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For enzymes, bigger is better

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Abstract

Previously published data are re-examined in order to address two fundamental questions concerning enzyme catalysis: Why are enzymes so big? How is the substrate binding energy realized in the transition state? Relationships are shown that demonstrate (1) an increased enzyme:substrate mass ratio is associated with greater stabilization of the transition state and with increased substrate binding energy, and (2) tighter substrate binding is associated with greater transition state stabilization. It is argued that the conventional view of enzyme catalysis cannot account for these trends while the Shifting Specificity Model can. It is postulated that enzymes have evolved to be massive so that the interaction of the substrate with the active site alters the global conformation of the enzyme in a meaningful way; that is, the interaction alters the active site from an initial substrate-specific geometry to a transition state-specific geometry. It is also postulated that strong enzyme–substrate interactions better facilitate this active site transformation, thus, providing a mechanism for the realization of the substrate binding energy in the transition state of the chemical transformation. © 1997 Elsevier Science B.V.

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1. Introduction

One of the more intriguing features of enzymes that has been recognized from their first physical characterization is their typical great size relative to the substrates upon which they act. The curiosity surrounding this aspect is often expressed in the question: Why are enzymes so big? meaning, What is the function, if any, of the bulk of the enzyme that does not constitute the active site? The currently accepted view of enzyme catalysis first offered by Haldane [1] and later popularized by Pauling [2,3] is that the bulk of the enzyme that does not constitute the active site exists for the purpose of maintaining the active site in a geometry faithful to the transition state structure of the reaction the enzyme has evolved to catalyze. This view implies a rather loose definition for substrate as any small molecular region that can diffuse into the active site and that can also assume the transition state geometry dictated by the active site environment.

Another aspect of enzyme catalysis that has long been unclear is how the substrate binding energy is realized in the stabilization of the transition state rather than in the stabilization of the enzyme/substrate complex. The conventional view that it is catalytically advantageous to bind substrates weakly [4] stems from the idea that the active site must

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remain faithful to transition state binding. Thus, if the active site is to bind the transition state structure strongly, it must necessarily relinquish affinity towards the ground state since it possesses a different electronic arrangement and the active site is not allowed to display multiple complementarities. This requirement of poor substrate affinity for the active site has been criticized [5–7] and data have been offered that demonstrate the opposite—strong substrate binding accelerates the rate-limiting step—applies [6,8].

It has now become clear, at least to this author, that the Haldane model is an inadequate description of enzyme catalysis. The Shifting Specificity Model (SSM) for enzyme catalysis [6] embraces data that the Haldane model is at odds with and is offered as an alternate general explanation for enzyme catalysis. Succinctly expressed, the following are the principles of the SSM.

(1) The enzyme active site, at its normal, physiologic temperature, is configured to bind ground state structures preferentially to transition state structures.

(2) Interaction of the substrate with the active site necessarily results in an alteration of the global structure of the enzyme which is associated with a repositioning of the active site functionalities. Nature has selected for the new active site specificity to be for the transition state.

(3) Since it is the extent of interaction of the substrate with the active site that gives the transition state-complementary conformer, strong active site/substrate interactions are favorable in that they more efficiently modulate the specificity of the active site from substrate-specific to transition state-specific.

To express these ideas even more succinctly, it may be said that enzymes have evolved to bind substrates and enzyme/substrate complexes have evolved to bind transition states. To place the SSM in a historical perspective, the first step (the binding of substrate to a substrate–complementary enzyme) is simply the 'lock and key' model of Fischer [9] for enzyme catalysis offered over a hundred years ago. The second step (the transformation of the enzyme from substrate-specific to transition state-specific) can certainly be thought of as an 'induced fit' [10] but it is one which must involve the *entire* enzyme molecule and one that is from an original, full-fledged substrate complementarity. Thus, the Haldane view that enzymes have evolved to bind transition states is not so incorrect as it is misleading.

From the point of view of the SSM, nature is then required to come up with a molecular machine that must bind two specific structures during the course of reaction—the ground state in recognizing and binding the substrate to initiate the reaction and the transition state in catalyzing the transformation to product. My purpose here is to present previously published kinetic and thermodynamic data that demonstrate: (1) the larger the enzyme relative to substrate, the greater both the transition state stabilization and substrate binding energy, and (2) the stronger the substrate binding to the active site, the greater the transition state stabilization. It is argued that the conventional view of enzyme catalysis cannot be reconciled with these data, taken as a whole, while the SSM can. In so doing, explanations for the great size of enzymes and for a mechanism of realization of the substrate binding energy in the transition state are offered.

2. Results and discussion

The data plotted in Fig. 1 were collected from many sources (Table 1) [11–27]. The criteria for inclusion in this table are: (1) the substrates must be clearly physiologically relevant since it is their catalysis which has presumably driven the evolution of each enzyme's efficiency, and (2) there must exist data on both the binding energy of the substrate to the active site and kinetic data which compares the enzyme-catalyzed rate to the non-catalyzed rate at comparable temperatures and pHs. This second point is necessary since the present study seeks to understand the interplay between ground state binding and transition state stabilization. In addition to these two criteria, it was preferred that the organismal source of the enzyme is identified so that the correct molecular weight of the enzyme could be employed. A computer search of the chemical abstracts revealed only fifteen enzyme/substrate systems for which the above considerations were satisfied (Table 1). The limiting factor to the number of data is the use of physiologically relevant substrates. For example, conspicuously absent from the table are information

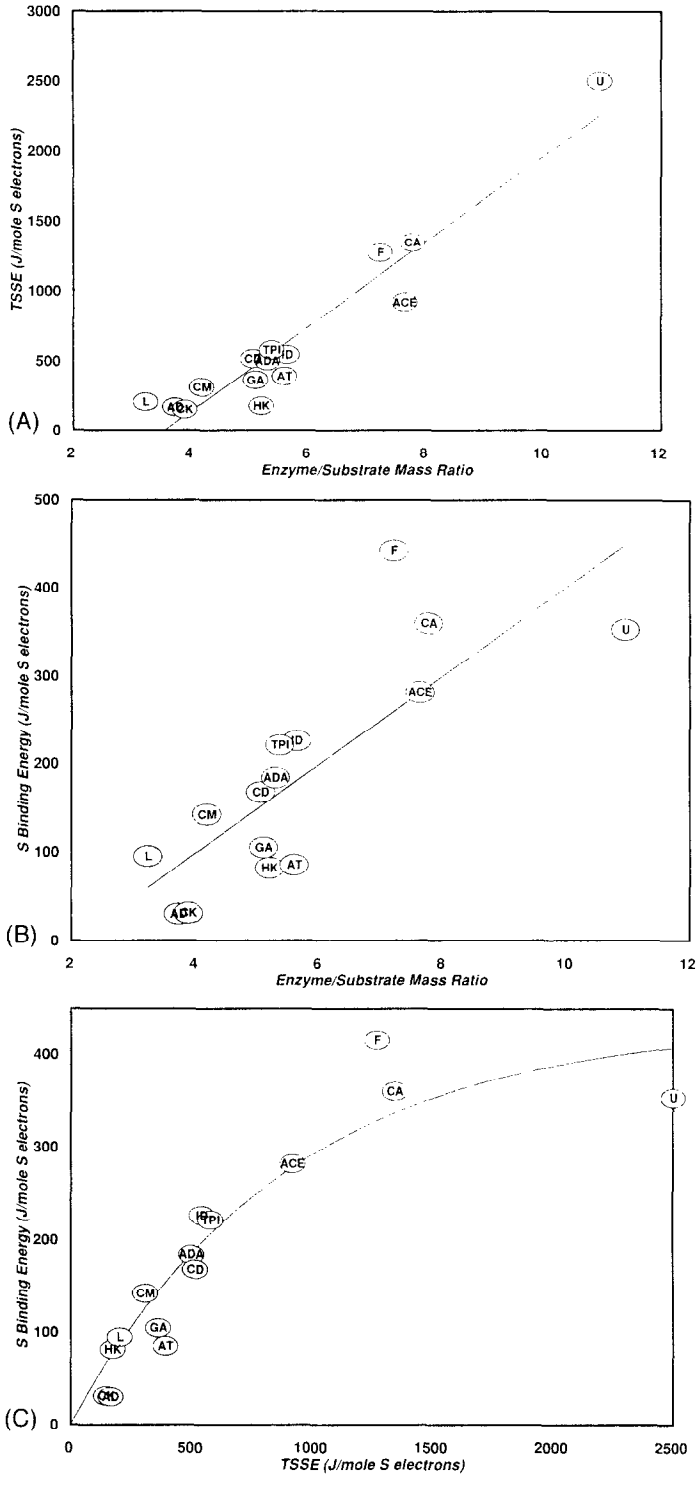

Fig. 1. Relationships between enzyme:substrate mass ratios, normalized transition state stabilization energies (TSSEs), and normalized substrate binding energies for data from Table 1. Mass ratios are the cube root of M_E/M_S. Labels refer to enzyme name abbreviations (see Table 1). (A) Normalized transition state stabilization energy vs. mass ratio (linear trend is shown). (B) Normalized substrate binding energy vs. mass ratio (linear trend is shown). (C) Normalized substrate binding energy vs. normalized transition state stabilization energy (single exponential trend is shown).

Table 1
Physical data of enzymes and substrates used in Fig. 1

Enzyme	Organism[a]	M_E[b]	Substrate	M_S	S_N	K_M[c]	$-\Delta G_b$[d]	$-\Delta G_b^{Norm}$[e]	TSSE[f]	TSSE^{Norm}[g]	$(M_E/M_S)^{1/3}$
Acetylcholinesterase (ACE)[h]	*T. californica*	65,906	Acetylcholine	147.2	82	9×10^{-5}	23	282	75	918	7.63
Glucoamylase (GA)[i]	*A. niger*	48,000	Maltose	360.3	182	4.6×10^{-4}	19	105	66	366	5.10
Lysozyme (L)[j]	Chicken	14,388	$(NAG)_2$	424.4	226	1.75×10^{-4}	21	95	46	204	3.23
Isocitrate dehydrogenase (ID)[k]	(Yeast)	46,562	Isocitrate	258.1	100	1.1×10^{-4}	23	226	54	544	5.64
Cytidine deaminase (CD)[l]	*E. coli*	31,539	Cytidine	243.2	128	1.74×10^{-4}	21	167	66	516	5.05
Chorismate mutase (CM)[m]	*A. aerogens*	16,659	Chorismate	226.2	116	1.3×10^{-3}	16	142	36	310	4.19
Urease (U)[n]	Jack bean	79,667	Urea	60.1	32	1.05×10^{-2}	11	352	80	2500	10.96
Adenosine deaminase (ADA)[o]	Rat	39,991	Adenosine	267.2	140	3.08×10^{-5}	26	184	70	500	5.30
Triosephosphate isomerase (TPI)[p]	Chicken	26,489	G-3-P	170.1	88	3.9×10^{-4}	19	221	51	580	5.37
Carbonic anhydrase (CA)[q]	Cow	28,890	HCO_3^-	61.0	32	9.6×10^{-3}	12	360	43	1344	7.78
Hexokinase (HK)[r]	Cow	103,064	Glucose and ATP	731.5	356	8.0×10^{-6}	29	82	62	174	5.19
Alcohol dehydrogenase (AD)[s]	Yeast	36,879	Ethanol and NAD	709.4	356	1.3×10^{-2}	11	30	59	166	3.73
Creatine kinase (CK)[s]	Rabbit	40,500	Creatine and ATP	682.5	330	1.6×10^{-2}	10	31	49	148	3.90
Fumarase (F)[t]	Pig	50,009	Malate	132.1	70	3.79×10^{-6}	31	442	89	1271	7.22
Glutamine-F6P-aminotransferase (AT)[u]	(*E. coli*)	66,735	Glutamine and F6P	379.2	214	6.5×10^{-4}	18	85	84	391	5.59

[a] For TSSE data where the nature of the organismal source of the enzyme is unknown or unclear, the organism listed in parentheses was used to obtain the M_E, K_M, or TSSE data unless otherwise noted.
[b] Enzyme molecular weight data obtained from Swiss–Prot (http://expasy.hcuge.ch/sprot/sprot-top.html) [12].
[c] K_M values obtained from Barman [13], except where noted and expressed in M.
[d] Binding energies estimated from $-RT(\ln K_M)$ and expressed in kJ/mol.
[e] Calculated as $-\Delta G_b/S_N$ and expressed in J/mol of substrate electrons.
[f] Values obtained from references concerning enzyme entries and expressed in kJ/mol.
[g] Calculated as TSSE/S_N and expressed in J/mol of substrate electrons.
[h] TSSE from Harel et al. [21]; K_M from *E. electricus*.
[i] TSSE and K_M from Olsen et al. [25]; M_E from *C. cerebella* [13].
[j] TSSE from Chipman [15]; M_E from Barman; K_M entry is actual K_S (Barman).
[k] TSSE data determined from unknown species [22]; all data obtained from yeast.
[l] TSSE from Frick et al. [19].
[m] TSSE from Andrews et al. [11].
[n] TSSE data from Creighton [17].
[o] TSSE from Frick et al. [19]; M_E from mouse; K_M from Fabianowski-Wajewska and Greger [18].
[p] TSSE from Hall and Knowles [20]; K_M from calf muscle.
[q] TSSE from Pocker and Meany [26].
[r] TSSE from Koshland [24].
[s] M_E from Barman.
[t] TSSE from Bearne and Wolfenden [14]; malate rather than fumarate considered as substrate based upon value of equilibrium constant.
[u] TSSE from Tempczyk et al. [27]; only stronger binding substrate (glutamine) considered in $-\Delta G_b$ calculation.

on the extensively studied digestive enzymes because almost all of the substrates used in their characterization, because they are not, say, animal muscle proteins, have no direct physiological relevance.

Fig. 1A is a plot of the normalized transition state stabilization energy (TSSE) vs. the cube root of the relative mass of the enzyme to substrate ($(M_E/M_S)^{1/3}$). The TSSEs were obtained from inferences in molecular modeling studies of the active sites or from direct comparisons of enzymatic rates to non-enzymatic rates.

To obtain meaningful comparisons between the TSSEs, a normalization is necessary to account for the fact that larger substrates possess the potential for greater interactions with the active site and, therefore, for greater overall transition state stabilization, than smaller substrates. The TSSE is a measure of the extent to which the individual active site groups are able to interact with regions of the substrate transition state structure via the various noncovalent interactions (H-bonding, van der Waals interactions, dipole interactions, etc.) that govern binding. Of interest here is not the overall magnitude of the binding affinity but the binding efficiency. Consider, for example, the case of a hydrogen bonding interaction. The contribution of this interaction to the total binding free energy is a function of the distance and relative orientation between the hydrogen bonding donor and acceptor orbitals, resulting in formation energies from 0 to -60 or so kJ/mol. An efficiently evolved enzyme orients active site groups to give the most energetically favorable interaction with the transition state.

The method used here is based on the method of Chaires et al. [28] where a comparison was made between the binding free energy of the *lac* repressor (MW = 153,400 Da) and of daunomycin (MW = 524 Da) to DNA. The *lac* repressor binds to its specific DNA site with a free energy of -17.3 kcal/mol [29] while the free energy of daunomycin binding is -8.7 kcal/mol. When the binding energies are normalized to the molecular weights of the ligands it is found that, on a per mass unit basis, the binding free energy of the small antibiotic is over 150 times that of the binding of the repressor, a reflection of the maximal interaction of the intercalator with DNA versus the interaction of only a relatively small portion of the repressor with DNA. The analogy in substrate binding to an enzyme active site would be a situation where a larger substrate is bound overall more tightly to the active site than a smaller substrate, though the individual interactions contributing to the binding would be significantly less efficient than the binding of the smaller ligand due to less optimal spatial orientation of the active site groups.

The method preferred here is to divide each TSSE by the total number of electrons in each substrate since it is this component of the molecular structure that participates in stabilizing interactions and not neutrons which contribute to molecular weights (Table 1). The mass comparison between enzyme and substrate is expressed as the cube root of the enzyme:substrate mass ratio to emphasize the volumetric character of the mass; that is, the cube root emphasizes maximizing contacts between enzyme and substrate requires the extension of the enzyme bulk in three dimensions. Plots employing M_E/M_S instead of $(M_E/M_S)^{1/3}$ and TSSEs normalized to molecular weights rather than the sum of the total substrate electrons do not alter the general trends observed.

The trend observed from Fig. 1A is for the TSSE to increase apparently linearly with $(M_E/M_S)^{1/3}$. The Haldane model, which tends to be unconcerned with any role for the bulk of the enzyme that does not constitute the active site, does not explicitly predict such an observed trend. However, these data, taken alone, support the Haldane model for enzyme catalysis in that the data suggest that large enzyme mass is needed to optimize the positioning of active site functional groups for maximum stabilization of the transition state. The SSM explicitly predicts such a trend for this reason.

Fig. 1B shows a plot of the $\Delta G_{binding}$ of substrates to their respective enzymes estimated from $\Delta G_{binding} = -RT(\ln K_M)$ where K_M is the apparent Michaelis constant. Again, normalization of $\Delta G_{binding}$ by the sum of the substrate electrons is necessary in order to obtain a meaningful comparison of the relative abilities of the enzymes to bind their substrates. For example, carbonic anhydrase, which is often referred to as a perfectly evolved enzyme, is considered to have a weak affinity for its HCO_3^- substrate based on the relatively high value of K_M for the interaction (9.6 mM) but a very high k_{cat} value of 4×10^5 s^{-1}. This is often offered as support for the Haldane

model of enzyme catalysis in that carbonic anhydrase apparently binds its substrate weakly yet turns it over very rapidly. But when it is considered that bicarbonate is a very small substrate, it is found that it is actually bound very strongly to the enzyme active site (Table 1); i.e., the specific enzyme/substrate stabilizing interactions in this case seem to be more efficient than for all but one of the other enzyme/substrate systems considered here (Fig. 1B). The trend observed is an increase in the binding energy with $(M_E/M_S)^{1/3}$. For the same reasons that a large enzyme is better able to bind a transition state, it is also better able to bind the ground state.

The Haldane model of enzyme catalysis makes no predictions concerning the relationship between the size of an enzyme and its ability to bind substrate. It is interesting to consider a corollary of the Haldane model which is widely believed by many enzymologists; namely, that weak-binding substrates are necessarily turned over more efficiently than strong-binding substrates, a concept that stems from the presumed relative inflexibility of the enzyme active site. Given the data in Fig. 1B, this point of view leads one to suspect that enzymes would evolve to be of a size on the order of only ten times that of the substrate since this condition leads to minimal binding affinities. The SSM maintains that the inability of small molecular weight enzyme mimics to both bind a substrate well and catalyze its conversion rapidly, stems from a lack of conformational flexibility of the mimics to bind both ground state and transition state structures efficiently.

The SSM maintains that enzymes have evolved to maximize interactions with substrates. Enzymes are very efficient catalysts. It stands to reason that any evolutionary process that results in a very efficient catalyst exploits all mechanisms that permit, first, development of an efficient physical step of recognizing and binding the appropriate substrate (manifest in a reduced K_M) and then an efficient chemical process of turning over the substrate (manifest in an enhanced TSSE). The most straightforward way begins with an active site initially configured for the accommodation of substrate. This is Fischer's lock-and-key model for enzyme catalysis which has the advantage of explaining the remarkable specificity of enzymes but the distinct shortcoming in that it says little about what happens after the substrate is bound to the active site. A mechanism for the transformation of this catalytically incompetent state to a transition state-binding state is obvious today from our knowledge of protein structure. We know now that substrates interact with the active site via the same relatively weak interactions (H-bonding, dipole–dipole interactions, van der Waals interactions, etc.) that govern interactions between enzyme domains. We also know that enzymes possess many large scale, low-frequency vibrations ($< 50\ cm^{-1}$) involving collective motions of the domains that often involve fluctuations centered around the active site [30–34]. It stands to reason that adding essentially what amounts to another domain to the enzyme (the substrate) via the same interactions that exist between enzyme domains will alter the overall structure of the complex.

The specific substrate/active site interactions will result in a global conformational change in the enzyme that will alter the geometry of the active site; i.e., the enzyme/substrate complex becomes essentially a different molecule—with a different global structure and different active site affinity—than the free enzyme molecule. The substrate cannot induce complementarity to itself as the free enzyme exists initially in a substrate complementarity. The substrate can only perturb the global structure. Nature has selected for a global conformational change that transforms the active site from substrate-specific to transition state-specific with a simultaneous alteration of the substrate from the ground-state structure to the transition state structure. Thus, it is the binding energy of the substrate which produces the transition state-binding conformer of the enzyme, which does not exist at physiologic temperatures in its absence. The SSM predicts that the ability of an enzyme to efficiently catalyze a reaction will increase with the mass ratio of enzyme to substrate. This is explained by the necessity of the enzyme to possess not one but two relevant specificities. A greater mass increases the likelihood that the global conformational rearrangement that must result from the binding of substrate will be a meaningful process that transforms the active site from substrate-specific to transition state-specific.

Inevitably, the issue of transition state analog binding to enzyme active sites arises here. The SSM does not challenge the notion that the active site

ultimately possesses a far greater affinity for the transition state of the reaction than for any ground state species, only that the enzyme is originally configured to bind substrate and it is specific substrate/active site interactions that give the transition state-binding conformer. It is well known that transition state analogs typically bind tightly to enzyme active sites and that this is generally offered as proof that enzyme active sites have evolved to bind transition states but what is less well known is that many bind very slowly, often with second order association constants one-hundredth or less than the association constants that describe the binding of corresponding substrates to enzymes [35]. The rate-limiting step in many cases seems to involve an alteration in the tertiary structure of the enzyme.

The relationships of the normalized TSSEs and of the normalized $\Delta G_{\text{binding}}$s upon $(M_E/M_S)^{1/3}$ implies a relationship between them. Fig. 1C shows a plot of normalized $\Delta G_{\text{binding}}$ vs. normalized TSSE for the enzymes listed in Table 1. A definite exponential dependence is obvious with an increase in normalized substrate binding energy associated with an increase in the normalized TSSE. The Haldane/Pauling model predicts the opposite trend —weak substrate binding gives greater TSSE. The SSM predicts this trend since it is the substrate binding interactions that are necessary to give the transition state-complementary conformer. Furthermore, the stronger the substrate interacts with the active site, the more efficient the global conformational alteration from substrate-specific to transition state-specific although the exponential nature of the data suggest there exists a limit to the TSSE that can result from favorable enzyme/substrate interactions. This can be explained by considering that there exists a point where the increased conformational flexibility that results from increased enzyme mass becomes superfluous in the enzyme's requirement to efficiently bind both the ground state and transition state structures.

3. Conclusion

In conclusion, the data presented here as a whole are better reconciled with the SSM view of enzyme catalysis than with the conventional (Haldane) view. The SSM has the advantage over the conventional view in that it offers an active role for the bulk of the enzyme that does not constitute the active site. Enzymes have evolved to be such massive structures so that the global conformational change that must result from substrate binding becomes a meaningful process; i.e., the conformational change transforms the active site from an initial substrate specificity to a transition state specificity, simultaneously transforming the substrate from the ground state to the transition state. The SSM, thereby, offers a mechanism for the realization of the substrate binding energy in the transition state—under physiologically relevant conditions, the transition state binding conformer can result only from substrate binding. The SSM is intended to be a general explanation for enzyme catalysis though the model collapses to the Haldane model under conditions where the ground state and transition state structures closely resemble.

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