Reconciliation of Split-Site Model with Fundamentalist Formulation Enabled by Equilibrium Assumption

Thong-Sung Ko,† Hyeong-Won Ryu, and Young Cho‡

†*Department of Biochemistry, Chungnam National University, Daejeon 305-764, Korea*
‡*1st R & D Center (#2-2), Agency for Defense Development, P.O. Box 35 Yuseong, Daejeon 305-600, Korea*
Received December 27, 2002

By the use of multi-loop thermodynamic boxes developed here by us, we show that models of enzyme catalysis (*e.g.*, split-site model) developed in an attempt to emphasize the importance of the reactant-state destabilization and, thus, demonstrate misleading nature of the fundamentalist position which defines Pauling's transition-state stabilization as the entire and sole source of enzyme catalytic power, should be reduced to the fundamentalist formulation which completely neglects dynamical aspects of mechanism between the reactant and the transition states and dwells only on events restricted to the reactant and transition states alone, because the split-site (and other canonical) formulations as well as fundamentalist formulations are based, in common, on equilibrium assumptions stipulated by the thermodynamic box logics. We propose to define the equilibrium assumptions as the requisite and sufficient conditions for the fundamentalist position to enjoy its primacy as central dogma, but not as sufficient conditions for its validity, because it is subjected to contradictions presented by existing data.

Key Words : Enzyme, Split-site, Transition-state, Catalysis

Introduction

In this paper, by the use of multi-loop thermodynamic boxes developed by us here, we will carry out thought experiment (numerical analysis) to show that even Menger's split-site model,[1] which was brought about in an attempt to demonstrate contradictions of the fundamentalist position of enzyme catalysis as expressed in the quote put forth by Showen,[2] "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.", eventually comes down to a fundamentalist formulation. Nevertheless, even if indeed all enzyme theories ultimately reduce to the language of transition-state stabilization as shown by Showen[2] and even the split-site model can not be an exception to this as will be shown in this paper, the fundamentalist position is still subjected to contradiction, *e.g.*, that raised by Britt,[3] who presents previously published data which show that strong reactant-state interaction is favorable for the enzyme catalysis. The present data obtained by virtue of the multi-loop thermodynamic boxes, in conjunction with the fact that all of them are based on the equilibrium assumption, explains how it is possible that everyone of the customary enzyme catalysis theories be reduced to the fundamentalist formulation, and for all that, why the latter which may now be considered dogma should still be subjected to contradictions.

Murphy[4] revisited the split-site and the fundamentalist formulations in order to resolve the apparent contradictions raised by Menger against the latter. According to Murphy, an important distinction is that Showen's paper analyzes ground-state interactions; Menger's paper analyzes ground-state effects, and the resolution of the contradictions can be attained by a rigorous definition of ground-state effect and ground-state interaction. Then the question arises: "Are the contradictions nothing else but matters of language and the two (split-site and fundamentalist) formulations actually equivalent otherwise?" For our purposes, we revisit not only the split-site and fundamentalist formulations but also the canonical formulations[5-9] which were so named and shown to be equivalent to the fundamentalist version by Showen.[2]

In Menger's development of the split-site model, reactant-state interaction (ΔG_{ES}) is subdivided into distinct binding (ES_B, stabilizing) and reactive (ES_R, destabilizing) entities, *viz.*, $\Delta G_{ES} = ES_B + ES_R$ ($ES_R = -ES_B + \Delta G_{ES}$). However, it will come out in this paper that this subdivision of ΔG_{ES} into ES_B and ES_R is essentially tantamount to the translation of transition-state (fundamentalist) formulations back to the canonical versions *via* restoring the "reactant-state destabilization" entity, *e.g.*, ΔG_D^* (= ES_R).

The fundamentalist notions can be quantitatively expressed by the Kurz's formulation[9] (eq. 4) which is based on the thermodynamic box of equilibrium (Fig. 1d) relating the equilibrium binding of enzyme with substrate in reactant-state (S) and in transition state ($S^{\ddagger}$). K_S and K_{TS} are dissociation constants of ES and $ES^{\ddagger}$ respectively; $K_U^{\ddagger}$ and $K_C^{\ddagger}$ are equilibrium constants for the formation of the transition states of the uncatalyzed and catalyzed reactions, $S^{\ddagger}$ and $ES^{\ddagger}$, respectively. One can get: $K_C^{\ddagger}/K_S = K_U^{\ddagger}/K_{TS}$ (eq. 1), *i.e.*, $\Delta G_{ES} + \Delta G_C^{\ddagger} = \Delta G_U^{\ddagger} + \Delta G_b^{\ddagger}$ (eq. 2) directly from this box of equilibria, since overall free-energy change (or equilibrium constant) must be the same regardless of the path ($E + S \rightarrow ES \rightarrow ES^{\ddagger}$ or $E + S \rightarrow E + S^{\ddagger} \rightarrow ES^{\ddagger}$).

*To whom correspondence should be addressed. Tel: +82-42-821-4383 Fax: +82-42-821-2391 e-mail: biochip@hanmail.net

Rearrangements of eqs. 1 and 2 and combination of eq. 1 with Eyring's equation ($k = k\nu K^{\ddagger}$)[11] leads immediately to $\Delta G_{cat} = \Delta G_U^{\ddagger} - \Delta G_C^{\ddagger} = -\Delta G_b^{\ddagger} + \Delta G_{ES}$ (eq. 3) and the Kurz's formulation, $k_C/k_U = K_C^{\ddagger}/K_U^{\ddagger} = K_S/K_{TS}$ (eq. 4), respectively. From these equations, one can define a quantity, $\Delta G_{cat} = \Delta G_U^{\ddagger} - \Delta G_C^{\ddagger} = RT \ln (k_C/k_U)$. This "catalytic free energy", tantamount to rate enhancement (k_C/k_U), must be a positive number which will be the larger as the rate enhancement is larger.[2] Eqs. 3 and 4, at which we have finally arrived under the conditions of equilibrium assumption, where ES level lies below (E + S) level (in this paper we are referring to these conditions unless mentioned otherwise), are quantitative formulations of the fundamentalist position of enzyme catalysis.

ΔG_{cat} combines two factors, $-\Delta G_b^{\ddagger}$ and ΔG_{ES}, that influence the catalysis (ΔG_{cat}) in opposite directions (catalytic effect by $-\Delta G_b^{\ddagger}$, and inhibitory effect by ΔG_{ES}). Thus, reactant-state stabilization (which is tantamount to ES lowering, stronger substrate-binding, and smaller K_m) necessarily lessens rate increase (*viz.*, ΔG_{cat} increase) which is brought forth by $-\Delta G_b^{\ddagger}$ increase outweighing the increase of the absolute value of ΔG_{ES}, in accordance with the fundamentalist notions.

That the canonical versions require much greater detail in the postulation of what occurs between reactant and transition states – dynamical aspects of mechanism (*e.g.*, reactant-state destabilization) – than the fundamentalist language, which completely neglects this dynamic aspect and resides only on reactant (initial) and transition (final) states alone, and accordingly, the equilibrium boxes and the free-energy diagrams for the canonical (*viz.*, split-site) descriptions should be more complicated, having extra links (closed loops) in addition to the simple quadrilateral fundamentalist boxes composed of the basic four lateral lines alone, prompted us to develop a new thermodynamic boxes, *i.e.*, multi-loop boxes for them as shown in Figure 1.

Methods

Construction of multi-loop thermodynamic boxes for canonical (*viz.*, split-site) models. In the canonical descriptions (*vid.* Fig. 1c), part of the intrinsic binding energy (ΔG_{int}) is utilized to cancel the unfavorable energy of the path (ΔG_D^*), while the remainder is released as net observed binding energy of substrate, ΔG_{ES} ($\Delta G_D^* = -\Delta G_{int} + \Delta G_{ES}$).[2] One may reason that this formulation is related to additional extra loops of the equilibrium boxes of the canonical (*viz.*, split-site) models, and that the formulation corresponds to that of Menger's split-site model, $ES_R = -ES_B + \Delta G_{ES}$ (ΔG_D^* and ΔG_{int} correspond to ES_R and ES_B, respectively, as shown

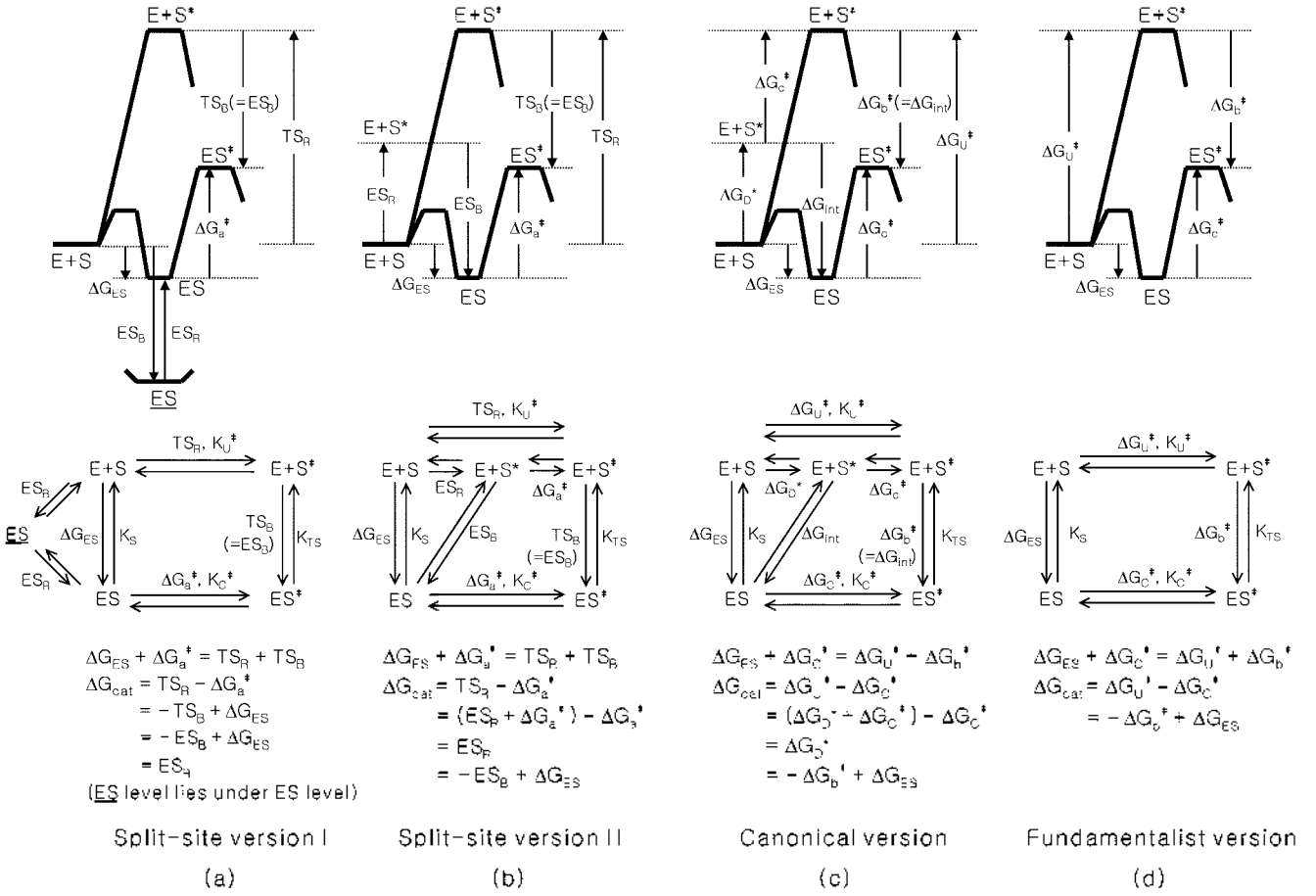

Figure 1. Thermodynamic boxes and free-energy diagrams illustrating: (1) split-site versions [I (a) and II (b)] of enzyme catalysis involving reactant-state destabilization embodying "conserved" interactions at the binding region, and (2) equivalency of the split-site versions to other canonical (c) and fundamentalist versions (d).

in Fig. 1). Evidently, the subdivision of ΔG_{ES} into ES_B and ES_R, which corresponds to the installation of the dynamical path in the thermodynamic box of the fundamentalist version, brings about modification of the conventional simple fundamentalist box to a looped one which should correspond to the split-site model (*vid.* Fig. 1a and 1b). The split-site version can be depicted in two ways, I and II, as shown in Figure 1a and 1b, respectively, according to the sequential order of the destabilization (ES_R) and stabilization (ES_B) of the reactant-state: (I) intrinsic binding (ES_B) of reactant-state S with E first and subsequently followed by destabilization (ES_R) of the ES complex (*vid.* Fig. 1a), (II) destabilization of the reactant state (ES_R) first to unstable poised structure (say S*) and subsequently followed by vertical intrinsic stabilizing binding (ES_B) by enzyme (that is, the same manner as in the case of canonical version illustrated by Showen). Split-site versions I and II look different in their overall free-energy diagrams and equilibrium boxes because of their different pathways resulting from their different sequential orders of stabilization (ES_B) and destabilization (ES_R). Nonetheless, regardless of such differences, both parts of the released intrinsic binding energy, ES_B, of stabilized reactant-state complex (ES in I) and of reactive complex (ES* in II) are utilized in the same way to compensate the unfavorable reactant-state destabilization (ES_R) while the remainder is released as net observed binding energy of substrate, ΔG_{ES} : $ES_R = -ES_B + \Delta G_{ES}$ in both versions. It is readily seen in Figure 1a and 1b for both I and II that catalytic acceleration, $\Delta G_{cat} = TS_R - \Delta G_a^{\ddagger}$ is equal to ES_R, *i.e.* $\Delta G_{cat} = -ES_B + \Delta G_{ES} = ES_R$.

Comparative examination of the multi-loop thermodynamic boxes with the conventional simple thermodynamic box. As well as fundamentalist formulations, split-site and other canonical formulations are based on equilibrium assumptions and can be illustrated by thermodynamic boxes in terms of the equilibrium thermodynamic arguments (equilibrium-box logics) that overall equilibrium constant (*viz.*, free energy change) is dependent only on the initial and final states of the system and is independent of the path or mechanism of changing from one state to another. Thus, all canonical formulations, including split-site formulation, irrespective of the intermediate dynamical devices, reduce to a single factor *viz.*, transition-state language which is relevant only to the initial and the final states. Namely, they are equivalent to the fundamentalist formulation which dwells on transition-state language to the exclusion of other descriptive apparatus. However, one should realize, through comparative inspection of the equilibrium boxes in Figure 1, that some of the identical species of catalysis factors are denoted differently in the two versions: the denotations, $\Delta G_U^{\ddagger}$, $\Delta G_b^{\ddagger}$, $\Delta G_C^{\ddagger}$, ΔG_{int}, and ΔG_D^{*} of the fundamentalist correspond to TS_R, TS_B, $\Delta G_a^{\ddagger}$, ES_B, and ES_R of the split-site,[1] respectively. One may notice, in the equilibrium boxes of the split-site model shown in Figure 1a and 1b, that their overall free energy change for the formation of $ES^{\ddagger}$, *viz.* kinetic aspects of mechanism [events restricted to initial (E + S) and final ($ES^{\ddagger}$) states alone] must be the same regardless of their difference in dynamic aspect of mechanism, *viz.*, path (*e.g.*, $E + S \rightarrow E + S^* \rightarrow ES \rightarrow ES^{\ddagger}$, $E + S \rightarrow \underline{ES} \rightarrow ES \rightarrow ES^{\ddagger}$, or $E + S \rightarrow ES \rightarrow ES^{\ddagger}$): $TS_R + TS_B = \Delta G_{ES} + \Delta G_a^{\ddagger} = ES_B + ES_R + \Delta G_a^{\ddagger}$ (eq. 3.0). Rearrangement of this equation leads to the fundamentalist eq. 3 ($\Delta G_{cat} = \Delta G_U^{\ddagger} - \Delta G_C^{\ddagger} = -\Delta G_b^{\ddagger} + \Delta G_{ES}$) in Menger's denotations, *i.e.*, $\Delta G_{cat} = TS_R - \Delta G_a^{\ddagger} = -TS_B + \Delta G_{ES}$ (eq. 3.1).

Thought experiment (numerical analysis) using the thermodynamic boxes. In the present study, with the use of equilibrium boxes of initial (E + S) and final ($ES^{\ddagger}$) state in common having multi-loop boxes developed here by us, we clarify the underlying conditions and mechanism – the equilibrium assumption and the equilibrium box logics – of their translation and equivalency. Having verified that split-site model as well as fundamentalist formulations is based on equilibrium assumption that can be explained by the equilibrium thermodynamic-box logics and is eventually equivalent to the latter, we will now proceed to demonstrate that the numerical analysis data of the split-site model laid out by Menger as his demonstration examples of the misleading nature of the fundamentalist position can be turned round to show the equivalency of the two versions.

In Menger's attempt to demonstrate the contradiction of the fundamentalist notion, in particular that "reactant-state interactions are by nature inhibitory and waste catalytic power", Menger made an example of rate increase irrespective of ES lowering (the comparison of case A with case F from Table IV of Menger's original paper).[1] We lay out both part from Menger's paper[1] and additional new cases to show the inhibitory and wasting nature of ΔG_{ES} more clearly, *i.e.*, in agreement with the fundamentalist position. In order to reconfirm that there can not be any conflict in evidence between the split-site and fundamentalist formulations, we reexamine Menger's Table IV data (Table 1i). (Menger's Table III and IV are attached at the end of this paper as Appendix.)

Results and Discussion

Thus, one may here draw a conclusion that a split-site model, regardless of either version I or II, can be translated into the fundamentalist version, and thus be equivalent to the latter. As we have shown above that $\Delta G_D = -\Delta G_{int} + \Delta G_{ES}$ corresponds to $ES_R = -ES_B + \Delta G_{ES}$, we can rewrite eq. 3 and 3.1 as: $\Delta G_{cat} = \Delta G_U^{\ddagger} - \Delta G_C^{\ddagger} = -\Delta G_b^{\ddagger} + \Delta G_{ES} = -\Delta G_b^{\ddagger} + \Delta G_{int} + \Delta G_D^{*}$ (eq. 3a) and $\Delta G_{cat} = TS_R - \Delta G_a^{\ddagger} = -TS_B + \Delta G_{ES} = -TS_B + ES_B + ES_R$ (eq. 3.1a), respectively.

The postulation (so-called "rule of conserved energies") that the transition-state binding energy be equal to the intrinsic binding energy, *i.e.*, $ES_B = TS_B$, is required by split-site model[1] just as it (*i.e.*, $\Delta G_b^{\ddagger} = \Delta G_{int}$) is required in the fundamentalist translation of canonical formulation.[2] This postulation requires automatically $\Delta G_{cat} = ES_R$ (*i.e.*, $\Delta G_{cat} = \Delta G_D^{*}$), which can be derived from the equilibrium-box logic, *viz.*, from eqs. 3.1a and 3a, respectively. That is, if $\Delta G_b^{\ddagger} = \Delta G_{int}$, then $\Delta G_{cat} = \Delta G_D^{*}$, and vice versa, in eq. 3a; and if $ES_B = TS_B$, then $\Delta G_{cat} = ES_R$, and vice versa, in eq. 3.1a. Thus,

one may notice that $\Delta G_b^{\ddagger} = \Delta G_{int}$ and $\Delta G_{cat} = \Delta G_D^*$, required by canonical formulations,[2] are based on equilibrium assumption, and that this requirement ($ES_B = TS_B$ and $\Delta G_{cat} = ES_R$) holds valid in split-site model too, as shown in Table 1i [Table IV in Menger's original paper[1]]. From the eq. 3a, eq. 3.1a, and the "rule of conserved energies" (*i.e.*, $ES_B = TS_B$), we get: $\Delta G_{cat} = -\Delta G_b^{\ddagger} + \Delta G_{ES} = -ES_B + \Delta G_{ES} = ES_R$ (eq. 3.2), which may demonstrate the equivalency of the two versions (split-site, as well as canonical, and fundamentalist versions).

Thus, we hereby find that the catalytic acceleration (ΔG_{cat}) is given by $-ES_B + \Delta G_{ES}$, *i.e.*, by the utilized part of the intrinsic binding energy ($\Delta G_{cat} = -ES_B + \Delta G_{ES}$) (split-site) or by $-\Delta G_b^{\ddagger} + \Delta G_{ES}$, *i.e.*, by the net catalytic binding energy ($\Delta G_{cat} = -\Delta G_b^{\ddagger} + \Delta G_{ES}$) (fundamentalist).

This conclusion is quite similar to the one suggested by Showen[2] two decades ago with respect to the fundamentalist translation of canonical formulations. Showen used free-energy diagrams in his making comparison and translation to clarify the equivalency of fundamentalist and canonical approaches.[2]

Recall that ΔG_{cat} combines $-\Delta G_b^{\ddagger}$ (*i.e.*, $-ES_B$) and ΔG_{ES} that influence catalysis oppositely – plus effect of $-\Delta G_b^{\ddagger}$ (*i.e.*, $-ES_B$) and minus effect of ΔG_{ES}. In moving from case A to case F, ES is lowered from −4 to −5, yet ΔG_{cat} increases from +3 to +4 thanks to the increase in $-\Delta G_b^{\ddagger}$ from +7 to +9 outweighing the ES lowering. Menger cites this as an example for a contradiction with the fundamentalist position that "reactant-state interactions are inhibitory and waste catalytic power", because ES is lowered yet rate increases.

$$\Delta G_{cat} = -\Delta G_b^{\ddagger} + \Delta G_{ES}$$
$$\Delta G_{cat} = -ES_B + \Delta G_{ES} = ES_R \quad \text{(eq. 3.2)}$$

Menger's: case A +3 = −(−7) + (−4) +3; case F +4 = −(−9) + (−5) +4

Authors': case F′ +5 = −(−9) + (−4) +5; case F″ +6 = −(−9) + (−3) +6

However, now we show that this can not be a source of the contradiction, because the rate increase is lessened by the ES lowering even though the rate is increased despite of ES lowering. In case F′ (modified case F) where there is no ES lowering, ΔG_{cat} (*viz.* rate) is increased. With smaller ES

Table 1. Conformity of examples from Menger[1] with fundamentalist formulations[a]

(i)

case	ΔG_{cat} = =	$-ES_B$ + $(\Delta G_b^{\ddagger}$ +	ΔG_{cat} = $\Delta G_{ES})$	ES_R	effect	"effect" in Menger's Table IV
A[b]		−(−7) +	(−4)	+3		
B		−(−8) +	(−4)	+4 (increase)	increase	increase
C		−(−6) +	(−4)	+2 (decrease)	decrease	decrease
D		−(−8) +	(−5)	+3 (no change)	no change	no change
E		−(−7) +	(−3)	+4 (increase)	increase	increase
F		−(−9) +	(−5)	+4 (increase)	increase	increase

(ii)

case	K_S	(ΔG_{ES})	K_{TS}	(TS)	K_S/K_{TS}	$(\Delta G_c^{\ddagger}, G_a^{\ddagger})$	effect	"effect" in Menger's Table IV
A[b]		(-4)		(+13)		(+17)		
B	no change	(-4)	decrease	(+12)	increase	(+16)	acceleration	acceleration
C	no change	(-4)	increase	(+14)	decrease	(+18)	deceleration	deceleration
D	decrease	(-5)	decrease	(+12)	no change	(+17)	no change	no change
E	increase	(-3)	no change	(+13)	increase	(+16)	acceleration	acceleration
F	decrease	(-5)	decrease	(+11)	increase	(+16)	acceleration	acceleration

(iii)

case	substrate state				transition state				effect	
	ES_B	ES_R	ΔG_{ES}	K_S	TS_B	TS_R	TS	K_{TS}	from Menger's Table III	from fundamentalist (equation 9)
A[b]	+3	+7	+10		+3	+20	+23			
B	+2	+8	+10	no change	+2	+20	+22	decrease	acceleration	acceleration
C	+4	+6	+10	no change	+4	+20	+24	increase	deceleration	deceleration
D	+2	+7	+9	decrease	+2	+20	+22	decrease	acceleration	acceleration
E	+3	+6	+9	decrease	+3	+20	+23	no change	none	none

[a](i) eq. 3.2, (ii) eq. 8, (iii) eq. 9 (and eq. 10). [b]Case A is the reference to which other cases are compared. [c](i) and (ii): Cases where ES is of lower free energy than (E +S). (iii): Cases where ES is of higher free energy than (E + S).

lowering as shown in case F", ΔG_{cat} is furthermore increased.

Thus, Menger's numerical analysis data laid out to show that there exists a contradiction with the fundamentalist position that "reactant-state interactions are inhibitory and waste catalytic power", can be turned round to the basis of the argument that defends the fundamentalist position from the contradiction raised by Menger.[1] Obviously, under the equilibrium assumptions substrate destabilization (ES_R) embodying conserved interactions ($ES_B = TS_B$ *i.e.*, $\Delta G_b^{\ddagger}$) at the binding region, can not be such a circumstance, as supposed by Menger,[1] which enable an evolving enzyme to increase both wasting (lowering K_m, *i.e.*, ΔG_{ES}) and catalytic rate (*viz.* ΔG_{cat}), but for increased source of catalytic power ($-\Delta G_b^{\ddagger}$ *viz.*, $-ES_B$) outweighing the wasting.

In our reexamination of Menger's Table IV data (Table 1i), we can reconfirm that there can not be any conflict in evidence between the split-site and fundamentalist formulations. Contrary to Menger's original attempt, we can be convinced again that when the inhibitory effect of enzyme on the reactant, *i.e.*, the free-energy expended in reactant-state stabilization (ΔG_{ES}) is subtracted from the transition-state stabilization energy, we once again find that the catalytic acceleration is given by $\Delta G_{cat} = -ES_B + \Delta G_{ES}$, that is utilized part of the intrinsic binding energy. Neither ΔG_{ES} nor ES_B alone but only the combination of them, $\Delta G_{cat} = -ES_B + \Delta G_{ES} = ES_R$, *i.e.*, the size of the utilized part of the intrinsic binding energy (split-site) or net stabilization of the transition state (fundamentalist) can be the criterion of the catalysis (ΔG_{cat}). Thus, Menger's claim for the existence of a contradiction with the fundamentalist position that "the reactant-state interactions are inhibitory and waste catalytic power", simply on the ground that ES is lowered yet the rate increases, can hardly be accepted to be fair, because it is the "alleviation" in the ES lowering (inhibition) and not ES lowering itself that brings forth the increased catalysis (ΔG_{cat}).

The fundamentalist approach, which completely neglects events between reactant and transition states, is purely kinetic, while such dynamical aspect of mechanism is central to the canonical version,[2] *viz.* the split-site model. Again let us look at another kind of Menger's contradiction examples (Table 1ii) in terms of the kinetic aspect to reconfirm the equivalency of the split-site to the fundamentalist formulations. But prior to this examination, we first derive proper kinetic equations based on the fundamentalist version from the equilibrium box (Fig. 1d). The Michaelis-Menten equation, $v = k_{cat}[E]_t[S] / (K_m + [S])$ (eq. 5), may be cast into $v = k_{cat}[E]_t$ (eq. 6) under the present conditions of consideration [*i.e.*, ES is lower energy than (E+S)]. On the other hand, Kurz's equation (eq. 4) can be rearranged to give $k_{cat} = (K_S/K_{TS})k_{un}$ (eq. 7). Upon introduction of eq. 7 into eq. 6, we get $v = (K_S/K_{TS})k_{un}[E]_t$ (eq. 8). Now we are ready to re-examine Menger's contrary position in Table 1ii [parts taken from Table IV in Menger's paper[1]] against eq. 8, a kinetic expression of the fundamentalist position. The differential stabilization of the transition state, K_S/K_{TS}, always gives the catalytic acceleration, as is expressed by the equivalent free-energy terms: $\Delta G_{cat} = -TS_B + \Delta G_{ES}$ ($\Delta G_{cat} = -\Delta G_b^{\ddagger} + \Delta G_{ES}$ in fundamentalist denotation). One may notice in Table 1ii that the examples of Menger's contrary position, are actually confirmatory to the fundamentalist position instead of being contradictory, betraying Menger's original attempt.

So far, in our argument, we have been referring to Menger's contradiction examples where ES is of lower energy than (E + S). Next, we move to those, where ES level is not lower than that of (E +S) (Table 1iii). In that case, we have the equations: $v = (k_{un}/K_{TS})[E][S]$ (eq. 9), $\Delta G_{cat} = -\Delta G_b^{\ddagger} = -ES_B$ (eq. 10). Now let us look at Menger's contrary examples in Table 1iii [parts taken from Table III of his original paper[1]]. One may notice that changes in the "effect" column is completely relevant to changes in ES_B (or ES_B-equivalent, *i.e.*, either TS_B or K_{TS}) alone, exactly as stipulated by equation 9 or 10. And the "effect" obtained here based on the fundamentalist position coincides with the "effect" in Table III of Menger's original paper based on his split-site model.[1] Thus, we may now ought to reconfirm our conclusion that there can not be any conflict in evidence between the split-site and the fundamentalist versions, and the contradictions raised by Menger against the fundamentalist position may simply be due to a matter of linguistics. Menger defines the ΔG_{ES} effect on the catalysis (*viz.*, ΔG_{cat}) without respect to the part of the TS_B (*i.e.*, ES_B) effect brought to the catalysis, while ΔG_{cat} is always determined by the combination of the two factors acting in the opposite way, but not by the ΔG_{ES} alone.

Conclusion

That current enzyme theories including even split-site model ultimately reduce to a single formulation, *viz.*, Pauling's transition-state binding, may reflect the fact that they, as well as the fundamentalist formulation, are all based on equilibrium assumptions that are illustrated by equilibrium logics of the looped thermodynamic boxes as shown in Figure 1. The translation of one version to another may correspond to simply a path change in the equilibrium box. Should the equilibrium assumptions be of oversimplification, the fundamentalist as well as other current enzyme theories including the split-site model must be subjected to contradictions revealed by experimentally observed enzyme data. In this respect, not only the contradiction of the fundamentalist position demonstrated by Britt[3] in terms of existing observed data of enzyme catalysis but also recently proposed machine-like mechanism of enzyme catalysis which requires non-equilibrium conditions of enzyme catalysis[12-14] are noticeable.

"Classical transition-state stabilization and an anti-Pauling effect are both capable of inducing rate accelerations."[1] The quote may well express an amended idea that internal molecular dynamics may also play a role in enzymic catalysis.[12] But, under the circumstances where the equilibrium assumption and thereby the rule of conserved interaction (*i.e.*, $ES_B = TS_B$) hold, this can not be the ground of the argument that "the Pauling idea of transition-state

binding is only partly correct",[1] because the equilibrium assumption requires that the net Pauling's transition-state stabilization and the anti-Pauling effect (*viz.*, reactant-state destabiliztion) be equivalent, but not be supplementary to each other, as formulated[2] in evidence: fundamentalist's net transition-state stabilization, *i.e.*, $-\Delta G_b^{\ddagger} + \Delta G_{ES}$ (= ΔG_{cat}), is equivalent to split-site (or canonical) utilized part of intrinsic binding energy, *i.e.*, $-ES_B + \Delta G_{ES}$ (= ΔG_{cat}), while the latter is equivalent to substrate-state destabilization, *i.e.*, ES_R (= ΔG_{cat}), *viz.*, anti-Pauling effect.

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Appendix

Table III. Analysis of the Split-Site Model in Cases Where ES Is of Higher Free Energy Than (E + S) as in Figure 1, Graph I

case	(E + S)	$ES_B^{\ddagger}$	ES_R	ES	TS_B	TS_R	TS_R	ΔG_a	effect[a]
A	0	+3	+7	+10	+3	+20	+23	+23	
B	0	+2	+8	+10	+2	+20	+22	+22	accel
C	0	+4	+6	+10	+4	+20	+24	+24	decel
D	0	+2	+7	+9	+2	+20	+22	+22	accel
E	0	+3	+6	+9	+3	+20	+23	+23	none

[a]Case A is the reference to which other cases are compared. [b]Values could have been made negative with no change in conclusions.

Table IV. Analysis of the Split-Site Model in Cases Where ES Is of Lower Free Energy Than (E + S) as in Figure 1, Graph II

case	(E + S)	ES_B	ES_R	ES	TS_B	TS_R	TS	ΔG_a	effect[a]
A	0	-7	+3	-4	-7	+20	+13	+17	
B	0	-8	+4	-4	-8	+20	+12	+16	accel
C	0	-6	+2	-4	-6	+20	+14	+18	decel
D	0	-8	+3	-5	-8	+20	+12	+17	none
E	0	-7	+4	-3	-7	+20	+13	+16	accel
F	0	-9	+4	-5	-9	+20	+11	+16	accel

[a]Case A is the reference to which other cases are compared. ΔG_a = TS – ES.