

From exchange factors to actomyosin-like energy transducing systems

The mechanism of muscle contraction and motility in muscle and other actomyosin-based systems involves cyclical changes in affinity between myosin and actin in the sense that the affinity between the two proteins is

Table 1. Equilibrium constants for the formation of the ternary complex between Ran, RCC1 and methylanthraniloyl derivatives of GDP and GTP. The constants are named according to Scheme 1

	mGDP (M^{-1})	mGTP (M^{-1})
K_N	1.6×10^{11}	7.4×10^{9}
K_L	4×10^{11}	4×10^{11}
K_L^N	1.4×10^{6}	1.9×10^{6}
K_N^L	5.4×10^{5}	3.4×10^{4}

Table 2. Equilibrium and rate constants for the formation of the ternary complex between Ran, RCC1 and methylanthraniloyl derivatives of GDP and GTP. Equilibrium constants are according to Scheme 1, while k_f and k_r refer to the forward and reverse rate constants of the respective step

	mdGDP	mdGTP
K_N (10^{10} M^{-1})	16	0.74
k_f (10^6 M^{-1} s^{-1})	2.4	1.9
k_r (10^{-5} s^{-1})	1.5	26
K_L (10^{11} M^{-1})	4.0	4.0
K_L^N (10^6 M^{-1})	1.35	1.85
k_f (10^6 M^{-1} s^{-1})	74.4	102
k_r (s^{-1})	55	55
K_N^L (10^5 M^{-1})	5.4	0.34
k_f (10^6 M^{-1} s^{-1})	11.4	0.65
k_r (s^{-1})	21.1	19

modulated by the presence and nature of the nucleotide at the myosin active site. Actin has the character of an exchange factor towards myosin with its properties (in fact, the combined properties of actin and the specific myosin involved) tuned to the task of energy transduction (production of mechanical from chemical energy) and fine-tuned to the specific requirements of the particular motile system. Thus, different muscle types (e.g. fast and slow skeletal muscle, heart muscle, smooth muscle) need to exhibit different properties, and while some of these are controlled at the level of regulation in the broadest sense of the term, certain basic properties arise from the exact nature of the actin-myosin interaction.

The enzymatic cycle of actomyosin has been characterized in great detail, so that the manner in which reciprocal changes in affinities of myosin for nucleotides and for actin changes during the cycle are well understood (e.g. Geeves et al. 1984). A specific property of the system is the special role of the myosin-ADP-P_i complex. This is a thermodynamically unstable but long-lived intermediate, with a half-life in the absence of actin in the range of tens of seconds. Its affinity to actin is very low, and similar to that of myosin-ATP, so that while the ADP.P_i state is the critical intermediate in force production, we will present arguments concerning the change of affinity of actin to myosin in the two forms, myosin-ATP and myosin-ADP, but bearing in mind that the myosin-ATP and myosin-ADP.P_i complexes could be interchanged in the relationships derived.

Considering the two separate "exchange" cycles for ATP and ADP, we can describe the equilibrium relationships in terms of the inverted Eq. (2):

$$\frac{K_L^{ND}}{K_L^{NT}} = \frac{K_{NT}K_{ND}^L}{K_{ND}K_{NT}^L} \tag{3}$$

This expression defines the change in affinity between actin and myosin which occurs on hydrolysis of ATP to ADP (after loss of phosphate). The striking difference to the simple exchange systems discussed above is that, for myosin, $K_{NT} \gg K_{ND}$ (ca. 10^5 greater; Goody et al. 1977). Since K_{ND}^L and K_{NT}^L have values of ca. 10^4 M, this means that $\frac{K_L^{ND}}{K_L^{NT}} = 10^5$. It is this difference of affinity, which occurs in the cross-bridge cycle as a result of ATP hydrolysis, which corresponds to the energy available for the power-stroke of muscle contraction. The system can be qualitatively characterized as one in which the exchange properties of actin towards myosin-ATP are strong, but weak towards ADP.

What are the limits to efficiency of such a system (i.e. could the equilibrium constants be manipulated to give a larger change in affinity?). One way to do this would be to make K_{ND}^L more nearly similar to K_{ND} (this means that actin would have no exchange activity with respect to ADP, only to ATP). While this would increase the available work in one cycle, it would mean that the system was then "stuck" in the L.P.D. state, since ADP exchange would not occur. Thus, there is a trade-off between getting the maximal affinity change of bound cross-bridges and having a reasonable speed of cycling (how these are balanced will depend on the required properties of the contractile system). This argument is reminiscent of that given for simple nucleotide exchange factors showing why a GDP-specific exchange would not have desirable properties. However, for energy transduction to occur using the structurally based mechanistic model which has been derived from extensive studies of the muscle system, the general exchange properties characteristic of pure exchange systems have to be modified towards specific exchange activity. In more general terms, the negative relationship between binding of nucleotide and partner protein (such as actin) to the motor protein must be significantly different depending on whether the nucleotide is ATP or ADP. As discussed below, this principle also applies to other motor protein systems, with the preferential binding to the ATP or ADP forms in one prominent case being reversed.

Nucleotide-dependent interaction with effectors and GAPs

Effectors

Common to many systems involved in signal transduction pathways and regulation is the property of nucleotide state-dependent interaction with the next molecule in the signal pathway or in the chain of events which are regulated. These molecules are often referred to as effectors. The term effector is used in a different sense to its older usage in describing substances which affect enzymatic or other activity by allosteric mechanisms. In general, the nucleoside triphosphate bound state of an NTPase is able to interact with the effector, while the NDP state is not. Equation (2) is the important one for this system, since it shows formally the relationship between affinities of the nucleotides in the binary and ternary complexes and the affinities of the effector to the respective nucleotide bound states. The change in affinity (in general P.NT is more strongly bound than P.ND to L) can be achieved by manipulation of the constants on the right hand side of the equation. In some systems (e.g. Ras-Raf; see below), K_{ND} and K_{NT} are similar, so that the ratio $\frac{K_L^{NT}}{K_L^{ND}}$ has to be increased by the same extent as $\frac{K_L^{NT}}{K_L^{ND}}$. This could be done by an increase (relative to the affinity in the binary complexes) in K_{NT}^L, a decrease in K_{ND}^L, or a combination of both. A decrease in GDP affinity in the ternary complex (compared to the binary) would imply that the effector binds strongly to the nucleotide-free GTPase. Thus, the effector would tend to act like an exchange factor (displacing GDP), which is not a desirable property, since the effector would switch the GTPase to the on state. Increase in GTP affinity but

lack of change in GDP affinity would achieve the desired result without unwanted consequences. This would imply that the effector binds strongly to G.GTP, but weakly to the free GTPase or its GDP complex.

A system which has been reasonably well studied is the Ras-Raf system. The proto-oncogene product Ras in its active (GTP) form appears to recruit Raf-kinase to the plasma membrane as a step in a signal transduction pathway which involves a kinase cascade. Accordingly, Raf binds with high affinity to Ras.GTP and low(er) affinity to Ras.GDP. Interaction of Raf with Ras.GTP decreases the rate of dissociation of GTP dramatically (Herrmann et al. 1995), implying an increase in GTP affinity in keeping with the second of the two possible mechanisms discussed above. The affinity of Raf for p21.GTP is $5\times10^7\ M^{-1}$. The affinity of Raf to p21.GDP is $5\times10^4\ M^{-1}$, meaning that there is a difference of ca. 1000 in affinities of Raf for Ras.GTP and Ras.GDP. However, note that GDP release is also retarded, although presumably less drastically than for GTP, but quantitative data are not available on this issue. According to these considerations, there is a limit to the amount of stabilization of effector binding by the presence of GTP which is likely to be achievable in such systems. Thus, if the main effect is due to a tightening of effector and GTP binding in the ternary complex, the fact that the GTP affinity is already very high in the binary complex is probably limiting.

GAPs (GTPase activating proteins)

Factors which accelerate the rate of GTP cleavage of a GTP-binding protein are an important feature of many signalling and regulatory systems. They are fundamentally different from actin in its effect on myosin ATPase, since the intrinsic rate of ATP hydrolysis by myosin is fast (ca. $100\ s^{-1}$), and actin increases the steady state ATPase by increasing the dissociation rates of the products P_i and ADP. In contrast, GAPs have a direct effect on the cleavage rate, in cases which have been well characterized by donation of catalytically active residues (Mittal et al. 1996; Scheffzek et al. 1997). Presently available evidence shows that GAPs for Ras and similar proteins bind with micromolar or sub-micromolar affinity to Ras.GTP, and several orders of magnitude less strongly to Ras.GDP. Since Equation (2) holds in this case, and $K_{ND} \approx K_{NT}$ (in the case of Ras), $\frac{K_L^{NT}}{K_L^{ND}}$ must be relatively large, but there is no evidence available on the manner in which this is achieved.

From exchange factors, effectors and classical GAPs to kinesin-like motile systems

It is of interest to note that a second class of motile systems is known, which will be referred to here as kinesin-like systems, and which also involve cyclic changes in affinity between a motor protein, typically kinesin, and a polymeric protein, in this case the microtubule filament (for a review see Mandelkow and Johnson 1998). Again, the affinity between the two proteins varies with the presence and nature of the nucleotide at the active site of the motor protein. However, in contrast to the actomyosin system, the exchange activity appears to be stronger towards ADP than ATP, exactly the opposite of the case with actomyosin. This results in the ADP form of kinesin being weakly bound to microtubules, and the ATP form being (relatively) strongly bound. As already discussed, an inherent property of proteins with thermodynamic exchange activity is that they are strongly bound to the nucleotide-free state of the nucleotide-binding protein, and this applies to the kinesin-microtubule system. Thus, in this system there is a large change (increase) in affinity between the motor protein and the polymeric protein on release of ADP, so that this can be identified as the likely place in the enzymatic cycle of ATPase activity in which the free energy of ATP cleavage is converted into mechanical energy. This is in contrast to actomyosin systems, where it is the release of phosphate from the actomyosin-ADP-Pi complex, and thus effectively represents the change in free energy associated with the transition between the weakly bound actomyosin-ATP state and the strongly bound actomyosin-ADP state. As discussed above, the currently accepted model of the actomyosin contraction cycle involves a coupling of this step in the enzymatic cycle with the so-called power stroke of the mechanical cycle. In the case of kinesin, the concept of a power stroke, in the sense that a change in structure of a kinesin head leads directly to a defined amount of movement, appears to be less appropriate, since it is becoming clear that the movement is expressed as a result of interactions between the two heads, but the driving force is still the tightening of the interaction between the two protein molecules on loss of ADP. The necessary step of head detachment, to allow sliding to occur, then occurs preferentially not after ATP binding (although this does appear to lead to some weakening of the affinity), but after ATP hydrolysis and loss of phosphate to give the weakly bound ADP state, which can then reattach, presumably at another tubulin binding site, this new anchoring point then being stabilized by loss of ADP.

There are two aspects of these interactions which are reminiscent of those seen for GTPases. On the one hand, the basic property of a relatively high affinity of microtubules to kinesin-ATP and a low affinity to kinesin-ADP is analogous to the situation with GTPase-effector interactions. In addition to this, although the rate of the ATP cleavage step is relatively high even in the absence of microtubules (ca. $10\ s^{-1}$, which is many orders of magnitude higher than that of the Ras-superfamily of GTPases and many other GTPases such as EF-Tu), this appears to be increased by ca. one order of magnitude on interaction with microtubules. Thus, this can be compared with the GTPase activating action of GAPs towards GTPases.

Conclusions

The formalism developed here to describe the reciprocal effects of different ligands to one protein gives insight into thermodynamic and kinetic constraints on the properties of such systems, and shows how principles which are effective in signal-transducing systems can lead to energy-transducing properties by quantitative modification of distinct aspects of the interactions. The question arises as to whether these systems have a common evolutionary background. The common principles at the level of basic thermodynamics and kinetics identified for the proteins described or mentioned in this article make this seem likely. At the level of three-dimensional structure, the GTPases show extensive sequence homology and the same fold of their GTP binding domain. There are common motifs involved in nucleotide binding, and it seems likely that the GTPase mechanism is similar in these related proteins. The basic fold is similar in myosin and kinesin, even if overlap in the secondary structure elements between these two proteins, on the one hand, and the GTPases, on the other hand, is less perfect (Kull et al. 1996). There is a striking similarity in the manner in which the nucleotide phosphates are bound between all of the proteins, including the strong interaction with the so-called P-loop and the interaction with the essential magnesium ion. All of the proteins displaying the properties discussed above, as well as some others, but not all ATP or GTP binding proteins in general, contain this P-loop, which has the conserved sequence GXXXXGKS/T. Further sequence elements with conserved properties and presumably function are referred to as the switch I and switch II regions. Switch II is involved in nucleotide hydrolysis, and switch I undergoes an important conformational change on hydrolysis, at least in GTPases (Milburn et al. 1990; Schlichting et al. 1990). In ATPases, it appears to be the change in the switch II region which is amplified into the power stroke in the muscle cross-bridge cycle (Geeves and Holmes 1999). The possibility that all of the systems discussed here have evolved from a common nucleotide binding core has been discussed on the basis of structural similarities and a possible common progenitor structure has been proposed (Kull et al. 1998).

The systems considered in detail (GTPases in signal transduction, ATPases in energy transduction) have turned out to have many similar features and structures of GTPases in complex with exchange factors and effectors have begun to reveal the mechanisms of interaction between nucleotide and protein ligand binding sites (Kawashima et al. 1996; Nassar et al. 1995; Renault et al. 2001). Analogous complex structures are not yet available at high resolution for the ATP-dependent motor systems but it is to be expected that similar structural and conformational changes underlie the similarities seen at the thermodynamic and kinetic levels.

References

Geeves MA, Holmes KC (1999) Structural mechanism of muscle contraction. Annu Rev Biochem 68:687–728

Geeves MA, Goody RS, Gutfreund H (1984) Kinetics of acto-S1 interaction as a guide to a model for the crossbridge cycle. J Muscle Res Cell Motil 5:351–361

Goody RS, Hofmann W, Mannherz GH (1977) The binding constant of ATP to myosin S1 fragment. Eur J Biochem 78:317–324

Gutfreund H (1995) Kinetics for the life sciences. Cambridge University Press, Cambridge, pp 95, 96

Herrmann C, Martin GA, Wittinghofer A (1995) Quantitative analysis of the complex between p21ras and the Ras-binding domain of the human Raf-1 protein kinase. J Biol Chem 270:2901–2905

Kawashima T, Berthet-Colominas C, Wulff M, Cusack S, Leberman R (1996) The structure of the *Escherichia coli* EF-Tu. EF-Ts complex at 2.5 Å resolution. Nature 379:511–518

Klebe C, Prinz H, Wittinghofer A, Goody RS (1995) The kinetic mechanism of Ran-nucleotide exchange catalyzed by RCC1. Biochemistry 34:12543–12552

Kull FJ, Sablin EP, Lau R, Fletterick RJ, Vale RD (1996) Crystal structure of the kinesin motor domain reveals a structural similarity to myosin. Nature 380:550–555

Kull FJ, Vale RD, Fletterick RJ (1998) The case for a common ancestor: kinesin and myosin motor proteins and G proteins. J Muscle Res Cell Motil 19:877–886

Mandelkow E, Johnson KA (1998) The structural and mechanochemical cycle of kinesin. Trends Biochem Sci 23:429–433

Milburn MV, Tong L, deVos AM, Brunger A, Yamaizumi Z, Nishimura S, Kim SH (1990) Molecular switch for signal transduction: structural differences between active and inactive forms of protooncogenic ras proteins. Science 247:939–945

Mittal R, Ahmadian MR, Goody RS, Wittinghofer A (1996) Formation of a transition-state analog of the Ras GTPase reaction by Ras-GDP, tetrafluoroaluminate, and GTPase-activating proteins. Science 273:115–117

Nassar N, Horn G, Herrmann C, Scherer A, McCormick F, Wittinghofer A (1995) The 2.2 Å crystal structure of the Ras-binding domain of the serine/threonine kinase c-Raf1 in complex with Rap1A and a GTP analogue. Nature 375:554–560

Prinz H, Striessnig J (1993) Ligand-induced accelerated dissociation of (+)-*cis*-diltiazem from L-type Ca^{2+} channels is simply explained by competition for individual attachment points. J Biol Chem 268:18580–18585

Renault L, Kuhlmann J, Henkel A, Wittinghofer A (2001) Structural basis for guanine nucleotide exchange on Ran by the regulator of chromosome condensation (RCC1). Cell 105:245–255

Scheffzek K, Ahmadian MR, Kabsch W, Wiesmuller L, Lautwein A, Schmitz F, Wittinghofer A (1997) The Ras-RasGAP complex: structural basis for GTPase activation and its loss in oncogenic Ras mutants. Science 277:333–338

Schlichting I, Almo SC, Rapp G, Wilson K, Petratos K, Lentfer A, Wittinghofer A, Kabsch W, Pai EF, Petsko GA, Goody RS (1990) Time-resolved X-ray crystallographic study of the conformational change in Ha-Ras p21 protein on GTP hydrolysis. Nature 345:309–315

Segel IH (1975) Enzyme kinetics. Wiley-Interscience, New York