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Revisiting Ground-State and Transition-State Effects, the Split-Site Model, and the "Fundamentalist Position" of Enzyme Catalysis

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ABSTRACT: In 1978 Schowen laid out the "fundamentalist position" of enzyme catalysis: "...the entire and sole source of catalytic power is the stabilization of the transition state; reactant-state interactions are by nature inhibitory and only waste catalytic power." In 1992 Menger developed the "split-site model" in order to demonstrate contradictions of the fundamentalist position. One of Menger's examples is recounted in which the energy of the enzyme−substrate complex (ΔG_{ES}) is lowered, yet the catalytic rate increases, incompatible with the claim that reactant- (*i.e.*, ground-) state interactions are inhibitory. A rigorous definition of ground-state effect is proposed which resolves the apparent contradictions. A ground-state effect ($\Delta\Delta G_{ES}$) is defined as one in which the energy of the ground state has changed, but the energy of the catalyzed transition state is unchanged when one enzyme is compared to another. This is a result of the constraint that the free energy of binding of the enzyme to the uncatalyzed reaction transition state, ΔG_b*, is constant. That ground-state interactions are inhibitory when a single enzyme-catalyzed reaction is considered has been proven by Schowen; this definition of ground-state effect achieves the result of making *additional* ground-state interactions inhibitory also. This definition is rather restrictive, however, and does not describe many of the possible changes in enzyme energy levels. A proposal is therefore put forth to simply explain the changes in terms of alterations in intrinsic and utilized binding energy.

In 1978 Schowen put forth the "fundamentalist position" of enzyme catalysis which showed that excessively tight substrate binding by an enzyme was detrimental to the catalytic cause and wasted some of the free energy available to an enzyme to accelerate the rate of its reaction. In a recent paper Menger (1992) analyzes ground-state and transition-state effects in terms of a "split-site" model in which the enzyme active site is mentally split into a binding region and a reactive region. Alterations in the energy levels at these regions are used to illustrate contradictions in the fundamentalist position, in particular the idea that "reactant-state interactions only waste catalytic power." The purpose of this paper is twofold, first to resolve the apparent contradictions raised by Menger by giving a restricted definition of "ground-state effect" and second to propose a more general and less controversial way to examine the effects of changes in the energetics of enzyme-catalyzed reactions.

Before taking a detailed look at Schowen and Menger's arguments, it is necessary to clarify some of the terminology. The terms "reactant state" and "ground state" appear to have the same meaning and are used here interchangeably. However, "ground-state *interaction*" and "ground-state *effect*" are not identical. A ground-state interaction refers to the energy of the ground state upon binding of substrate to enzyme for a particular enzyme−substrate pair. Ground-

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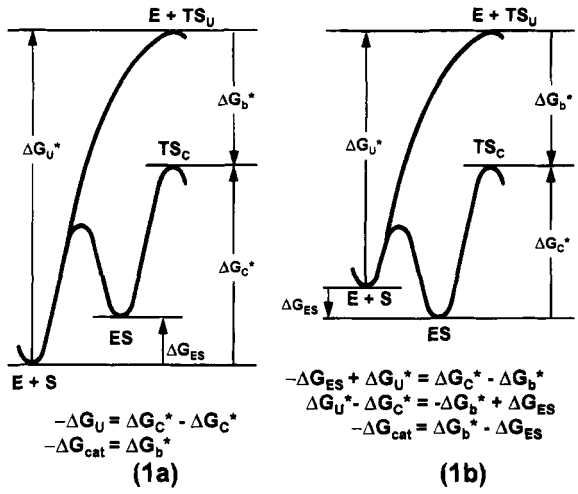

FIGURE 1: Free energy diagrams for uncatalyzed and enzyme-catalyzed reactions of the substrate S at different standard-state concentrations of S. (a) The concentration of S is low relative to K_m so that no substantial concentration of enzyme−substrate complex builds up. (b) The concentration of S is high compared to K_m so that the enzyme is primarily complexed with substrate. For detailed analysis and other insights, see Schowen (1978). Figure used with permission from the publisher.

state effect implies the effect of some *change* in the enzyme or substrate and therefore should be restricted to describing those cases where one is comparing two different enzyme or substrate species. Therefore, ground-state interactions are described by ΔG_{ES}, whereas ground-state effects are described by $\Delta\Delta G_{ES}$. An important distinction is that Schowen's paper analyzes ground-state interactions; Menger's paper analyzes ground-state effects.

The fundamentalist position "cast in high relief" by Schowen (1978) proposes "...that the entire and sole source of catalytic power is the stabilization of the transition state; that reactant-state interactions are by nature inhibitory and only waste catalytic power."

This conclusion is based on an analysis of the free energy diagrams shown in Figure 1. ΔG_{cat} is defined as the difference in energy between the uncatalyzed transition state and the catalyzed transition state: $\Delta G_{cat} = \Delta G_U^* - \Delta G_C^*$. The definition of ΔG_b^* is subtly, but meaningfully different. ΔG_b^* is the energy released from the theoretical interaction of the enzyme with the transition state of the reaction (*i.e.*, the free energy of transition-state binding by the enzyme), and it is the ultimate mechanism by which an enzyme is able to achieve catalytic rate enhancement (Pauling, 1946, 1948; Schowen, 1978). In Figure 1a substrate is subsaturating (ES is higher in energy than E + S), and the binding energy ΔG_b^* is fully utilized as catalytic power: $-\Delta G_{cat} = \Delta G_b^*$. All of the free energy gained from association of the reaction transition state with the enzyme goes into accelerating the reaction. In Figure 1b the substrate is saturating (ES is lower in energy than E + S), and consequently, some of the binding energy is "wasted" upon binding the substrate: $-\Delta G_{cat} = \Delta G_b^* - \Delta G_{ES}$. This equation is proof of the conclusion that reactant state interactions waste catalytic power. Note that each case, 1a or 1b, is analyzed independently. The free energy changes on the left side of each figure are set equal to the free energy changes on the right side, and the appropriate algebraic manipulations are performed to isolate $-\Delta G_{cat}$.

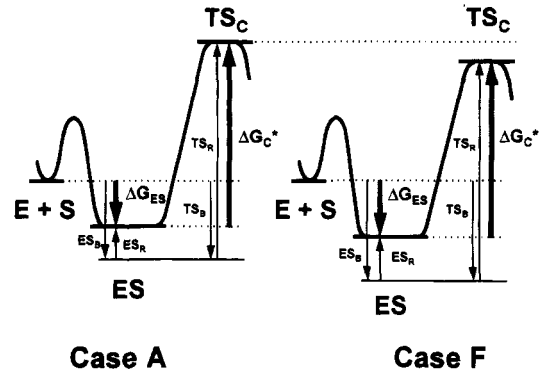

FIGURE 2: Free energy diagrams of cases A and F from Table 4 of Menger (1992). Energies of subsites, ES_B, ES_R, TS_B, and TS_R, are shown as thin arrows; energies of full sites, ΔG_{ES} and ΔG_C^*, are shown as thick arrows. The summations of ES_B plus ES_R to yield ΔG_{ES}, and TS_B plus TS_R to yield TS_C, are apparent. For the change from enzyme A to enzyme F, ES is lowered by 1 kcal and TS_C by 2 kcal. Figures are to scale. Values are in the text and Table 1.

Menger (1992), in order to point out the misleading nature of the fundamentalist notions, developed the "split-site model". According to Menger's model "...substrates and cofactors can be subdivided into a binding site and a reactive site. Substrate association is describable, therefore, as the sum of interactions with the enzyme at the distinct binding and reactive sites ($\Delta G_{ES} = ES_B + ES_R$)". The corresponding definition holds for the transitions state ($TS_C = TS_B + TS_R$); it follows that $ES_B = TS_B$ since no change is happening at this site during the reaction. In Menger's paper a number of examples are given where changes in the energies of ES_B and ES_R are made and the effect on the catalytic rate is determined. One example will suffice here. The comparison of case A with case F from Table 4 of the original paper illustrates much of Menger's contrary position and is drawn out in Figure 2. In moving from case A to case F, ES_B decreases from −7 to −9, while ES_R increases from +3 to +4; TS_R is constant and equal to +20 in both cases. ΔG_{ES} thus decreases from −4 to −5 while TS_C decreases from +13 to +11, yielding a net *increase* in rate because the energy to get from ES to TS_C has decreased from +17 to +16. Thus there exists a contradiction with the fundamentalist position that "reactant-state interactions are inhibitory and waste catalytic power" because ES is lowered, yet the rate increases. Menger concludes that "Owing to the rule of conserved energies, an adjustment of ES_B necessarily leads to a modification of TS. They are inseparable. One can call this a ground-state effect or a transition-state effect; it makes no difference."

It is argued here that it in fact *does* make a difference; a rigorous definition of a ground-state effect would avoid confusion and contradiction. Described next are two possible definitions for the energetic changes of a ground-state effect. They are illustrated in Figures 3 and 4. (The changes illustrated in Figure 2 are actually intermediate between these two limiting cases.) In Figure 3 the ground state moves in the change from 3a to 3b, but the catalyzed transition state does not. Thus ΔG_b^*, the free energy of enzyme binding to the transition state, remains *un*changed. (This change mirrors increasing the substrate concentration, as depicted in Figure 1.) In Figure 4 ES and TS_C move in parallel and ΔG_C^* is constant. This behavior has been described by Albery and Knowles (1976) as uniform binding. The careful reader will

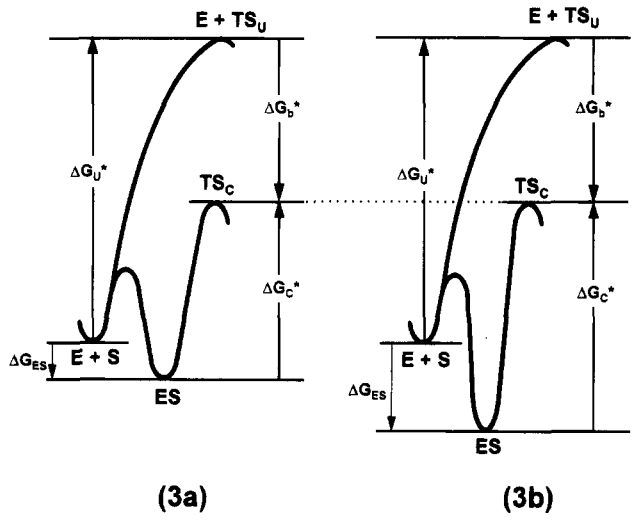

FIGURE 3: Free energy diagrams for the case where ES is lowered and TS_C is constant; $\Delta\Delta G_b^* = 0$. It is argued that this should be the definition of a ground-state effect.

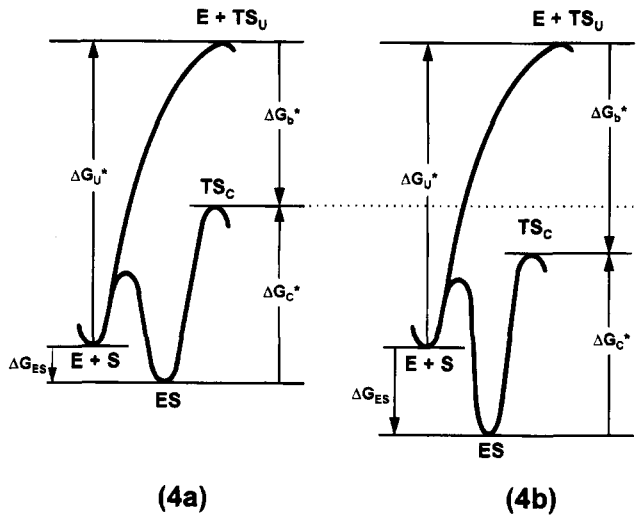

FIGURE 4: Free energy diagrams for the case where ES and TS_C are lowered by the same amount: $\Delta\Delta G_C^* = 0$. This is a uniform binding change as described by Albery and Knowles (1976).

note that an increase only in ES_B, with no change in ES_R, is another way of describing uniform binding. Note also that ΔG_b^* has increased. The crucial difference between the two is which thermodynamic parameter is constant, ΔG_b^* in Figure 3 or ΔG_C^* in Figure 4.

So which definition, that depicted in Figure 3 or that in Figure 4, should be chosen as the definition for a ground-state effect? This author proposes Figure 3 for two reasons: First, Schowen's position that "reactant-state interactions are inhibitory and only waste catalytic power" is true when analyzing a particular enzyme-catalyzed reaction; adoption of this definition will make it valid when a *change* in enzyme energetics is considered (something not directly addressed in the 1978 paper). In other words, if reactant- (*i.e.*, ground-) state interactions are inhibitory, then *additional* interactions should also be inhibitory. With the constraint of constant ΔG_b^*, they are. The second justification is elegantly laid out by Menger in the example above: the apparent contradiction between increased ground-state interactions and increased catalytic rate. By adopting constant ΔG_b^* as the definition of a ground-state interaction, no contradiction will exist. This is because if ΔG_b^* increases,

Table 1[a]

case	E + S	ΔG_{ES}	TS_C	ΔG_C^*	ΔG_b^*	effect
A[b]	0	−4	+13	+17	20	
B	0	−4	+12	+16	21	accel
C	0	−4	+14	+18	19	decel
D	0	−5	+12	+17	21	none
E	0	−3	+13	+16	20	accel
F	0	−5	+11	+16	22	accel

[a] Parts taken from Menger (1992). [b] Case A is the reference to which other cases are compared. $\Delta G_U^* = 33$. $\Delta G_C^* = TS_C - \Delta G_{ES}$. ΔG_C^* is called ΔG_a in Menger (1992).

then it is possible to increase the catalytic rate, even while increasing binding to the ground state. Increased interactions with the substrate are not wasteful if they are utilized for catalysis.

However, keeping ΔG_b^* or ΔG_C^* constant is a rather restrictive constraint and does not describe many of the possible differences in energetics between two enzymes, as exemplified in Figure 2. The following proposal is put forth to allow for the description of the changes in any situation and to avoid the temptation to use terms such as "pure" or "partial" ground-state effect. It makes use of the concepts elucidated by Jenks (1975) in his classic treatise on the *circe* effect.

Proposal for the Interpretation of Free Energy Changes between Different Enzymes: (1) determine the change in apparent binding energy of the substrate(s), $\Delta\Delta G_{ES}$, (2) determine the change in the catalytic rate(s), $\Delta\Delta G_{TS}$, and (3) analyze the *fate* of the intrinsic binding energy; what percentage of any change is utilized for catalysis. The percentage of any additional intrinsic binding energy utilized for catalysis can of course vary between 0 and 100%. (It should be pointed out that intrinsic binding energy can also be used to accelerate the rate of noncovalent steps, but this analysis will be restricted to the single catalytic step for simplicity.) To examine this proposal in action, several examples are laid out in Table 1. To enable a direct comparison, these are the same examples used by Menger in Table 4 of his paper with the additional parameter ΔG_b^*. All changes are referenced to case A.

Case B. (1) There is no change in apparent binding energy, ΔG_{ES}, (2) there is a 1 kcal decrease in the energy required to reach the transition state, ΔG_C^*, and (3) since the apparent binding energy is unchanged but the activation energy is lower, 100% of the 1 kcal of additional intrinsic binding energy is utilized for catalysis. This is referred to by Albery and Knowles (1976) as catalysis of the elementary step. This can also serve as the definition of transition-state effect.

Case C. (1) There is no change in apparent binding energy, (2) there is a 1 kcal increase in the activation energy to reach the transition state, and (3) 100% of the 1 kcal decrease in intrinsic binding energy is lost from catalysis.

Case D. (1) There is a 1 kcal increase in the apparent binding energy, (2) there is no change in the activation energy required to reach the transition state, and (3) 0% of the 1 kcal of additional intrinsic binding energy has been utilized for catalysis (all of it appears as $\Delta\Delta G_{ES}$). This is uniform binding.

Case E. (1) There is a 1 kcal decrease in apparent binding energy, (2) there is a 1 kcal decrease in the energy required to reach the transition state, and (3) intrinsic binding energy

is unchanged, but 1 kcal of intrinsic binding energy has been shifted from binding to catalysis. This is the proposed definition of ground-state effect (ΔG_b^* is unchanged). One kilocalorie of binding energy which was wasted by tighter substrate binding by enzyme A is no longer wasted by enzyme D.

Case F. (1) There is a 1 kcal increase in the apparent binding energy, (2) there is a 1 kcal decrease in the energy required to reach the transition state, and (3) 50% of the 2 kcal of additional intrinsic binding energy has been utilized for catalysis. This is the example drawn out in Figure 2.

Several authors have used this proposal in practice, if not in exact wording [for a leading review, see Benkovic and Johnson (1990)]. A relevant example comes from the work on tyrosyl-tRNA synthetase (Wells & Fersht, 1986). The mutant Y34F removes a hydrogen bond from the substrate tyrosine and leads to a 0.5 kcal decrease in the substrate affinity but no change in the catalytic rate; *e.g.*, (1) there is a 0.5 kcal decrease in the apparent binding energy, (2) there is no change in the activation energy required to reach the transition state, and (3) 0% of the 0.5 kcal of lost intrinsic binding energy has been lost from catalysis. Another mutant, C35S, lengthens the hydrogen bond between the enzyme and the ribose of ATP. There is no change in the binding energy of the substrates, but the catalytic rate has decreased from 38 to 4.7 s^{-1}; *e.g.*, (1) there is no change in apparent binding energy, (2) there is a 1.2 kcal increase in the activation energy to reach the transition state, and (3) 100% of the 1.2 kcal decrease in intrinsic binding energy is lost from catalysis.

In the context of the evolutionary path an enzyme may take, uniform binding and catalysis of the elementary step involve changes in intrinsic binding energy, whereas a ground-state effect as defined above involves no change in intrinsic binding energy but rather a redistribution of that energy between binding and catalysis. A ground-state change therefore formally represents a third change to an evolving enzyme which can improve its catalytic function. The effect of a useful ground-state change would be to match the enzyme binding affinity and natural substrate concentration and thereby avoid wasting catalytic power.

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