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Substrate Ground State Binding Energy Concentration Is Realized as Transition State Stabilization in Physiological Enzyme Catalysis

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Previously published kinetic data on the interactions of seventeen different enzymes with their physiological substrates are re-examined in order to understand the connection between ground state binding energy and transition state stabilization of the enzyme-catalyzed reactions. When the substrate ground state binding energies are normalized by the substrate molar volumes, binding of the substrate to the enzyme active site may be thought of as an energy concentration interaction; that is, binding of the substrate ground state brings in a certain concentration of energy. When kinetic data of the enzyme/substrate interactions are analyzed from this point of view, the following relationships are discovered: 1) smaller substrates possess more binding energy concentrations than do larger substrates with the effect dropping off exponentially, 2) larger enzymes (relative to substrate size) bind both the ground and transition states more tightly than smaller enzymes, and 3) high substrate ground state binding energy concentration is associated with greater reaction transition state stabilization. It is proposed that these observations are inconsistent with the conventional (Haldane) view of enzyme catalysis and are better reconciled with the shifting specificity model for enzyme catalysis.

Keywords: Enzyme catalysis, Ground state binding energy, Shifting specificity model, Transition state stabilization

Introduction

The purpose of this paper is to demonstrate that the binding of a substrate ground state to an enzyme active site may be thought of as the introduction of a concentration of energy to the enzyme. Substrate ground state binding energies to enzyme active sites are calculated using $\Delta G_b = -RT\ln(K_m)$ where ΔG_b is the binding free energy gained by the association of an enzyme with the substrate ground state and K_m is the Michaelis constant. The free energy reduction observed upon binding reflects the sum of all noncovalent interactions between the substrate and active site, which are more favorable than the interaction between each and the solvent.

However, this analysis fails to consider that larger substrates have an intrinsically higher binding potential than smaller substrates since they possess more atoms, which might also be involved in these interactions. It is possible that a more favorable ΔG_b results from a larger substrate interacting poorly with an enzyme than a smaller substrate, which ostensibly interacts better on an atom-for-atom basis.

A simple way to estimate the potential with respect to the binding of a substrate on an atom by atom basis is to normalize the binding energy versus some aspect of the substrate molecular structure. A good measure of how much a substrate puts into binding can be obtained from $P_{GS} = RT\ln K_m/V_{M(S)}$ where $V_{M(S)}$ is the molar volume of the substrate calculated by summing all atomic volumes, as determined from van der waals radii, and P_{GS} is the binding energy concentration of the interaction (unit analysis of P_{GS} gives J/L or 1000 N/m^2). The binding interaction may therefore be thought of from the substrate perspective as an introduction of an energy concentration or an applied interaction pressure.

As an application of this approach the binding affinities of hexokinase and urease for their physiological substrates were compared. Hexokinase exhibits an apparently much stronger affinity for glucose ($\Delta G_b = -29.1$ kJ/mole) than urease does for urea ($\Delta G_b = -11.3$ kJ/mole) (Barman, 1969). However, when these binding energies are normalized with respect to substrate molar volumes it is found that urea has the higher concentration of binding energy with $P_{GS} = 197$ kJ/L for the urea/urease interaction versus $P_{GS} = 162$ kJ/L for the hexokinase/glucose interaction.

Likewise, the binding energy concentration between an enzyme and a reaction transition state may be calculated using

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Table 1. Enzyme/substrate physical data

Enzyme	Enzyme empirical formula	$V_{E(M)}$ (L)	Substrate	$V_{S(M)}$ (L)	P_{GS} (kJ/L)	P_{TS} (kJ/L)
lysozyme (LYS)[a]	$C_{630}H_{961}N_{177}O_{190}S_{13}$	15.5	$(NAG)_2$	0.436	49.1	105
acetylcholinesterase (ACE)[b]	$C_{3269}H_{4954}N_{850}O_{978}S_{30}$	79.1	acetylcholine	0.132	175	569
urease (URE)[c]	$C_{4000}H_{6404}N_{1112}O_{1120}S_{36}$	99.2	urea	0.0574	197	1390
glucoamylase (GCA)[d]	$C_{3013}H_{4570}N_{782}O_{1009}S_{13}$	73.7	maltose	0.342	55.6	193
isocitrate dehydrogenase (ICD)[e]	$C_{2145}H_{3435}N_{575}O_{627}S_{17}$	52.7	isocitrate	0.199	115	271
cytidine deaminase (CD)[f]	$C_{1406}H_{2196}N_{382}O_{421}S_{11}$	34.4	cytidine	0.240	89.0	274
adenosine deaminase (ADA)[g]	$C_{1821}H_{2834}N_{484}O_{552}S_{14}$	44.5	adenosine	0.263	97.8	266
triosephosphate isomerase (TPI)[h]	$C_{1163}H_{1877}N_{327}O_{351}S_6$	28.8	G3P	0.127	153	402
carbonic anhydrase (CA)[i]	$C_{1285}H_{1961}N_{353}O_{393}S_3$	31.3	bicarbonate	0.0433	266	993
creatine kinase (CK)[j]	$C_{1882}H_{2941}N_{527}O_{573}S_{17}$	46.4	creatine, ATP	0.477	21.4	103
fumarase (F)[k]	$C_{2209}H_{3540}N_{616}O_{665}S_{20}$	54.7	malate	0.111	278	800.
Gln-F6P-aminotransferase (GFA)[l]	$C_{2953}H_{4737}N_{831}O_{896}S_{17}$	73.2	glutamine	0.151	121	557
alcohol dehydrogenase (ALD)[m]	$C_{1636}H_{2574}N_{442}O_{488}S_{14}$	40.1	ethanol	0.0597	177	987
arginine decarboxylase (ARD)[n]	$C_{3758}H_{5734}N_{1010}O_{1134}S_{38}$	91.6	arginine	0.191	93.4	593
hexokinase (HK)[o]	$C_{4522}H_{7274}N_{1272}O_{1369}S_{53}$	113	glucose	0.180	162	345
ketosteroid isomerase (KI)[p]	$C_{1178}H_{1931}N_{335}O_{358}S_{13}$	29.5	5-androstene-3,17-dione	0.366	57.1	180
orotidine-5-monophosphate	$C_{1296}H_{2078}N_{356}O_{392}S_{10}$	32.1	orotidine-5-monophosphate	0.284	124	346

K_m valules (M) from Barman except where noted (1969)
[a]TSSE (transition state stabilization energy) from Chipman, 1971; K_m is actual K_s from Barman (1969); enzyme from dog milk
[b]TSSE from Harel *et al.* (1996); enzyme from cotton aphid;
[c]TSSE from Creighton; enzyme from jack bean
[d]TSSE and K_m from Olsen *et al.* (1992); enzyme from human
[e]TSSE from Hurley and Remington, (1992); all other data from bakers yeast
[f]TSSE from Frick, 1987; enzyme from *E. coli*
[g]TSSE from Frick *et al.* (1987); K_m from Fabianowski-Wajewska and Greger (1992); enzyme from rat
[h]TSSE from Hall and Knowles (1975); enzyme from chicken
[i]TSSE from Pocker and Meany (1967); enzyme from human
[j]TSSE from Creighton;enzyme from rabbit
[k]TSSE from Bearne and Wolfenden (1995); enzyme from pig
[l]TSSE from Tempczyk *et al.* (1992); enzyme from *E. coli*
[m]TSSE from Creighton; enzyme from yeast
[n]TSSE from Wolfenden (2000); enzyme from *E. coli*
[o]TSSE from Koshland (1956); enzyme from cow
[p]TSSE and K_m from Radzicka and Wolfenden (1995); enzyme from *Pseudomonas testosteroni*
[q]TSSE and K_m from Radzicka and Wolfenden (1995); enzyme from bakers yeast

$P_{TS} = RT\ln(k_{cat}/k_{uncat})/V_{M(S)}$ where k_{cat} and k_{uncat} are the rate constants for the conversion of substrate to product for the catalyzed and uncatalyzed reactions, respectively.

When the interactions of reaction ground states and transition states with enzymes for physiologically relevant reactions are considered in this manner several interesting features emerge. First, it is found that P_{GS} drops off exponentially with substrate volume, demonstrating that smaller substrates interact more strongly with enzymes on an atom-for-atom basis. Second, it is observed that the larger an enzyme is relative to its substrate the greater are the interactions of the reaction ground and transition states with the enzyme. Third, it is found that a strong ground state interaction with the enzyme is directly correlated with the stabilization of the reaction transition state.

Finally, it is argued that these results are inconsistent with the conventional (Haldane) view of enzyme catalysis and more consistent with a view that invokes the active participation of the entire enzyme molecule in the catalytic event and that recognizes that a strong ground state interaction with the enzyme favors catalysis (Britt, 1993; Britt, 2004). The ideas presented here expand on those presented earlier by the author on this topic (Britt, 1997).

Materials and Methods

We consider only interactions between enzymes and their physiological substrates since this has driven the evolution of each enzyme's structure and function. Presented are previously published data from 17 such systems (Table 1). Enzyme empirical formulas were obtained using the ProtParam tool at the ExPASy Molecular

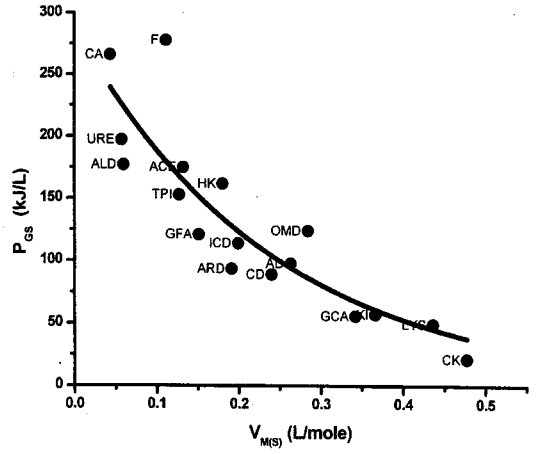

Fig. 1. Plot of P_{GS} vs subtrate molar volume $V_{M(S)}$. The data trend is described as $P_{GS} = 288e^{-4V}$ where V is the substrate molar volume ($R^2 = 0.77$).

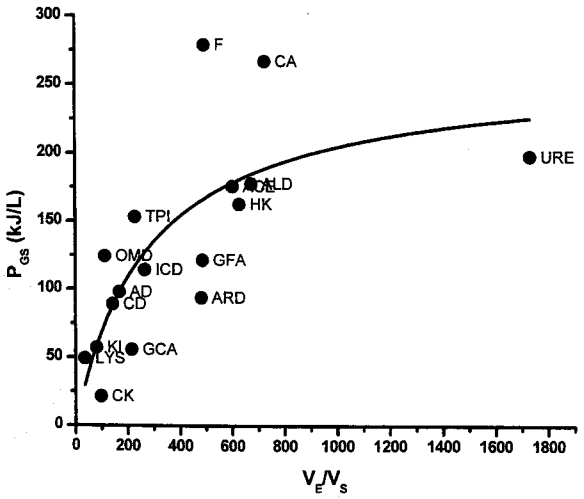

Fig. 2. Plot of P_{GS} vs. V_E/V_S. The data trend is described by $P_{GS} = 261(V_E/V_S)/[281 + (V_E/V_S)]$ ($R^2 = 0.57$).

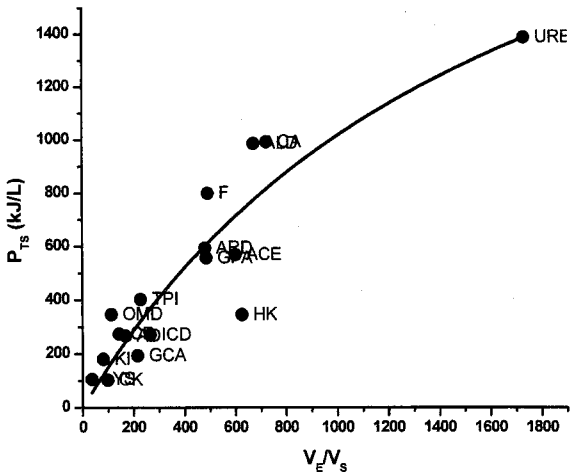

Fig. 3. Plot of P_{TS} vs. V_E/V_S. The data trend is described by $P_{TS} = 2795(V_E/V_S)/[1749 + (V_E/V_S)]$ ($R^2 = 0.83$).

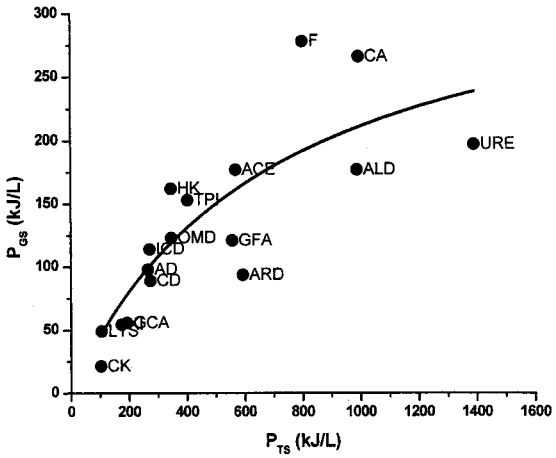

Fig. 4. Plot of P_{GS} vs. P_{TS}. The data are described by $P_{GS} = 357P_{TS}/(691 + P_{TS})$ ($R^2 = 0.71$).

Biology Server website. Enzyme and substrate volumes were calculated using the following van der Waals radii in pm: C (170), H (120), N (155), O (152), S (180), P (180). Volumes presented are the sums of the elemental van der Waals volumes for each enzyme or substrate. The trends presented in Figs. 1-4 were obtained using the curve fitting software provided in Microcal Origin.

Results

Figure 1 shows how the substrate ground state binding concentration or the applied interaction pressure P_{GS} falls exponentially upon increasing substrate volume. Constraining the fit of the exponential to go to zero binding energy concentration for infinite volume yields a trend of $P_{GS} = 288e^{-4V}$ where V in this equation is the substrate molar volume. These data illustrate that smaller substrates bring more binding energy concentrations to the enzyme than larger substrates. The trend in Fig. 1 suggests that the maximal affinity a substrate can bring to the active site is approximately 300 kJ/mole or 3×10^5 N/m^2 or 3 atm.

It is interesting to consider the effect on ground state binding of the relative size of an enzyme to its substrate. Figure 2 shows how substrate ground state binding energy concentration increases hyperbolically with increasing V_E/V_S. The most straightforward explanation for the observed trend is that larger enzymes are better able to tweak active site groups for optimal interaction with substrates than are smaller enzymes.

As the interaction of the reaction ground state with the enzyme may be considered a binding energy concentration effect, so can the interaction of the transition state. Again, we ask what relationship exists between the relative size of an enzyme and its ability to interact with and thus stabilize the transition state. Figure 3 shows how P_{TS} increases hyperbolically

with V_E/V_S. It appears that larger enzymes (relative to their substrates) are better able to bind the reaction transition state. Again, the most straightforward explanation for this effect is that the increased enzyme mass allows for an optimization of contacts with the transition state. It is important to note that the conventional explanation of enzyme catalysis has no active role for the bulk of the enzyme not constituting the active site, and therefore, allows no predictions based on relative sizes and the abilities of enzymes to bind ground and transition states.

Finally, Fig. 4 shows how strong ground state binding is correlated with greater transition state stabilization.

Discussion

The data presented here are not easily interpreted in terms of the Haldane model for enzyme catalysis, which allocates no active role for the enzyme mass not constituting the active site, and which maintains that poor ground state binding is associated with enhanced transition state stabilization (Fersht, 2000). Instead, the data support an alternate view of enzyme catalysis (Britt, 1993; Britt, 1997; Britt, 2004), which requires the participation of the entire enzyme molecule in the catalytic event, and which maintains that strong ground state interactions favor catalysis. This view advocates that the physiological enzyme exists in a relatively high energy conformation and has an active site geometry that favors interaction with the reaction ground state over the transition state. Interaction of the ground state with the enzyme via the same noncovalent interactions that govern protein tertiary structure necessarily induces a global conformational change, which transforms the active site from a ground state complementarity to a transition state complementarity, and in the process transforms the substrate from the ground state to the transition state. The reaction is facilitated by the transient adoption of a more stable enzyme global conformation in the transition state; that is, though the total energy of the system must be, by definition, at a maximum, the enzyme-localized energy achieves an energy minimum at this point. The substrate ground state binding energy is thus used to trigger this conformational change. The greater the interaction of the ground state with the enzyme the more efficient the enzyme conformational relaxation and the more efficient is the catalysis. This model therefore correlates strong substrate ground state interactions with the enzyme with enhanced catalysis.

This model also suggests an explanation for the correlation between catalytic efficiency and increased enzyme mass. First, a larger enzyme mass increases the probability that the enzyme possesses two catalytically relevant conformations because there is greater potential for the tweaking of active site functionalities. Secondly, the mechanism for stabilization of one folded protein form relative to another involves the optimization of nonpolar side chain contacts. Obviously, a larger enzyme possesses an intrinsically greater potential for energy minimization.

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