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On the attribution and additivity of binding energies

(proteins/ligands/entropy/enzymes)

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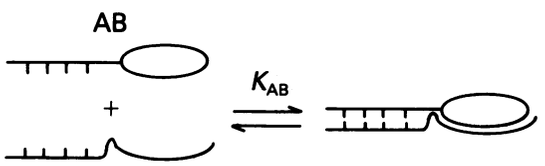
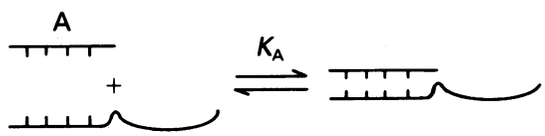
Contributed by William P. Jencks, March 9, 1981

ABSTRACT **It can be useful to describe the Gibbs free energy changes for the binding to a protein of a molecule, A—B, and of its component parts, A and B, in terms of the "intrinsic binding energies" of A and B, ΔG^i_A and ΔG^i_B, and a "connection Gibbs energy," ΔG^s that is derived largely from changes in translational and rotational entropy. This empirical approach avoids the difficult or insoluble problem of interpreting observed ΔH and $T\Delta S$ values for aqueous solutions. The ΔG^i and ΔG^s terms can be large for binding to enzymes and other proteins.**

Consider the question of what forces are responsible for the binding to a protein of a molecule A—B that contains a region capable of forming hydrogen bonds, A, and a nonpolar hydrophobic region, B. This problem could be approached by comparing the observed binding of A—B and of the small molecule A (Eqs. 1 and 2).



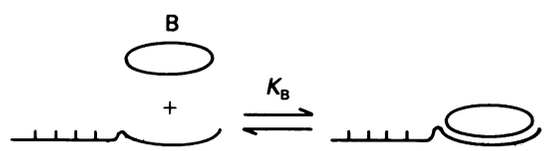
[1]



[2]

If the binding constants for A—B and A are 10^3 M^{-1} and 1 M^{-1}, respectively, corresponding to standard Gibbs free energy changes of $\Delta G^0_{AB} = -RT\ln K_{AB} = -4.1$ kcal mol^{-1} and $\Delta G^0_A = 0$ kcal mol^{-1}, it might be concluded that some kind of hydrophobic or dispersion interaction of the protein with the B moiety is primarily responsible for the binding of A—B.

There is no valid basis for this conclusion. Suppose that the binding of B (Eq. 3) had been examined first and found to give $K_B = 1$ M^{-1} and $\Delta G_B = 0$ kcal mol^{-1}.



[3]

The above argument would then lead to the conclusion that hydrogen bonding to the A moiety is primarily responsible for the binding of A—B.

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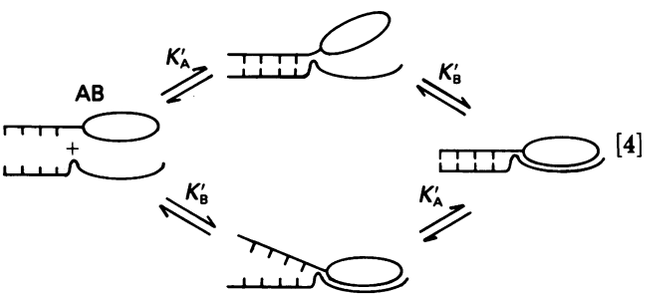
To reach a meaningful conclusion about this problem (and to avoid conclusions that depend on the sequence in which experiments are carried out), it is necessary to find a way of relating the observed binding of A and B to that of A—B (1–5). In one simple approach to this problem, the binding of A—B is considered to occur in two steps, in which the initial binding of the A or B moiety is followed by the binding of the rest of the molecule (Eq. 4).



[4]

If it is assumed that the equilibrium constants for the initial binding steps are the same as those for the binding of A and B, the intramolecular binding of B and A in the second step occurs with equilibrium constants of $K''_B = 10^3$ and $K''_A = 10^3$ and with standard Gibbs energy changes of $\Delta G^i_B = -4.1$ kcal mol^{-1} and $\Delta G^i_A = -4.1$ kcal mol^{-1}. The binding energy for any group X is given by Eq. 5, and ΔG^i_A and ΔG^i_B may be taken as estimates of the "intrinsic binding energies" of A and B. The intrinsic binding energy of X is

$$\Delta G^i_X = \Delta G^0_{AX} - \Delta G^0_A, \qquad [5]$$

defined as the Gibbs free energy change for the binding of X in the absence of strain and losses in translational and rotational entropy (1). Observed values of ΔG^i_X give limiting values for the intrinsic binding energy because these conditions can never be completely met in a real system.

It is useful to divide the contributions to the observed binding of A—B at a given temperature into three parts: (*i*) the observed intrinsic binding energy of the A moiety, ΔG^i_A; (*ii*) the observed intrinsic binding energy of the B moiety, ΔG^i_B; and (*iii*) a connection Gibbs energy, ΔG^s, that represents the change in the probability of binding that results from the connection of A and B in A—B. This is largely, but not entirely, an entropy term that would correspond to a change in translational and overall rotational entropy in the gas phase; in solution, it is partly transformed into other degrees of freedom and does not necessarily appear as a change in the observed $T\Delta S$ (1). It may also include any Gibbs energy that is required (*i*) to bring about a conformation change in the protein if the same conformation change is required for the binding of A, B, and A—B, such as the opening of a cleft for binding, or (*ii*) to overcome strain or destabilization on binding A or B *individually* that is relieved in bound

A—B. The latter effect is not expected to be important unless there is a large change in the structure of A and B when they are converted into A—B.†

These contributions are related by Eq. 6:

$$\Delta G^0_{AB} = \Delta G^i_A + \Delta G^i_B + \Delta G^s. \quad [6]$$

We consider, as a first approximation, the case in which the binding energies of A, B, and the A and B moieties of A—B are independent, the probability terms for the binding of A, B, and A—B are the same and equal to ΔG^s, and the loss of internal entropy in the K''_A and K''_B steps is negligible. This approximation is far from exact, but it is better than nothing. The losses of translational entropy on binding A, B, and A—B are nearly the same because the translational entropy has only a small dependence on molecular size, the differences in overall rotational entropy are not large for nonlinear molecules of moderate size, and the entropy of a single internal rotation is small compared with the translational and overall rotational entropy (7, 8).

When the binding of A, B, or A—B has the same probability or loss of entropy, corresponding to ΔG^s, ΔG^0_{AB} for the binding of A—B will be more favorable than $\Delta G^0_A + \Delta G^0_B$ by an amount corresponding to this entropy loss. The entropy barrier for binding, ΔG^s, is given by the difference between the intrinsic binding energies, $\Delta G^i_A + \Delta G^i_B$, and the observed binding energy, ΔG^0_{AB} (Eq. 6) or by the equivalent Eq. 7.

$$\Delta G^s = \Delta G^0_B + \Delta G^0_A - \Delta G^0_{AB}. \quad [7]$$

The ΔG^s barrier of 4.1 kcal mol^{-1} must be overcome for the binding of A or B, as well as for A—B. The intrinsic binding energies are additive and, once the entropy barrier has been overcome [K'_A or K'_B (Eq. 4)], can be expressed as increases in the observed binding energies.

The economics for the binding of A—B is illustrated in Fig. 1. The intrinsic binding energy of A—B, ΔG^i_{AB}, is the sum of the intrinsic binding energies of the A and B moieties, $\Delta G^i_A + \Delta G^i_B$. Part of it is used to overcome the loss of entropy on binding, ΔG^s, and what is left over appears as the observed binding, ΔG^0_{AB}. Thus, the observed binding of A—B represents $\Delta G^i_A = -4.1$ kcal mol^{-1} from hydrogen bonding and $\Delta G^i_B = -4.1$ kcal mol^{-1} from hydrophobic interactions, which overcome a ΔG^s term of +4.1 kcal mol^{-1}.

A second approximation would take account of differences in ΔG^s for the binding of A, B, and A—B according to Eq. 8 and (in the general case) possible differences in ΔG^i_X and in the loss of internal entropy in the intermolecular (K_X) and the intramolecular (K''_X) binding of X.

$$\Delta G^0_X = \Delta G^i_X + \Delta G^s_X. \quad [8]$$

Binding of the B moiety of A—B (K''_B) may decrease the freedom of movement of the A moiety or, if B is not optimally aligned with the binding site, may require overcoming some strain or destabilization, so that ΔG^i_B for this process is less favorable than that for the binding of free B. Binding of the B moiety of A—B also requires loss of internal entropy of A—B that is not required for the binding of free B. This term is not large for a single internal rotation of a typical organic ligand but it may become large when A and B are macromolecules if binding of the B moiety of A—B results in loss of the entropy of a number of low-frequency motions of A—B. These factors will have the effect of making ΔG^i_B less negative. The observed binding will also be weaker if the binding site is not complementary to the ligand initially, so that binding requires overcoming strain or a conformation change. Destabilization of A—B that does not occur for A and B individually or nonproductive binding will result in underestimation of $-\Delta G^i$ and ΔG^s [if nonproductive binding of A is observed, productive binding must be weaker, so that $-\Delta G^i_B$ will be underestimated (Eq. 5)]. Thus, experimental data are likely to provide only lower limits for the intrinsic binding energies $-\Delta G^i_A$ and $-\Delta G^i_B$ and for ΔG^s.

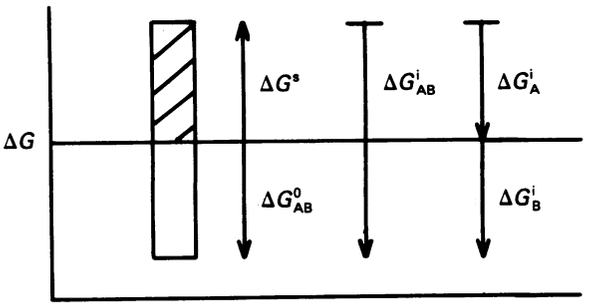


FIG. 1. Balance of Gibbs free energies for the binding of A—B to a protein. The observed binding, ΔG^0_{AB}, is the difference between the intrinsic binding energy, ΔG^i_{AB}, and the loss of entropy on binding that is given by ΔG^s. The intrinsic binding energies of A and B are additive to give ΔG^i_{AB}.

It is not unusual to find that the binding of individual molecules A and B is weak or negligible but A—B binds well, so that the whole appears to be greater than the sum of its parts. Anionic dyes bind tightly and stoichiometrically to cationic groups on proteins, for example (9), although small anions bind weakly or not at all to ammonium ions in water and the nonpolar moiety of the dye does not provide enough binding energy to cause significant binding to uncharged regions of the protein. The binding of small nonpolar molecules or amides to each other in water is weak or negligible, but nonpolar interactions and hydrogen bonding provide the binding energy that holds proteins in their native structure when these groups are connected in a peptide chain (1, 10–12). The same phenomenon is well known in the binding of chelating agents to metals (7).

ΔG^s and the additivity of binding energies

It is frequently assumed that the observed Gibbs binding energies of two molecules, A and B, are additive in the molecule A—B, so that $\Delta G^0_{AB} = \Delta G^0_A + \Delta G^0_B$. There is no basis for this assumption (1, 5). The addition of Gibbs energies is equivalent to the multiplication of binding constants and, if K_A, K_B, and K_{AB} are expressed in molarity, the equation $K_A K_B (M^{-1})^2 = K_{AB}(M^{-1})$ is meaningless. Use of the mole fraction or unitary scale is better for some purposes (13, 14), but this scale also does not take account of differences in entropy from rotations, translations, and low-frequency motions on formation of complexes that have various degrees of tightness. The loss of entropy on combining A and B by a covalent bond to form A—B can be as much as −40 or −32 cal mol^{-1} K^{-1} for molar and mole fraction standard states, respectively, and some unpredictable fraction of this difference will appear in the binding of A—B compared with that of A and B (7).

An empirical way of dealing with this problem is provided by use of the ΔG^s term according to Eqs. 6 and 7. This term is equal to $RT\ln(K_{AB}/K_A K_B)$ and can be regarded as a crude measure of the intrinsic entropy changes in a set of binding re-

† Both a conformational change and the entropy contribution can give a favorable ΔG for the binding of A—B relative to A and B, but only the former will give a stronger binding of B to AP than to the free protein, P. This provides an experimental test for distinguishing the two contributions, provided that nonbonded interactions do not interfere with the concurrent binding of A and B to P. Such interactions may sometimes be avoided by substituting a smaller ligand for A or B, such as phosphite for phosphate (6).

actions. Although ΔG^s values are far from exact, they are better than assumptions of additivity of binding energies based on molar or mole fraction standard states. Values of ΔG^s can, in principle, correspond to values of K_{AB}/K_AK_B = <1 M to 10^8 M, depending on the tightness of binding in a complex, whereas the mole fraction standard state corresponds to the single value of 55 M.

If there is not an exact tight fit, the values of both ΔG^s and $-\Delta G^i$ will be small. Large values of $-\Delta G^i_{AB}$ and ΔG^s are not desirable in the ground states of most biological systems because they give rise to tight binding and slow desorption of A—B; they are more likely to be found in the transition states of enzymic reactions and the activated states of receptor–effector complexes. A value of ΔG^s = 5.9 kcal mol^{-1} has been found for the tight binding to avidin of desthiobiotin, with $\Delta G^0_{AB} = -16.9$ kcal mol^{-1} compared with its component parts 4-methylimidazolidone ($\Delta G^0_A = -6.1$ kcal mol^{-1}) and hexanoate ($\Delta G^0_B = -4.9$ kcal mol^{-1}); this corresponds to $K_{AB}/K_AK_B = 2 \times 10^4$ M (15). The other extreme is represented by the binding of the two heads of heavy meromyosin to actin with $\Delta G^s = -5.3$ kcal mol^{-1} and $K_{AB}/K_AK_B \cong 10^{-4}$ M, based on $\Delta G^0_{AB} = -12.9$ kcal mol^{-1} and $\Delta G^0_A = \Delta G^0_B = -9.1$ kcal mol^{-1} for binding the individual heads of the S-1 subunits (15).‡ The unfavorable binding of the second head may reflect strain and a large loss of entropy on binding (16, 18, 19).

Intrinsic binding energies

There are a number of examples of ΔG^i values for binding in stable complexes and in transition states of enzyme-catalyzed reactions that suggest that intrinsic binding energies for substituent groups can be considerably larger than has generally been believed when the conditions for tight binding are optimal. The conditions are usually not optimal, especially in enzyme–substrate complexes—a characteristic property of enzymes is that they must bind substrates and products much less tightly than transition states (1, 20, 21).

Reported values of $-\Delta G^i$ for small groups include (in kcal mol^{-1}) the following: CH_3, 2.0–3.9 (22–24); HS, 5.4–9.1 (25, 26); CH_3S, 4.9 (27); H_2N, 4.5 (28); cyclopropanol, 8 (29); HO, 8 (30, 31); H_3N^+, 6.7; and COO^-, 4.3 (32); in each case, the indicated group was substituted for H. Some of these examples could represent special effects, such as destabilization of a polar or charged group in the binding site by the reference compound, but the large values for nonpolar groups suggest a strong favorable interaction that presumably involves dispersion forces.

Thermodynamic parameters

If ligands could bind to rigid proteins in the gas phase, the binding energy and losses of translational and rotational entropy on binding would appear directly in the observed ΔH and $T\Delta S$ parameters. There would then be no need for ΔG^i and ΔG^s, which represent crude approximations to what the binding forces and translational–rotational entropy terms would be if there were no perturbing and partially compensating contributions to the observed thermodynamic parameters from solvent effects and conformational changes in the protein (1, 32–34). In all but the simplest cases, these contributions make it difficult or impossible to interpret observed thermodynamic parameters in aqueous solution in terms of the actual driving forces and overall rotational–translational entropy contributions that are responsible for the observed binding.

It is well known that hydrophobic interactions and electrostatic bonding are usually accompanied by the release of constrained water molecules that give a positive contribution to the observed ΔS and may give positive ΔH terms (14). Conformational changes in proteins can also give large and unpredictable changes in thermodynamic parameters due to changes in solvent interactions, as well as to the complex structure of the protein itself. However, it is not widely appreciated how easily the compensation of ΔH and ΔS can lead to misinterpretation of observed thermodynamic parameters.

Suppose that molecule A binds to a protein in an enthalpy-driven process for which $\Delta G^0 = -4$ kcal mol^{-1}, $\Delta H = -4$ kcal mol^{-1}, and $T\Delta S = 0$ and that transfer of another molecule, B, from water to ethanol is a satisfactory model for its transfer to the binding site. The transfer of ethanol from water to ethanol occurs with $\Delta G = -0.8$ kcal mol^{-1}, $\Delta H = 2.4$ kcal mol^{-1}, and 298 $\Delta S = 3.2$ kcal mol^{-1} (35). If substituent B, having the same properties as ethanol, is added to A to give A—B, the binding of A—B would then show approximate thermodynamic parameters of $\Delta G^0 = -4.8$ kcal mol^{-1}, $\Delta H = 1.6$ kcal mol^{-1}, and $T\Delta S = 3.2$ kcal mol^{-1}. Although substituent B makes a small additional contribution to the observed binding, ΔG^0, it gives larger, compensating changes in ΔH and $T\Delta S$. The resulting thermodynamic parameters for the binding of A—B might then be mistakenly interpreted as evidence for entropy-driven binding, when in fact most of the binding arises from an enthalpy-driven process of the A moiety (36).

Separation of observed thermodynamic parameters into intrinsic and solvent terms has been shown by Hepler and others to be useful in the interpretation of ionization reactions for similar reasons (37). An electron-donating substituent that increases the pK_a of an oxygen acid, for example, will also cause stronger hydrogen bonding of water molecules to the anionic base, with resulting negative changes in ΔH and ΔS that largely compensate. These compensating changes result in the appearance of the substituent effect as a less favorable entropy rather than enthalpy of ionization.

For these reasons, and also because of the large changes in heat capacity that are found in aqueous systems, the binding of ligands to proteins is likely to change with temperature in ways that are difficult to interpret or predict. The ΔG^i and ΔG^s terms are empirical parameters that can be useful for describing the binding of ligands at a given temperature.

Enzymes

Both ΔG^i and ΔG^s can, and in some cases do, make larger contributions to catalysis by enzymes than has been generally believed. Some examples of ΔG^i values for small groups have been noted above. The advantage from ΔG^s can be as large as $\approx 10^8$ (M) and has been shown to be $\approx 10^5$ M.

There are many intramolecular model reactions that exhibit rate increases of $\approx 10^5$ M compared with corresponding bimolecular reactions, in which the reacting groups have the freedom to undergo independent translation and rotation; in some cases, rate increases of $\approx 10^8$ M have been observed in the absence of strain (7, 8, 38). If an enzyme can bring about a similar restriction to motion, it can cause a similar rate acceleration for its bound substrates (e.g., by a factor of 10^5 M if it can hold an acyl group and a nucleophile as rigidly relative to each other as they are held in a succinate half-ester.

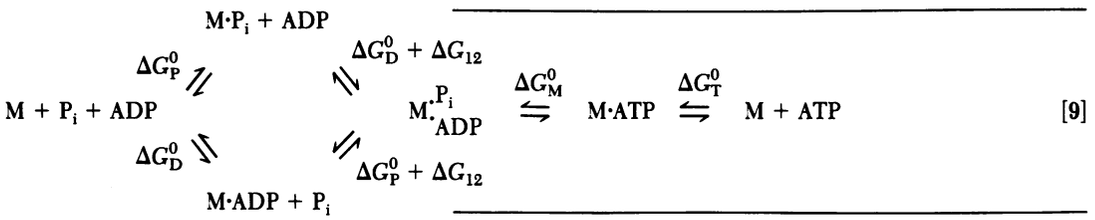
The strongest evidence that this kind of advantage is significant in an enzymic reaction is still Thompson's demonstration of a factor of $\approx 10^5$ M for binding of the peptide aldehyde Ac-Pro-Ala-Pro-AlaCHO to elastase (39). The peptide aldehyde is an A—B system in which the peptide, A, binds to a series of subsites and the aldehyde group, B, binds covalently (and reversibly) to the serine hydroxyl group to form a hemiacetal. This hemiacetal is analogous to the addition intermediate in the hy-

‡ Equilibrium constants that give $K_{AB}/K_AK_B = 10^{-5}$ M and $\Delta G^s = -6.3$ kcal mol^{-1} have also been reported (17, 18).

drolysis of acyl compounds. Comparison of the observed binding of the aldehyde ($\Delta G^0_{AB} = -8.9$ kcal mol^{-1}), binding of the corresponding peptide alcohol ($\Delta G^0_A = -4.5$ kcal mol^{-1}), and an estimate for hemiacetal formation ($\Delta G^0_B = +2.5$ kcal mol^{-1}) gives $\Delta G^i_A = -11.4$ kcal mol^{-1}, $\Delta G^i_B = -4.4$ kcal mol, and $\Delta G^s = 6.9$ kcal mol^{-1}. Thus, initial binding of the peptide chain makes formation of the hemiacetal more favorable by a factor of $\approx 10^5$ M and formation of the hemiacetal makes binding of the peptide chain more favorable by the same factor, compared with the corresponding bimolecular reactions. There is evidence that rate accelerations are brought about by this mechanism in the normal enzyme-catalyzed reaction (39, 40).

A similar factor is manifested at the active site of myosin (M) ATPase, which makes the hydrolysis of ATP readily reversible and thereby preserves the large Gibbs energy change from this reaction for release or energy transduction when the products dissociate. Comparison of the observed binding of ATP ($K_{AB} = 3 \times 10^{11}$ M^{-1} and $\Delta G^0_{AB} = -15.6$ kcal mol^{-1}), ADP ($K_A = 10^6$ M^{-1} and $\Delta G^0_A = -8.2$ kcal mol^{-1}), and inorganic phosphate ($K_B = 670$ M^{-1} and $\Delta G^0_B = -3.8$ kcal mol^{-1}) gives $\Delta G^i_A = -11.8$ kcal mol^{-1}, $\Delta G^i_B = -7.4$ kcal mol^{-1} and $\Delta G^s = 3.6$ kcal mol^{-1} (41–43). The large intrinsic binding energies of ADP and of P_i are used to overcome the ΔG^s term when each species binds individually; they are manifested to a greater extent as observed binding in ATP and thereby favor ATP formation.§

A more complete analysis takes into account directly the facile formation of M·ATP from M·ADP·P_i (Eq. 9) (42).



[9]

This is made possible by the tighter binding of ATP than of ADP and P_i (at some standard state), which occurs because much of the binding energy of ADP and P_i is used in an interaction energy, ΔG_I, that reflects the loss of entropy and destabilization of ADP and P_i on binding (Fig. 2A) (1, 44). This interaction energy is given by Eq. 10,

$$\begin{aligned}\Delta G_I &= \Delta G^0_N - \Delta G^0_M = \Delta G^0_{PD} - \Delta G^0_T \\ &= \Delta G^0_D + \Delta G^0_{P_i} + \Delta G_{12} - \Delta G^0_T \\ &= \Delta G^s + \Delta G_{12},\end{aligned} \qquad [10]$$

in which ΔG^0_N and ΔG^0_{PD} are the standard Gibbs energy changes for the synthesis of ATP from 1 M ADP and P_i in solution and

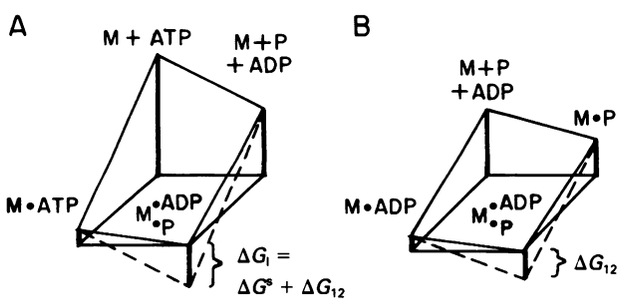


FIG. 2. Gibbs energy diagrams for binding to myosin to show (*A*) the relationship of energies for binding and ATP hydrolysis and (*B*) the binding of ADP and P_i separately and together. ATP synthesis on myosin is favored by the interaction energy ΔG_I, to which ΔG_{12}, from mutual destabilization of bound ADP and P_i, makes a contribution. M, myosin.

the binding of both ADP and P_i, respectively. It is apparent that ΔG_I differs from ΔG^s by ΔG_{12}, an interaction or "coupling" term (45, 46) that describes any destabilization of the binding of ADP caused by the presence of bound P_i or destabilization of the binding of P_i caused by the presence of ADP (Fig. 2*B*). This could result from electrostatic or van der Waals repulsion, an additional freezing and loss of entropy for the binding of one compound when the other is already present, or conformational changes. The ΔG_{12} term may also include weak attractive interactions between bound A and B. The loss of entropy and relief of unfavorable interactions that are included in ΔG_{12} can provide a driving force for the synthesis of ATP from bound ADP and P_i.

The binding of P_i to M·ADP ($K = 5$ M^{-1} and $\Delta G^0_P + \Delta G_{12} = -1.0$ kcal mol^{-1}) is weaker than that to M ($K = 670$ M^{-1} and $\Delta G^0_P = -3.8$ kcal mol^{-1}) by $\Delta G_{12} = 3.8 - 1.0 = 2.8$ kcal mol^{-1} (41, 43).§ Thus, the facile synthesis of ATP at the active site of myosin results from an interaction Gibbs energy of $\Delta G_I = 6.4$ kcal mol^{-1} (Eq. 10). If the interaction energy, ΔG_{12}, contributes directly to ATP synthesis,¶ the driving force for the reaction may be attributed to comparable contributions from $\Delta G^s = 3.6$ kcal mol^{-1} and $\Delta G_{12} = 2.8$ kcal mol^{-1}.

Destabilization by compression can be significant for reactions in which the product is considerably smaller than the reactants, as in the synthesis of ATP from ADP and P_i. However, it is unlikely to make a large contribution to enzymic catalysis by overcoming van der Waals repulsions between bound reactants because of the small changes in distances in going from reactants to transition state and the relatively small force constants that can be developed by proteins (47). Therefore, the main role of strain by compression in causing rate accelerations is presumably to make the reaction more probable by decreasing the entropy of the bound reactants (1). This kind of strain or destabilization can appear in the ΔG_{12} term and can also make an important contribution to catalysis by freezing the motions of A and B individually.

Binding of substrates to enzymes differs from binding to other proteins in that the geometry and charge distribution of the ligand change on conversion to the transition state and products. Therefore, destabilization of bound A or B individually (as well as through ΔG_{12}) by strain, loss of solvation, or electrostatic repulsion may be relieved in the transition state and product and can contribute to the observed energy balance of

§ These equilibrium constants are based on total myosin concentrations, including all conformational states. A simple thermodynamic box shows that, if binding of a ligand forces a protein to change conformation from its thermodynamically most stable state P to a less stable state P*, some of the binding energy of the ligand will be used up to cause this change; binding to isolated P*, if it could be observed, would be correspondingly stronger. The entropy loss and destabilization energy included in ΔG^s and ΔG_{12} are certainly manifested in altered conformational states of myosin (e.g., M*, M**).

¶ Loss of entropy and destabilization of bound ADP·P_i that is relieved on ATP synthesis will favor the reaction. Any destabilization that is not relieved on ATP synthesis will also reduce ΔG^s and requires that there be a correspondingly larger contribution to ΔG_I of entropy loss and other factors that would appear in the ΔG^s term in the absence of such destabilization.

the reaction. For the enzyme-catalyzed conversion of A + B into A—B, such destabilization will appear in the ΔG^s term. Therefore, in reactions such as ATP synthesis from bound ADP and P_i, it is difficult or impossible to distinguish between destabilization and entropic contributions to ΔG^s (or to ΔG_{12} and ΔG_I); it is virtually certain that both are significant.

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