

Analyzing the Reaction of Orotidine 5'-Phosphate Decarboxylase as a Way to Examine Some Key Catalytic Proposals

Mojgan Asadi and Arie Warshel*

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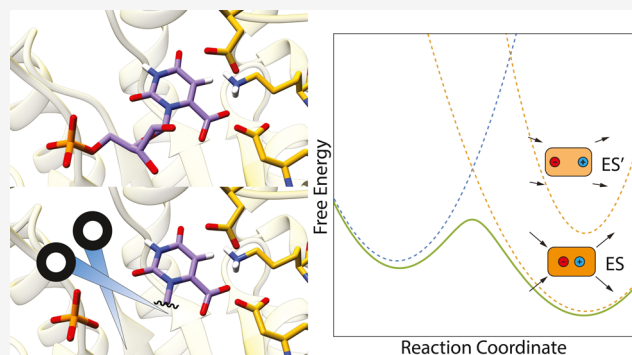


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ABSTRACT: This study analyzes the origin of enzyme catalysis by focusing on the reaction of orotidine 5'-phosphate decarboxylase (ODCase). This reaction involves an enormous catalytic effect of 23 kcal/mol that has been attributed to reactant state destabilization associated with the use of binding energy through the so-called Circe effect. However, our early studies and subsequent key experiments have shown that the presumed effect of the binding energy (namely, the strain exerted by a bond to a phosphate group) does not contribute to the catalysis. In this study, we perform quantitative empirical valence bond calculations that reproduce the catalytic effect of ODCase and the effect of removing the phosphate side chain. The calculations demonstrate that the effect of the phosphate is due to a change in reorganization energy and should not be described as an induced fit effect. Similarly, we show that the overall catalytic effect is due to electrostatic transition state stabilization, which again reflects the smaller reorganization energy in the enzyme than in water. We also elaborate on the problems with the induced fit proposal, including the fact that it does not serve to tell us what the actual origin of the action of the catalytic effect is. In addition to the above points, we use this paper to discuss misconceptions about the meaning of the preorganization effect, as well as other misunderstandings of what is being done in consistent calculations of enzyme catalysis.



1. INTRODUCTION

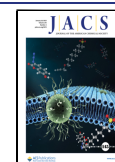
The search for the origin of the catalytic power of enzymes has a very long history, leading to many proposals (for reviews, see refs 1–9). The validity of the key proposals has been explored by computer simulations and experimental studies.^{7,8} However, despite rather solid conclusions from some computational studies, a large part of the community has continued to wonder about the contributions of different catalytic effects. Unfortunately, the attribution of such contributions involves proposals that do not reflect any physical-based analysis. This general criticism is even applied to the frequent assertion that Pauling⁹ offered the solution to the problem of enzyme catalysis. It is true that Pauling had the great insight to state that enzymes catalyze reactions by reducing the activation barrier. He even mentioned that this is achieved by reducing the transition state energy. However, he was not able to describe how this can be done, and his actual suggestion involved using van der Waals interactions that can only lead to ground state destabilization. In fact, almost all the subsequent proposals from the chemical and biochemical community that attempted to assign specific catalytic factors involved ground state destabilization effects. This included the strain proposal, the entropic proposal, the desolvation proposal, the Circe proposal, and more (see ref 3 for a review).

In view of the controversy about the origin of the catalytic power of enzymes, it is very useful to revisit cases where the

catalytic effect is very large and the origin of the large effects is unlikely to be accounted for by incorrect proposals. Such a case is provided by the reaction of orotidine 5'-phosphate decarboxylase (ODCase).¹⁰ This enzyme involves one of the largest catalytic effects; about a 23–24 kcal/mol difference between the activation barrier between the catalyzed enzymatic reaction and the reference reaction in water (see the analysis of ref 11). We must clarify here that we define catalysis, as it should be, as the difference between the reaction in a water cage and the barrier associated with k_{cat} , namely, the barrier of the chemical step starting from the enzyme-substrate complex (ES). As we explained before,¹¹ the puzzle about enzyme catalysis is not related to $k_{\text{cat}}/K_{\text{M}}$ (or defined accordingly as is done in ref 12) because the factors that control the binding energy are quite clear. At any rate, ODCase includes a substrate with a negatively charged carboxylate group that is converted to CO_2 , while the substrate ring becomes negatively charged while accepting a proton from a Lys residue (Figure

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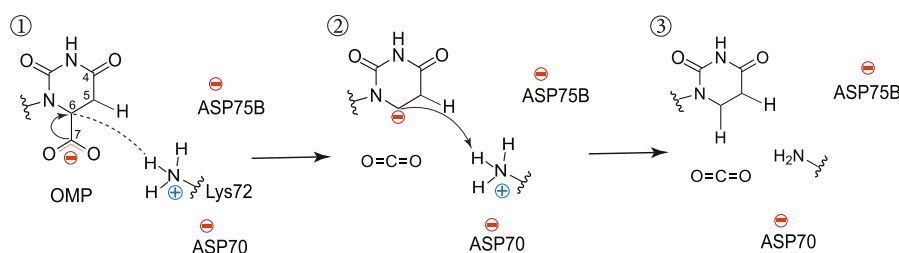


Figure 1. Describing the reaction steps of ODCase along a stepwise pathway for our 3-state EVB calculations. The presence of two charged Asp residues is strong evidence that the active site of ODCase is not an oil drop.

1). An early attempt to rationalize the action of this enzyme¹³ involved the classical desolvation idea,^{2,7,8,14} suggesting that the enzyme active site is like an oil drop that destabilizes the charged reactant state and thus increases the ground state energy and reduces the activation barrier. Following the publication of this proposal,¹³ we¹⁵ repeated our early considerations, emphasizing that enzyme active sites are not oil drops and predicted that when the enzyme structure is elucidated, it will be found to have a very polar (rather than oily) active site.

When the structure of ODCase was solved,^{16,17} it was found (as predicted in ref 15) to be very polar, with an aspartic group pointing toward substrate negatively charged carboxylate (Figure 2). At this point, Gao and co-workers¹⁷ proposed

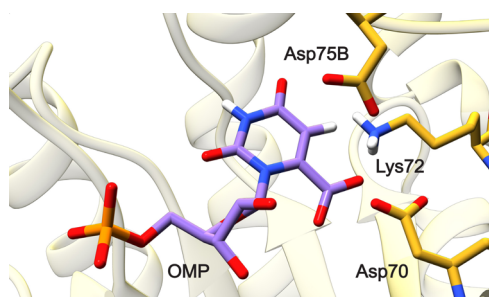


Figure 2. Active site and substrate of ODCase are shown with Lys72, which is involved in the catalytic reaction, as well as the Asp70 and Asp75B residues that are assumed to destabilize the carboxylate.

that the catalysis is due again to reactant state destabilization (RSD) but this time due to the repulsion between the ionized Asp 70 and the substrate carboxylate.

However, the above proposal overlooked the simple fact that if the ionized Asp repels the substrate's carboxylate, the repulsion will force the Asp to accept a proton (and thus lose the RSD) since the Asp pK_a value would be shifted by the interaction with Asp 70. Furthermore, careful calculations of the reaction in the enzyme and solution,¹¹ which took into account the fact that the reaction in the enzyme includes a positively charged lysine residue, accounted for the catalysis with a very large transition state stabilization (TSS).

Despite the above work, the idea of RSD in ODCase continued to be popular. In particular, it was thought by some that the side chain of the ring, which is terminated by a phosphate group, pulls the ring and its carboxylate to a place where it is very unstable.¹⁷

However, an important experiment conducted by Richard and co-workers¹⁸ has basically destroyed this proposal. That is, these workers removed the bond between the phosphate and the ring and found a major reduction in catalysis (which seems consistent with the RSD proposal), but upon addition of

inorganic phosphate to the system, the catalysis returned (see Section 4). This established that the strained bonding between the phosphate and the ring is not the reason for any RSD or catalysis. Although this should have put to rest the RSD idea, related proposals emerged by changing the original meaning of the Jencks proposal of converting binding energies to RSD-induced catalysis (see below).

Despite the major decrease in supporters of the explicit idea that ODCase uses RSD, it has been difficult for some to use the logical interpretation of the effect of phosphate removal and re-insertion experiment, perhaps due to the difficulty to fully understand the preorganization idea or the insistence on using other proposals. This led, for example, to the recent proposal that ODCase is using the induced fit effect.¹⁹

As is shown below, this idea is basically a poorly defined catalytic proposal that was reincarnated inconsistently by ref 20 as an RSD idea. This point is established in Section 3. At any rate, our point is not to disprove various proposals but rather to establish that without consistent energy considerations, it is impossible to establish any catalytic proposal. Thus, we will consider carefully the induced fit and the Circe proposals and show that they are either invalid or not defined in a way that can tell us on the origin of the catalytic effect. In doing so, we will focus on a careful analysis of the energetics of the reaction of ODCase with and without the bound phosphate. This analysis will start from the experimental facts (Figure 3) and then use the empirical valence bond (EVB) to reproduce the experimental facts and to elucidate the origin of the catalytic effect.

2. RESULTS

2.1. Evaluating the Free Energy Profiles. Our analysis starts with careful EVB calculations of the reaction of ODCase in the enzyme and in solution. The calculations were performed by the EVB method that is described in great details in refs 21–23 and also outlined in the Supporting Information. The EVB parameters used were similar to those used in ref 11 and implemented with the program Q6.²⁴

The results of the calculations are summarized in Table 1 and shown in Figure 4. The calculated barriers were analyzed using the representation given in Figure 5 that leads to the approximated expression²⁵

$$\Delta G_{1 \rightarrow 2}^\ddagger \approx \frac{(\Delta G_0 + \lambda)^2}{4\lambda} - |H_{12}(X^\ddagger)| + \frac{H_{12}^2(X_0)}{(\Delta G_0 + \lambda)} \quad (1)$$

We note that there is a good agreement between the calculated EVB activation barriers and the corresponding observed values. This means that we reproduced the observed catalytic effect, including the effect of the phosphate (which is not a minor accomplishment). The agreement between the

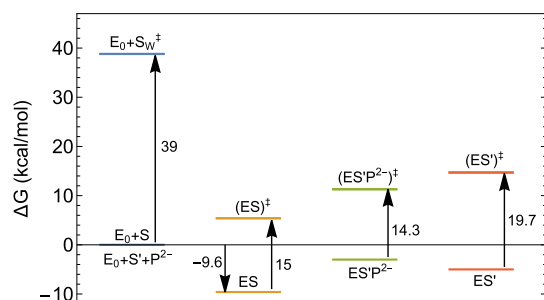


Figure 3. Key experimental information about the catalytic reaction of ODCase. E_0 is the unbound ODCase. ES is the ODCase with the bound OMP substrate. ES' is the ODCase with a bound OMP where the side chain with the phosphate is removed. $ES'P^{2-}$ is ES' in the presence of inorganic phosphate. The uncatalyzed reaction in water has a barrier of 39 kcal/mol. The binding affinity of OMP in ODCase is -9.6 kcal/mol, while the catalyzed reaction has a chemical barrier of 15 kcal/mol. The observations of Richard et. al.¹⁸ show that removing the phosphate tail increases the barrier of the catalytic reaction to 19.7 kcal/mol. However, the catalysis is recovered even in the presence of inorganic phosphate with a barrier of 14.3 kcal/mol.

Table 1. Calculated Estimates of the Activation Barriers and the Corresponding Observed Results^a

system	$\Delta G_{0,EVB}$	λ^b	$\Delta G_{eq1}^\ddagger$	$\Delta G_{EVB}^\ddagger$	$\Delta G_{obs}^\ddagger$
water	30.0	352	63.4	40	38.0
ES'	14.5	250	32.4	24	19.7
ES	6.0	228	23.9	20	15.0

^aAll energies are in unit of kcal/mol. $H_{I \rightarrow II} = 45$. ^bWhen λ is very different from $\lambda_{II \rightarrow I}$, we use their average as λ . ^c $\Delta G_{eq1}^\ddagger$ is evaluated using eq 1. ^dObserved values from ref 18.

$\Delta G_{I \rightarrow 2}^\ddagger$ evaluated by eq 1 and the observed value is much less satisfactory. This reflects the fact that the reorganization energies are not identical in the forward and backward directions. Obviously, we could obtain a refined “effective” reorganization energy by fitting eq 1 to reproduce the observed activation barrier. However, the qualitative values of the reorganization energies are sufficient for our purpose.

At any rate, the key question in this study is the origin of the catalytic effect of the phosphate and the overall catalytic effect. We start by commenting on the relationship between the reorganization energy and $\Delta G_{I \rightarrow 2}^\ddagger$. We first note that the reorganization energy along the reaction coordinate is evaluated from the actual full diabatic curves and is given in Figure S1 and listed in Table 1. With the values of the reorganization energies and the ΔG_0 , we can analyze the factors that determine the changes in the activation barriers. We can start our analysis by considering the largest effect, which is the change in the activation barrier that occurs upon moving the substrate from solution to the enzyme–substrate complex (ES). In the case of the solution reaction, ΔG_0 is very large and we are at the limit of $\Delta G_0 \gg 0$, where $\Delta \Delta G^\ddagger$ is approximately $\Delta \Delta G_0$. In this case, we should ask what is leading to the very large change in ΔG_0 (a calculated reduction of 24 kcal/mol). Interestingly, this effect is not due to the reorganization energy along the reaction coordinate (λ). Nevertheless, it still reflects a reduction in a reorganization energy. That is, it appears that upon moving from state I to state II, the Lys residue moves closer to C6, whereas in water, it stays at a larger distance. Now, the reduction in the ion pair distance can lead to a significant stabilization inside protein's

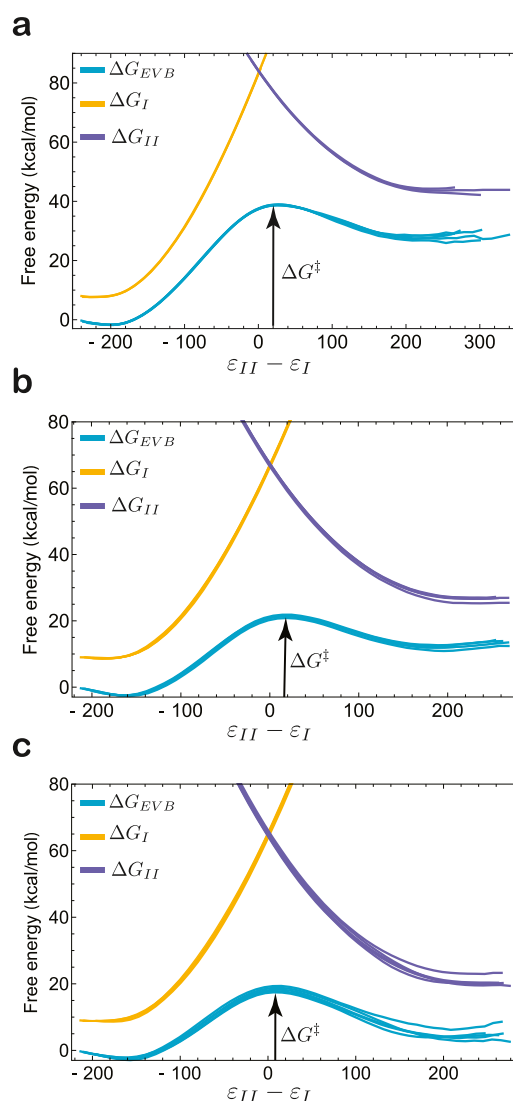


Figure 4. EVB calculations of the reaction of ODCase (energies in kcal/mol). Each figure describes the free energy functionals of the two diabatic states and the adiabatic state obtained from their mixing ΔG_{EVB} . The intersection of the diabatic states gives the diabatic barrier $\Delta G^\ddagger$ and the maximum of the adiabatic functional gives the actual barrier. Figures (a–c) describe, respectively, the EVB results for the reaction in water, the reaction of ES' , and the reaction of ES . The calculations evaluated the free energy barrier for the first step (I–II), which provides a reasonable estimate for the barrier of the concerted path (see the Supporting Information). The full figure that allows us to determine the reorganization energy is given in the Supporting Information.

active sites and much less in water.²⁶ This reflects a smaller effective dielectric for charge–charge interaction in the protein than in water due to a smaller reorganization of the polar environment in the protein. The fact that we have less reorganization in the protein (and thus less solvation reduction as a response to the reduction in the ion pair distance) is reflected in a significantly lower reorganization energy along the solvation coordinate in the protein than in water, as documented in Table 1.

Next, we turn our attention to the change in the activation barrier upon moving from the complex without the phosphate tail (ES') to ES , reflecting the effect of the phosphate. In this case, we note that ΔG_0 is smaller than that in the water case

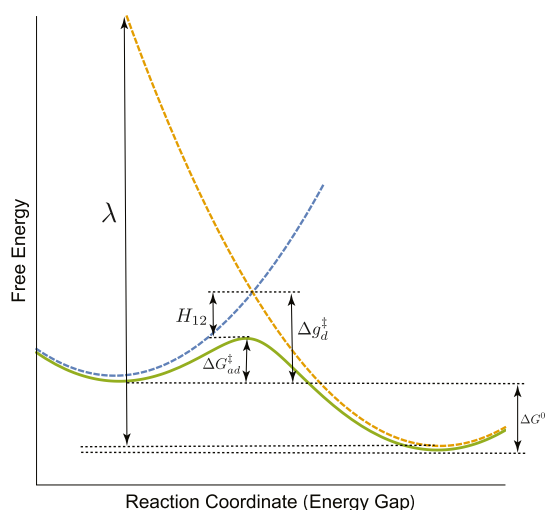


Figure 5. Describing the relationship between the diabatic and adiabatic curves and the meaning of the reorganization energy.

and thus the direct effect of the change in λ in eq 1 is significant, although it does not reach the limit of $\Delta G_0 \ll \lambda$, where eq 1 can be approximated by $\Delta G^\ddagger \approx \lambda/4$. Apparently, we have here a combined effect of the change in λ and in ΔG_0 . Thus, it is useful to consider both factors while using eq 1. In doing so, we note that our calculated change in ΔG_0 is consistent with the experimental estimate of ref 27. At any rate, the overall decrease in the activation barrier is due to the direct reduction in the reorganization energy and the related reduction in ΔG_0 . This reduction also reflects a reduction in reorganization energy as discussed above.

2.2. Induced Fit Does Not Explain Enzyme Catalysis Nor the Effect of the Phosphate in ODCase. Recently,²⁰ it was proposed that ODCase catalysis reflects the induced fit effect. This proposal¹⁹ has been shown before to be extremely poorly defined and very problematic (see ref 28). Briefly, the induced fit proposal attributes the catalysis to the structural change associated with the substrate binding. Now, first, the issue has always been what is the origin of the reduction of the activation barrier for the chemical step starting from the ES state (where the substrate is already bound). Second, the induced fit proposal provides zero information of how the catalysis is accomplished, and finally, in most cases, most of the catalysis is obtained by the preorganization of the enzyme before the binding of the substrate.

Regardless of the above arguments, it is very useful to consider the induced fit proposal in the case of ODCase, where the RSD is now attributed to the enzyme rather than to the bond to the phosphate. That is, ref 20 argued that the catalytic reaction of ODCase can be accounted for by the energy diagram presented in Figure 6.

Apparently, the diagram and the corresponding concepts have major problems. This starts with trivial issues, such as the fact that if the $E_O S$ is lower in energy than $E_C S$, then the system will remain at $E_O S$ (unless we have a very unrealistic conformational barriers). Furthermore, we face much more fundamental problems. First, the assumption that we have the same TS energy at different enzyme conformations [which is similar to the common incorrect assumption that the TS energy is the same in the enzyme and in water (e.g., ref 2)] is fundamentally incorrect. Second, the assumption that the motion to E_C leads to a major instability in the enzyme does

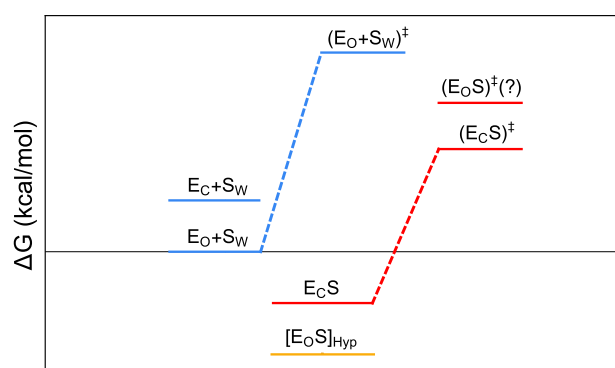


Figure 6. Schematic reproduction of the induced fit diagram of ref 20. In this proposal, the substrate binding energy stabilizes the open state E_O and drives the conformation to the closed state forming ($E_C S$), which in turn catalyzes the reaction moving the system to ($E_C S^\ddagger$). The energy of the complex with the open state is indicated by $[E_O S]_{Hyp}$. The energy of the transition state with the open state ($E_O S^\ddagger$) is not indicated in the original diagram and is probably assumed to be the same as ($E_C S^\ddagger$).

not mean that this instability only occurs at the reactant state. Thus, the proposal of ref 20 does not provide clues about the presumed way by which the conformational change is supposed to stabilize the TS (this is needed since the presumed RSD is not sufficient). Finally, we really have to account for the enormous difference between the activation barrier in water and in the enzyme (about 23 kcal/mol). If it is due to the binding energy, then we would need at least 23 kcal/mol interaction between the substrate and the binding site, which is hard to rationalize (see Section 4). In fact, one of the largest binding energies seen in biochemistry is found in acetylcholinesterase inhibitors, which go up to 18 kcal/mol.²⁹ At this point, it is useful to point out that the recent promotion of the induced fit effect in ODCase has been focused on the phosphate effect. However, the largest catalytic effect is due to the change between water and ES' and thus not due to any structural change induced by the phosphate. In fact, in many enzymes, the catalysis is due to the preorganization to the folded structure before the binding of the substrate.

Finally, we may comment on the idea that it is possible to classify our preorganization concept as an induced fit effect. Here, the fundamental difference is that the preorganization concept clearly defines and quantifies the catalytic effect, where the induced fit statement does not tell us anything about the nature of the contribution of the substrate binding to catalysis. Furthermore, most of the preorganization effect is already determined by the folding of the protein in the unbound state.

2.3. Circe Effect Does Not Account for Enzyme Catalysis. The catalysis of ODCases has been vigorously propagated as an example of the famous “Circe proposal” of Jencks.² According to this proposal, enzymes work by RSD (see clarification below) and the binding of a nonreactive part of the substrate pulls the chemical part of the substrate into the active site (in the same way that Circe from the Greek mythology lured Odysseus’s men into her mansion, drugged them, and transformed them into pigs). The Circe proposal has been very popular and used as a general explanation for the catalytic power of enzymes (without providing clear any correct thermodynamic considerations).

In order to prevent incorrect arguments, we like to point out that Jencks defined the Circe effect as “the utilization of string

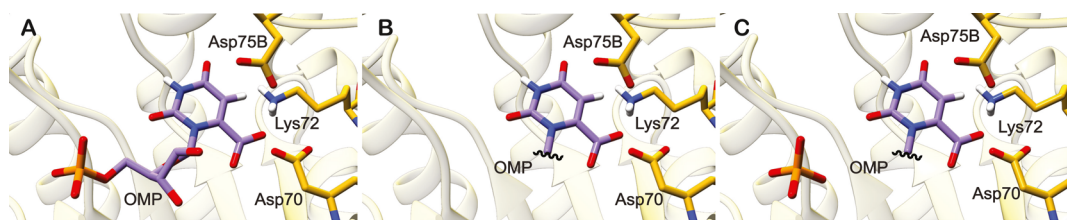


Figure 7. Description of the experiment that actually disproved the Circe RSD effect. (a) Describing the ODCase enzyme substrate system, which is an extremely powerful catalyst, where the Circe proposal assumes that the binding of the phosphate tail pushed the substrate to a state with a very large RSD (due to the presumed repulsion between the substrate carboxylate and Asp 70). (b) After chopping the tail group, the catalysis stops, which seems fully consistent with the Circe proposal since the presumed RSD disappears when the tail does not pull the substrate to the position where it has a large RSD. (c) Surprisingly, adding inorganic phosphate restores the catalysis, and this means that the catalysis is not related to the strain between the tail and the reactive part. The fact that we do not have RSD is established in the simplest way by seeing that the observed binding free energy of the substrate without the chain with the phosphate (S') is negative, as shown in Figure 3.

attractive force to lure a substrate into a site in which it undergoes an extraordinary transformation” (which does not explain how the transformation is performed). However, it is clear that his writing has always involved the RSD proposal where the binding of the unreactive part brings the substrate chemical part to an unfavorable configuration where the activation barrier is reduced due to RSD (see the appendix of ref 31). Thus, arguing that Jencks’ proposal involves the use of the binding energy for TSS is simply misleading.

Now, we can return to the ODCase issue. That is, as shown in Figure 2, the enzyme includes a substrate with a negatively charged carboxylate group that is converted to CO_2 , while the substrate ring becomes negatively charged. Here, it is tempting to suggest, following the Circe proposal, that the chemical part is forced into a destabilizing environment by the binding energy of the phosphate group of the substrate, which is not a part of the chemical region.

Fortunately, as mentioned above, Richard and his co-workers,¹⁸ who originally supported the Circe idea in its RSD version, performed a great relevant experiment, illustrated in Figure 7. That is, they chopped the substrate tail and indeed found that the catalysis disappeared, which seems to be consistent with the RSD Circe idea since without the presumed distanced strain, the substrate should lose the destabilization of the chemical part and the catalytic effect. However, they then performed an additional experiment and added inorganic phosphate instead of the tail, and then suddenly, the enzyme started to catalyze the reaction. This has shown that the bond to the phosphate (and the corresponding presumed distanced strain) is not the reason for the catalysis. In other words, the enzyme does not work using distanced binding energy in an RSD mechanism and a Circe effect.

An excellent example of the influence and problem with the Circe idea is the analysis of the catalysis of cytidine deaminase. The corresponding catalytic effect was attributed to the entropic loss that is paid by the binding energy. However, the careful study of Åqvist and co-workers³⁰ found that the apparent catalytic effect is due to a change in the reaction mechanism between the solution and the enzyme active site and is due to preorganization rather than the Circe effect.

3. DISCUSSION

This study starts by providing a quantitative and unique reproduction of the catalytic effect of the phosphate as well as the overall catalysis, while showing that the catalytic effect is due to electrostatic effects. Perhaps the most interesting finding is the establishment that the catalytic effect of the

phosphate is due to the preorganization induced by its negative charge. Interestingly, our accomplishment allows us to demonstrate what are the main factors in enzyme catalysis and to address some problems with the proposal that are not based on energy considerations or clear verifiable logic. To further elaborate on our points, we will consider below some instructive points.

3.1. General Consideration of the Problems with the Induced Fit Catalytic Proposal. As we argued before,²⁸ the induced fit proposal has never been more than the realization that enzymes can change their structure upon substrate binding, and obviously, the resulting ES structure is likely to be the structure that was optimized for catalysis. However, this proposal could not describe consistently how the conformational change would contribute to catalysis. This proposal does not provide information about the relationship between the structural change upon the formation of the ES and the activation barrier of the chemical step. In fact, the catalysis of the chemical step is defined by the difference between the activation barrier in the ES complex and the barrier for the reference reaction in water. Otherwise, enzyme catalysis can be considered relative to any structure during the folding to the ES state and not just relative to the structure before the substrate binding (in general, the folding energy is invested in reorganizing the active site¹).

While the above points might sound somewhat philosophical, it is crucial to realize that the current work took the case of the phosphate-induced catalysis, which was brought up as an example of an induced fit effect and provided quantitative evidence for its true origin. That is, as shown above, the structural change due to the phosphate charge changes the catalytic effect by a very well-defined change in electrostatic preorganization, and trying to label this effect as an induced fit (which might be formally reasonable for the differential phosphate effect) only serves to obscure the understanding of what is the actual effect of the structural change induced by the phosphate.

3.2. Clarifying Misunderstanding about the Preorganization Effect and Other Catalytic Factors. The crucial preorganization concept is not completely intuitive, and its understanding may require familiarity with the nature of solvation effects. However, misrepresentation of this effect does not advance the understanding of enzyme catalysis. A recent example of the misunderstanding of the preorganization effect is provided by the studies of Menger and Nome (MN),^{32,33} who ventured to assert that the calculations of the preorganization effect by our group ignored the evaluation of

the interaction between the environment and the reacting system. This mischaracterization is carefully discussed in ref 34, clarifying that MN ignored the explanation that the interactions were always considered, but they appeared to be very similar in the enzyme and in solution and thus are not the origin of the catalytic effect. However, the original misunderstanding was followed by a new “review” of MN³³ that overlooked energy-based calculations, which had been used very reliably in the last 40 years. Reference 33 appeared to spread misleading information, despite the clarification that our studies (e.g., ref 1) considered very carefully general acid-based catalysis (see below) and other catalytic factors. The readers of ref 33 were told that no such effect has been considered. Similarly, the readers were told that major catalytic factors were never considered by us.

Reference 33 continues to assert that we proposed that the catalysis is due to “water molecules preorganized in the active site”. This is an alarming misunderstanding since we have calculated the activation free energies and the reorganization energies in the bulk water and in the protein active site and compared them. It was found that *in the bulk water* (without the protein), the activation free energy is much larger due to the reorganization energy (of the water in the bulk), while in the protein active site, the protein polar groups (not water molecules) cannot reorganize significantly, and this is the reason for the catalytic effect. This point has been completely clarified in previous occasions. Of course, MN never tried to estimate reorganization energies by simulations (where our methods are now used by many groups) and failed to follow our studies on this issue. They also missed the fact that estimating reorganization energy without considering the electronic mixing term (the H_{12} term in eq 1 and Figure 5) gives extremely small and incorrect reorganization energies (see refs 35 and 36).

Unfortunately, ref 33 was unable or unwilling to follow our studies of any enzyme. For example, in the case of ketosteroid isomerase (KSI), they stated that “the first step in the enzyme reaction involves an aspartate carboxylate catalyzing the enolization of a ketone, with the barrier diminishing from 21.9 kcal/mol in water to 10.3 kcal/mol within the enzyme”. The origin of this catalytic effect was attributed, in part, to the “electrostatic stabilization” of the enolate by hydrogen bonds to Tyr57 and Asp103. Bio-organic chemists call the effect a “general-base/general-base catalysis”, although the term *per se* is not found in the Warshel article. Unfortunately, the issue in the field is not the names of different effects but the ability to understand and include such effects in the given study. Probably, MN were confused by the term “catalysis” in the name general acid/base catalysis and missed the fact that the same effect (e.g., having a base in the system) is shared by both the enzyme and the solution reactions. The term “catalysis” is with regard to a reference state without a base. The confusion about the nature of the general base effect is the consequence of not trying to move from descriptive names to quantitative energy surfaces. This problem is particularly obvious when ref 33 presumes that the 21.9 to 10.3 kcal/mol observed catalytic effect of KSI reflects general acid/base catalysis, completely failing to realize that the general acid/base effect designates a mechanism that already exists in the reference reaction in water and thus has not been considered in the actual enzyme catalytic effect.¹ Unfortunately, ref 33 substitutes mentioning names of different physical organic concepts with the ability to evaluate the contributions of such effects. For example, the

issue with the enolate contribution in KSI is not just its existence, which was objected by many workers, but the difference between the energy of the enolate in the protein and in water, which is mainly an electrostatic preorganization effect (see ref 37). Obviously, MN are unaware of the ways of evaluating such effects and replace this inability with the sound of the term “general acid/base catalysis”. As stated above, the word “catalysis” in general acid catalysis “has very little to do with actual enzyme catalysis”.

As much as ignoring general acid/base catalysis, our works studied and quantified many general acid/base reactions and of course studied the reference reactions in water and reproduced known experimental facts. Some have presumed that including a classical name from physical organic chemistry in a calculation without calling it a given name meant that it has been ignored by us. However, our studies always considered general acid/base catalysis and related effects including covalent catalysis and included it, as it should in the reference solution reaction. The same analysis has, of course, been performed by physical organic chemistry workers such as Bruice.³⁸ This issue has been clarified in many of our papers, including a major part of the introduction to our review.¹

Reference 33 also ventured to talk on predictability, arguing that the preorganization concept is just some theoretical concept that has made no predictions. First, we made many predictions and reproduced the catalytic power of many enzymes (e.g., ref 1). The predictions have been made by models that included all contributions to the rate constant, and only then were the results then analyzed to see which contribution is the major one (this appeared to be the preorganization effect). On the other hand, we are not aware of any prediction made by MN regarding the rate of a single enzymatic reaction, whereas our models reproduced quantitatively many enzymatic reactions, and this model (program) can predict very well the effect of mutation and absolute catalysis, showing that it is quantitatively related to the preorganization effect. Of course, our models can also be used to simulate organic model compounds, including those studied by MN, and predict their activity as we did in a recent paper.³⁴ Probably what ref 33 calls prediction is just a suggestion that something will help catalysis.

4. CONCLUDING REMARKS

The present study focused on the enzyme ODCase and its enormous catalytic power, trying to establish several important points about the key factors in enzyme catalysis. Special instructive emphasis is placed on the effect of the phosphate, which provides a very instructive information. This includes the analysis of the fact that the effect of cutting the bond between the phosphate and the ring is restored by inorganic phosphate. This finding leads to several major conclusions. First, it is established that the catalytic effect is not due to the RSD Circe effect, where it is postulated that the phosphate binding energy is used to hold the ring in a very unstable reactant state configuration.¹⁷ In this respect, we would like to note that the recent tendency to relate the use of binding energy for TSS is highly inconsistent with the original Circe proposal and with the very long history of promoting RSD.

Next, we demonstrated that the difference between the rate constant of the system with and without the phosphate is accounted quantitatively by the electrostatic reorganization energy and not by any induced fit or other concepts. In this respect, we note that the induced fit proposal can also be

coached as a version of the use of binding energy in catalysis. Here, we first repeat the point that the idea of using binding energy was always converted to an RSD idea in any attempt to actually rationalize this proposal.

However, we also have an additional instructive concept. That is, the use of binding energy in the Circe proposal is rationalized by repeating Jencks' statement that presumed strain is being released in TS and suggesting that Jencks supported TSS.¹² However, although folding energy and sometimes binding energy are used to preorganize the active site, the corresponding energy is not "released" upon moving to the TS but actually less energy is invested in moving to the TS than in the corresponding process in water. Furthermore, Jencks and others could never propose an actual catalytic mechanism that is not based on RSD. In this respect, we also repeat our comment that crediting Pauling in having the TSS proposal¹² misses the key point that he could not envision any way by which an enzyme can actually stabilize the TS. Here, as we emphasized repeatedly, the key question has always been how do enzyme catalyze their reactions, rather than what are the names of the different possibilities.

At any rate, the present study provides an excellent example of what can be achieved with reliable computational approaches in analyzing enzyme catalysis. Having an unbiased computer program that reproduces catalytic effects is the best (and arguably the only) way of assessing the importance of catalytic effects.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/jacs.2c11728>.

Details of computational methods, description of the EVB method and parameters used in the simulations, and the table of observed experimental parameters (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Arieh Warshel – Department of Chemistry, University of Southern California, Los Angeles, California 90089-1062, United States; orcid.org/0000-0001-7971-5401; Email: warshel@usc.edu

Author

Mojgan Asadi – Department of Chemistry, University of Southern California, Los Angeles, California 90089-1062, United States; orcid.org/0000-0002-1356-4900

Complete contact information is available at:

<https://pubs.acs.org/doi/10.1021/jacs.2c11728>

Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Warshel, A.; Sharma, P. K.; Kato, M.; Xiang, Y.; Liu, H.; Olsson, M. H. M. Electrostatic Basis for Enzyme Catalysis. *Chem. Rev.* **2006**, *106*, 3210–3235.
- (2) Jencks, W. P. Binding Energy, Specificity, and Enzymic Catalysis: The Circe Effect. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1975**, *43*, 219–410.
- (3) Fersht, A. *Enzyme Structure and Mechanism*; W. H. Freeman, 1977.
- (4) Martí, S.; Roca, M.; Andrés, J.; Moliner, V.; Silla, E.; Tuñón, I.; Bertrán, J. Theoretical Insights in Enzyme Catalysis. *Chem. Soc. Rev.* **2004**, *33*, 98–107.
- (5) Mulholland, A. J. Computational Enzymology: Modelling the Mechanisms of Biological Catalysts. *Biochem. Soc. Trans.* **2008**, *36*, 22–26.
- (6) Nagel, Z. D.; Klinman, J. P. A 21st Century Revisionist's View at a Turning Point in Enzymology. *Nat. Chem. Biol.* **2009**, *5*, 543–550.
- (7) Dewar, M. J.; Storch, D. M. Alternative View of Enzyme Reactions. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 2225–2229.
- (8) Crosby, J.; Stone, R.; Lienhard, G. E. Mechanisms of Thiamine-Catalyzed Reactions. Decarboxylation of 2-(1-Carboxy-1-Hydroxyethyl)-3,4-Dimethylthiazolium Chloride. *J. Am. Chem. Soc.* **1970**, *92*, 2891–2900.
- (9) Pauling, L. Molecular Architecture and Biological Reactions. *Chem. Eng. News* **1946**, *24*, 1375–1377.
- (10) Radzicka, A.; Wolfenden, R. A Proficient Enzyme. *Science* **1995**, *267*, 90–93.
- (11) Warshel, A.; Štrajbl, M.; Villà, J.; Florián, J. Remarkable Rate Enhancement of Orotidine 5'-Monophosphate Decarboxylase Is Due to Transition-State Stabilization Rather Than to Ground-State Destabilization. *Biochemistry* **2000**, *39*, 14728–14738.
- (12) Amyes, T. L.; Richard, J. P. Specificity in Transition State Binding: The Pauling Model Revisited. *Biochemistry* **2013**, *52*, 2021–2035.
- (13) Lee, J. K.; Houk, K. N. A Proficient Enzyme Revisited: The Predicted Mechanism for Orotidine Monophosphate Decarboxylase. *Science* **1997**, *276*, 942–945.
- (14) Cohen, S. G.; Vaidya, V. M.; Schultz, R. M. Active Site of α -Chymotrypsin Activation by Association-Desolvation. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *66*, 249–256.
- (15) Warshel, A.; Florián, J. Computer Simulations of Enzyme Catalysis: Finding out What Has Been Optimized by Evolution. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 5950–5955.
- (16) Appleby, T. C.; Kinsland, C.; Begley, T. P.; Ealick, S. E. The Crystal Structure and Mechanism of Orotidine 5'-Monophosphate Decarboxylase. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 2005–2010.
- (17) Wu, N.; Mo, Y.; Gao, J.; Pai, E. F. Electrostatic Stress in Catalysis: Structure and Mechanism of the Enzyme Orotidine Monophosphate Decarboxylase. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 2017–2022.
- (18) Amyes, T. L.; Richard, J. P.; Tait, J. J. Activation of Orotidine 5'-Monophosphate Decarboxylase by Phosphite Dianion: The Whole Substrate Is the Sum of Two Parts. *J. Am. Chem. Soc.* **2005**, *127*, 15708–15709.
- (19) Thoma, J. A.; Koshland, D. E., Jr. Competitive Inhibition by Substrate during Enzyme Action. Evidence for the Induced-Fit Theory 1,2. *J. Am. Chem. Soc.* **1960**, *82*, 3329–3333.
- (20) Richard, J. P. Enabling Role of Ligand-Driven Conformational Changes in Enzyme Evolution. *Biochemistry* **2022**, *61*, 1533–1542.
- (21) Kamerlin, S. C. L.; Warshel, A. Multiscale Modeling of Biological Functions. *Phys. Chem. Chem. Phys.* **2011**, *13*, 10401–10411.
- (22) Åqvist, J.; Warshel, A. Simulation of Enzyme Reactions Using Valence Bond Force Fields and Other Hybrid Quantum/Classical Approaches. *Chem. Rev.* **1993**, *93*, 2523–2544.
- (23) Warshel, A.; Weiss, R. M. An Empirical Valence Bond Approach for Comparing Reactions in Solutions and in Enzymes. *J. Am. Chem. Soc.* **1980**, *102*, 6218–6226.

(24) Bauer, P.; Barrozo, A.; Purg, M.; Amrein, B. A.; Esguerra, M.; Wilson, P. B.; Major, D. T.; Åqvist, J.; Kamerlin, S. C. L. Q6: A Comprehensive Toolkit for Empirical Valence Bond and Related Free Energy Calculations. *SoftwareX* **2018**, *7*, 388–395.

(25) Warshel, A. *Computer Modeling of Chemical Reactions in Enzymes and Solutions*; Wiley: New York, 1991.

(26) Warshel, A. Energetics of Enzyme Catalysis. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5250–5254.

(27) Goryanova, B.; Amyes, T. L.; Gerlt, J. A.; Richard, J. P. OMP Decarboxylase: Phosphodianion Binding Energy Is Used To Stabilize a Vinyl Carbanion Intermediate. *J. Am. Chem. Soc.* **2011**, *133*, 6545–6548.

(28) Ram Prasad, B.; Warshel, A. Prechemistry versus Preorganization in DNA Replication Fidelity. *Proteins: Struct., Funct., Bioinf.* **2011**, *79*, 2900–2919.

(29) Nair, H. K.; Lee, K.; Quinn, D. M. M-(N,N,N-Trimethylammonio)Trifluoroacetophenone: A Femtomolar Inhibitor of Acetylcholinesterase. *J. Am. Chem. Soc.* **1993**, *115*, 9939–9941.

(30) Kazemi, M.; Himo, F.; Åqvist, J. Enzyme Catalysis by Entropy without Circe Effect. *Proc. Natl. Acad. Sci. U.S.A.* **2016**, *113*, 2406–2411.

(31) Jencks, W. P. *Catalysis in Chemistry and Enzymology*; Courier Corporation, 1987.

(32) Souza, B. S.; Mora, J. R.; Wanderlind, E. H.; Clementin, R. M.; Gesser, J. C.; Fiedler, H. D.; Nome, F.; Menger, F. M. Transforming a Stable Amide into a Highly Reactive One: Capturing the Essence of Enzymatic Catalysis. *Angew. Chem.* **2017**, *129*, 5429–5432.

(33) Menger, F. M.; Nome, F. Interaction vs Preorganization in Enzyme Catalysis. A Dispute That Calls for Resolution. *ACS Chem. Biol.* **2019**, *14*, 1386–1392.

(34) Jindal, G.; Warshel, A. Misunderstanding the Preorganization Concept Can Lead to Confusions about the Origin of Enzyme Catalysis. *Proteins: Struct., Funct., Bioinf.* **2017**, *85*, 2157–2161.

(35) Schutz, C. N.; Warshel, A. Analyzing Free Energy Relationships for Proton Translocations in Enzymes: Carbonic Anhydrase Revisited. *J. Phys. Chem. B* **2004**, *108*, 2066–2075.

(36) Kong, Y. S.; Warshel, A. Linear Free Energy Relationships with Quantum Mechanical Corrections: Classical and Quantum Mechanical Rate Constants for Hydride Transfer between NAD⁺ Analogs in Solutions. *J. Am. Chem. Soc.* **1995**, *117*, 6234–6242.

(37) Warshel, A.; Sharma, P. K.; Chu, Z. T.; Åqvist, J. Electrostatic Contributions to Binding of Transition State Analogues Can Be Very Different from the Corresponding Contributions to Catalysis: Phenolates Binding to the Oxyanion Hole of Ketosteroid Isomerase. *Biochemistry* **2007**, *46*, 1466–1476.

(38) Rogers, G. A.; Bruice, T. C. Synthesis and Evaluation of a Model for the So-Called Charge-Relay System of the Serine Esterases. *J. Am. Chem. Soc.* **1974**, *96*, 2473–2481.

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