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Understanding Enzyme Structure and Function in Terms of the Shifting Specificity Model

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The purpose of this paper is to suggest that the prominence of Haldane's explanation for enzyme catalysis significantly hinders investigations in understanding enzyme structure and function. This occurs despite the existence of much evidence that the Haldane model cannot embrace. Some of the evidence, in fact, disproves the model. A brief history of the explanation of enzyme catalysis is presented. The currently accepted view of enzyme catalysis -- the Haldane model -- is examined in terms of its strengths and weaknesses. An alternate model for general enzyme catalysis (the Shifting Specificity model) is reintroduced and an assessment of why it may be superior to the Haldane model is presented. Finally, it is proposed that a re-examination of many current aspects in enzyme structure and function (specifically, protein folding, x-ray and NMR structure analyses, enzyme stability curves, enzyme mimics, catalytic antibodies, and the loose packing of enzyme folded forms) in terms of the new model may offer crucial insights.

Keywords: Enzyme catalysis, Haldane, Shifting specificity model

Enzyme Catalysis

History of enzyme catalysis models By any criteria, particularly industrial ones, enzymes are remarkable catalysts. They tremendously accelerate reactions under mild temperature and pressure conditions to the extent that many reactions essentially do not occur in their absence. They also display an amazing specificity for not only the nature of the reaction but for which particular molecular geometry will be permitted to undergo the reaction.

Understanding the fundamental processes which allow these macromolecules to function so efficiently has long intrigued scientists. In 1894, Emil Fischer offered the first popular model to explain enzyme function (Fischer, 1894) (Fig. 1). This model came from his fascination with certain glycolytic enzymes and their abilities to distinguish between different sugar stereoisomers. In his textbook 'lock-and-key' analogy, the enzyme is the 'lock' that is capable of accepting only one or a very few 'keys'. While suggesting that the enzyme active site is complementary to the reaction ground state, thereby offering an explanation for the remarkable specificity of enzymes, this model is grossly oversimplified in that it says nothing about what happens after the enzyme binds to the substrate. Questions concerning the means by which the bound substrate is transformed to the transition state are ignored. However, we should remember that the prevailing view at the time was that enzymes were essentially carbohydrate in nature.

This view of enzyme catalysis predominated until 1930 when J.B.S. Haldane turned his thoughts to the subject (Haldane, 1930). Though the proteinaceous nature of enzymes had still not been established, it was acknowledged that they were massive molecules and that "chemistry" must be confined to a small region, coined the 'active site'. Ground state specificity is fruitless, Haldane thought, recognizing what conventional thinking in enzyme catalysis today still regards as the crippling liability of Fischer's model. In his reasoning, it made more sense for the active site to exist, not in a ground state complementarity, but in a complementarity to the reaction transition state as its stabilization resulted in rate enhancement. He suggests no active role for the bulk of the enzyme that does not constitute the active site, and it is generally believed that the large enzyme mass is necessary for the optimal orientation of the active site functionalities for binding the transition state. As has been pointed out by Menger (1992), essentially all explanations for enzyme catalysis, regardless of their nuances, embrace this central idea which is the foundation for the currently-accepted picture of enzyme catalysis. The only significant modification to

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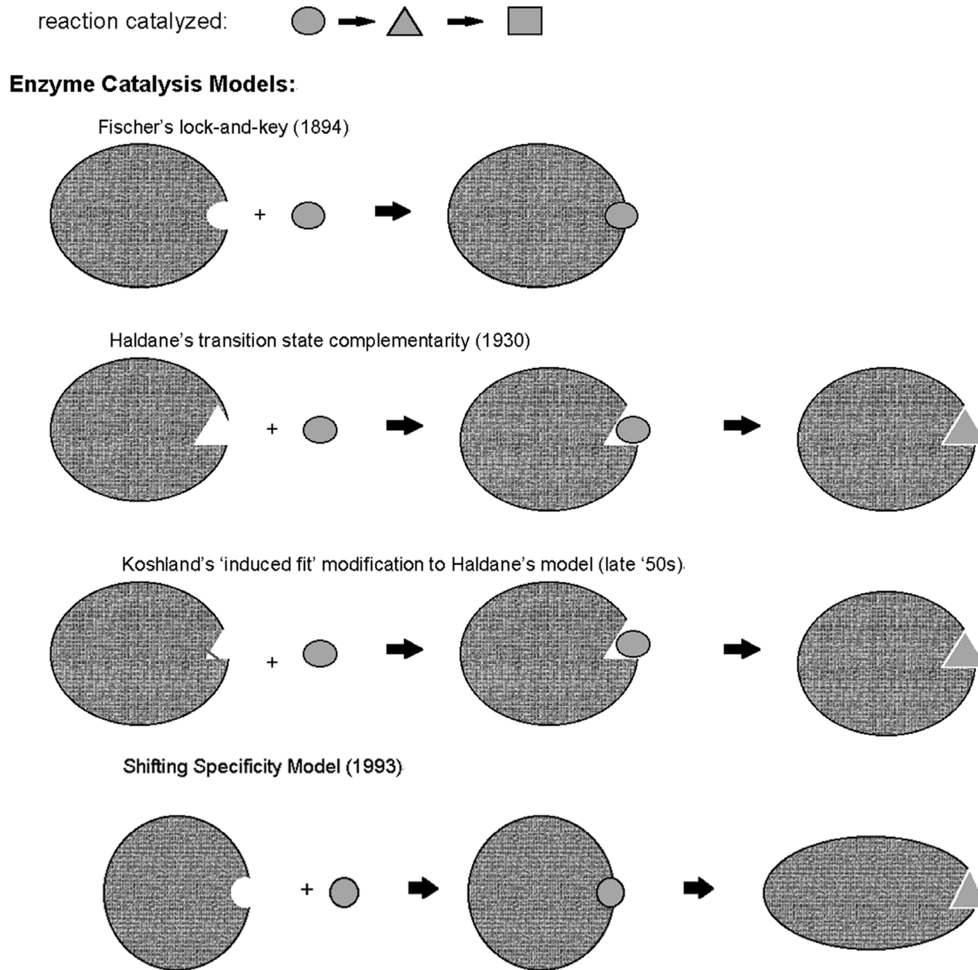

Fig. 1. Schematic Representations of Models for Enzyme Catalysis. The schematic considers the conversion of a circular substrate to a square product via the formation of a triangular transition state. Fischer's model considered an enzyme as a lock capable of accommodating only one or a few keys (substrates). Haldane realized that the source of the catalytic power must ultimately arise from a marked affinity of the enzyme active site for the reaction transition state. Koshland suggested that the enzyme should be so only after interaction of the ground state with the enzyme. The SSM calls for a facilitation of the reaction by a shift of specificity of the active site from a ground state complementarity to a transition state complementarity accompanied by a global conformational relaxation at the reaction transition state. Note that the first step of the SSM is simply Fischer's model.

augment this picture is the 'induced fit' idea proposed by Koshland (Koshland, 1960) (Fig. 1). In the Haldane/Koshland model, the enzyme catalytic groups are not quite optimized for stabilization of the transition state until the ground state binds. This idea is a substantial improvement in that it recognizes that the chemical force fields of the enzyme and substrate must influence one another in the binding process. Thus, in the Haldane/Koshland view, the substrate induces the transition state complementarity via local rearrangements of the active site functionalities, is subjected to this transition state stabilizing force, and converted to the product. This modification is deficient, however, in that it ignores the great potential for reaction facilitation via global enzyme conformational changes, unreasonably restricting the adjustment of the enzyme conformation to the enzyme active site.

Deficiencies in the currently-accepted view of enzyme catalysis The Haldane model therefore leaves us with a picture of enzymes as highly efficient yet strangely static and passive catalysts. In the Haldane model, the bulk of the enzyme exists solely as a scaffold for the active site functionalities to exist, with Koshland's caveat, in a faithful transition state complementarity. Due to the weak nature of the interdomain interactions, it seems quite likely that enzymes may alter their global conformations upon some stimulus, such as substrate binding; however, generally speaking, this is not allowed. Since the enzyme must choose, as it were, between a transition state specificity and a ground state specificity, and since only the former permits catalysis, it follows from conventional thinking that it is catalytically advantageous to bind the ground state as weakly as possible. Thus, the enzyme is an apparently overly massive,

conformationally inert structure and the substrate is any collection of atoms that can wander into the active site and also assume the appropriate transition state geometry.

The primary strength of the Haldane model is that it recognizes the essential quality an enzyme must possess; namely, an ultimate markedly enhanced affinity for the reaction transition state over any other phase of the reaction and is, in this sense, far superior to the Fischer conjecture. And while this view is supported by much data (such as the very tight binding of transition state analogues), there persists several disturbing peculiarities and inconsistencies.

One of the strangest, which was previously discussed (Menger, 1992), is that the enzyme must necessarily minimize its interaction with the substrate since it is not a transition state structure. This is the result of the view of the enzyme as a static, conformationally inflexible entity. In this view, the enzyme may bind the ground state or it may bind the transition state, but it may not bind both, though this scenario would provide for the most efficient catalysis. The enzyme must always maintain its presumed transition state affinity and is not allowed to display multiple conformations during the course of reaction. A weak ground state affinity therefore implies a strong transition state affinity. This has led to all sorts of rationalizations for poor ground state binding.

Arguments for this position rely primarily on considerations of the relative values of the substrate concentration [S] to the enzyme/substrate dissociation constant K_m. The logic seems to be that strong ground state binding cannot help and, in fact, can actually impede catalysis, so the best thing to do is to bind the substrate weakly (Menger, 1992). This position has been previously criticized (Menger, 1992; Britt, 1993; Britt, 1997; Castro and Britt, 2001; Strohmeyer *et al*, 2002). There are in fact sound reasons why strong ground state binding may facilitate catalysis. In fact, this is actually the case, as revealed by plots of K_s vs. k_{cat} for many enzymes with related substrates (Britt, 1993). This is also true when the comparison is made between enzymes with their physiological substrates (Fig. 2) (Britt, 1997). The trend in Fig. 2 clearly indicates that enzymes that are better able to bind the ground state are also better able to stabilize the transition state. This is very strong evidence against the Haldane model, which predicts the opposite trend.

A question that has generated much discussion (almost since the macromolecular nature of enzymes was discovered) concerns the role, if any, that the bulk of the enzyme that does not constitute the active site plays during catalysis. In the Haldane view, there is no active role. Its only role is a passive one: a framework for the optimal positioning of the active site groups for transition state complementarity. The assumption that the bulk of the enzyme is largely superfluous to function spawned the research area of enzyme mimics - the design of small molecular weight molecules intended to achieve the catalytic efficiency of enzymes. Enzyme mimics have never been able to approach the catalytic efficiency of enzymes, however. This suggests that the enzyme bulk has some important, active function.

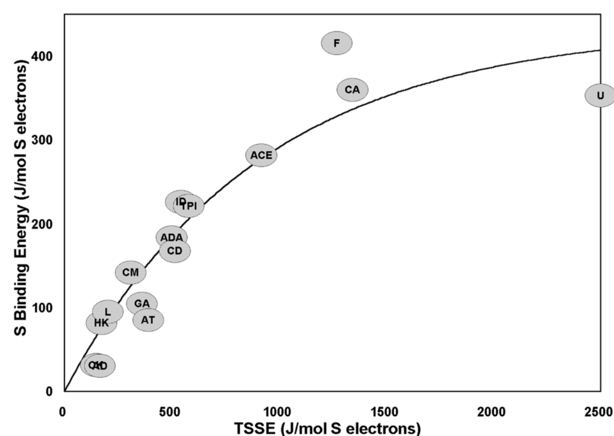

Fig. 2. A plot of the normalized ground state binding energy versus the normalized transition state stabilization energy (TSSE) for enzymes with their physiological substrates. Binding energies are normalized to the sum of the electrons comprising the substrate to compensate for the fact that larger substrates possess an inherently greater capacity for binding interactions (i.e., a larger substrate with fewer enzyme contacts may display a greater binding energy than a smaller substrate that interacts more extensively with the enzyme). Normalizing the binding energies in this way provides a better measure of the extent of interaction between substrate and enzyme (Britt, 1997, for a further explanation and for an explanation of the symbols which are enzyme abbreviations).

The Haldane model also does not offer a satisfactory explanation for the realization of the ground state binding energy in the reaction transition state. Enzymes interact favorably with their substrates (i.e., substrate binding is associated with a favorable free energy change). Yet this is in fact an unstable situation as the substrate is quickly converted to the transition state. Much of the confusion is due to the misconception that strong substrate binding inhibits catalysis.

There also exists much data that simply cannot be reconciled with the Haldane model. For example, the binding of transition state analogues to enzyme active sites is typically very strong. This is often considered proof for the validity of the Haldane model. However, transition state analogues quite often bind very slowly to active sites. Substrates and ground state analogues typically bind much more rapidly, often in the diffusion limit, suggesting that enzymes are initially optimized for interaction with the ground state rather than the transition state.

Given these insufficiencies, it has become clear that the Haldane model is fundamentally lacking as a general description of enzyme catalysis.

The shifting specificity model for enzyme catalysis A new model is required. The model proposed (Britt, 1993; Britt, 1997; Castro and Britt, 2001; Anderson and Britt, 2002; Strohmeyer *et al.*, 2002) is called the Shifting Specificity

Model (SSM) to emphasize the key feature of the model: the enzyme global conformation shifts from a ground state specificity to a transition state specificity to facilitate the reaction. Considering enzymes in this way explains much of the data that is at odds with the Haldane model and may offer insights into current issues in enzyme structure and function.

The basic aspects of SSM are: The native enzyme (i.e., the folded form at its physiological pressure, temperature, and pH) exists in a conformation that possesses an active site that is complementary to the ground state of the catalyzed reaction. There exists no other orientation of atoms, including the transition state or transition state analogue, that has a higher affinity for the active site in this conformation. This provides for a specific, nonrandom means for the initiation of the reaction. This form of the enzyme exists only in the free state under its normal, physiological conditions and is designated E_{GS}.

Binding of the ground state necessarily induces a global conformational change in the enzyme. This occurs because the substrate interacts with the active site via the same weak, noncovalent forces that govern enzyme tertiary structure. Introducing mass (ground state) into, typically, a cleft in the enzyme must result in a global conformational change. If this seems too far-fetched, one need only consider the conformational changes that occur in hemoglobin upon binding its small ligand. This conformational change alters the geometry of the active site functionalities thereby altering their specificity. The evolution of enzyme catalysis has selected for a conformational transition which shifted the active site specificity from the ground state to the transition state. The transition state binding form of the enzyme is designated E_{TS} and can only fleetingly exist under normal, physiological conditions at the reaction transition state, or to a fair approximation when bound to a transition state analogue. The conversion of the substrate from the ground state to the transition state structure is coincidental with the conversion of the enzyme from E_{GS} to E_{TS}.

The conformational change that transforms the enzyme from E_{GS} to E_{TS} is one to lower conformational energy. An enzyme-catalyzed reaction is therefore greatly facilitated by attaining an enzyme-localized conformational energy minimum at the reaction transition state.

Since it is the interaction of the ground state with the enzyme that produces the transition state binding conformer, strong enzyme/ground state interactions better facilitate this conformational change and therefore directly accelerate catalysis.

Placing this model in a historical perspective, the first step in SSM catalysis is simply Fischer's lock and key analogy (Fig. 1). We now know that this does not necessarily imply a dead end for the reaction, since enzymes possess the potential for adopting different conformations. Binding of the ground state then results in an active site geometry which prefers the binding of the transition state, as Haldane requires. There is an "induced fit" of sorts, but it is one from a full-fledged ground state complementarity and one that actively involves the entire enzyme molecule. It is clear that Koshland's induced-fit concept implicated quite localized changes and did not involve a massive conformational change. Furthermore, the conformational change is one that leads to a significantly lower enzyme-localized energy at the transition state. We recently demonstrated that the binding of the transition state analogue coformycin to bovine adenosine deaminase under physiological conditions is associated with a stabilizing conformational change in the enzyme (Fig. 3).

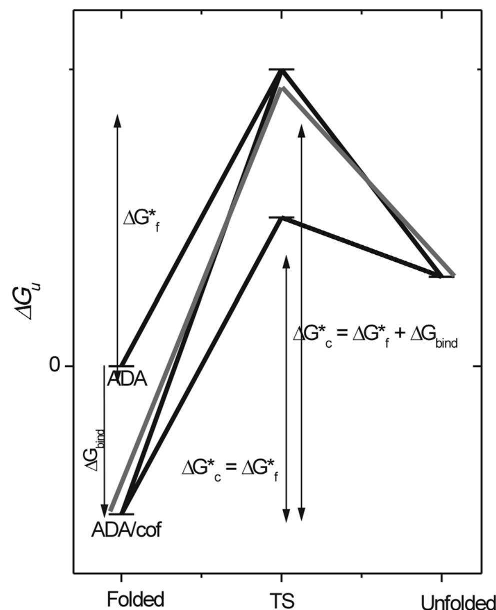

Fig. 3. Rationale for determination of P_B^{global}, the fraction of coformycin binding energy which results from a stabilizing, global enzyme conformational change. Shown are the free energies of unfolding (ΔG_u) for free ADA and the ADA/coformycin complex. Coformycin binding (ΔG_{bind}) stabilizes the folded form of the enzyme, regardless of whether a stabilizing enzyme conformational change results. One limiting case of coformycin binding energy partitioning occurs when the activation free energy for unfolding of the free enzyme (ΔG^*_f) equals that of the complex (ΔG^*_c). In this case, the interaction energy must be purely local (confined to the active site) and $P_B^{global} = 0$. The other limiting case occurs when the unfolding activation free energy of the complex equals the sum of the unfolding activation energy of the free enzyme and the coformycin binding energy. In this case, binding must result exclusively from a stabilizing global enzyme conformational change and $P_B^{global} = 1$. If binding is partitioned between local and global interactions the most likely scenario -- then $\Delta G^*_f < \Delta G^*_c < \Delta G^*_f + \Delta G_{bind}$ and, for a generalized case, $P_B^{global} = -(\Delta G^*_c - \Delta G^*_f)/\Delta G_{bind}$ and can take a value between 0 and 1. The grey line shows the results of our measurements and indicates that ~90% of the binding energy of coformycin to ADA is attributable to a global conformational relaxation of the enzyme (See Strohmeyer *et al*., for a more detailed explanation).

Enzyme catalysis mechanisms that invoke a relatively high

energy free enzyme that unleashes its stored-up energy at the transition state to facilitate catalysis have traditionally been dismissed on the grounds that such an event is thermodynamically improbable as it implies an usually unknown process by which the enzyme is 'reloaded' for the next round of catalysis (Jencks, 1975). The faulty assumption here is that the free enzyme cannot innately possess a relatively high conformational energy (i.e., the free enzyme must exist at its overall conformational energy minimum). SSM recognizes that the enzyme-localized energy always tends towards its energy minimum given its specific bound state. Though the enzyme-localized energy of the free enzyme is indeed greater than that of the transition state-bound enzyme, it is at the most stable conformation that is accessible. It is impossible for E_{GS} to transform to E_{TS} under physiological conditions without the presence of a transition state structure -- either the reaction transition state or a transition state analogue -- at the active site. E_{TS} results from the ground state binding energy and has only a transient lifetime since its existence is coincidental with the formation of the reaction transition state. Though in SSM the E_{TS} is of a lower conformational energy than E_{GS}, the total reaction energy is maximal. The most stable enzyme conformation therefore exists only at the reaction energy maximum.

To make a more succinct comparison, the Haldane model maintains that enzymes have evolved to bind transition states, but SSM maintains that enzymes have evolved to bind ground states and that enzyme/ground state complexes have evolved to bind transition states (Fig. 1). Therefore, from the SSM point-of-view, the Haldane model is not so much incorrect as it is oversimplified and misleading. SSM converges to the Haldane model under conditions where the ground state and transition state geometries are identical or nearly so, as in metal redox reactions.

A superior model to the Haldane model should meet the following minimum criteria as SSM does.

1) It should encompass those features of the Haldane model which have either been proven or for which no contrary evidence exists. The most important of these is the requirement for transition state stabilization by a complementary active site. There can be no doubt that the crucial aspect of enzyme catalysis is the ultimate preference of the enzyme for the transition state rather than for any other reaction intermediate.

2) It should require that enzymes be large molecules and implicate the bulk of the enzyme directly in the catalytic event. It is probably safe to assume that if there was a more economical way to achieve biological catalysis than investing the energy and material in forming large enzyme structures, then these smaller molecules would exist. The fact that all of the enzyme catalysts are large molecules implies a catalytic function for the enzyme mass that does not constitute the active site. SSM maintains that enzymes are necessarily very large structures for two reasons. First, since an enzyme is required to show preferential binding to not one (as the

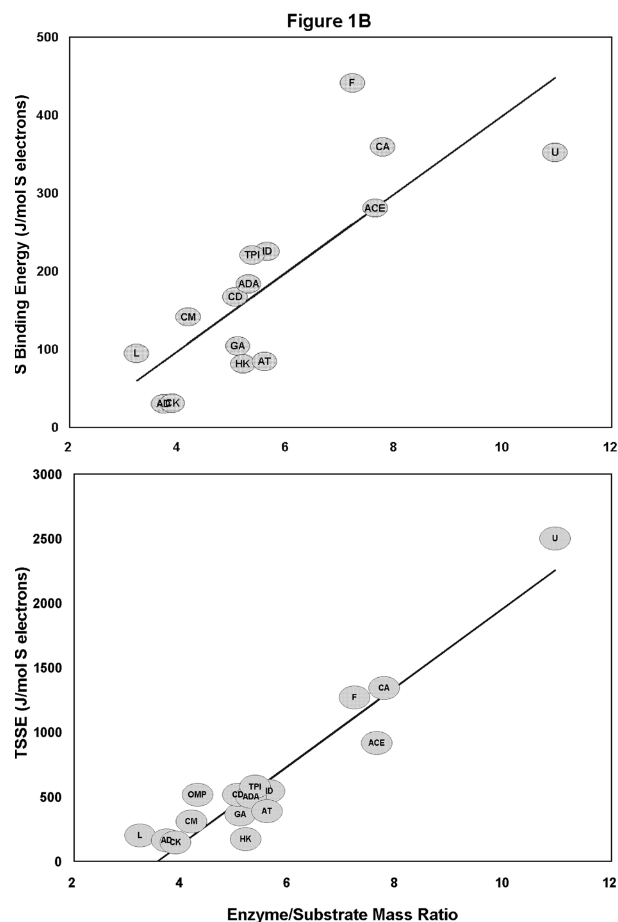

Fig. 4. Plots of substrate binding energies and transition state stabilization energies versus the enzyme/substrate mass ratio of several enzymes with their physiological substrates. Each energy is normalized to the sum of the electrons on the substrate. Substrate binding energies are calculated from $\Delta G = -RT\ln(K_m)$ where K_m is the Michaelis constant. TSSEs are calculated from comparison of the nonenzymatic and enzymatic rates. The enzyme/substrate mass ratio (x-axis) is the cube root of the molecular weight of the enzyme divided by the molecular weight of the substrate (see Britt, 1997, for further details).

Haldane model requires) but two structures during catalysis, a large enzyme not only optimizes the positioning of the active site functionalities for a particular ligand geometry, but also increases the likelihood that any conformational change that occurs upon ligand binding will be a catalytically meaningful one (i.e., will also display a catalytically relevant specificity -- in the case of SSM, from a complementarity to the reaction ground state to the transition state). Secondly, an increased enzyme size allows for greater rate acceleration via the adoption of a more stable conformational energy minimum at the transition state. Lower conformational energies in enzymes are primarily achieved through the optimization of nonpolar amino acid side chain contacts. Since rate acceleration is proportional to the decrease in enzyme

conformational energy in going from E_{GS} to E_{TS}, a larger enzyme (relative to its physiological substrate) should be better able to facilitate the reaction as it possesses more nonpolar surface area. Larger enzymes are better catalysts than smaller enzymes in that they bind their substrates more strongly and achieve better transition state stabilizations (Britt, 1997) (Fig. 4).

3) It should provide a concrete means by which the ground state binding energy can lead to rate enhancement. In SSM, the free enzyme is not in a transition state complementarity. Binding of the substrate provides the activation energy to achieve E_{TS} under normal, physiological conditions. Thus, it is the ground state binding energy that directly results in E_{TS} and the reaction transition state.

4) It should recognize that strong ground-state interactions facilitate catalysis. Since the ability of the substrate to facilitate this conformational change is proportional to the extent of the ground state/active site contacts, SSM predicts that stronger ground state binding is associated with more rapid substrate turnover. This facet of the model has been demonstrated (Fig. 4) and cannot be reconciled with the Haldane model.

In addition, a superior explanation for enzyme catalysis should call for a re-evaluation of some basic assumptions concerning investigations into enzyme structure and function and related areas, as well as provide alternate explanations and suggest more fruitful avenues of investigation.

Implications of SSM in areas of enzyme structure and function, predictions

Efforts to predict the functional (physiologic) folded form of an enzyme by conformational energy minimization calculations are invalid In the Haldane view, the functional enzyme is at a conformational energy minimum. In the SSM view, the conformational energy minimum is obtained under physiological conditions only at the transition state of the reaction or when bound to a transition state analogue. Current algorithms that produce the most stable enzyme structure, therefore, tend to give E_{TS} and not the intended result, E_{GS}, the free, physiological enzyme structure. The most stable enzyme conformation is relatively inert as it lacks the conformational potential to stabilize the reaction transition state via an enzyme conformational relaxation.

SSM predicts a strong correlation between the conformational energy difference in E_{GS} and E_{TS} and the amount of transition state stabilization that is provided by the enzyme. If so, then successful folding strategies for predicting the physiological structure should search for a conformational energy that is increased by some factor of this amount from the conformational energy minimum. As there are likely to be many conformations of this energy, it may be possible to select the correct one by starting from the minimal energy conformation and then inputting the appropriate energy.

In the absence of the bound transition state or transition state analogue at the physiologic temperature, E_{TS} may be artificially induced by lowering the temperature a few degrees below the physiological temperature The functional enzyme E_{GS} exists on the thermodynamic brink of assuming E_{TS}, requiring only substrate binding to provide the activation energy for its formation. Under physiological conditions, E_{TS} exists only fleetingly at the reaction transition state or permanently, to a fair approximation, when bound to a transition state analogue. SSM predicts that E_{TS} may be artificially induced without the influence of ligands by lowering the enzyme a few degrees below the optimal thriving temperature of the organism from which the enzyme is isolated, thereby exploiting the tendency of enzymes as catalysts to assume the transition state binding conformer. The transition from E_{GS} to E_{TS} may be sharp and perhaps better described as a phase change in the respect that the enzyme is either completely in one form or the other and not partitioned in a Boltzmann distribution, for example. It is also quite likely to be associated with a very high activation energy (Anderson and Britt, 2002). This idea is born from a wealth of evidence, much in the form of nonlinear Arrhenius plots, for sharp, temperature-induced conformational transitions for many enzymes which suggest conformational changes. SSM predicts that this is a general feature of enzymes which will be confirmed with further study.

Traditional x-ray and NMR structural analyses do not reveal the physiological structure of the enzyme In the Haldane view, there is no difference in the conformations of the free, ground state-, or transition state-bound enzyme. It is further assumed in the methods employed in modern x-ray and NMR structure determination that the experimentally-determined structure, typically determined at a temperature well below the physiological temperature, is the physiological structure. For those enzymes for which there has been clear demonstrations of nondenaturational, temperature-induced conformational changes between the temperatures of structure determination and the physiological temperatures, this is certainly not the case. It seems quite likely that this may be a general feature of enzymes, particularly given the wealth of nonlinear Arrhenius plots. Though these low-temperature structures are not the physiological structures, in the SSM view, they possess catalytic relevance. They reveal the structure of E_{TS} since this structure predominates at low temperatures. This is supposed to be the case for avian and mammalian enzymes where typical normal body temperatures are around 40°C and for enzymes from thermophilic organisms. The only exception to this rule may involve enzymes from psychrophilic organisms where the x-ray diffraction temperatures happen to coincide with near-physiological temperatures.

This incorrect assumption that the low-temperature structures are the physiological structures has two unfortunate consequences: it has led to the widespread belief that enzymes

are essentially rigid molecules and that enzyme active sites are complementary to reaction transition states. The general observation that the addition of transition state analogues to enzyme crystals are less likely to crack the crystals than are the addition of ground state analogues, which is understandable in the Haldane context, has strongly reinforced this latter point. Though crystallographers have traditionally examined the transition state analogue-bound forms by infusion of the analogues into preformed crystals, thereby dismissing the possibility for a ligand-induced conformational change rather than crystallizing the complex, SSM predicts that the use of either method will yield the same result. If one wants to determine the structure of the physiological form, it is then necessary to form the crystals at, or only a few degrees below, the species optimal thriving temperature. This is probably impossible given the difficulties that are encountered with attempts to crystallize proteins, even at low temperatures. It is also not likely that the physiological form could be achieved by infusing ground state analogues into low-temperature crystals as the crystal packing forces are likely to prevent the conformational change that would otherwise result. It appears, therefore, that the best x-ray crystallography can do, as currently practiced, is to give us only half the picture, albeit an important half, the structure of E_{TS}. In the future, the best determinations of physiological enzyme structures and of their complexes with catalytically relevant ligands will probably come from NMR studies, though care should be taken to work at or near the physiological T and a structural similarity between differently-bound states should never be presumed. Recently, a comparison of the structures of human lysozyme between 4ºC and 35ºC revealed significant differences in the overall structure of the enzyme, particularly in the active site area (Kumeta *et al*, 2003). SSM predicts that similar studies with other enzymes will yield similar results.

Enzyme stability curves are biphasic It has long been known that enzyme stability curves -- plots of ΔG_U, the free energy of unfolding, vs. temperature -- are markedly nonlinear, with both hot and cold unfolding temperatures either demonstrated or inferred (Becktel and Schellman, 1987). This is a consequence of the large increase in heat capacity upon forming the unfolded state. A common strategy for deriving these curves is to obtain equilibrium-unfolding data at or near the heat denaturation temperature, and then calculate the entire curve from this data (Feller *et al*, 1999; Kumar *et al*, 2001). In all cases, the thermodynamic parameters directly determined in the high temperature regime are presumed meaningful in the lower temperature regime. SSM clearly predicts that the ‘stability curve’ of an enzyme will be revealed as the actual sum of two stability curves (Fig. 5) with the low-temperature form being the more stable. The enzyme which predominates in the physiological regime is E_{GS}. The enzyme that predominates at low temperature is E_{TS}. Thermodynamic analyses of the two curves may then yield

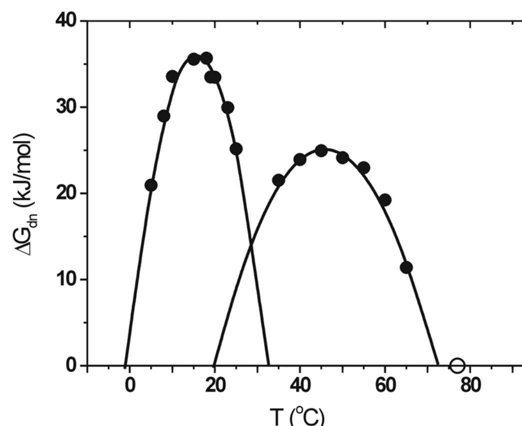

Fig. 5. Bimodal stability curve of bovine adenosine deaminase (see Anderson and Britt, 2002, for further details).

insights into the structural differences of the two conformers. We found in the case of bovine adenosine deaminase, for example, that the low-temperature conformer is a much more compact structure (Anderson and Britt, 2002).

Enzymes are not necessarily “inefficiently packed” Studies of pressure-induced conformational changes of enzymes have led some to conclude that enzymes are “inefficiently packed”, because there is often a substantial void volume element within the enzyme interior (see Royer, 2002 for a review). Although I realize that these individuals are merely emphasizing that there may exist alternate conformations that could substantially reduce this void volume, this loose packing may have a direct consequence in the catalytic event. To suggest that there is something inefficient in this may be misleading. Perhaps it is this initial loose packing that gives E_{GS} the initial high energy. Perhaps upon assuming the transition state binding conformer, the void volume is greatly reduced as the enzyme assumes a more stable conformation. SSM predicts that there exists a relationship between the extent of this void volume and the ability of enzymes to stabilize the transition state of the physiologically relevant reaction when pressure-induced unfolding measurements are made under physiological conditions.

Small molecular weight molecules and catalytic antibodies cannot achieve the catalytic efficiency of enzymes These strategies for imitating the catalytic efficiency of enzymes provide interesting tests for discerning between the Haldane model and SSM. The notion that only the enzyme active site is necessary for catalysis has spawned the field of artificial enzymes or enzyme mimics. The premise in this field is that the bulk of the enzyme that does not constitute the active site is unnecessary for catalysis and may be safely eliminated. The general failure of this strategy may be attributed to an engineering-out of the conformational flexibility that is

required to bind both the ground state and the reaction transition state, and for the reaction to be facilitated by a conformational relaxation. For these small molecular weight catalysts, it is typically found that the ground state is bound weakly while the transition state is bound strongly or vice versa. Enzymes bind both states strongly to facilitate the reaction.

The sad story of catalytic antibodies offers another interesting insight into SSM. The primary idea behind the development of catalytic antibodies is that since enzymes function by exclusively binding the reaction transition states, then it should be possible to create a powerful catalyst for a specific reaction by raising an antibody to a cleverly-designed transition state analogue. The general failure of catalytic antibodies to achieve their desired objectives has been blamed on several aspects (Hollfelder *et al*, 1996; Hilvert, 2000). I would like to suggest that the failure may instead be largely attributable to the subconscious engineering out of the essence of enzyme catalysis; namely, the ability of the enzyme to relax to a more stable global conformation at the reaction transition state. If the Haldane model is correct, then catalytic antibodies should exhibit rate enhancements on par with enzymes. As it stands, the general failure of catalytic antibodies indicts the Haldane model and suggests that SSM may be more valid.

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