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Reexamination of Induced Fit as a Determinant of Substrate Specificity in Enzymatic Reactions

Carol Beth Post*

Department of Medicinal Chemistry, Purdue University, West Lafayette, Indiana 47907-1333

William J. Ray, Jr.

Department of Biological Sciences, Purdue University, West Lafayettte, Indiana 47907-1392

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ABSTRACT: It has been argued that a substrate-induced conformational change involving the orientation of catalytic groups cannot affect the specificity for two substrates in an enzymatic system where the chemical step is rate limiting, because such an induced fit would alter the catalytic efficiency for both to an equal extent. To the contrary, the generalized induced-fit treatment described here shows that when critical substrate-specific conformational changes in the enzyme persist in the transition state, specificity is linked to conformational differences between the reactive complex for a good substrate and the related complex for a poor one. Conformational differences are a determinant of specificity when the reaction proceeds via an "induced-fit" transition state. Our treatment also shows that such conformational changes can enhance the specificity of an enzyme with suboptimal catalytic efficiency. If substrate-dependent conformational differences in a primative enzyme can enhance specificity, evolutionary pressure to increase specificity could inseparably link enzymatic specificity to induced conformational changes.

In a provocative 1958 paper, Koshland suggested that conformational changes induced by substrate binding could orient functional groups on an enzyme so as to enhance the efficiency of the subsequent chemical process and that such conformational changes could serve as a basis for substrate specificity when the ability of a good substrate to properly align enzymatic groups exceeds that of a poor one with similar chemical reactivity. The suggestion that "induced fit" could serve as a basis for specificity was challenged by Fersht (1974, 1985), who concluded that substrate-induced conformational changes would affect equally the relative catalytic efficiency toward two substrates when the chemical step is rate limiting. However, Fersht's conclusion rests on the assumption that the reactive conformation of the enzyme is unique, *i.e.*, that an identical alignment of catalytic groups must be achieved in the transition state for any substrate to react. This critical assumption is not widely recognized. The more general formulation of the reaction pathway for an induced-fit enzyme described here allows different substrates to induce nonidentical forms of the activated enzyme such that substrate-dependent conformational differences persist in the transition state and leads to a conclusion more consistent with Koshland's earlier suggestion about induced-fit specificity (Koshland, 1958; Wolfenden, 1974; Wolfenden & Kati, 1991). In this formulation, differences in the catalytic efficiency toward two substrates can originate, in part, from specific, substrate-induced conformational changes, even when the chemical step is rate limiting. Our analysis also shows how conformational activation can either enhance substrate specificity or mediate against it, depending on one's reference. That an induced conformational change can affect

* To whom correspondence should be addressed.

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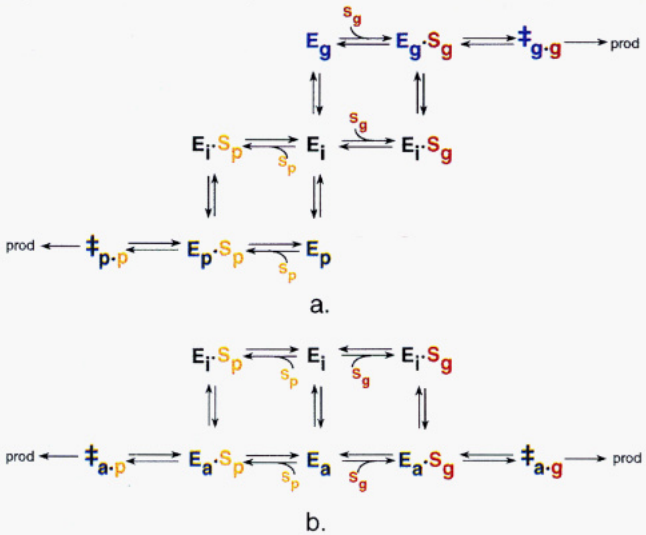
Scheme 1: Thermodynamic Binding Cycles To Represent Induced-Fit Processes That Include a Conformational Change of the Catalytically Inactive Enzyme, E_i, to the Active Forms, E_g, E_p, or E_a, in the Reaction of the Good Substrate, S_g, or Poor Substrate, S_p[a]



[a] Panels: (a) Generalized induced-fit model where the active form of the enzyme for a good and poor substrate is E_g and E_p, respectively. Each of the three forms of the enzyme is shown on a separate horizontal line. (b) Coupled induced-fit scheme where the reaction proceeds via a conformationally unique form of the enzyme, E_a, regardless of which substrate is bound. Only two horizontal lines are needed to represent the enzymatic states.

specificity, be it an increase or a decrease, contrasts with Fersht's conclusion [see also Herschlag (1988)].

The Link between Substrate-Induced Conformational Changes and Specificity. The thermodynamic binding cycles in Scheme 1, introduced by Fersht (1985), provide the basis for assessing the effect of conformational activation of an enzyme by isolating the conformational change in what may be a virtual step: conversion of the energetically stable but inactive form of the enzyme, E_i, to an activated form, E_a, E_p, or E_g, prior to substrate binding (middle vertical arrows). [Here, along with Fersht, we consider only schemes where the rate limitation is the catalytic step, as opposed to other steps (Herschlag, 1988; Johnson, 1993).] Scheme 1a shows our generalized reaction pathway formulated for comparing the catalytic efficiency (k_{cat}/K_M) for a good substrate, S_g, and a poor one, S_p. Separate thermodynamic cycles for substrate binding that include conformationally different forms of the enzyme, E_g and E_p, are constructed for each substrate. In this scheme, processes involving the three different forms of the enzyme, E_i (black), E_g (blue), and E_p (green), are represented on three different horizontal lines, respectively.

The essential element in Scheme 1a is the distinction between the conformational form of the enzyme that produces the chemical transformation when alternative substrates are bound. The energetically favorable complex produced when the good substrate, S_g, occupies the active site is $E_g{\cdot}S_g$, whereas the favored complex when the poor substrate, S_p, binds is $E_p{\cdot}S_p$. In this generalized scheme, a chemically important conformational distinction between the activated enzyme in $E_p{\cdot}S_p$ and $E_g{\cdot}S_g$ persists in the transition states $‡_{p\cdot p}$ and $‡_{g\cdot g}$, respectively.[1]

By contrast, Fersht's formulation for an induced-fit enzyme (1985) requires that the active conformation of the enzyme producing the chemical transformation be identical for both substrates. Therefore, Scheme 1b, which represents this formulation, includes only two states, E_i (black) and E_a (green), and only two horizontal lines. Here, the difference between the two substrates is the ability of S_g to stabilize E_a and the failure of S_p to do so. A process energetically equivalent to $E_i \rightleftharpoons E_a$ is common to the reaction pathway for both substrates (Fersht, 1985; Herschlag, 1988) and must occur somewhere along the reaction coordinate between (E_i + S_g) or (E_i + S_p) and their respective transition states, $‡_{a\cdot g}$ and $‡_{a\cdot p}$. The commonality of this process couples the binding cycles for S_g and S_p, as shown in Scheme 1b.

The significant difference between Scheme 1a, where the substrate binding cycles are not coupled, and Scheme 1b, where they are coupled, is that substrate-induced activation differentially affects the catalytic efficiency of the enzyme toward the two substrates. In Scheme 1a, specificity is linked to an induced conformational change; if that step is eliminated, enzymatic specificity is altered. However, if the same change is made in Scheme 1b, specificity is unchanged. Thus, for specificity to be inseparably linked to a substrate-induced fit, critical conformational differences in the enzyme must persist in the transition states $‡_{p\cdot p}$ and $‡_{g\cdot g}$.

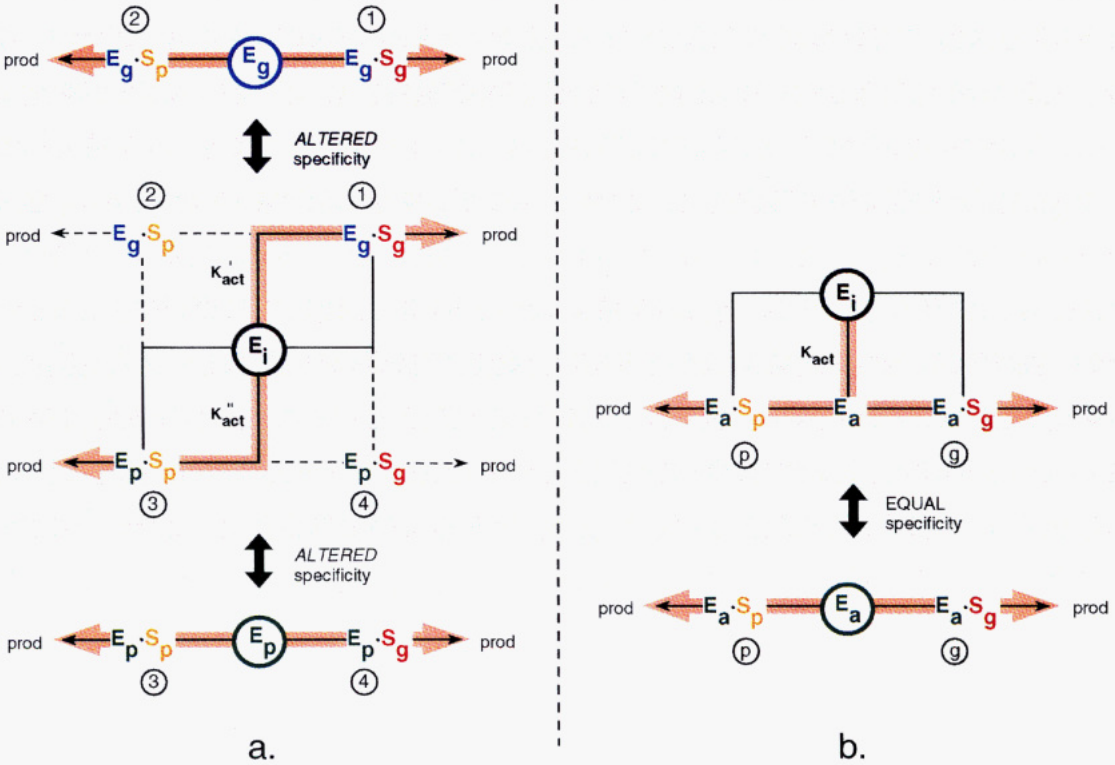
The link between specificity and induced fit can be seen by eliminating the activation step, using an hypothetical "rigid-reference" enzyme where the alignment of groups critical to the chemical transformation is the same in the presence or absence of bound substrates, as well as in the transition state for both substrates.[2] The reaction sequences for hypothetical enzymes of this type are shown in Scheme 2a (top, bottom) and Scheme 2b (bottom) with single horizontal lines, since there is only one conformational state for each such enzyme. The reaction sequences for induced-fit enzymes are drawn in Scheme 2 (middle) with simple squares that indicate thermodynamic cycles like those shown in detail in Scheme 1. The active enzyme−substrate complex is identified explicitly to distinguish each square. In addition to the cycles from Scheme 1 (drawn with solid lines in Scheme 2), the generalized induced-fit sequence also includes cycles with the complexes $E_g{\cdot}S_p$ and $E_p{\cdot}S_g$ for completeness, although, by definition, neither contributes significantly to catalytic efficiency.[3] As usual, substrate specificity is defined by the ratio of k_{cat}/K_M values for two substrates. The k_{cat}/K_M values for S_g and S_p in a particular sequence are identified by the two pathways shown in red, beginning with the circled form of the enzyme and ending with products. In the case of an induced-fit enzyme, the

[1] The significant energy of nonbonding interactions and the conformational flexibility of proteins, demonstrated by different ground-state structures of the enzyme when alternative substrates occupy a catalytic site, support our suggestion that the conformation of an enzyme in the transition state can depend on the structure of the substrate [*cf.* Wolfenden and Kati (1991)].

[2] "Rigid" is used herein to describe the lack of conformational activation and the lack of structural variability of the enzyme in transition states for the reactions with different substrates. It is unrelated to whether a given transition state is "loose" or "tight" [*cf.* Page (1987)].

[3] The generalized scheme is formulated so that obtaining the optimal k_{cat}/K_M for S_g involves expending the binding energy required to produce conformation E_g from E_i. But for S_p, the catalytic advantage gained via conformation E_g does not compensate the cost for conformational activation. Hence, for S_p, the lower energy transition state will involve E_p.

Scheme 2: Comparison of Specificity for Induced-Fit Enzymes and the Related "Rigid-Reference" Enzymes[a]



[a] A simple square represents a binding cycle for an induced-fit reaction sequence like those shown in detail in Scheme 1. Red-arrow pathways define the k_{cat}/K_M values whose ratio is the specificity ξ. The k_{cat}/K_S value for substrate binding and catalysis by an activated form of the enzyme is represented by ①, ②, ③, and ④ in (a) or ⓟ and ⓖ in (b). (a) Middle: uncoupled induced-fit pathway, as in Scheme 1a, but including the catalytically unimportant species $E_g{\cdot}S_p$ and $E_p{\cdot}S_g$ (dashed lines); ξ_{if} is given by eq 4. Top and bottom: the related rigid-reference pathways; ξ_{rr} is given by eqs 5 and 6. (b) Top: coupled induced-fit pathways as in Scheme 1b. Bottom: the related rigid-reference pathway. ξ_{if}, given by eq 3, is equal to ξ_{rr}, given by eq 2.

pathways where activation precedes binding are marked, although the actual binding process likely involves the other limb of the cycle. (Since the binding cycle is taken as an equilibrium process, which pathway is used in deriving relationships is immaterial.) The specificity ratio of an enzyme that utilizes the induced-fit sequence in Scheme 2a (middle) *differs* from that of either of the two corresponding rigid-reference enzymes (Scheme 2a, top or bottom); *i.e.*, the specificity ratio for S_g and S_p that characterizes the induced-fit enzyme cannot be reproduced by a rigid-reference enzyme in either the E_g or E_p forms. Thus, the conformational activation does indeed alter the specificity relative to any enzyme that does not undergo a conformational change. On the other hand, for the induced-fit process illustrated in Scheme 2b, where a unique arrangement of chemically important enzymatic groups is required, regardless of what conformational changes are favored by substrate binding, the specificity ratio for the enzyme that utilizes conformational activation (Scheme 2b, top) is the same as the ratio for the enzyme that always is present in the activated form (Scheme 2b, bottom), as Fersht correctly concludes.

Quantifying the Link between Substrate-Induced Conformational Changes and Specificity. The distinction between induced-fit enzymes with coupled and uncoupled binding cycles can be quantified by considering the reaction sequences in Scheme 2 specified by red arrows. Here, like Fersht (1974, 1985), we separate the activation step from the chemical process by factoring $1/K_M$ into the product of the equilibrium constants for the first two steps along the induced-fit pathways: activation in the absence of substrate, $\mathcal{K}_{act}$, and substrate binding, $1/K_S$ (where $\mathcal{K}_{act} = [E_{active}]/[E_{inactive}]$ and $1/K_S = [E_{active}{\cdot}S]/[E_{active}][S]$). Hence, the catalytic efficiency of any induced-fit enzyme is

$$k_{cat}/K_M = \mathcal{K}_{act}(k_{cat}/K_S) \qquad (1)$$

According to the induced-fit paradigm, $\mathcal{K}_{act}$ (or K_{act}, K'_{act}, K''_{act} in Scheme 2) is less than 1. In Scheme 2, the circled numbers and letters refer to the k_{cat}/K_S value for a given active form of the enzyme; *i.e.*, ⓧ $\equiv (k_{cat}/K_S)_x$ for the sequence $(E_{active} + S)_x \rightarrow$ product.

For the rigid-reference enzyme (rr) in Scheme 2b, bottom, the specificity ratio, ξ, for substrates S_g and S_p, defined by the red paths beginning at E_a, is

$$\xi_{rr} = \frac{(k_{cat}/K_S)_g}{(k_{cat}/K_S)_p} \equiv \frac{ⓖ}{ⓟ} \qquad (2)$$

where the circled notation, defined above, is used. For an induced-fit enzyme (if) with coupled binding cycles, Scheme 2b, top, the specificity defined by the red paths beginning with E_i is

$$\xi_{if} = \frac{K_{act}(k_{cat}/K_S)_g}{K_{act}(k_{cat}/K_S)_p} \equiv \frac{K_{act}\,ⓖ}{K_{act}\,ⓟ} \qquad (3)$$

The value of ξ in eqs 2 and 3 is the same, since in Fersht's treatment a unique alignment of catalytic groups is required for either S_g or S_p to react. Because ξ is the same for an induced-fit enzyme and its rigid-reference counterpart,

substrate specificity can be explained without regard to the induced-fit process. The conclusion that an induced conformational change cannot serve as the basis for substrate specificity (Fersht, 1985; Herschlag, 1988) thus is correct, *if the equilibrium constant for activation, $\mathcal{K}_{act}$, is independent of the substrate.*

When the conformational activation of the inactive enzyme is described by two different equilibria, $K'_{act} = [E_g]/[E_i]$ and $K''_{act} = [E_p]/[E_i]$, as in Scheme 2a, middle, substrate-driven conformational changes will contribute to specificity. In such a case, the specificity ratio is

$$\xi_{if} = \frac{K'_{act}\,①}{K''_{act}\,③} \tag{4}$$

For the related rigid-reference enzymes, where a unique alignment of catalytic groups persists throughout the catalytic process, the specificity is ξ^g_{rr} (top) or ξ^p_{rr} (bottom):

$$\xi^g_{rr} = \frac{①}{②} \tag{5}$$

$$\xi^p_{rr} = \frac{④}{③} \tag{6}$$

Equation 4 obviously differs from either eq 5 or eq 6. Thus, no rigid-reference enzyme can mimic the specificity of an induced-fit enzyme in the generalized scheme, since the effect of conformational activation on (k_{cat}/K_M) cannot be factored to give a common $\mathcal{K}_{act}$. *Hence, the conformational activation induced by a substrate can serve as a determinant of specificity if $\mathcal{K}_{act}$ is substrate dependent such that an induced-fit transition state is involved.*

Since induced conformational changes can indeed alter specificity, we next consider whether such changes might enhance specificity, or diminish it, again by comparing the middle with the top and bottom parts of Scheme 2a. This comparison is facilitated by recognizing that since the activated enzyme, E_g or E_p, middle, is identical to the rigid-reference enzymes, top or bottom, respectively, there are only four unique sequences of the type $E_{active} + S \rightarrow$ prod, where k_{cat}/K_m values are labeled ① through ④. Moreover, by definition of the generalized induced-fit sequence, the reaction involving $E_p{\cdot}S_g$ is less efficient than that involving $E_g{\cdot}S_g$, and that involving $E_g{\cdot}S_p$ is less efficient than the reaction involving $E_p{\cdot}S_p$.[3] Thus

$$K''_{act}\,④ < K'_{act}\,① \tag{7}$$

and

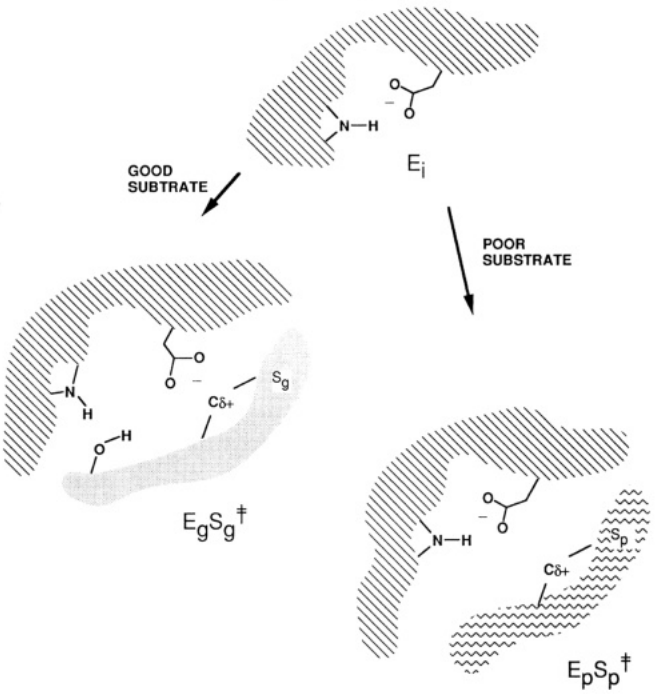
$$K'_{act}\,② < K''_{act}\,③ \tag{8}$$

Given the specificity ratios from eqs 4−6, it follows that

$$\xi^p_{rr} < \xi_{if} < \xi^g_{rr} \tag{9}$$

Thus, conformational activation can increase the specificity of the rigid-reference enzyme E_p, whose catalytic efficiency toward the good substrate is suboptimal. On the other hand, as a rigid-reference enzyme, E_g has a higher degree of specificity than can be obtained with a comparable induced-fit enzyme. That conformational activation can alter specificity is the point missing from Fersht's treatment. That it can decrease specificity is the point missing from Koshland's treatment.

Scheme 3: Simple Example of How Differences in Binding-Induced Conformational Changes Could Lead to Alternative Enzymatic Forms in the Transition State and Thus Provide a Basis for Specificity[a]



[a] Here, S_p can be considered as either a noninducing substrate or one that induces a less active form of the enzyme. See text for explanation.

An Induced Fit Mechanism in Structural Terms. Scheme 3 is a cartoon that shows how differences in enzyme−substrate interactions could lead to nonidentical transition states for good and poor substrates. Here, more efficient catalysis occurs when a carboxylate side chain at the active site of an enzyme stabilizes a positive charge that develops in the transition state but is not essential for catalysis. In the free enzyme, the carboxylate group participates in an intramolecular hydrogen bond that is replaced by an enzyme−substrate hydrogen bond when S_g binds. The carboxylate group then adopts a different conformation that, in stabilizing the transition state (lower left), more than compensates for loss of the intramolecular hydrogen bond. In contrast, S_p cannot replace the intramolecular hydrogen bond and thus cannot recruit the carboxylate group to provide significant transition-state stabilization (lower right). For S_p, the energetic cost of breaking the hydrogen bond to the carboxylate group is greater than the energetic gain in stabilizing the developing positive charge. Hence, the alignment of catalytic groups will differ in the transition states for S_g and S_p, and a smaller k_{cat}/K_M for S_p will arise partly from its failure to induce a conformational change in the enzyme[4] due to its smaller intrinsic binding energy (Jencks, 1975).

[4] The misorientation of a single catalytic residue in the case of a poor substrate is unlikely to serve as the basis for a high induced-fit specificity. More likely, the misorientation of a number of residues would be required. Hence, the Scheme 3 cartoon must be considered as a simplistic model for the more intricate structural rearrangements that can be induced by substrate binding.

Schemes 2 and 3 also show the generality of the principle that an induced fit can contribute to specificity. Such contributions are not limited to exceptional circumstances, such as reaction schemes where the conformational change is rate limiting, or envelopment of the substrate (Herschlag, 1988; Johnson, 1993), and can provide a rationale for the discrimination against water as a substrate. The absolute requirement is that substrate-specific differences in the conformation of the enzyme affect the energy of the transition state.

Rigidity versus Flexibility. There are two reasons why nature might favor an enzyme lacking induced-fit capabilities. An induced conformational change requires energy (Fersht, 1974, 1985) and reduces the catalytic efficiency that would accrue if the activation step were circumvented; *i.e.*, if E_a becomes the stable form of the enzyme, k_{cat}/K_M would increase because the energetically unfavorable step, $E_i \rightleftharpoons E_a$, is eliminated (see eq 1, where $\mathcal{K}_{act} < 1$). Second, as noted above, the requirement for a unique alignment of catalytic groups in the transition state can produce a higher specificity than can an induced conformational change. Nonetheless, conformational flexibility of proteins is well recognized (Herschlag, 1988; Kraut, 1988; Bone *et al.*, 1989; Wolfenden & Kati, 1991; Johnson, 1993; Creighton, 1993), and the number of examples where ligand binding and solvation alter three-dimensional structure seems to increase proportionally with the information available from structural biology. In this paper, we are concerned not about rationalizing the existence of conformational flexibility in enzymes but about how this variability might affect enzymatic action.

Despite the general recognition of conformational flexibility in enzymes, the invariance of the transition state in Fersht's treatment of induced-fit specificity often is overlooked. Thus, from the standpoint of specificity, a flexible enzyme that responds differentially to substrate binding but requires an identical alignment of enzymatic groups in the transition state, independent of the substrate, can be considered as rigid.[2] Although the variations in enzyme structure that can be observed by physical methods involve ground-state complexes, our treatment, with its induced-fit transition-state conformations, represents a reasonable extension of such observations. Indeed, the specificity pattern for some induced-fit enzymes may require consideration of a family of conformationally different enzymatic forms in the transition state. With the generalized induced-fit proposal described here, we have established a sound thermodynamic basis for linking induced conformational changes with specificity, even when the chemical step is rate limiting. We also want to emphasize the possibility that such a linkage can enhance the specificity of an enzyme with suboptimal catalytic potential.

Concluding Remarks. The fundamental difference between the generalized induced fit scheme presented here and commonly accepted formulations of induced fit (Fersht, 1985; Herschlag, 1988; Price & Stevens, 1989; Johnson, 1993) is that the different alignments of the catalytic groups for a poor substrate and a good one are extended to an induced-fit transition state. If this difference affects catalytic efficiency, substrate specificity will be determined, in part, by the conformational change induced by the substrate, even with rate-limiting chemistry.

The possibility that substrate-specific structural differences in an enzyme persist in the transition state may be related simply to altered "solvation" of the transition state for the substrate (as in Scheme 3), as well as to changes in residues with a direct chemical role (as might be detected by differences in the position of bond making and bond breaking along the reaction coordinate). The elusive nature of transition states makes it difficult to provide rigorous proof that the generalized induced-fit model holds for any enzyme. Nevertheless, we point out that some substrate-induced effects in the phosphoglucomutase reaction seem more readily rationalized in terms of what Koshland posed, originally, than in terms of Fersht's scheme (Ray *et al.*, 1993). We also note that mutagenesis studies have provided evidence that a unique form of an enzyme is not required for efficient catalysis of a reaction. Thus, changes in residues which participate directly or indirectly in bond making and breaking can be compensated by other residues of the enzyme [*cf.* Komives *et al.* (1991) and Huang *et al.* (1994)]. But these and other studies, where mutant enzymes with altered k_{cat}/K_M values have been produced, do not rigorously establish that the transition state of a *particular* enzyme is different for two substrates, even when altered conformations of the enzyme and altered substrate specificity have been obtained. Structural studies of inactive enzyme complexes also do not provide direct information on the transition state. As such, Fersht's claim that the same specificity ratio would be obtained in the absence of substrate-induced changes still could be made for the studies of which we are aware. Nevertheless, this report establishes a thermodynamically sound basis for an induced-fit contribution to substrate specificity, although our proposal for a generalized induced-fit model is difficult to test critically.

Finally, we suggest that induced conformational changes in enzymes that alter specificity could serve as an evolutionary basis for producing an inseparable link between specificity and induced conformational changes. We make this suggestion in spite of the notion that, in theory, a precise transition-state template produces maximal specificity.

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