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Hypothesis

Transduction of enzyme-ligand binding energy into catalytic driving force

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We propose a testable general mechanism by which ligand binding energy can be used to drive a catalytic step in an enzyme catalyzed reaction or to do other forms of work involving protein molecules. This energy transduction theory is based on our finding of the widespread occurrence of ligand binding-induced protein macrostate interconversions each having a large invariant $\Delta H°$ accompanied by a small but highly variable $\Delta G°$. This phenomenon, which can be recognized by the large $\Delta Cp°$'s it generates, can provide the necessary energy input step but is not in itself sufficient to constitute a workable transduction mechanism. A viable mechanism requires the additional presence of an 'energy transmission step' which is terminated to trigger the 'power' stroke at a precise location on the reaction coordinate, followed by an energetically inexpensive 'return' step to restore the machine to its initial conditions. In the model we propose here, these additional steps are provided by the existence of ligand inducible 2-state transitions in the free enzyme and in each of the enzyme complexes that occur along the reaction coordinate, and by the selective blocking of certain of these interconversions by high energetic barriers. We provide direct experimental evidence supporting the facts that these additional mechanistic components do exist and that the liver glutamate dehydrogenase reaction is indeed driven by just such machinery. We describe some aspects of the chemical nature of these transitions, and evidence for their occurrence in other systems.

Energy transduction; Enzymatic catalysis; Interaction parameter; Molecular machine

1. INTRODUCTION

The idea that enzyme-linked binding energy can somehow be converted into a catalytic driving force, originally suggested by Pauling [1], is now widely accepted, and a number of elegant theories based on this idea have been developed [2,3] but, by their very nature, they are not of a form that is subject to experimental testing. Lumry [4] has suggested a mechanism for such energy transduction in which ligand-induced changes in protein free energy are arranged so as to be complementary to the corresponding features for the chemical reaction coordinate (Fig. 1). The basis on which this scheme rests is the dissection of the free energy along the reaction coordinate into two separate components: (1) that of the substrate system; and (2) that of the protein molecule itself (indicated by the dashed line). Here again, however, direct experimental testing of the scheme on real enzymatic systems is obviated by the lack of any realistic method of determining the protein component curve.

We have now found a specific enthalpic signal which permits us to track a ligand-induced, highly energetic protein macrostate transition in pyridine nucleotide dehydrogenases which appears to fulfill Lumry's criterion of complementarity. We propose a directly testable model for such energy transduction and provide some preliminary evidence in its support.

Dedicated to Prof. Rufus Lumry on his 70th birthday.

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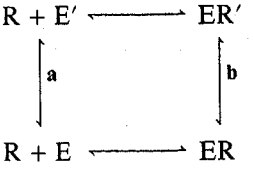
2. THE HIGH ENTHALPY, LIGAND-INDUCED TRANSITIONS

These phenomena are described in detail in [5], and are summarized below. Each of the many binary and ternary complexes of glutamate dehydrogenase (GDH) appears to be characterized by the presence of a 2-state transition whose $\Delta H°$ is invariably 22 000 cal·mol^{-1}, but whose $T°$ values vary widely among the various enzyme forms. ($T°$ is defined as the temperature at which $\Delta G° = 0$ for that transition.) Evidence for the existence of such transitions is provided by the observation of the temperature-dependences of the $\Delta H°$ of the binding of ligands to an enzyme. An example of the phenomenon is shown in Fig. 2. The filled circles in the figure represent the observed ΔH of the binding of NADPH (R) to liver glutamate dehydrogenase (E). The solid line represents the observed ΔH for the system:



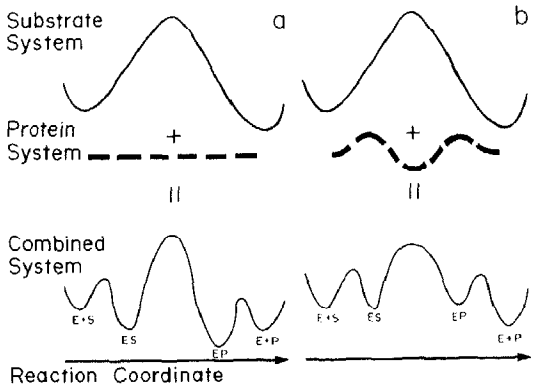


Fig. 1. The Lumry 'free-energy complementarity' concept. (a) An example where no alteration in the activation free energy of the substrate process occurs. (b) An example where free energy changes in the protein itself are partially complementary to those of the substrate system. The dashed line represents the level of ligand binding energy store in the protein molecule itself.

where transitions **a** and **b** each have the same ΔH° (22 000 cal·K^{-1}·mol^{-1}) but quite different ΔG°'s, as expressed by the large difference in T° values.

$$\Delta H(T) = \Delta H_2 + \Delta H^\circ \left[\frac{1}{(1 + K_a)} - \frac{1}{(1 + K_b)} \right] \quad (1)$$

where ΔH_2 is the temperature-independent component of the binding enthalpy, ΔH° is the enthalpy of the protein transition, $K_a = \exp [\Delta H^\circ (T - T^\circ_a)/RTT^\circ_a]$ and K_b is similarly defined. Using this definition of K_i as a function of T°_i we can express the change in the free energy of any given form of the protein itself induced by a shift in the equilibrium constant of its 2-state high enthalpic transition as:

$$\Delta G^\circ_i = \Delta H^\circ (1 - T/T^\circ_i) \quad (2)$$

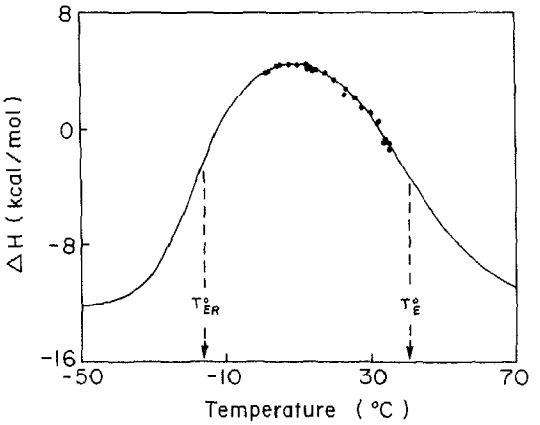


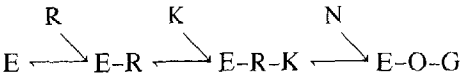
Fig. 2. Thermodynamic parameters for a ligand-induced 2-state protein transition. Temperature dependene of ΔH_{obs} for the binding of NADPH to glutamate dehydrogenase (●) experimental points; the solid line corresponds to eqn. 11 of [5]; arrows indicate values of T° (the temperature at which $\Delta G^\circ = 0$ for an E E′ transition) for the enzyme-ligand complex and for the free enzyme, respectively.

(2)

The isothermal forms of eqns. (1) and (2) which indicate the protein macrostate thermodynamic components observed at 25°C are indicated in Fig. 3. The distinctive shape of the temperature dependence of the enthalpy from such 2-state transitions as seen in Fig. 2, permits the assignment of the changes in ΔG° of the enzyme molecule itself induced by the various ligands (coenzymes, substrates, modifiers, H^+, etc.) as shown by the dashed line in Fig. 3. This set of such protein-component ΔG°'s obtained for the various enzyme complexes that occur along the reaction path provides us for the first time with the opportunity to actually isolate the heretofore hypothetical component shown as the dashed line in Fig. 1. It is this feature which lies at the heart of the formulation which follows.

3. THE GLUTAMATE DEHYDROGENASE CATALYZED REDUCTIVE AMINATION REACTION

We will use experimental data obtained from this system to test our transduction model. The reaction, expressed in terms of the major intermediates which are known to accumulate in substantial amounts is:



where E is the free enzyme, R and O are the reduced and oxidized pyridine nucleotide coenzymes, respectively, K is α-ketoglutarate; N is ammonia; and G is L-glutamate [6].

Experimental ΔG° values for the enzyme component in the various reactive species calculated at 25°C, along with the fraction of enzyme in the high enthalpy state, are listed in Table I. The values for E and E–R are obtained directly from the data shown in Fig. 2 and are calculated from eqns. (1) and (2). The value for E–R–K is based on the T° value obtained from the location of its enthalpic interaction parameters, ΔH_I and ΔCp_I on the phase diagram shown in Fig. 12 of [5]. The value for the reactive E–O–G complex is estimated from the remarkably high $\Delta Cp^\ddagger$ of the E–O–G ERK + N step as measured by the temperature-dependences of the transient state kinetic parameters of the enzymatic reaction [7]. Such a $\Delta Cp^\ddagger$ indicates the presence of a 2-state transition having an enthalpy of at least 20 kcal·mol^{-1}.

4. ENERGY TRANSDUCTION MACHINERY IN ENZYME REACTIONS

It is easily shown from formal thermodynamic considerations that the preferential binding of a ligand to one form of an enzyme which itself undergoes a 2-state

transition must increase the $\Delta G°$ of the protein at the expense of some of the $\Delta G°$ of binding of the ligand [8]. Such an event does not, however, constitute 'storage' in any real sense and is not in itself sufficient to constitute an energy transduction machine.

An operable machine which does have such a capability requires a minimum of the following features: (i) an energy input step. The binding of a ligand to a 2-state protein, described above does suffice for this step; (ii) one or more 'transmission' steps. This kind of step, in a protein machine of the type considered here, would involve the conversion of one enzyme-ligand complex to a second such complex without the dissociation of the ligand, and thus without the loss of a substantial portion of the now-stored original binding energy. The necessity of such a step has been inferred by Kodama [15] who has used the term 'locking step' for such a process; (iii) a power stroke. In this step a major portion of the stored energy is released to compensate for the large intrinsic energy of activation of the chemical bonding changes of the normal reaction. The timing of the termination of the transmission step and the onset of the catalytic step is crucial. We imagine that the 'trigger' which establishes this critical point along the reaction might be some highly specific but low energy chemical step, such as the gain or loss of a particular proton; (iv) the return step. Here, the enzyme is returned to its original starting point, a process which should occur with only a minimal further loss of energy. An obvious feature of such a step, therefore, is the dissociation of the products in such a way that no substantial change in the enzyme 2-state transition is incurred.

We visualize the essential features of our machine to occur in the following way. If each of the enzyme forms along the reaction pathway has the particular '$T°$' necessary at that point in the sequence, then that series of interconversions between their high and low enthalpy forms can constitute the linked 4-step machine necessary for the transduction of binding energy into catalytic driving force. A necessary feature of this machine is the blockage of such interconversions by high energy barriers at certain critical points in the sequence, and the absence of such barriers at other such points.

With this said, we now proceed to use the data from Table I to determine whether the glutamate dehydrogenase reaction does in fact exhibit the features of such a ligand-binding energy transduction machine. The $\Delta G°$ values and degree of displacement of the E E′ equilibrium for the various complexes are shown in Fig. 4. It is important to note that the heights of the columns representing the various enzyme forms, (and, therefore, the dashed strip representing the reaction coordinate) reflect only the $\Delta G°$ values of the protein itself.

A. Energy input step. The binding of R (NADPH) to the enzyme raises the free energy of the protein by about 5 kcal·mol^{-1} at the expense of a correspondingly weakened binding of the coenzyme.

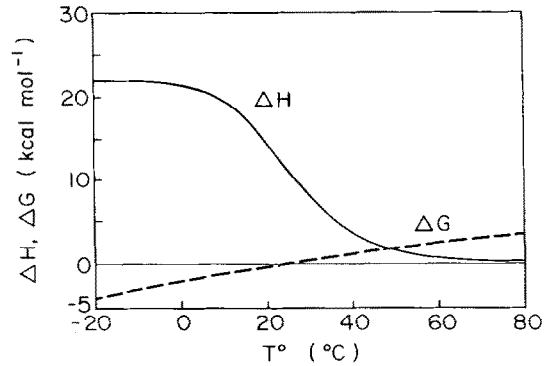


Fig. 3. The dependences of ΔH (solid line) and of ΔG (dashed line) of the enzyme component observed at 25°C on $T°$ (adapted from [5]).

B. Transmission step. The binding of K (α-ketoglutarate) to the high energy ER form releases only a part of the energy induced in the system. This partial energy release accomplishes two functions: it provides a kinetic force to drive the overall reaction at the necessary speed and it also happens to tighten the binding of K at the expense of R, a local energy transduction feature.

C. Power stroke. The addition of N (NH_3) to ERK initiates the actual chemistry of the reaction, releasing most of the remainder of the original energy input and thereby driving the catalytic step.

D. Return step. The energy of the protein in the enzyme–product complex is only slightly higher than that of the free enzyme itself, satisfying the necessary condition here.

We may now compare the experimentally defined protein component of the reaction coordinate shown as the dashed line in Fig. 4 with the corresponding dashed line of Lumry's complementarity scheme shown in Fig. 1. It is apparent that the liver glutamate dehydrogenase catalyzed reaction does indeed appear to exhibit the essential feature of the Lumry theory; ligand binding energy is stored in the protein and is released at precisely the right step so as to offset a significant portion of the activation energy involved in the chemical events of the reaction.

5. THE NATURE OF THE 2-STATE TRANSITION IN THE LIVER GLUTAMATE DEHYDROGENASE MOLECULE

While we have by no means completely characterized this transition at this time, we have determined a number of phenomena whose occurrence appear to be so tightly coupled to the transition that they can be said to indicate at least its general nature. The ligand-binding-induced transitions are closely linked to a complex set of proton ionizations [9]. The transitions themselves, and the linked proton ionizations, are controlled by the state of occupancy of a double anion binding site which is, in turn, allosterically affected by acetate [10]. The binding of acetate alone appears to lower the $T°$ of the

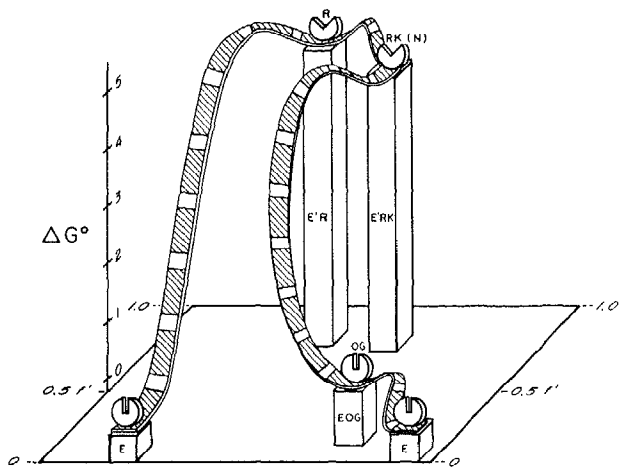


Fig. 4. The liver glutamate dehydrogenase reaction as a ligand-binding energy transduction machine. The dashed band corresponds to the relative $\Delta G°$ of the *protein component only*, corresponding to the dashed line of Fig. 1. Values of $\Delta G°$ and %E′/(E+E′) have been calculated from experimental data provided in Table I. The $T°$ values of the E and E–R forms were obtained from the data shown in Fig. 2. The $T°$ value for the E–R–K form was calculated from ΔCP_i and ΔH_i interaction parameters as shown in Fig. 12 of [5]. The $T°$ value for the reactive E–O–G form was calculated from the temperature-dependence of transient-state kinetic parameters described in [7]. E and E′ represent the low and high enthalpy states of the various enzyme forms. R, NADPH; K, α-ketoglutarate; N, ammonia; O, NADP; G, L-glutamate.

enzyme, and this effect is further enhanced synergistically by the binding of NADPH. Thus, the high enthalpy 2-state protein transition participates in a complex tightly coupled control mechanism. In the context of the argument presented here, this mechanism has the properties required to serve as a device to regulate the energetic and kinetic properties of the enzymatic machine and may provide a linking function between these two aspects.

A particularly striking characteristic of the 2-state transition is that it clearly seems to involve a substantial change in the gross structure of the protein itself. Direct evidence of this behavior is provided by the observation that the free enzyme, which has a $T°$ of 43°C is stable for at least 2 min at 56°C (indeed such a step is used in its purification) while the E–NADPH form of the enzyme ($T°$< 0°C) begins to denature rapidly at 37°C. Furthermore the free enzyme in Tris-acetate buffer in the absence of added phosphate denatures at a considerable rate at temperatures as low as 15°C. diPrisco and Strecker [11] some years ago observed that beef liver glutamate dehydrogenase is stable in Tris buffer only if 0.01 M phosphate is present. This observation is now easily understood in view of the effects of the anion-binding site loading described above. More quantitative (but yet preliminary) studies support the notion that the stability of the glutamate dehydrogenase molecule is a function of the $T°$ of the particular complex or form the enzyme occupies. The rate of denaturation of the various forms of the enzyme measured at 47°C, for example, increase in the same order as the $T°$ values of those complexes decrease. A particularly interesting example of that fact is that the E–ADP complex has a $T°$ higher than that of the free enzyme [5], and, as predicted, the E–ADP complex requires a higher temperature than does E itself for denaturation to occur. It appears, then, that the 2-state transition described here must involve a considerable loosening of its internal structure. Presumably this substantial change in protein structure may involve the exposure of a number of hydrophobic groups to the solvent, thus accounting for the unusually large positive $\Delta H°$ of these transitions.

It is essential to note that the 'machine' described here is in no sense a heat engine. In our experiments (and presumably in the cell) the reactions occur under essentially isothermal conditions. The large temperature-dependences of binding enthalpies we observe serve simply as a signal which, by accompanying the protein transitions, permit us to identify and measure them. Indeed, it appears that in general the large endothermic transitions of the protein are largely compensated for by the tightly coupled exothermic binding steps that induce them. Thus the overall system appears to proceed without very much transfer of heat to or from the solvent. Whether this fact is significant or merely coincidental is not clear at this time.

It is useful at this point to consider the extent of the evidence that might indicate the presence of transduction machinery of the sort described here in other systems. It is admittedly quite meager at this time. Since we have shown that enthalpic phenomena qualitatively similar to those described here appear to occur quite generally among the pyridine nucleotide dehydrogenases, it may be presumed that the other members of this class of proteins also possess the elements of such machinery, although in each individual case, the transduc-

Table I

Predenaturational transition temperatures of glutamate dehydrogenase and its reaction intermediates

Enzyme form	$T°$[a] (°C)	$\Delta G°$[b] (kcal·mol^{-1})	$\Delta\Delta G°$[c] (kcal·mol^{-1})	f′[d]
E	43 ± 1	1.2 ± 0.07	0	0.10
E–R	−15 ± 5	−3.4 ± 0.5	4.6	1.00
E–R–K	−10 ± 5	−2.9 ± 0.5	4.1	0.99
E–O–G	35 ± 10	0.7 ± 0.7	−0.5	0.23

All data were obtained at pH 7.6 in 0.1 M phosphate buffer.

[a]Sources of individual values are described in the text.
[b]Calculated from eqn. (2). By definition, a negative value of $\Delta G°$ implies an E′/E ratio >1.
[c]The sign of $\Delta\Delta G°$ is the reverse of that of $\Delta G°$ and indicates the free energy that must be located in the given enzyme form relative to that of the free enzyme form.
[d]Calculated from $f' = \frac{E'}{E_{total}} = \frac{K}{K+1}$

tive events comprising the machinery would be expected to be arranged in a unique pattern to fit the specific function of that enzyme. Beyond this, then, what evidence exists that is at once more direct and which also arises from proteins outside of this rather specialized class? There are, to our knowledge, two sets of studies that have been published thus far. Differential scanning calorimetric (DSC) studies, which measure the temperature-dependence of the ΔCp of a protein directly, show that predenaturational ΔCp phenomena do occur quite commonly in proteins in general. Privalov [12], whose elegant studies have explored this phenomenon most thoroughly, has shown that in a number of cases these phenomena appear to have shapes consistent with 2-state transitions.

A second and more direct piece of evidence of ligand-induced energy transduction through displacement of 2-state highly enthalpic transitions in another (and quite different) enzyme system has been obtained from studies on myosin ATPase. This system is, of course, clearly implicated in an important biological energy transduction process. Sykes and Shriver [13] interpreted their observations on temperature-dependent NMR signals on this system in terms of a conceptual scheme not unlike that shown here in Fig. 4. Indeed, these authors used the term 'power stroke' in precisely the same sense we have employed. Kodama [14] carried out direct calorimetric measurements on the binding of ATP to myosin–ATPase and observed temperature-dependent $\Delta H°$ of binding which closely resembled the data we had presented in our earlier study of the binding of NADPH to liver glutamate dehydrogenase [15]. Based on this finding, Kodama proposed a 4-step machine quite similar to the one described here.

However, aside from the naivety inherent in basing a theory intended to explain the behavior of a complex system on a single pair of assumptions, recent experiments similar to that shown in Fig. 2 but carried out on ternary complexes of the same enzyme over a wider temperature range suggest a more complex behavior than can be accounted for by a single 2-state transition. We now suspect, in fact, that the enzyme is capable of at least two such transitions, each affected by some ligands but not others, and that these two transitions become coupled only under particular conditions. While we conclude that the picture we present is therefore far from a realistic one, the general formulation does provide a conceptual framework for understanding the thermodynamic properties of energy transduction and is, at the very least, capable of making the specific experimentally testable predictions whose results will lead to a detailed understanding of energy transduction in protein systems.

In the enzymatic machine we have just described, ligand binding energy is converted into chemical work. The induced conformational change which must be presumed to accompany the demonstrated structural change appears here only as a necessary byproduct of the process. However, one can equally well imagine similar processes in which either the shape of a biological macromolecule is changed at the expense of ligand binding energy without the production of chemical changes in the ligand, or its converse, the transduction of chemical reaction energy into such specific changes in molecular shape. Such machines might well be involved in the membrane-bound proteins which are thought to be responsible for the control of ion-specific gated channels, for example. Thus, the concept set forth here may be applicable in its general form to any of a wide variety of biological energy transduction processes.

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