BIOORGANIC CHEMISTRY 16, 62–96 (1988)

REVIEWS

The Role of Induced Fit and Conformational Changes of Enzymes in Specificity and Catalysis

DANIEL HERSCHLAG

Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02254

Received April 21, 1987

The induced fit mechanism and conformational changes of some enzymes are reviewed. Although it is maintained in the literature that induced fit provides specificity, one simple model for induced fit does not provide specificity, according to the usual definition of specificity: the discrimination by an enzyme between the reactions of two competing substrates. Discrimination is measured by the ratio of the values of k_{cat}/K_m for the two substrates under both k_{cat}/K_m and k_{cat} conditions, and induced fit decreases k_{cat}/K_m by the same factor for both a good substrate and a poor substrate when a chemical or other central step is rate limiting. For a good substrate, this decrease in k_{cat}/K_m can result from an increase in K_m without a change in k_{cat}, while for a poor substrate the decrease can result from a decrease in k_{cat} without a change in K_m. It is reasonable that specificity results primarily from specific interactions of an enzyme with its substrate, which can be described in terms of "intrinsic binding energy." There are, however, exceptions that can give specificity from induced fit relative to a hypothetical non-induced fit enzyme. When a conformational change results in the substrate being surrounded on all sides by the enzyme there is additional intrinsic binding energy that can enhance catalysis of the reaction of a specific substrate. A second exception can occur when a binding step is rate limiting for the good substrate while a chemical step is rate limiting for an alternative poor substrate. In this case induced fit can enhance specificity between the good and the poor substrate by slowing the reaction of the poor substrate and not the rate-limiting binding of the good substrate. Another exception in which induced fit can enhance specificity occurs when product release is the rate-limiting step for the good substrate and a chemical step is rate limiting for the alternative poor substrate. In this case the rate of reaction of the poor substrate is slowed, while the rate for the good substrate is not slowed due to the introduction of an alternate pathway for product dissociation. In addition, it is shown that an induced fit enzyme can provide specificity under three types of conditions that are conceivable *in vivo*. Specificity can occur (i) when the concentration of the substrate oscillates or changes with time; (ii) when the substrate for the induced fit enzyme is provided at a constant rate from a preceding metabolic step; or (iii) when enzyme is in excess of substrate. Induced fit can provide substrate synergism in binding and reactivity. However, it is shown that this provides no advantage in specificity relative to a non-induced fit mechanism, in general. An advantage in specificity can occur when the active site surrounds the substrate, or when the steady-state concentration of a reactive enzyme/substrate binary complex is lowered during turnover relative to its equilibrium concentration. All of these analyses pertain to competition with hydrolysis in a two-substrate reaction as well as to competition with an alternate substrate, despite the fact that water is always present at the active site in the absence of bound substrate.

0045-2068/88 $3.00

INTRODUCTION AND SCOPE OF THE REVIEW

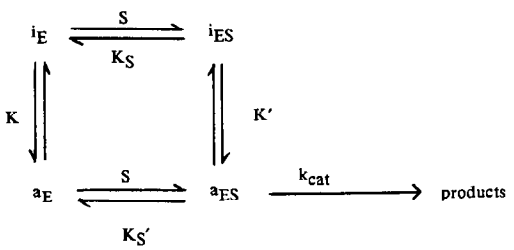
It is often suggested that conformational changes occur in the course of an enzymatic reaction, and there is strong evidence for conformational changes in several cases. It is difficult to imagine how some enzymes could function without one or several conformational changes. Enzymes expected to require conformational changes include allosteric enzymes, which bind a substrate or effector that causes a change in the catalytic properties at a different site on the enzyme, and enzymes that couple a chemical reaction and work, such as the pumping of an ion against a concentration gradient, which must change chemical specificity and ion-binding specificity during turnover (*1*).

It is more difficult to imagine the role of conformational changes in enzymatic reactions other than coupled vectorial processes and reactions that are regulated. Before evidence had been obtained for conformational changes, Koshland suggested that the rate of reaction of a specific substrate could be enhanced compared to the rate for a smaller compound of similar reactivity by a conformational change that properly aligns groups of an enzyme for catalysis upon binding of the specific substrate (*2*). This insightful mechanism, which has been called "induced fit," was seminal to the research and analysis of enzymatic conformational changes. Although induced fit can play roles in phenomena such as allosteric control, only the possible roles of induced fit and conformational changes in enyzme specificity and catalysis are discussed here.

One aim of this review is to clarify apparent contradictions in the literature. Fersht has argued that the induced fit mechanism cannot provide specificity (*3, 4*). Despite these arguments, several textbooks and reviews include induced fit as a mechanism for enzymatic specificity (*5–7*).

A second aim is to integrate the ideas of several investigators in order to evaluate the consequences of conformational changes. Conformational changes that surround the substrate on all sides have been discussed by Wolfenden (*8*). The roles of conformational changes are discussed in terms of intrinsic binding energy, which provides a means of relating structural changes to the enzymatic functions of specificity and catalysis (*6*). A conformational change in which the enzyme surrounds the substrate can provide additional intrinsic binding energy, thus enhancing specificity and aiding catalysis (*8*).

A third aim is to discuss *in vivo* conditions that would render the induced fit mechanism capable of enhancing specificity. Fersht (*3, 4*) has shown that at any



SCHEME 1

constant concentrations of two competing substrates an enzyme that exhibits induced fit does not increase specificity relative to an analogous enzyme that does not follow the induced fit mechanism when the central conversion of the enzyme–substrate to the enzyme–product complex is rate limiting. The analysis herein shows that this induced fit mechanism can provide specificity under *in vivo* conditions in which the concentration of a substrate varies or is exceeded by the concentration of enzyme.

THE CASE IN WHICH INDUCED FIT DOES NOT PROVIDE SPECIFICITY

The induced fit mechanism. It is easy to understand why a molecule that is larger than an enzyme's substrate, but of similar chemical reactivity, will not react with the enzyme: it will not fit into the enzyme's active site. It is harder to understand why a molecule that is smaller than the substrate does not react.

Koshland (*2*) offered the following theory to explain the inability of molecules smaller than the substrate to participate in enzymatic reactions: (1) precise orientation of catalytic groups is required for enzyme action; (2) the binding of substrate causes a change in the three-dimensional structure of the enzyme at the active site; and (3) changes caused by binding the substrate result in proper orientation for reaction, whereas a nonsubstrate in the active site does not cause these changes. This theory has been called "induced fit."

One specific model for induced fit is depicted in Scheme 1 (*2–4, 6*). In this scheme ^{i}E is the stable conformation of the free enzyme ($K < 1$), and ^{a}E is the catalytically active conformation of the enzyme.[1] Upon binding a good substrate the active conformation, ^{a}ES, is favored ($K' > 1$), while the inactive conformation, ^{i}ES, is favored upon binding a poor substrate ($K' < 1$).

In order to simplify descriptions of reactions, limiting cases of this model are discussed in which one of three processes is completely rate limiting under k_{cat}/K_m conditions: (1) the central conversion[2] of ES to EP; (2) the binding of substrate; or (3) the release of product. That is, the barrier for the single rate-limiting step is much higher on a free energy-reaction profile than the positions of the other barriers. Situations in which more than one of these steps contributes significantly to the observed rate will exhibit behavior that is intermediate between those for the limiting cases.

The first limiting case will be referred to as the "C induced fit mechanism," for chemical or central step rate limiting. In the C mechanism there is rapid equilib-

[1] Throughout this review, the abbreviation ^{a}E is used to represent the active conformation of an enzyme, and the abbreviation ^{i}E is used to represent the inactive conformation of an induced fit enzyme.

[2] The rate constant k_{cat} (e.g., Scheme 1) can be the rate constant for a single step, such as a chemical conversion or conformational change, or it can be an apparent rate constant for several central steps that are subsequent to substrate binding and prior to product dissociation. Although the case with product dissociation partially or totally rate limiting could also be described by this apparent rate constant, this case is treated separately in order to simplify the analysis of specificity.

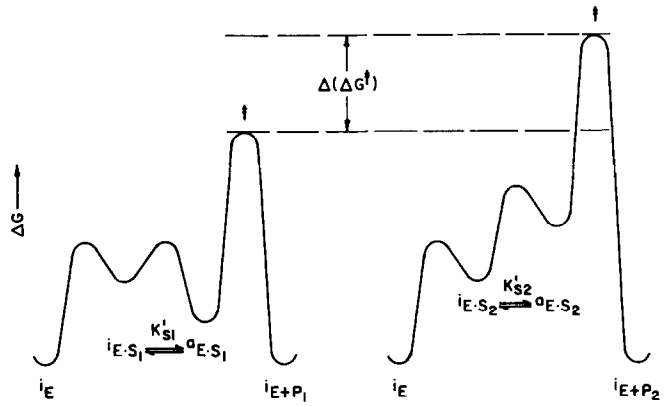


FIG. 1. Free energy profile with [S] < K_m for the reaction of an induced fit enzyme with a good substrate, S_1 (A) and a poor substrate, S_2 (B). The conformational change of the enzyme is shown after binding of substrate.

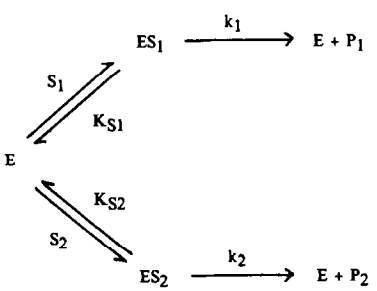
rium binding of substrate, and the rate constant k_{cat} can represent any central step in the reaction path.[2] The cases with rate-limiting release of product and rate-limiting binding of substrate are discussed in subsequent sections. In all cases the conformational change is fast relative to the rate-limiting step.

The free energy profile for the reaction of a good substrate, S_1, with a C induced fit enzyme is shown in Fig. 1A for $[S_1] < K_m$. The enzyme binds S_1 to form $^iES_1$, but $^iES_1$ does not react. Rather, $^iES_1$ undergoes a rapid energetically favorable conformational change to $^aES_1$, which then reacts to form product, P_1.

An analogous free energy profile for the reaction of a poor substrate, S_2, is shown in Fig. 1B. Here, with the poor substrate, the conformational change of $^iES_2$ to form $^aES_2$ and correctly align S_2 in the active site is thermodynamically unfavorable.

The conformational change to form the active conformation of the enzyme, ^aE, is more favorable with S_1 bound than with S_2 bound ($K'_{S1} > K'_{S2}$, Fig. 1) and S_1 is a good substrate, while S_2 is a poor substrate ($\neq$, Figs. 1A and B). However, this induced fit mechanism does not provide specificity, as is explained below.

Analysis of induced fit: The C mechanism does not provide specificity between two competing substrates. The analysis by Fersht (*3, 4*) which shows



SCHEME 2

that the C induced fit mechanism does not enhance specificity is described with a heuristic argument, and then quantitatively. Specificity must first be defined.

Discrimination by an enzyme between catalysis of the reaction of two potential substrates in the presence of both compounds is the biologically relevant measure of specificity, because this is the situation that a cell must face (*9*). This discrimination at any constant concentrations of the two competing substrates can be derived from Scheme 2 (*10*). The rate of formation of products from S_1 and S_2 (v_1 and v_2) can in general be expressed in terms of the concentration of free enzyme, the concentration of the respective substrate, and the apparent second-order rate constant for reaction of the free enzyme and the free substrate, k_{cat}/K_m:[3]

$$\begin{aligned} v_1 &= (k_{cat}/K_m)_1[E_{free}]\,[S_1]: \\ v_2 &= (k_{cat}/K_m)_2[E_{free}]\,[S_2]. \end{aligned} \qquad [1]$$

In taking the ratio of rates for the two substrates, v_1/v_2, the dependence on enzyme concentration drops out:

$$v_1/v_2 = \frac{(k_{cat}/K_m)_1[E_{free}]\,[S_1]}{(k_{cat}/K_m)_2[E_{free}]\,[S_2]} = \frac{(k_{cat}/K_m)_1}{(k_{cat}/K_m)_2} \times \frac{[S_1]}{[S_2]}. \qquad [2]$$

It is seen that the discrimination at any given concentrations of competing substrates is determined by the ratio of the values of $\{k_{cat}/K_m\}$ for the two substrates. Thus, this specificity ratio (Eq. [2]) holds under both k_{cat}/K_m and k_{cat} conditions. The ratio is determined by the difference in free energy of the transition states ${}^aES_1^{\neq}$ and ${}^aES_2^{\neq}$ (see Fig. 1; $\Delta(\Delta G^{\neq})$.[4]

Since discrimination is determined by the difference in the free energy of the rate-limiting transition states for the competing substrates, the order of steps leading to this transition state can be changed without affecting the specificity; Fig. 2 describes the same induced fit reaction as is drawn in Fig. 1, but with the conformational change to form ${}^aE$ prior to the binding of substrate, S_1 or S_2, instead of a conformational change subsequent to binding for purposes of illustration. The difference in the free energy of the rate-limiting transition states of S_1 and S_2 is $\Delta(\Delta G^{\neq})$. If there were no induced fit mechanism (and thus no ${}^iE$) ${}^aE$ would be the only conformation of the enzyme, and the reaction would be ${}^aE + S \rightleftarrows {}^aES \rightleftarrows {}^aES^{\neq} \rightarrow$ products (Fig. 2). For this non-induced fit mechanism the difference in the free energy of the transition states for the good and poor substrates is also $\Delta(\Delta G^{\neq})$, which is the same as that for the induced fit mechanism (Figs. 1 and 2). As stated above, the difference in the free energy of the transition

[3] The fact that the second-order rate constant for reaction of free enzyme and free substrate is k_{cat}/K_m can be derived from the Michaelis–Menten equation, $v = k_{cat}\,[S]\,[E]_{tot}/([S] + K_m)$. At low substrate concentration ($[S] \ll K_m$) all of the enzyme is free and the Michaelis–Menten equation reduces to $v = (k_{cat}/K_m)\,[S]\,[E]_{tot}$. At high substrate concentration ($[S] \gg K_m$) all of the enzyme is in the form ES and the Michaelis–Menten equation reduces to $v = k_{cat}\,[E]_{tot}$.

[4] It should be noted that the value of k_{cat}/K_m can be related to the free energy of a single transition state only when the free energy of a single transition state on a reaction profile with $[S] \ll K_m$ is much greater than the free energy of all other transition states, as is assumed in the analyses of specificity in this review. The analogous situation for the value of k_{cat} holds for the free energy-reaction profile with $[S] \gg K_m$.

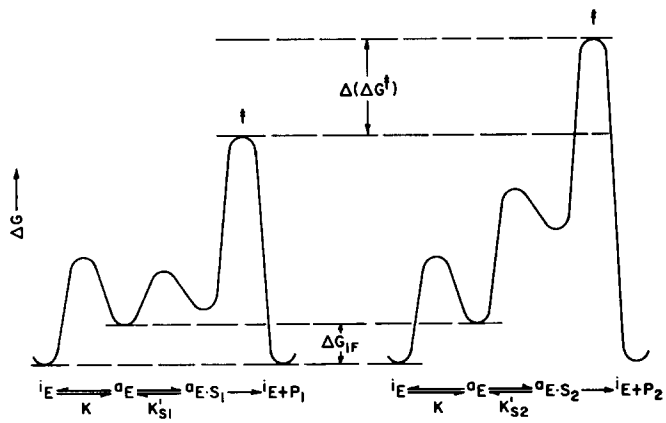


FIG. 2. Free energy profile with [S] < K_m for the reaction of an induced fit enzyme with a good substrate, S_1 (A) and a poor substrate, S_2 (B). The same reactions as in Fig. 1 are shown, but with the conformational change prior to substrate binding in this figure, rather than subsequent to binding as in Fig. 1.

states for two substrates determines specificity. Thus the induced fit mechanism, with its conformational change ($^iE \rightleftarrows {}^aE$), does not enhance specificity over that for the non-induced fit mechanism, with only ^aE and no conformational change.

This inability of induced fit to enhance specificity is apparent from the free-energy-reaction profile shown in Fig. 2. The equilibrium of ^aE with ^iE increases the free energy of the rate-limiting transition state by the same amount, ΔG_{IF}, for good substrate (S_1) and the poor substrate (S_2). The difference between the free energy of the rate-limiting transition states, $\Delta(\Delta G^{\neq})$, is not affected. Again, the discrimination between two substrates is the same whether or not induced fit occurs.

The inability of the C induced fit mechanism to provide specificity at any constant concentrations of two competing substrates can be shown quantitatively (*4*). The values of k_{cat}/K_m for the induced fit and non-induced fit mechanism are determined because, as described above, the ratio of values of k_{cat}/K_m determines specificity under both k_{cat}/K_m and k_{cat} conditions. For the non-induced mechanism,

$$^aE + S \underset{K'_S}{\rightleftarrows} {}^aES \xrightarrow{k_{cat}} \text{products},$$

the value of $(k_{cat}/K_m)_{obsd}$ is k_{cat}/K'_S. This mechanism is the lower line from the induced fit mechanism of Scheme 1. In the induced fit mechanism the predominant form of the free enzyme is ^iE rather than ^aE, so that K is less than 1 (Scheme 1). Since the concentration of active enzyme is reduced by the amount K relative to the non-induced fit mechanism, the induced fit mechanism gives $(k_{cat}/K_m)_{obsd} = K(k_{cat}/K'_S)$ with $K \ll 1$. The equilibrium constant for the conformational change of the free enzyme, K, is independent of the substrate. Thus the dependence on K of the specificity ratio, v_1/v_2 (Eq. [2]), is canceled when the ratio of values of $(k_{cat}/K_m)_{obsd}$ for two substrates is taken:

$$(v_1/v_2)_{\text{induced fit}} = \frac{(k_{cat}/K_m)_1\,[S_1]}{(k_{cat}/K_m)_2\,[S_2]} = \frac{K(k_{cat}/K'_S)_1\,[S_1]}{K(k_{cat}/K'_S)_2\,[S_2]} = \frac{(k_{cat}/K'_S)_1\,[S_1]}{(k_{cat}/K'_S)_2\,[S_2]}. \quad [3]$$

The specificity ratio, v_1/v_2, is independent of the equilibria involving the conformational changes, K and K' (Scheme 1). The same specificity ratio is obtained from the mechanism without a conformational change,

$$^{a}E \underset{K'_S}{\rightleftarrows} {}^{a}ES \xrightarrow{k_{cat}} \text{product},$$

as is shown:

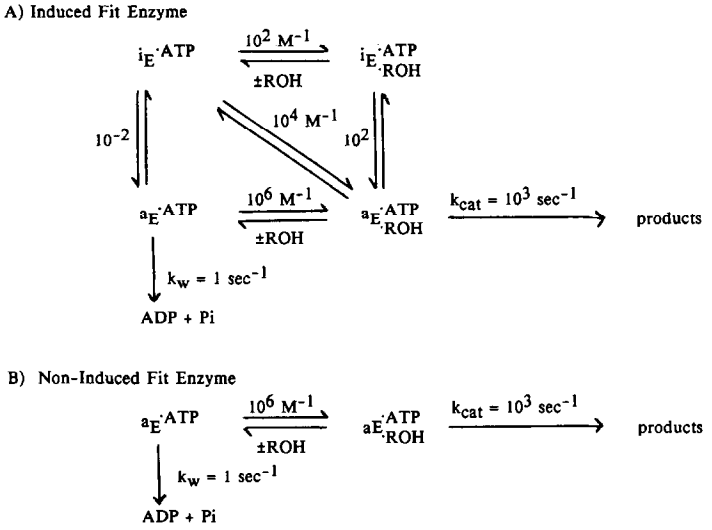
$$(v_1/v_2)_{\text{non-induced fit}} = \frac{(k_{cat}/K_m)_1\,[S_1]}{(k_{cat}/K_m)_2\,[S_2]} = \frac{(k_{cat}/K'_S)_1\,[S_1]}{(k_{cat}/K'_S)_2\,[S_2]}. \quad [4]$$

The above describes Fersht's comparison of the induced fit enzyme, ^{i}E, with the hypothetical enzyme, ^{a}E. The basic question in evaluating induced fit is whether or not an advantage is gained by introducing a conformational change, $^{i}E \rightleftarrows {}^{a}E$. That is, is there a reason that ^{i}E should have evolved as the stable form of the enzyme rather than the hypothetical ^{a}E? This hypothetical enzyme has the same conformation as the active enzyme conformation of the induced fit enzyme (^{a}E). Thus the two enzymes that are compared have the same catalytic moieties, and these catalytic moieties are arranged the same in the transition state ($^{a}ES^{\neq}$), but not in the ground state (^{i}E vs ^{a}E).

Fersht's analysis suggests that the C induced fit mechanism does not play a role in specificity between two potential substrates. In addition, it has been suggested that the induced fit mechanism is useful in providing specificity against reaction with solvent and in increasing K_m for a reaction. The following analyses suggest that the C induced fit mechanism also provides no advantage for these processes.

The C induced fit mechanism also does not enhance specificity compared to water. In some two-substrate reactions, water competes with the second substrate; for example, in the hexokinase reaction water competes with glucose for reaction with ATP. Water is present in an enzyme's active site whenever substrate is not bound. It has been suggested that induced fit can enhance specificity against hydrolysis. However, the following analysis shows that the C induced fit mechanism does not enhance specificity against hydrolysis at any constant concentration of substrate.

In order to discuss the water reaction, a hypothetical kinase reaction, which is shown in Scheme 3, is introduced. Mechanism A (Scheme 3) is the induced fit mechanism; it has both the active and inactive enzyme conformations (^{a}E and ^{i}E, respectively). Binding of the second substrate, ROH, stabilizes the active conformation of the enzyme. Mechanism B (Scheme 3) is the non-induced fit mechanism for comparison. The catalytically active enzyme conformation from mechanism A, ^{a}E, is the stable species in mechanism B (Scheme 3). Thus the non-induced fit mechanism (Scheme 3B) is simply the lower line from the induced fit mechanism (Scheme 3A), and the rate constants for the two mechanisms correspond to the same interactions of enzyme and substrate in the transition state. For simplicity, the case with saturating ATP is considered in the following comparisons.



SCHEME 3

A numerical comparison of the induced fit and the non-induced fit mechanisms of Schemes 3A and 3B shows that the induced fit mechanism does not enhance specificity for the reaction of substrate (ROH) relative to the reaction with water. The steady-state kinetic parameters, k_{cat}/K_m and k_{cat}, for the induced fit and the non-induced fit mechanisms in Scheme 3A and 3B, are given in Table 1. The observed rate constant for hydrolysis of ATP in the absence of substrate ROH, $(k_w)_{obsd}$, is also given in Table 1.

The observed rates of reaction of ATP with ROH and with water can be calculated at varying concentrations of ROH from the kinetic parameters in Table 1; these rates are listed in columns A and B of Table 2. Specificity can be defined as the ratio of the observed rates of reaction of ATP with ROH and water, which is the value given in column A divided by the value in column B (Table 2). This specificity ratio is given in column C of Table 2. The ratio is the same for the induced fit and the non-induced fit mechanisms. This shows that induced fit does

TABLE 1

Comparison of the Kinetic Parameters for Specificity and Catalysis for the Induced Fit and the Non-Induced Fit Kinase from Scheme 3

	k_{cat} (s^{-1})	K_m(ROH) (M^{-1})	k_{cat}/K_m (M^{-1} s^{-1})	$(k_w)_{obsd}$[a] (s^{-1})	Specificity ratio $\frac{(k_{cat}/K_m)[ROH]}{(k_w)_{obsd}}$ (M^{-1})
Induced fit enzyme from Scheme 3A	10^3	10^{-4}	10^7	10^{-2}	10^9 [ROH]
Non-induced fit enzyme from Scheme 3B	10^3	10^{-6}	10^9	1	10^9 [ROH]

[a] Rate constant for hydrolysis of ATP in the absence of the second substrate, ROH.

TABLE 2

Comparison of Rates of Reaction with Substrate (ROH) and Water for the Induced Fit and Non-Induced Fit Kinase of Scheme 3 with Varying [ROH] and Saturating ATP[a]

	Induced fit enzyme			Non-induced fit enzyme		
[ROH] M	(A) Rate of ROH reaction[b] (μM s^{-1})	(B) Rate of water reaction[c] (μM s^{-1})	(C) Specificity ratio ROH/water reactions[d]	(A) Rate of ROH reaction[b] (μM s^{-1})	(B) Rate of water reaction[c] (μM s^{-1})	(C) Specificity ratio ROH/water reactions[d]
10^{-8}	1.0×10^{-4}	1.0×10^{-5}	10	9.9×10^{-3}	9.9×10^{-4}	10
10^{-6}	9.9×10^{-3}	9.9×10^{-6}	10^3	0.50	5.0×10^{-4}	10^3
10^{-5}	9.1×10^{-2}	9.1×10^{-6}	10^4	0.91	9.1×10^{-5}	10^4
10^{-4}	0.50	5.0×10^{-6}	10^5	0.99	9.9×10^{-6}	10^5
10^{-3}	0.91	9.1×10^{-7}	10^6	1.0	1.0×10^{-6}	10^6

[a] Rate calculated with 10^{-9} M enzyme.
[b] Rate calculated using the equation: rate = $k_{cat}/(K_m + [ROH])\ [Enzyme]_{tot}[ROH]$, with k_{cat} and K_m from Table 1.
[c] Rates calculated using the equation: rate = $k_w[^aE]_{free}$, with $[^aE]_{free}$ calculated from $[E]_{total\ free} = K_m/([ROH] + K_m)$ and $[^aE]_{free} = 10^{-2}[^iE]_{free}$ for the induced fit enzyme and $[^aE]_{free} = [E]_{total\ free}$ for the non-induced fit enzyme (Scheme 3) and with K_m and k_w from Table 1.
[d] The specificity ratio is the ratio of rates from columns A and B.

not enhance specificity relative to the hydrolysis reaction. For example, at 10^{-4} M ROH the ratio of rates for the ROH and water reactions for the induced fit mechanism is $0.50/(5.0 \times 10^{-6}) = 10^5$, and the ratio for the non-induced fit mechanism is also 10^5: $0.99/(9.9 \times 10^{-6}) = 10^5$.

This specificity for reaction of ROH relative to the reaction with water is given by the ratio $(k_{cat}/K_m)\ [ROH]/(k_w)_{obsd}$. This ratio is entirely analogous to the specificity ratio, v_1/v_2, shown in Eq. [2] for competition between two substrates, S_1 and S_2. It was shown above that the specificity ratio for competition between S_1 and S_2 is the same for the C induced fit and the non-induced fit mechanisms. The specificity ratio for competition of ROH and water, $(k_{cat}/K_m)[ROH]/(k_w)_{obsd}$, can be calculated for the induced fit and non-induced fit mechanisms of Scheme 3 from the kinetic parameters given in Table 1. This specificity ratio is 10^9 [ROH] for both mechanisms (Table 1; for example, for the induced fit mechanism (k_{cat}/K_m) $[ROH]/(k_w)_{obsd} = (10^7/10^{-2})\ [ROH] = 10^9\ [ROH]$). Thus, at 10^{-4} *M* ROH the observed specificity is 10^5 for both the induced fit and the non-induced fit mechanism, as was determined in the numerical example above (Table 2). Again, the C induced fit mechanism does not enhance specificity.

Effect of induced fit on K_m. Catalysis is stabilization of the transition state of a reaction. As is described below, destabilization of the ground state enzyme/substrate complex relative to the transition state is also necessary for enzymatic catalysis; this prevents accumulation of the enzyme in the enzyme/substrate complex. It has been suggested that the induced fit mechanism can aid catalysis by providing ground state destabilization relative to the transition state. The following analysis shows that the C induced fit mechanism can increase the value of K_m, which reflects the energy of enzyme/substrate complexes, but that no catalytic advantage is provided.

The requirement for destabilization of the enzyme/substrate ground state is

described first. This requirement is illustrated by the reaction catalyzed by the two hypothetical enzymes in Fig. 3 (*6, 11*). Figure 3A shows the energetics for the uncatalyzed reaction of substrate S in solution. Figure 3B shows the reaction when the enzyme stabilizes the ground state and the transition state (ES and $ES^{\neq}$) to the same extent; the enzymatic ground state, ES, is more stable than free S by the amount ΔG_B, and the transition state, $ES^{\neq}$, is more stable than the free transition state, $S^{\neq}$, by the same amount (Fig. 3). For the enzymatic reaction of Fig. 3B the rate-limiting step is ES $\rightleftarrows$ EP, and the energetic barrier for this step is ΔG_A. This is the same barrier as for the reaction of free S. Thus the enzyme depicted in Fig. 3B does not catalyze the reaction, even though the transition state is stabilized by this enzyme.

Figure 3C shows what the enzyme must do: the transition state complex ($ES^{\neq}$) is stabilized as in Fig. 3B, but in addition, the ground state complex (ES) is destabilized relative to that in Fig. 3B by the amount ΔG_D. The energetic barrier for the reaction in Fig. 3C, ΔG_C, is much less than the barrier for free S (Fig. 3A; ΔG_A) or the barrier for the enzyme of Fig. 3B (also ΔG_A). The destabilization of the ground state relative to the transition state that is brought about by the enzyme of Fig. 3C is necessary for catalysis. This destabilization corresponds to an increase in K_m while the value of k_{cat}/K_m remains constant.

Enzymatic catalysis will be increased by increasing the energy of the ES complex as long as the transition state energy is not also increased; again, this corresponds to an increase in K_m at constant k_{cat}/K_m (*3, 4*). The increased efficiency from increasing K_m can be explained as an advantage in minimizing the amount of enzyme tied up as ES and thus maximizing the amount of enzyme available for catalysis of the reaction: E + S $\rightleftarrows$ E + P. The increase in he observed rate from increasing K_m at constant k_{cat}/K_m is large when the substrate concentration is near

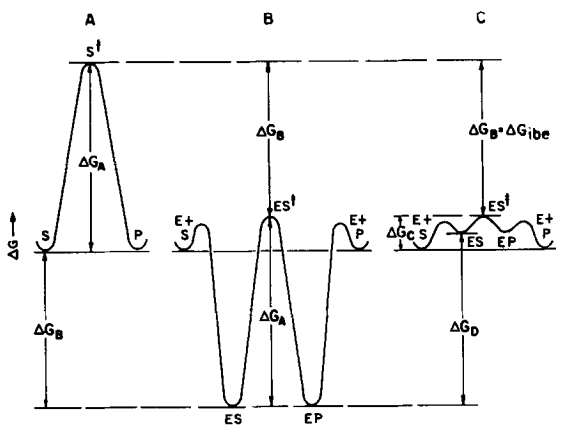


FIG. 3. Comparison of the free energy profiles for the nonenzymatic reaction of substrate S (A); for the enzymatic reaction of S in which the ground state and transition complexes are stabilized to the same extent (B); and the enzymatic reaction of S in which the transition state complex is stabilized to a greater extent than the ground state complex (C). Catalysis occurs in the enzymatic reaction (C), but not in the enzymatic reaction (B). All three reaction profiles are for the same standard state, which is a concentration of S that is less than K_m for the enzyme of (C).

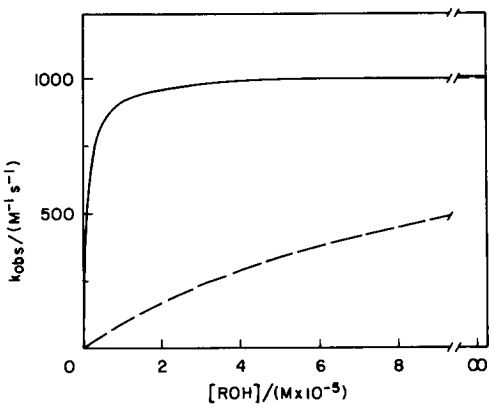


FIG. 4. Effect of substrate concentration on the observed rate constant for an induced fit enzyme (---) and for the analogous non-induced fit enzyme (—) from Schemes 3A and 3B. Rate constants are calculated from $k_{obsd} = k_{cat}[ROH]/([ROH] + K_m)$ and the values of k_{cat} and K_m in Table 1.

or above K_m, because enzyme that is complexed with substrate when K_m is low becomes available for catalysis. When the substrate concentration is well below K_m a further increase in K_m at constant k_{cat}/K_m will yield no advantage because essentially all of the enzyme is free and already catalyzing the reaction with the apparent second-order rate constant k_{cat}/K_m.[3]

Although an increase in K_m at constant k_{cat}/K_m is useful for catalysis, the C induced fit mechanism increases K_m but decreases k_{cat}/K_m; this actually lessens catalysis (*4*).[5] The hypothetical kinase reaction shown in Scheme 3 is used to illustrate this point numerically. The kinetic parameters for the induced fit mechanism (Scheme 3A) are compared to the kinetic parameters for the non-induced fit mechanism (Scheme 3B); the rationale for this comparison is described above. The rate and equilibrium constants given in Schemes 3A and 3B have been used to calculate the values of K_m, k_{cat}, and k_{cat}/K_m listed in Table 1 for the induced fit and the non-induced fit mechanisms. The value of K_m is 10^2-fold larger for the induced fit mechanism relative to the non-induced fit mechanism (Table 1; $10^{-4}/10^{-6} = 10^2$). However, the second-order rate constant, k_{cat}/K_m, is decreased 10^2-fold by introduction of induced fit (Table 1; $10^7/10^9 = 10^{-2}$).

Induced fit is unable to provide an advantage via this increase in K_m since the induced fit enzyme is slower than the non-induced fit enzyme at all concentrations of substrate. This is shown in Fig. 4 by the dependence on substrate concentration (ROH) of rate of reaction for the induced fit mechanism (dashed line) and for the analogous non-induced fit mechanism (solid line) from Scheme 3. The rate decrease occurs because k_{cat}/K_m is decreased while k_{cat} is unchanged. It should be noted that an increase in K_m could increase the concentrations of substrate that give a change in rate with a change in substrate concentration.

The decrease in k_{cat}/K_m for the induced fit enzyme relative to the non-induced fit enzyme arises because some of the energy that is used to stabilize the transition

[5] Fersht (*4*) has corrected an earlier suggestion that induced fit could increase both K_m and k_{cat} (*3*).

state for the non-induced fit enzyme must be used to drive the conformational change of the induced fit enzyme. An equivalent explanation is that the decrease in k_{cat}/K_m arises from a lower concentration of the active enzyme conformation, ^aE, in the induced fit mechanism.

Effect of induced fit on k_{cat}. Although the C induced fit mechanism decreases k_{cat}/K_m by the same extent for a good and a poor substrate, this decrease results from an increase in K_m for the good substrate, but results from a decrease in k_{cat} for a poor substrate.

The above analysis (Effect of induced fit on K_m) is for a good substrate, i.e., a substrate that binds to the active enzyme conformation, ^aE, better than to the inactive enzyme conformation, ^iE. This is depicted in Fig. 1A, which shows the induced fit mechanism with a good substrate; the equilibrium $^iES_1 \rightleftarrows {}^aES_1$ favors $^aES_1$ ($K'_{S1} > 1$). As explained above, induced fit decreases k_{cat}/K_m by increasing K_m while not affecting k_{cat}. This lack of an effect on k_{cat} is apparent in Fig. 1A; the energetic barrier for k_{cat} is that for the process $^aES_1 \rightleftarrows {}^aES_1^{\neq}$ both with and without induced fit.

For a "poor" substrate, formation of the complex with inactive enzyme, ^iE, would be favored over formation of the complex with the active enzyme, ^aE. This is because the poor substrate lacks the specific binding interactions needed to stabilize the active conformation. The situation with the poor substrate is depicted in Fig. 1B, with the equilibrium $^iES_2 \rightleftarrows {}^aES_2$ favoring $^iES_2$ ($K'_{S2} < 1$).

This unfavorable equilibrium with a poor substrate results in a decrease in the value of k_{cat} from induced fit. The value of k_{cat} represents the energy of going from the ES complex to the transition state, which is $^aES_2 \rightarrow {}^aES_2^{\neq}$ for the non-induced fit enzyme (Fig. 1B). In the induced fit mechanism the transition state has the same energy, but the ground state complex, $^iES_2$, is more stable than $^aES_2$ (Fig. 1B). The value of k_{cat} is decreased by induced fit since the energetic barrier for $^iES_2 \rightleftarrows {}^aES_2 \rightarrow {}^aES_2^{\neq}$ is greater than the barrier without induced fit ($^aES_2 \rightarrow {}^aES_2^{\neq}$).

The C induced fit mechanism can therefore increase the difference between the values of k_{cat} for a good and poor substrate. Jencks (*6*) has described how induced fit can give this difference in k_{cat} for the reaction of hexokinase with the good substrate, glucose, and the poor substrate, water. However, as described above, discrimination between two competing substrates is determined by k_{cat}/K_m under both k_{cat}/K_m and k_{cat} conditions so that the C induced fit mechanism does not enhance this discrimination. Also, the C induced fit mechanism decreases k_{cat}/K_m, and thus catalysis, through an increase in K_m with a good substrate and a decrease in k_{cat} with a poor substrate.[6]

EXCEPTIONS: CONFORMATIONAL CHANGES THAT PROVIDE SPECIFICITY

A conformational change that surrounds the substrate on all sides can provide specificity. The above analysis shows that the C induced fit mechanism (1) does

[6] This comparison between a good and poor substrate is a limiting case, in which K_m and not k_{cat} changes for the good substrate, and k_{cat} and not K_m changes for the poor substrate. Intermediate cases are possible.

not enhance specificity compared with a second potential substrate; (2) does not enhance specificity compared to a side reaction with water; and (3) decreases catalysis at all concentrations of substrate. However, conformational changes do occur. The rest of this discussion addresses possible roles for conformational changes. This section discusses a potentially important exception to Fersht's analysis: a conformational change in which the active site surrounds the substrate. This type of conformational change, which has been described in detail by Wolfenden (*8*), can increase both catalysis and specificity.

Surrounding the substrate gives more enzyme–substrate interactions. Maximal binding to the transition state, and thus maximal catalysis, should occur when the interactions with the enzyme occur on all sides of the substrate. In order for an enzyme to surround its substrate in the transition state there must be a conformational change that allows binding of substrate and release of product; an enzyme with an active site that is accessible to substrate cannot interact with the substrate on all sides (*8, 12, 13*). Therefore an enzyme that undergoes a conformational change can increase catalysis relative to an enzyme with no conformational change in its mechanism.

When the active form of the enzyme, ^{a}E, surrounds the substrate, the comparisons made above between the induced fit enzyme (^{i}E) and non-induced fit enzyme (^{a}E) are no longer possible because ^{a}E cannot be the sole form of the free enzyme. If ^{a}E were the only form of the free enzyme, substrate could never gain access to the active site and catalysis would not occur. Thus the conclusion that introduction of a conformational change provides no specificity and hinders catalysis does not apply when the conformational change causes the enzyme to surround the substrate.

The usefulness of surrounding the substrate on all sides for both catalysis and specificity can be explained in terms of "intrinsic binding energy" (*6, 11*). Intrinsic binding energy is the energy from binding that is used to stabilize the transition state. The intrinsic binding energy is depicted in Fig. 3C; the transition state of the enzymatic reaction is stabilized relative to the transition state for the nonenzymatic reaction by an amount of energy ΔG_{ibe}, the intrinsic binding energy. This energy is not fully expressed in the enzyme–substrate complex; rather, it is realized in the transition state complex.

Specific interactions of an enzyme and substrate provide intrinsic binding energy for catalysis of the reaction of that substrate, and not the reaction of other potential substrates lacking the moieties for these interactions (*6*). Thus, specificity occurs from specific interactions, and not simply from the ability of a substrate to fit into the active site of an enzyme. For example, a substrate that has many specific interactions with an enzyme can be held in the proper position to react with a second substrate; in contrast, a substrate with few specific interactions cannot be held precisely and will thus derive less catalysis from "induced intramolecularity." The maximum rate enhancement from induced intramolecularity in solution is about 10^8 relative to 1 M reactants, and with enzymes there is evidence for induced intramolecularity that would correspond to rate enhancements of about 10^4 (*6, 14*).

A conformational change that surrounds the substrate provides catalysis for the

"right" substrate, and thus specificity, by making intrinsic binding energy available that could not be utilized without a conformational change. Many enzymes have active sites in crevices or cavities, which are often between two domains, and active sites have been shown to surround the substrate in several cases (see "Some Examples of Conformational Changes"). An advantage will occur only if the intrinsic binding energy realized from this conformational change is greater than the energy needed to drive the conformational change (*8*). In contrast, other types of conformational changes decrease catalysis by using energy to provide a conformational change rather than to stabilize the transition state, and these conformational changes do not enhance the specificity (*4*).

Pauling and others have shown that enzymatic catalysis will, in general, be greatest when the free enzyme is complementary to the transition state for reaction of its substrate (*15, 16*). This still holds when the enzyme surrounds the substrate in the transition state. That is, the rate constant for reaction of free enzyme and free substrate, k_{cat}/K_m, is greatest when the closed conformation of the enzyme is more stable than the open conformation. This can be understood as follows. The value of k_{cat}/K_m is represented by the free energy difference between the ground state and the rate-limiting transition state. If the closed conformation, ^cE, is the stable ground state, then this free energy difference is that for $^cE + S \rightarrow {}^cE \cdot S^{\neq}$. However, if the open conformation, ^oE, is the stable ground state conformation, then the conformational change $^oE \rightleftarrows {}^cE$ is unfavorable, and the free energy difference from the ground state to the transition state is that for $^oE + S \rightarrow {}^cE \cdot S^{\neq}$, which is greater than the free energy difference for $^cE + S \rightarrow {}^cE \cdot S^{\neq}$. Thus, even in this system with a conformational change that contributes to catalysis, the conclusion holds that an enzyme should be complementary to the transition state. This is the case as long as the binding of substrate and the conformational change between the open and closed forms of the enzyme do not provide kinetic barriers that are rate limiting.

Another exception: Induced fit can provide specificity if the rate-limiting step is a binding step for one substrate and a chemical step for a competing substrate. The analysis by Fersht which showed that induced fit cannot provide specificity was for the C mechanism; it assumed that a binding step was not rate limiting for either the good or the poor substrate (Fig. 2, S_1 and S_2). In this section the case is considered with a binding step rate limiting for the good substrate (S_1) and a central step rate limiting for the poor substrate (S_2). The greater intrinsic binding energy for a good substrate compared with a poor substrate allows a step that is rate limiting for the poor substrate to be faster for the good substrate. This greater intrinsic binding energy for the good substrate arises from the specific interactions of the good substrate with the active site of the enzyme. The question is asked whether or not introducing induced fit can enhance specificity in this case, and the following analysis shows that it can. Binding of substrates is assumed to occur with the same rate constant (e.g., diffusion controlled) for all of the reactions compared below.

Reaction profiles for a non-induced fit enzyme with $[S] < K_m$ are shown in Figs. 5A and 5B. The reaction with a good substrate, S_1, is shown in Fig. 5A; the rate-limiting step is binding of S_1 as is depicted by "$\neq$" in the reaction profile. The

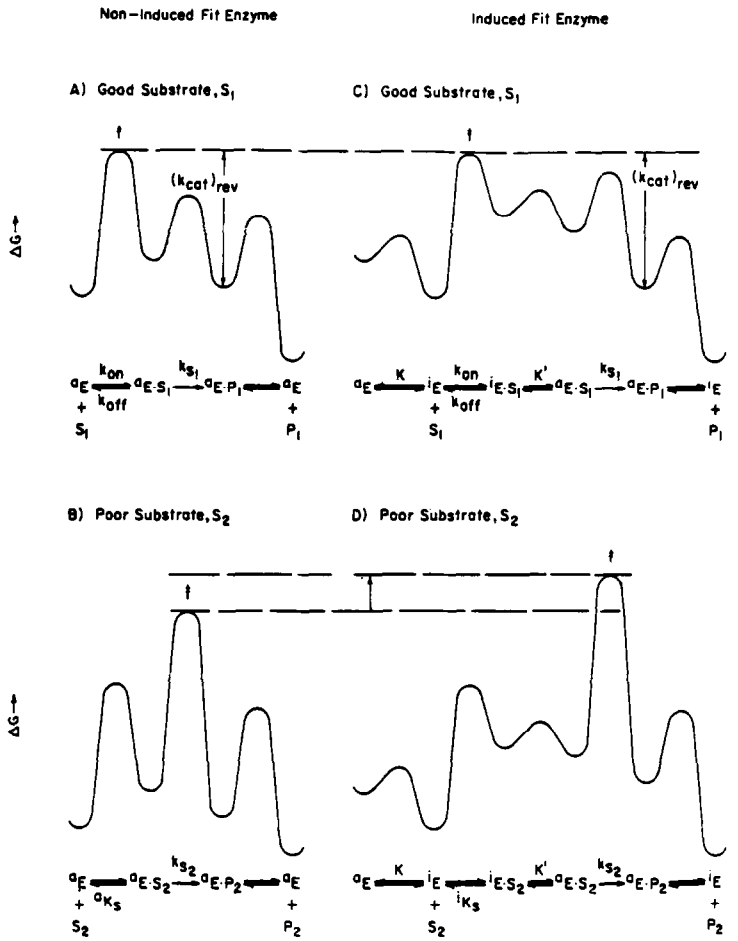


FIG. 5. Comparison of free energy profiles ([S],[P] < K_m) with a non-induced fit enzyme ((A) + (B)) and an induced fit enzyme ((C) + (D)) for the reaction of a good substrate, S1, with rate-limiting binding, ((A) + (C)) and for the reaction of a poor substrate, S2, with a rate-limiting chemical step ((B) + (D)).

reaction of a poor substrate, S_2, with the same non-induced fit enzyme is shown in Fig. 5B; in contrast to the reaction with S_1, the rate-limiting step for the reaction with S_2 is a central step (e.g., the chemical conversion[2]). This step: $^aES_2 \rightarrow {}^aEP_2$, is depicted by "$\neq$" in the reaction profile of Fig. 5B.

Figures 5C and 5D show reaction profiles for the induced fit enzyme that is analogous to the non-induced fit enzyme of Fig. 5A and 5B. As in the earlier comparisons, ^aE is the same for the induced fit and non-induced fit mechanisms but ^iE is the stable, ground state form of the induced fit enzyme, and the conformational changes are assumed to be fast. The reaction of this induced fit enzyme with the good substrate, S_1, is shown in Fig. 5C; the rate-limiting step is diffusion-controlled binding ($\neq$ in Fig. 5C), as for the non-induced fit enzyme ($\neq$ in Fig. 5A). The reaction with the poor substrate, S_2, is shown in Fig. 5D; the rate limiting step is the conversion $^aES_2 \rightarrow {}^aEP_2$ ($\neq$ in Fig. 5D).

The induced fit and non-induced fit enzymes can now be compared. For the good substrate, S_1, the energy in going from the ground state of free enzyme and free substrate to the transition state of the rate limiting step ($\neq$ in Figs. 5A and 5C) is the same for the non-induced fit and the induced fit enzymes since binding is assumed to have the same rate constant in both cases. This is shown by the single dashed line in Figs. 5A and 5C. Thus, introduction of induced fit does not change the rate of reaction with the good substrate.

For the poor substrate, S_2, the energy of the rate-limiting central conversion is greater for the induced fit enzyme than for the non-induced fit enzyme; this is shown by the dashed line in the comparison of Fig. 5D with Fig. 5B. Thus introduction of the induced fit mechanism slows the reaction that has a rate-limiting central step.

Specificity can be defined as the ratio of rate constants for the good substrate and the poor substrate with $[S] \ll K_m$ (k_{cat}/K_m conditions), as described above. The rate constant for the poor substrate is decreased by the introduction of the induced fit mechanism, while the rate constant for the good substrate is unaffected. Therefore introduction of induced fit increases specificity. An additional advantage from induced fit can be obtained when binding is rate limiting if binding to ^{i}E is faster than binding to ^{a}E; this could result from steric or electrostatic differences in ^{i}E and ^{a}E. It should be noted that the conformational change of ^{i}ES to ^{a}ES need not be less favorable for the poor substrate in order for specificity to result from the conformational change.

This enhancement of specificity by introduction of a conformational change can be shown formally. The non-induced fit enzyme exhibits Briggs–Haldane kinetics with the good substrate, S_1. That is, all subsequent steps are faster than the dissociation of the ES complex (Fig. 5A: e.g., $k_{S1} \gg k_{off}$). Thus $k_{cat}/K_m = k_{on}$ and $k_{cat} = k_{S1}$. In the induced fit mechanism with the good substrate (Fig. 5C) Briggs–Haldane kinetics still hold, and the kinetic parameters are unaffected.

In contrast, the poor substrate (S_2) exhibits Michaelis–Menten kinetics, with the central step slower than the rate constant for dissociation (Fig. 5B: $k_{S2} \ll k_{off}$). Thus in the non-induced fit mechanism with the poor substrate $k_{cat}/K_m = k_{S2}/{}^{a}K_S$ and $k_{cat} = k_{S2}$. In the induced fit enzyme the equilibrium constant to form the active conformation of the free enzyme, K, is unfavorable, but the active form of the enzyme is the same as for the non-induced fit enzyme (Fig. 5D). Thus $k_{cat}/K_m = K(k_{S2}/{}^{a}K_S)$, with $K \ll 1$.

The specificity ratio, which is the ratio of values of $\{k_{cat}/K_m\}$ (Eq. [2]), is greater for the induced fit enzyme (Eq. [5]) than for the non-induced fit enzyme (Eqs. [6] and [7]):

$$(v_1/v_2)_{\text{induced fit}} = \frac{k_{on}}{K(k_{S2}/{}^{a}K_S)} \times \frac{[S_1]}{[S_2]}; \quad K < 1 \qquad [5]$$

$$(v_1/v_2)_{\text{non-induced fit}} = \frac{k_{on}}{K_{S2}/{}^{a}K_S} \times \frac{[S_1]}{[S_2]} \qquad [6]$$

$$(v_1/v_2)_{\text{induced fit}} > (v_1/v_2)_{\text{non-induced fit}}. \qquad [7]$$

This holds as long as the conformational change, $^{i}ES_1 \rightleftarrows {}^{a}ES_1$, does not become rate limiting; it must be fast relative to the off rate (k_{off} in Fig. 5C).

Induced fit can also provide specificity if the rate-limiting step is product dissociation for one substrate and a chemical step for a competing substrate. Specificity can also be enhanced by introduction of the induced fit mechanism when dissociation of product is rate limiting; this is the reverse of the reactions shown in Fig. 5. Note that the rate-limiting transition state is the same for the forward and reverse reaction with $[S] < K_m$ (e.g., "≠" in Fig. 5A) so that any change in k_{cat}/K_m for the forward reaction will obtain for the reverse reaction as well. This can also be seen from the Haldane equation, $K_{eq} = [P]/[S] = (k_{cat}/K_m)_{for}/(k_{cat}/K_m)_{rev}$. Since K_{eq} is not affected by the enzyme, any change in the forward rate under k_{cat}/K_m conditions is also reflected in the rate of the reverse reaction. Thus, the changes in values of $\{k_{cat}/K_m\}$ that give enhanced specificity for the forward reaction upon introduction of induced fit also give enhanced specificity for the reverse reaction.

In addition, when dissociation of product is rate limiting induced fit can provide an increase in k_{cat}. Figure 5 shows free energy profiles with $[S] < K_m$ and $[P] < K_m$. Under k_{cat} conditions the concentration of substrate in the reverse direction (P) is much greater than K_m, so that the energy of the free enzyme and substrate species (E + P) is raised and the barrier for binding to form EP is insignificant.

Figure 5A shows a non-induced fit mechanism for substrate S_1, in which binding is the rate-limiting step. In the reverse reaction dissociation of the product (S_1) is the rate-limiting step. The value of k_{cat} is estimated from the energy difference between the most stable enzyme/substrate species and the transition state of highest energy; this is shown by $(k_{cat})_{rev}$ in Fig. 5A.[7]

The same reaction with the induced fit enzyme is shown in Fig. 5C. The complex $^{a}EP_1$ is destabilized in the induced fit mechanism relative to the non-induced fit mechanism (Figs. 5A and 5C), while the free energy of the rate-limiting transition state ("≠") is the same for both the induced fit and non-induced fit enzymes (Figs. 5A and 5C; dashed line). Thus, $(k_{cat})_{rev}$ is greater for the induced fit enzyme than for the non-induced fit enzyme (Figs. 5A and 5C). This increase in the value of $(k_{cat})_{rev}$ results from a lower barrier for the rate-limiting dissociation of S_1 relative to the ground state $^{a}E \cdot P_1$ complex for the induced fit enzyme. The induced fit enzyme avoids the large barrier for dissociation from the non-induced fit enzyme ($^{a}E \cdot S_1 \rightarrow S_1$; Fig. 5A) by following an alternate dissociation pathway that includes the conformational change $^{a}E \cdot S_1 \rightarrow {}^{i}E \cdot S_1$, prior to the more facile dissociation $^{i}E \cdot S_1 \rightarrow {}^{i}E + S_1$ (Fig. 5C).

Since k_{cat} is increased and k_{cat}/K_m is unaffected by the conformational change when product dissociation is rate limiting, the value of K_m increases for the induced fit enzyme. The utility of an increase in K_m at constant k_{cat}/K_m is discussed above. Alber (*17*) has discussed the analogous increase in k_{cat} with rate-limiting dissociation from the specific conformational change of a flexible free enzyme going to a rigid active enzyme upon binding of substrate.

Reactions with substrate binding, product dissociation, and the central conversion rate limiting have been analyzed in the preceding sections. Specificity relative

[7] As in other analyses, a single rate-limiting transition state is assumed (see footnote 4).

to a second substrate with a rate-limiting central conversion is unaffected by the C induced fit mechanism, which has the central conversion rate limiting for the good substrate as well. However, specificity can be enhanced relative to this second substrate when substrate binding or product dissociation is rate limiting for the good substrate. When a single step is not fully rate limiting under k_{cat}/K_m conditions intermediate results will obtain.

SITUATIONS IN WHICH INDUCED FIT CAN PROVIDE SPECIFICITY *IN VIVO*

Fersht's analysis shows that the C induced fit mechanism does not provide specificity at any constant concentration of two competing substrates. Exceptions to this analysis have been discussed above. In addition, there are three situations which might result in greater specificity *in vivo* for a C induced fit enzyme than for the analogous non-induced fit enzyme. The first occurs when the concentration of substrate oscillates or changes with time, the second occurs when the desired substrate is generated at a constant rate in a prior metabolic step, and the third occurs when enzyme is in excess of substrate.

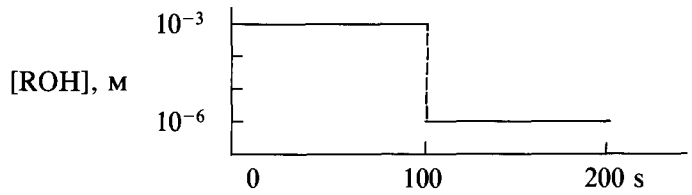
The specificity of a kinase for the reaction of ATP with a substrate compared to reaction with water in a kinase reaction is described for simplicity (Scheme 3). This treatment is analogous to that for specificity between two substrates.

Temporal oscillations or changes in substrate concentration. Oscillations or changes in substrate concentration with time provide a situation in which induced fit could give specificity *in vivo*. This result is most dramatic and most easily understood in the case of an oscillation in substrate concentration from saturating to subsaturating.

The greater specificity of the induced fit enzyme over a cycle of changing substrate concentration can be shown with a numerical example. The induced fit and non-induced fit enzymes of Scheme 3 are compared over the cycle with 10^{-3} M substrate (ROH) for 100 s and then 10^{-6} M ROH for a second 100 s (Scheme 4).

The amount of product formed and the amount of ATP hydrolyzed with 1 nM enzyme are shown in Scheme 4. The rates of product formation and of ATP hydrolysis for both the induced fit enzyme and the non-induced fit enzyme (Schemes 3A and 3B, respectively) are given in Table 2, as was discussed above. From these rates, the amount of product formed and the amount of ATP hydrolyzed in each of the time intervals of the cycle are determined from the rates in Table 2 and are listed in Scheme 4. For example, for the induced fit enzyme 91 μM product is formed with 10^{-3} M ROH for 100 s (0.91 μM s^{-1} × 100 s = 91 μM), and 9.1×10^{-5} μM ATP is hydrolyzed. For the non-induced fit enzyme 100 μM of product is formed with 10^{-3} M ROH for 100 s, and 1.0×10^{-4} μM ATP is hydrolyzed. The values listed in Scheme 4 for product formation and ATP hydrolysis at 10^{-6} M ROH in the second 100 s are similarly obtained from Table 2.

The amount of product formed and ATP hydrolyzed over the 200-s cycle obtained by summing over the cycle is listed in the last column of Scheme 4 for the induced fit enzyme and for the non-induced fit enzyme. The specificity ratio,



	t = 0–100 s	t = 100–200 s	Total
Induced fit enzyme			
Product formed (μM)	91	0.99	92
ATP hydrolyzed (μM)	9.1×10^{-5}	9.9×10^{-4}	1.1×10^{-3}
Specificity ratio	10^6	10^3	9×10^4
Non-induced fit enzyme			
Product formed (μM)	100	50	150
ATP hydrolyzed (μM)	1.0×10^{-4}	5.0×10^{-2}	5.0×10^{-2}
Specificity ratio	10^6	10^3	3×10^3

SCHEME 4

which is the ratio of product formed to ATP hydrolyzed, is 9×10^4 for the induced fit enzyme and 3×10^3 for the non-induced fit enzyme over the cycle (Scheme 4). Thus the induced fit enzyme provides greater specificity over the cycle than is provided by the non-induced fit enzyme. The enhanced specificity occurs despite the identical specificity of the two enzymes at each concentration of substrate (see Table 2, specificity ratio). This mechanism will hold for specificity between two substrates if one substrate concentration varies with time while the other remains constant.

The specificity of the induced fit enzyme is increased because product formation occurs at maximal velocity at high substrate concentration for both enzymes (k_{cat} is not changed by induced fit) and hydrolysis is turned off at low substrate concentrations only for the induced fit enzyme (most of the induced fit enzyme is in the inactive conformation, ^iE).

The above conclusion that induced fit increases specificity when there are temporal changes in substrate concentration can be described as follows. The induced fit enzyme and the analogous non-induced fit enzyme of Schemes 3A and 3B have the same value of k_{cat} and the same specificity at a given concentration of substrate (ROH), as was described above (Table 2). Therefore, in the first time interval of Scheme 4 at saturating substrate both enzymes will form nearly equal amounts of product, and nearly equal amounts of ATP will be hydrolyzed in the reaction with water.

The induced fit enzyme has a lower value of k_{cat}/K_m than the non-induced fit enzyme since most of the induced fit enzyme is in an inactive conformation in the absence of substrate (ROH) (see Table 1). Therefore, in the second time interval of Scheme 4 at subsaturating substrate the non-induced fit enzyme will give more product than the induced fit enzyme. However, the amount of product formed in the second time interval is small relative to that formed in the first time interval, so

both the induced fit and the non-induced fit enzymes give about the same amount of phosphorylated product over the cycle.

In contrast, the hydrolysis of ATP is greater for both enzymes in the second time interval with subsaturating substrate than in the first time interval with saturating substrate. This occurs because less of the enzyme is tied up with bound substrate. ROH, in the second time interval, while in the first time interval the high concentration of substrate inhibits the reaction with water by competing for the active site. Furthermore, the non-induced fit enzyme gives much more ATP hydrolysis in the second time interval than the induced fit enzyme as it is all in an active conformation (^{a}E), whereas the induced fit enzyme is nearly all in an inactive conformation (^{i}E).

The non-induced fit enzyme thus gives significantly more ATP hydrolysis than the induced fit enzyme over the cycle, but about the same amount of product, as was shown numerically in Scheme 4. Again, the ratio of product formed to ATP hydrolysis is greater for the induced fit enzyme than for the non-induced fit enzyme so that the induced fit enzyme provides greater specificity than the non-induced fit enzyme over the cycle of substrate concentration.

Generation of substrate at a constant rate. When a substrate is generated at a constant rate, a cell containing the induced fit enzyme will have a higher steady state concentration of substrate than a cell containing the analogous non-induced fit enzyme. This buildup of substrate in a cell containing the induced fit enzyme results in greater specificity relative to a cell with the non-induced fit enzyme.

This enhanced specificity with the induced fit enzyme can be shown numerically. As in the above analyses, the induced fit enzyme of Scheme 3A is compared with the analogous non-induced fit enzyme of Scheme 3B. For this case, substrate ROH is generated at a constant rate $k_1[A]$ (Scheme 5; the concentration of A is constant). The rate constant k_2 in Scheme 5 is smaller for an induced fit enzyme (Scheme 3A) than for the analogous non-induced fit enzyme (Scheme 3B). That is, the induced fit enzyme is slower than the non-induced fit enzyme at all concentrations of ROH, as is shown by Fig. 4 and by the rates of reaction for the two mechanisms at various concentrations of ROH listed in Table 2. Since ROH is generated at a constant rate and k_2 is smaller for the induced fit enzyme than for the non-induced fit enzyme, ROH will accumulate in the steady state to a greater extent with the induced fit enzyme. For example, when ROH is generated at a rate of 9.9×10^{-3} μM s^{-1} the concentration of ROH is 10^{-8} M with the non-induced fit enzyme; this is the concentration of ROH that gives the reaction rate for ROH that matches the rate of formation of ROH (Table 2). At this concentration of ROH (10^{-8} M) the specificity ratio is 10 (Table 2). With the induced fit enzyme, the concentration of ROH that matches the rate of flux of 9.9×10^{-3} μM s^{-1} is 10^{-6} *M*, and the specificity is 10^3 (Table 2). Thus the specificity with the induced fit enzyme is 10^2-fold greater than with the non-induced fit enzyme.

$$A \xrightarrow{k_1} ROH \xrightarrow{k_2} ROPO_3^{=}$$

SCHEME 5

Two factors are responsible for the enhanced specificity with the induced fit

enzyme: (1) the presence of the slower induced fit enzyme results in a higher steady-state concentration of the substrate than occurs in the presence of the non-induced fit enzyme, and (2) specificity increases with increasing concentration of substrate (Table 2, specificity ratio).

It could be argued that this mechanism for specificity may be of limited utility because lowering the concentration of the non-induced fit enzyme in a cell would also increase the specificity. Lowering the concentration of enzyme would lower the rate of the reaction; thus more substrate would accumulate (factor 1 above). In addition, the higher concentration of substrate gives greater specificity since there is less free enzyme to catalyze the side reaction (factor 2 above). Although this is true, the induced fit enzyme might be more advantageous than a lower concentration of the non-induced fit enzyme because the induced fit enzyme at higher concentration would provide a greater maximal rate of turnover with saturating substrate. In addition, the concentration of an enzyme may be set by the formation of complexes with other enzymes or by the coordination of its synthesis with that of other enzymes.

The values above were calculated for discrimination against water, but specificity between two substrates via this mechanism would also be obtained, provided that the concentration of only one of the substrates is determined primarily by the rate constant, k_2, of the enzyme in question.

Enzyme in excess of substrate. Determinations of the intracellular concentrations of enzymes and their substrates suggest that the concentration of an enzyme is often greater than the concentration of its substrate (*18*). When the enzyme is in excess of substrate, induced fit can increase discrimination between a good substrate that operates under k_{cat} conditions and a poor substrate that operates under k_{cat}/K_m conditions.

When the enzyme is in excess of substrates the reactions of each substrate are independent. Thus, the decrease in k_{cat}/K_m for a poor substrate upon introduction of the induced fit mechanism and the lack of an effect on k_{cat} for a good substrate result in greater discrimination against the poor substrate with induced fit. In contrast, with substrate in excess of enzyme the ratio of values of k_{cat}/K_m for two competing substrates, which determines discrimination (Eq. [2]), is unaffected by induced fit, as described above; the values of k_{cat} alone are not pertinent to specificity when substrate is in excess.

The example shown in Fig. 6 is used to illustrate the increased specificity from induced fit when enzyme is in excess. For an enzyme without induced fit, the rates as a function of enzyme concentration at a given concentration of a good substrate, S_1, and a poor substrate, S_2, are shown by the solid lines in Fig. 6. At enzyme concentration $[E]_1$ substrate S_1 is saturating (the enzyme is under k_{cat} conditions) and substrate S_2 is subsaturating (the enzyme is under k_{cat}/K_m conditions). The ratio of rates that determines discrimination, v_1/v_2, is determined from the rates in Fig. 6 for each substrate in the absence of the other substrate. This is because each substrate ties up an insignificant amount of enzyme, leaving the two reactions essentially independent even though they occur in the same vessel. Thus, at enzyme concentration $[E]_1$ the specificity ratio is v_1'/v_2' (Fig. 6).

With induced fit k_{cat}/K_m is decreased for both substrates, but k_{cat} for the good

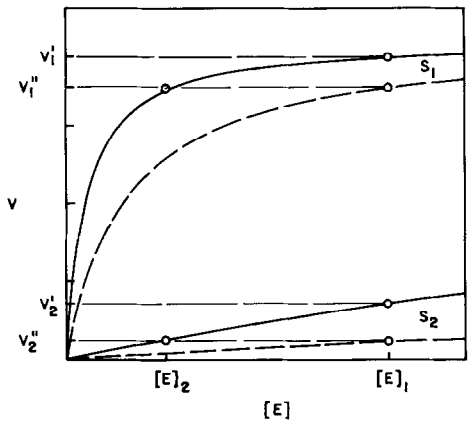


FIG. 6. Rate with varying concentration of enzyme with a good substrate, S_1, and with a poor substrate, S_2, for a non-induced fit enzyme (—) and for an induced fit enzyme (– – –). The concentrations of S_1 and S_2 are fixed such that $[E] \gg [S_1] = [S_2]$, $E_1 > K_m(S_1)$, and $E_1 < K_m(S_2)$.

substrate, S_1, is unaffected, as described above (see Effect of induced fit on K_m). This is shown by the heavy dashed lines in Fig. 6. At enzyme concentration $[E]_1$ the rate for S_1 (v_1'') is only slightly affected by induced fit, while the rate for S_2 (v_2'') is decreased by greater than twofold. Thus, discrimination is greater with induced fit than without induced fit ($v_1''/v_2'' > v_1'/v_2'$).

It should be noted that the specificity ratio can also be increased by lowering the concentration of the non-induced fit enzyme. For example, lowering the concentration from $[E]_1$ to $[E]_2$ gives nearly the same specificity as is given by introducing induced fit (v_1''/v_2'', Fig. 6). As described in the previous section, the introduction of induced fit can still be useful.

Other possible roles for conformational changes. In the above analyses, hypothetical enzymes that do and do not undergo conformational changes have been compared in order to determine which gives the greatest specificity and catalysis. However, there may be situations in which it is not possible to construct an enzyme that does not undergo a conformational change during catalysis. For example, it may not be possible for the peptide backbone of an enzyme to arrange catalytic groups as precisely and as rigidly as is required for optimal catalysis. Some fine tuning of alignment and "freezing" of catalytic groups might occur only after binding of substrate (*6*). This is reasonable since the active site of the free enzyme is solvated by water while the active site of the enzyme with bound substrate is "solvated" by the substrate.

The requirement of complementarity to the transition state and not to the ground state for catalysis may lead to conformational changes upon binding of substrate (*3, 6, 15, 16*).

Conformational changes may occur in the course of successive steps of a reaction when the individual steps have different requirements for catalysis (*12*). Such multiple conformations could include a conformation that optimizes the rate of substrate binding, even if the substrate is not subsequently surrounded by the enzyme. In order to gain an advantage from multiple conformations the rate

advantage from each conformation must outweigh the rate disadvantage from lowering the concentration of conformations that catalyze other steps of the reaction.

Enzymes are composed of domains, and there are similarities in domains of related enzymes that are consistent with a comon evolutionary origin. For example, there are similarities in the ATP binding domain of several kinases and in the NAD binding domain of several dehydrogenases (*7, 19* and see below). A simple way for one domain, e.g., an ATP binding domain, to interact with a domain that binds a second substrate may be via a conformational change that brings the two domains together.

It has been suggested that slow enzymatic reactions are necessary for control phenomena (*20*). As shown above, conformational changes can slow enzymatic reactions by decreasing k_{cat}/K_m and k_{cat}. Another possible role of a conformational change in metabolic control is to increase the value of K_m in order to change the range of substrate concentration that gives the greatest change in rate.

SOME EXAMPLES OF CONFORMATIONAL CHANGES

Evidence for conformational changes of several enzymes is reviewed briefly in this section; detailed reviews have been published (*7, 21*). Possible roles for enzymes surrounding their substrates are emphasized.

Kinases. Hexokinase is the classic example of an enzyme that undergoes a conformational change. Crystal structures of similar isozymes of hexokinase have been solved and revealed "closed" and "open" forms of the enzyme with and without bound glucose, respectively (*22*). The bound glucose molecule is virtually buried from solvent in the closed structure (*23, 24*), and it is unlikely that it could dissociate without a conformational change of the enzyme. Furthermore, a conformational change upon glucose binding has been observed by small angle X-ray scattering in solution, and the change in the radius of gyration is that expected based on the open and closed crystal structures (*25*). The increase in ATP hydrolysis catalyzed by hexokinase with bound lyxose or xylose is consistent with a sugar-induced conformational change that activates the enzyme (*26*).

Evidence for a conformational change of phosphoglycerate kinase upon addition of phosphoglycerate and ATP has also been obtained from small angle X-ray scattering in solution (*27*). Furthermore, it has been suggested, based on similarities of X-ray crystallographic structures of hexokinase, phosphoglycerate kinase, and adenylate kinase, that kinases may generally have active sites in deep clefts and that these clefts may close around the substrate upon binding, as for hexokinase (*23*). Recent reports of similarities in the primary sequence of binding sites for ATP (and GTP) in several enzymes are consistent with similar modes of catalysis (*28*).

The conformational changes of kinases that surround the substrate on all sides may provide greater intrinsic binding energy than would otherwise be available in order to enhance catalysis and specificity, as described above.

It has been suggested that the exclusion of water upon surrounding the sub-

strates can lower the dielectric constant of the active site in kinase, dehydrogenase, and decarboxylase reactions (*23, 29, 30*). However, the exclusion of water upon surrounding the substrate may in general be a by-product of the need to establish specific interactions for catalysis. The exclusion of water could provide catalysis in some instances. For example, although there is no direct evidence for surrounding the substrate in decarboxylase reactions, there is chemical precedent for a rate increase from lowering the dielectric constant (*31*). Also, lowering the dielectric constant might increase the rate of a kinase reaction by stabilizing a dissociative, metaphosphate-like transition state, in which there is electron donation from the phosphoryl oxygen atoms, although the rate of hydrolysis of acetyl phosphate is insensitive to changes in solvent (*32*). However, water is excluded whenever an enzyme–substrate complex is formed. Thus, the exclusion of water is inextricable from the establishment of specific enzyme–substrate interactions.

Triose phosphate isomerase (TIM).[8] X-ray crystallographic analysis of TIM suggests the presence of a flexible region of 10 amino acid residues (*12, 33*). The structure in the presence of substrate suggests that this flexible region behaves as a flap: it closes over the substrate and becomes rigid. Thus, a conformational change may be required to provide access to the active site (*12*), and TIM may derive some of its intrinsic binding energy from the additional interactions formed by surrounding its substrate.

In addition, Alber has pointed out that this conformational change of flexible to rigid can enhance catalysis for TIM (*17*). In the conversion of dihydroxyacetone phosphate to glyceraldehyde-3-phosphate the dissociation of glyceraldehyde-3-phosphate is partially rate limiting, and in the reverse reaction under k_{cat}/K_m conditions the binding of glyceraldehyde-3-phosphate is partially rate limiting (*34*). As explained above, when product dissociation is rate limiting the introduction of a conformational change can increase both specificity and the value of k_{cat}; in the reverse direction, with rate-limiting binding, the conformational change can increase specificity.

It is of interest to ask whether or not a flexible enzyme in general provides a catalytic advantage relative to a rigid enzyme that has the same catalytic groups and the same conformation in the transition state. The conclusions from the analysis of the C induced fit enzyme, that specificity is not increased and catalysis is decreased, can be generalized to the flexible enzyme, which behaves in exactly the same way. In the present comparison, in which a central step is rate limiting (Fig. 7), the rigid enzyme, E_R, is the active enzyme in both cases; the flexible enzyme, E_F, is the stable species of one enzyme, while E_R is the stable species of the other enzyme. The induced fit enzyme and the flexible enzyme both undergo an unfavorable conformational change prior to adopting the active catalytic conformation, ^aE or E_R (cf. Figs. 7B and 2).

The situation can be described as follows. Intrinsic binding energy from the interaction of substrate with enzyme is used to convert the enzyme from a flexible

[8] Abbreviations used: TIM, triose phosphate isomerase; OAA, oxaloacetate; AcCoA, acetyl coenzyme A.

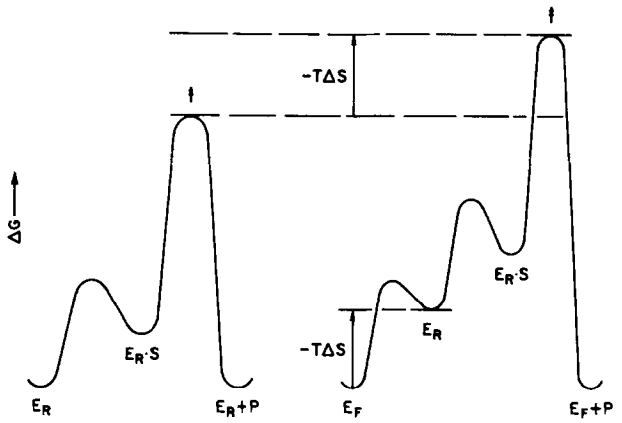


FIG. 7. Comparison of free energy profiles with [S] < K_m for the reaction catalyzed by a rigid enzyme (A) with the reaction catalyzed by a flexible enzyme that undergoes a conformational change to a catalytically active rigid form (B).

to a rigid state that is adopted in the E_RS and $E_RS^{\neq}$ complexes. Energy is required because entropy is lost in converting E_F to E_R, the change from a flexible to a rigid conformation. This loss occurs in forming both E_RS and $E_RS^{\neq}$ (Fig. 7; $-T\Delta S$). Thus, creating a more flexible enzyme decreases binding of the substrate and transition state relative to that for the hypothetical rigid enzyme, E_R; introduction of the flexible enzyme or the induced fit enzyme results in the unproductive use of intrinsic binding energy. The value of k_{cat}/K_m for the flexible enzyme is decreased relative to that for the rigid enzyme, so that introducing the initial step decreases, rather than increases, catalysis. In addition, the energy for conversion of E_F to E_R is the same for all substrates so that specificity is unaffected.

Although the above argument might suggest that a rigid enzyme would be more efficient in general than a flexible enzyme, Petsko and co-workers have suggested that advantages for flexible enzymes include the ability to catalyze more than one step in a reaction and the ability to allow a fast conformational change upon substrate binding (*12*). As described above, a conformational change is required when the enzyme's active site surrounds the substrate.

Dehydrogenases. X-ray crystal structures of lactate dehydrogenase, liver alcohol dehydrogenase, and glyceraldehyde-3-phosphate dehydrogenase have been solved in the presence and absence of bound substrate analogs (*7, 29*). Differences between the structures with and without bound ligands suggest that a conformational change takes place for each enzyme. These conformational changes result in the active site surrounding the substrates.

Surrounding the active site could result in increased intrinsic binding energy, and thus enhanced catalysis and specificity. A second role of surrounding the active site could be to slow the dissociation of products of the reaction, the utility of which is described below.

Bernhard and co-workers have shown that NADH can be transferred directly between dehydrogenases of opposite stereospecificity toward NAD/NADH (*18, 35, 36*). Slower dissociation of NADH could result in more transfer of NADH to a

second dehydrogenase relative to dissociation, and a conformational change, such as surrounding the active site, could slow dissociation of NADH. In order to provide an advantage toward transfer of NADH over dissociation, energy from the interaction of the two enzymes must be used to facilitate the conformational change. NADH dissociation or a conformational change preceding dissociation is rate limiting for several dehydrogenases under k_{cat} conditions (*37*). Evidence for an analogous transfer of diphosphoglycerate between phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase has also been obtained (*35*).

Slower dissociation of intermediates, which would be advantageous in preventing the release of a reactive species, might also result from conformational changes. Intrinsic binding energy can be used to stabilize enzyme-bound intermediates that are unstable in solution (*3*); this stabilization slows the dissociation of an intermediate. The intrinsic binding energy from surrounding a substrate could, of course, contribute to this slow dissociation. In addition, a conformational change on a reaction path could provide a kinetic barrier to dissociation of an intermediate. In the reverse reaction, which can be approximated by the binding of a transition state analog, association could be slowed. Apparent association rate constants well below diffusion-controlled have been observed for many transition state analogs (*8*).

There is evidence that the periplasmic arabinose-binding protein and related binding proteins undergo conformational changes in order to surround their ligands (*38*). These proteins transfer their ligands to membrane transport proteins. The closed conformation could increase the intrinsic binding energy and thus decrease the loss of ligand to solvent and could provide a signal for binding of the membrane transport protein, which internalizes the ligand. As in the case of NADH transfer, energy from the protein–protein interactions must be used to facilitate the conformational change in order to provide an advantage for ligand transfer over ligand dissociation.

CITRATE SYNTHASE AND SUBSTRATE SYNERGISM

Citrate synthase provides an example of an enzyme that undergoes a conformational change and exhibits substrate synergism. It catalyzes the conversion of oxaloacetate (OAA) and acetyl coenzyme A (AcCoA) to citrate and CoA. The enzyme has been crystallized, and X-ray analysis reveals open and closed conformations (*39*). There is a deep cleft between the two domains in the open form, and the two domains surround the active site in the closed form. The conformational change to form the closed enzyme can be described by an 18° rotation of the domains (*39*), and has also been described in more detail (*40*).

There is substrate synergism for both binding and reactivity with citrate synthase. When OAA is bound to citrate synthase the apparent association constant for AcCoA is increased at least 20-fold relative to the association constant for AcCoA with free enzyme (*41*). No enolization of AcCoA occurs in its binary complex with enzyme, as determined by the absence of exchange of the methyl

protons with solvent. However, upon addition of malate, a nonreactive analog of OAA, enolization of AcCoA does occur (*42*).

Induced fit and substrate synergism. Substrate synergism is usually considered to be a mechanism for specificity because a partial reaction involving just one of the substrates is slowed in the absence of the second substrate. For citrate synthase, substrate synergism might prevent a detrimental side reaction of AcCoA by preventing the stabilization of an enol intermediate or enol-like transition state until the second substrate, which normally reacts with the enol intermediate or enol-like transition state, is bound.[9] Also, for a kinase the hydrolysis of ATP from a binary complex might be prevented.

It has been suggested that induced fit can provide substrate synergism. However, the following analysis shows that although the C induced fit mechanism can give synergism in binding and reactivity, no advantage for specificity with respect to a side reaction is provided in general. This inability of the C induced fit mechanism to increase specificity against a side reaction is similar to the inability of the C induced fit mechanism to increase specificity against a competing substrate that is described above.

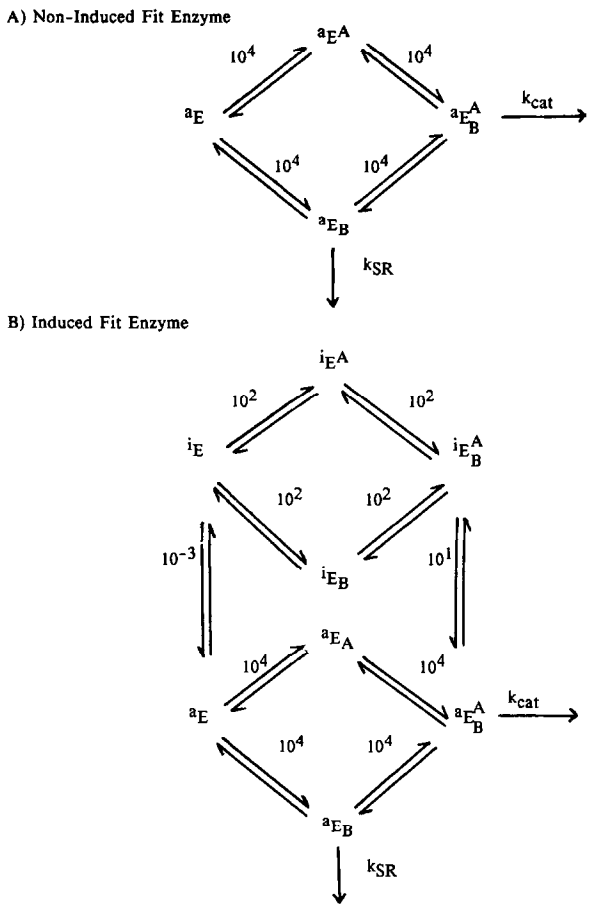
The non-induced fit mechanism and the induced fit mechanism for comparison are shown in Schemes 6A and 6B, respectively.[10] As in the comparisons above, the active form of the enzyme, ^{a}E, is the same in both mechanisms, but the induced fit mechanism has an inactive form of the enzyme, ^{i}E, as the stable form of the free enzyme. The species $^{a}E \cdot B$ can undergo a side reaction, such as an addition reaction of the enol of AcCoA for citrate synthase.

The ability of induced fit to give substrate synergism in binding can be shown using the numerical example in Scheme 6B. Substrates A and B each bind to the inactive enzyme, ^{i}E, with low affinity (10^2 M^{-1}), and bind to the active enzyme, ^{a}E, with high affinity (10^4 M^{-1}). Thus, although the free enzyme is more stable in the inactive conformation, the ternary complex, E · A · B, favors the active conformation of the enzyme ($^{i}E \rightleftarrows {}^{a}E$, $K = 10^{-3}$; $^{i}E \cdot A \cdot B \rightleftarrows {}^{a}E \cdot A \cdot B$, $K = 10^{1}$; Scheme 6B). The first substrate (A or B) binds with an observed association constant of $10^2\ M^{-1}$ (e.g., $^{i}E + A \rightleftarrows {}^{i}E \cdot A$, $K = 10^2\ M^{-1}$; Scheme 6B). The second substrate binds to form the ternary complex with an observed association constant 10^3 M^{-1} (e.g., $^{i}E \cdot B + A \rightleftarrows {}^{a}E \cdot A \cdot B$, $K = 10^3$ M^{-1}; Scheme 6B). Each substrate has a higher association constant when it binds to the binary complex, rather than to the free enzyme. Thus there is synergism in binding.

There is also substrate synergism in reactivity with induced fit. The ternary complex, E · A · B, favors the active enzyme conformation ($^{i}E \cdot A \cdot B \rightleftarrows {}^{a}E \cdot A \cdot B$, $K = 10$; Scheme 6B). In contrast, the inactive conformation of the enzyme is favored in the binary complex, E · B ($^{i}E \cdot B \rightleftarrows {}^{a}E \cdot B$, $K = 10^{-1}$; Scheme 6B). Thus, a reaction involving substrate B, such as enolization of AcCoA, occurs to a significant extent only with both substrates bound. Intrinsic binding energy from both A and B is necessary to stabilize the active conformation of the enzyme.

[9] It should be noted that it is not known if the enol of AcCoA is an intermediate in the formation of citrate or if proton abstraction and carbon–carbon bond formation are concerted.

[10] The constants listed in Schemes 6 and 7 are association constants, with units M^{-1}, for binding steps, or unitless constants for the interconversion of inactive enzyme to active enzyme.



SCHEME 6

Although induced fit provides synergism in binding and reactivity, there is no additional specificity over that for the non-induced fit enzyme. Discrimination between the desired reaction and the side reaction of substrate B is determined from the ratio of the concentrations of the species that give the reactions [^{a}E · A · B]/[^{a}E · B]. This ratio, and thus specificity, is the same for the non-induced fit and the induced fit mechanisms: ^{a}E · A · B/^{a}E · B = 10^4[A] (^{a}E · B + A $\rightleftarrows$ ^{a}E · A · B, $K = 10^4$; Schemes 6A and 6B).[11]

The induced fit mechanism in Scheme 6B is unable to enhance the specificity of citrate synthase against enolization of AcCoA because introduction of the conformational change, ^{i}E $\rightleftarrows$ ^{a}E, merely lessens the amount of active enzyme; the concentrations of all of the active forms of the enzyme are decreased proportionally by induced fit, which leaves unaffected the ratio of catalysis of the desired reaction to catalysis of the side reaction. The decrease in rates of reactions occurs

[11] The rate constants k_{cat} and k_{SR} are also involved in the discrimination ratio $v_{cat}/v_{SR} = k_{catl}$[^{a}E · A · B]/$k_{SR}$[^{a}E · B], but these rate constants are the same for the non-induced fit and the induced fit mechanisms of Schemes 6A and 6B.

because the induced fit enzyme uses intrinsic binding energy to drive the conformational change of inactive to active enzyme (${}^{i}E \rightleftarrows {}^{a}E$) rather than to stabilize the transition state of the reaction, as for the non-induced fit enzyme. This is analogous to the decrease in k_{cat}/K_m described above for a hypothetical kinase reaction (see Effect of induced fit on K_m).

There is an exception to the above analysis that allows an increase in specificity relative to random equilibrium binding of substrates, which can occur when the binding and conformational change steps are not all rapid relative to catalysis. This could result in a lower steady-state concentration of the binary complex, ${}^{a}E \cdot B$, which undergoes the side reaction, relative to the concentration of ${}^{a}E \cdot B$ with equilibrium binding, and thus could lessen a side reaction. This lower steady-state concentration of $E \cdot B$ occurs, for example, with rate constants and concentrations such that binding of A to $E \cdot B$ and reaction of $E \cdot A \cdot B$ are both fast relative to dissociation of B from $E \cdot B$; in this case as soon as $E \cdot B$ is formed it binds A and goes to product, so that $E \cdot B$ does not accumulate. With induced fit the steady-state concentration of ${}^{a}E \cdot B$ could be lowered still further relative to random equilibrium binding due to the additional pathway for reaction of ${}^{a}E \cdot B$ of conversion to ${}^{i}E \cdot B$; in this case the steady-state concentration of ${}^{a}E \cdot B$ could be lowered by (1) rapid conversion of ${}^{a}E \cdot B$ to ${}^{i}E \cdot B$ relative to rate of dissociation of B from ${}^{a}E \cdot B$ and (2) a preferred partitioning of ${}^{i}E \cdot B$ to products rather than to ${}^{a}E \cdot B$, via ${}^{i}E \cdot A \cdot B$ and ${}^{a}E \cdot A \cdot B$.

Substrate synergism with a conformational change that causes the active site to surround the substrate. Citrate synthase could increase the intrinsic binding energy relative to a hypothetical enzyme with only an open conformation by surrounding the substrate and could thereby increase the specificity against a side reaction. This is in addition to the increase in catalysis and in specificity against a competing substrate from surrounding the substrate that was described above.

The enhanced specificity against a side reaction can be described with reference to the numerical example of Scheme 6. The mechanism of Scheme 6A has only an active enzyme conformation; the enzyme cannot surround the substrate as this requires a conformational change. Mechanism B (Scheme 6) as written has the same binding energy as the mechanism without a conformational change (Scheme 6A); but, when the conformational change is to a closed enzyme conformation there can be additional binding energy. Thus, when ${}^{a}E$ in Scheme 6B is a closed enzyme conformation the association constants for substrates could be, for example, $10^6\ M^{-1}$, instead of the values of $K = 10^4\ M^{-1}$ that are listed in Scheme 6 (e.g., ${}^{a}E + A \rightleftarrows {}^{a}E \cdot A$, $K = 10^6\ M^{-1}$ when ${}^{a}E$ is the closed enzyme, and $K = 10^4\ M^{-1}$ when ${}^{a}E$ is the open enzyme; Scheme 6B). Therefore the ratio ${}^{a}E \cdot A \cdot B/{}^{a}E \cdot B$, which determines the specificity for the desired reaction relative to the side reaction, is $10^6[A]$ for the closed enzyme and only $10^4[A]$ for the open enzyme. Specificity is increased for the enzyme that surrounds its substrate relative to the enzyme with an open active site. In addition, the value of k_{cat} could also increase from the specific interactions that occur upon surrounding the substrate to give a further advantage for the desired reaction over the side reaction.

In summary, although introduction of a conformational change can give substrate synergism in binding and reactivity, an increase in specificity relative to an

enzyme with no conformational change does not occur in the simple case. However, specificity is increased when the conformational change provides additional intrinsic binding energy or when kinetic factors decrease the steady-state concentration of an enzyme/substrate complex that undergoes a side reaction.

Conformational change with coupled or independent binding sites. A mechanism with independent conformational changes in the binding sites for each substrate gives less specificity and less catalysis than the mechanism considered above with no conformational change or the mechanism with a single coupled conformational change. Thus, a conformational change that causes the enzyme to surround its substrates in order to give more intrinsic binding energy provides more specificity and catalysis when there is a coupled conformational change than when there are independent conformational changes.

The advantage from the coupled conformational change, which gives substrate synergism, relative to independent conformational changes, which does not give substrate synergism, is shown by the numerical example of Scheme 7 with random equilibrium mechanisms.[10] Scheme 7A shows an enzyme in which the conformational change involving the binding sites for substrates A and B are coupled. This mechanism gives substrate synergism, as is described above for the analogous mechanism of Scheme 6B.

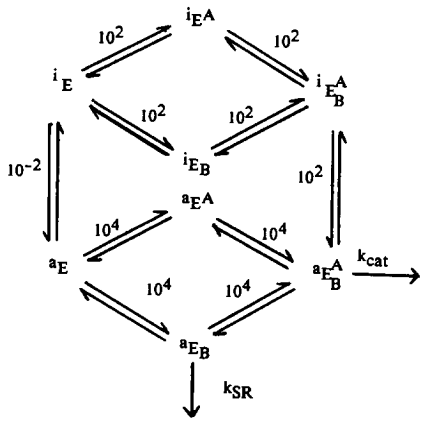
Scheme 7B shows a mechanism in which the conformational change involving each substrate is independent. This mechanism does not exhibit substrate synergism, since the binding constant for each substrate is the same whether it binds first or second (e.g., $K = 10^3$ M^{-1} for both ${}^{\cdot}_{\cdot}\mathrm{E} + \mathrm{B} \rightleftarrows {}^{\cdot}_{a}\mathrm{E} \cdot \mathrm{B}$, and ${}^{a}_{\cdot}\mathrm{E} \cdot \mathrm{A} + \mathrm{B} \rightleftarrows {}^{a}_{a}\mathrm{E} \cdot \mathrm{A} \cdot \mathrm{B}$). The binding site for substrate A is represented by the superscript and the binding site for substrate B is represented by the subscript; each site favors the active conformation when its ligand is bound.

The enzyme with coupled binding sites and the enzyme with independent binding sites can be compared since the total binding energy and the binding energy used to give the conformational change are the same for the two enzymes; the conformations ${}^{a}_{a}\mathrm{E}$ and ${}^{a}\mathrm{E}$ are identical, and the values of k_{cat} and k_{SR} are the same for the two mechanisms. For the enzyme with independent sites, binding energy from each substrate is used to provide the energy to surround only that substrate; i.e., there are two distinct conformational changes. In contrast, for the enzyme with coupled sites, binding energy from each substrate is used to provide some of the energy for surrounding both substrates; i.e., there is an all or none conformational change.

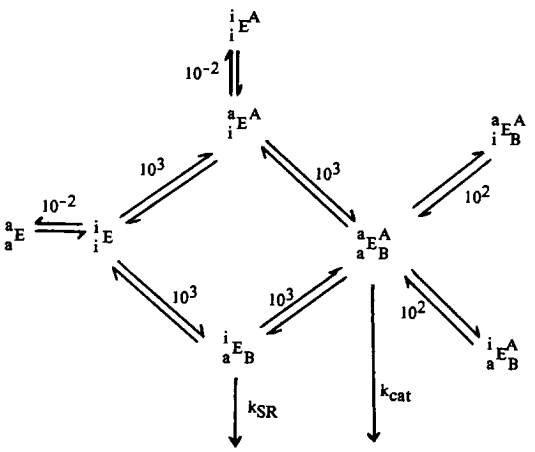
Comparison of the specificity for the enzymes with coupled and independent binding sites shows that coupling the sites provides discrimination against a side reaction. Again, discrimination is determined by the ratio $[{}^{a}\mathrm{E} \cdot \mathrm{A} \cdot \mathrm{B}]/[{}^{a}\mathrm{E} \cdot \mathrm{B}]$. The equilibrium constant for: ${}^{a}\mathrm{E} \cdot \mathrm{B} + \mathrm{A} \rightleftarrows {}^{a}\mathrm{E} \cdot \mathrm{A} \cdot \mathrm{B}$ is $K = 10^4$ M^{-1} for the enzyme with coupled sites, which is greater than the value $K = 10^3$ M^{-1} for the enzyme with independent sites (Schemes 7A and 7B). This gives more ${}^{a}\mathrm{E} \cdot \mathrm{A} \cdot \mathrm{B}$ relative to ${}^{a}\mathrm{E} \cdot \mathrm{B}$ with the coupled enzyme. Thus, there is greater discrimination against the side reaction by the enzyme with coupled binding sites than by the enzyme with independent binding sites.

Two factors lead to the enhanced specificity for the coupled enzyme, which

A) Enzyme with Coupled Binding Sites



B) Enzyme with Independent Binding Sites



SCHEME 7

exhibits substrate synergism. (1) The concentration of the binary complex, E · B, which gives the side reaction, is lower with coupled binding sites than with independent sites. This occurs because the binding of both substrates is favored over the binding of just one substrate with coupled sites, but not with independent sites (i.e., there is synergism in binding). (2) In addition, a significant amount of this binary complex is in the inactive conformation with the coupled enzyme, but not with the enzyme with independent binding sites (i.e., there is synergism in reactivity). When binding energy must be used to provide a conformational change, for example, in surrounding the substrate, it is possible to use this energy to also provide substrate synergism and an advantage in specificity relative to a mechanism without substrate synergism.

It should be noted that, under certain conditions, the coupled enzyme provides greater catalysis than the enzyme with independent binding sites (Schemes 7A and

7B). At saturating A the observed value of k_{cat}/K_m with varying concentration of B is $k_{cat}/(10^{-4}$ M) with the coupled enzyme (Scheme 6C), which is greater than the value $k_{cat}/K_m = k_{cat}/(10^{-3}$ M) for the enzyme with independent binding sites. Since the value of k_{cat} is the same for both enzymes, catalysis at saturating A and B is the same. At concentrations of A and B well below the apparent binding constants the observed rates of reaction are also the same.

Hysteresis and specificity. A conformational change that gives hysteresis can enhance specificity against a competing substrate.

Hysteresis, which is a slow response to a rapid change in ligand concentration, has been reported in several systems (*43*). It has been suggested that conformational changes that are slow relative to catalysis cause the hysteresis observed with β-lactamase (*44*) and rhodanese (*45*), although at least some of the hysteresis with β-lactamase can be explained by the formation of a long-lived acyl-enzyme species (*46*).

The ability of a hysteretic conformational change to switch an enzyme on in the presence of substrate and off in the absence of substrate can provide an advantage in specificity that is similar to the advantage in specificity from induced fit with changing concentration of substrate that was described above. In the absence of the desired substrate, an inactive conformation of the enzyme can predominate in order to limit an undesirable reaction of a competing substrate. When the desired substrate is introduced the hysteretic conformational change can put the enzyme in an active conformation so that there is rapid catalysis.

Enzymes with broad specificity cannot have complementarity to the transition states for all of their substrates so that some substrates may cause conformational changes; hysteresis occurs when such conformational changes are slow.

Myoglobin. X-ray crystallographic studies have shown that myoglobin totally encloses ligands such as oxygen and azide (*47*). The absence of a path for binding of a ligand to myoglobin, rapid kinetic measurements with varying temperature and viscosity following flash photolysis of myoglobin/ligand complexes, and molecular dynamics have led to the suggestion that a series of small conformational changes, each involving the concerted motion of a number of residues, allows binding and dissociation to occur (*48*).

Several small fluctuations in conformation provide a mechanism for an enzyme to surround its substrate that is an alternative to a single conformational change from an open to a closed enzyme conformation. Wolfenden has suggested that the changes in conformation that lead to an enzyme surrounding its substrate could occur in steps that are simultaneous to the chemical reaction rather than prior to the chemical conversion (*8*); this would result in the expression of the extra intrinsic binding energy from surrounding the substrate in the transition state and not in the ground state. As described above, enzymatic catalysis requires expression of binding energy in the transition state and not in the ground state.

Perspective. Conformational changes appear to be common. Some possible roles of conformational changes have been discussed in this review, and ideas of several investigators have been synthesized within the framework of the intrinsic binding energy concept. The concept of intrinsic binding energy is useful in understanding that specific interactions of an enzyme with its substrate can provide

catalysis for reaction of that substrate and thus can provide specificity as well. It is hoped that understanding possible roles of conformational changes will lead to experimental tests to determine what roles these changes actually play in catalysis and specificity.

The introduction of an induced fit conformational change does not provide an increase in specificity in general; each situation must be analyzed. It is hoped that this review will provide a basis for such analyses. Conformational changes that surround substrates on all sides can, in principle, contribute to catalysis and specificity by providing intrinsic binding energy that would not be available in the absence of a conformational change. In contrast, induced fit, with a central conversion rate limiting, does not provide specificity at constant concentrations of competing substrates and also lessens catalysis by decreasing k_{cat}/K_m. However, some conditions that could occur *in vivo* result in specificity from this induced fit mechanism. In addition, introduction of a conformational change can increase catalysis and specificity when the release of product is rate limiting and can increase specificity when the binding of substrate is rate limiting.

ACKNOWLEDGMENTS

I thank William Jencks for numerous discussions and probing questions, which helped clarify my thoughts about this material and its presentation. I also thank Eric Furfine for helpful discussions. This work has been supported by a Fellowship from the Gillette Foundation and by grants from the National Institutes of Health (GM20888) and the National Science Foundation (PCM8117816) to Dr. Jencks.

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