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Theoretical Investigation of the Mechanisms of ERK2 Enzymatic Catalysis

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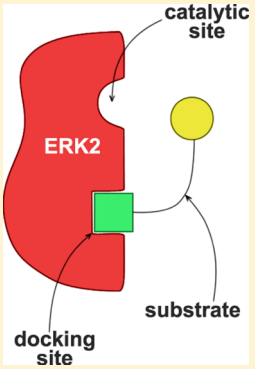
ABSTRACT: ERK2 are protein kinases that during the enzymatic catalysis, in contrast to traditional enzymes, utilize additional interactions with substrates outside of the active sites. It is widely believed that these docking interactions outside of the enzymatic pockets enhance the specificity of these proteins. However, the molecular mechanisms of how the docking interactions affect the catalysis remain not well understood. Here, we develop a simple theoretical approach to analyze the enzymatic catalysis in ERK2 proteins. Our method is based on first-passage process analysis, and it provides explicit expressions for all dynamic properties of the system. It is found that there are specific binding energies for substrates in docking and catalytic domains that lead to maximal enzymatic reaction rates. Thus, we propose that the role of the docking interactions is not only to increase the enzymatic specificity but also to optimize the dynamics of the catalytic process. Our theoretical results are utilized to describe experimental observations on ERK2 enzymatic activities.



INTRODUCTION

Traditionally, biochemists assume that enzymatic catalytic processes are fully determined by processes that take place in the active sites of enzymes.[1] However, there are several protein species, such as some protein kinases, phosphatases, and ubiquitin ligases, that employ additional interactions with the substrates outside of the enzymatic pockets.[2,3] It has been argued that these additional interactions, which are known as docking interactions, help enzymes to increase their specificity in a very large pool of chemically similar substrates in cells.[2,3] At the same time, molecular mechanisms of enzymatic catalysis in the presence of docking interactions remain poorly understood.[4]

One of the most important enzymes that employ the docking interactions during the catalysis is an ERK2 protein.[5] It belongs to a family of mitogen-activated protein kinase (MAPK) enzymes,[6,7] and it catalyzes the reaction of phosphorylation of OH groups in serine or threonine amino acid residues. MAPKs are responsible for a large number of critical processes in the living cells by transmitting extracellular signals from hormones and growth factors via cascades of sequential phosphorylations of the corresponding proteins.[1,5] ERK2 is part of this signaling cascade and its activity, in turn, is also controlled by the phosphorylation: unphosphorylated enzyme is 3 orders of magnitude less active than the phosphorylated molecule.[6,7] Activation is achieved through phosphorylation of Tyr^{185} and Thr^{183} residues, but the exact details of this process are still unknown. There is evidence that deviations in the regulation of the activity of ERK2 enzymes can lead to the development of broad spectrum of cancers and other diseases.[5]

ERK2 is a 42 kDa protein consisting of two domains: N-terminal, which is formed mainly by β-strands, and C-terminal, which is mainly α-helical. The structure of this protein is shown in Figure 1. The catalytic site is located in a cleft between two

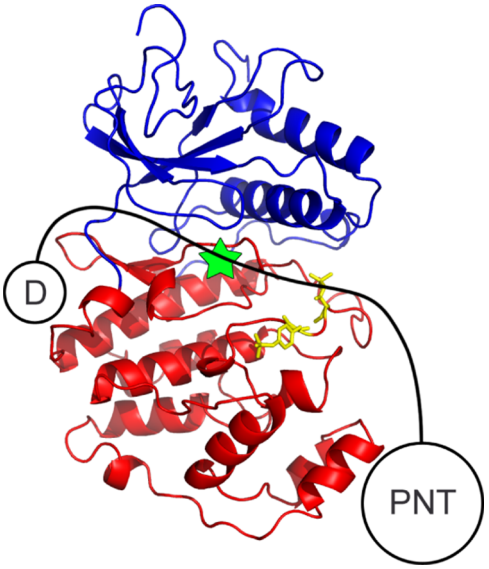


Figure 1. Cartoon representation of the activated ERK2 enzyme (2P-ERK2, PDB entry 2ERK) in a binding mode with a substrate *Ets*Δ138 (Ets-1 protein with only first 138 amino acid residues left). The N-terminal domain of ERK2 is shown in blue, and the C-terminal domain is shown in red. Two residues that are being phosphorylated upon activation are shown in yellow. Green star shows an approximate position of the active site. PNT stands for the corresponding domain of EtsΔ138 that participates in docking interactions, whereas D designates the part of the EtsΔ138 molecule that binds to an additional D-recruiting site of ERK2.

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domains. Phosphorylation puts negative charges on modified residues, creating local strong electrostatic interactions. These new interactions apparently change the relative position of the domains, but the exact mechanism is yet to be discovered. Latest research suggests that the activation opens the access to the active site, allowing the proper orientation of adenosine triphosphate (ATP) molecule relative to the substrate, and it also induces some conformational changes in the docking region.[8−11] ERK2 is a proline-directed protein kinase. This means that it phosphorylates only the residues with a proline in a proper position.[12,13] This has a very dramatic effect on the activity of this enzyme. Interactions in the catalytic site are relatively weak, which implies a need for additional interactions outside of its enzymatic pocket, the so-called docking sites, to control the substrate specificity. This unusual mode of action allows ERK2 to phosphorylate incredibly large range of substrates with a variety of different structures. Although the need for a separate docking site can be explained by a requirement to phosphorylate many different substrates, the quantitative molecular description of the enzymatic catalysis by ERK2 protein still does not exist.[5]

In this article, we present a simple theoretical approach for understanding the enzymatic catalysis in ERK2 proteins. Our goal is to develop a minimalist quantitative model that might clarify the role of the docking site and the catalytic site interactions with the substrates. We also intend to provide a molecular description of the chemical-kinetic experiments on the enzymatic activities of the ERK2 proteins. Our main finding is that the docking interactions outside of the catalytic site not only improve the enzymatic specificity but also optimize the dynamics of ERK2-catalyzed processes.

THEORY

We introduce a simplified discreet-state model of ERK2 activities as schematically shown in Figure 2. For simplicity, it is assumed that there are only two sites of interactions between the enzyme and the substrate: in the docking site and in the catalytic site. Thus, we omit the additional interactions at the D-recruiting site. But note that our theoretical model can be easily extended to take into account any additional biochemical states.

We define a state 0 as the one in which the substrate is not associated at all with the enzyme, and this is the starting point of all enzymatic processes. With rate u_0, part of the substrate binds to the docking site first, leading to state 1 (see Figure 2). From state 1, the system can transfer into state 2 via the substrate binding to the enzyme in the catalytic site with rate u_1. The reverse rate back to state 0 is equal to w_1. From state 2, the substrate can be modified to yield the final product with rate u_2, transforming the system into state 3. The substrate can also fall back into state 1 with rate w_2; see Figure 2. State 3 in our model is identical to state 0, and the enzymatic cycle consists of three states. More states and transitions can be added, but the main features of the enzymatic catalysis in this system should be captured by this simple model.

To analyze the dynamics of this system, we employ a first-passage process approach that has been successfully used to describe various phenomena in chemistry, physics, and biology.[14,15] The method is based on analytically evaluating a probability function, $F_n(t)$, to complete the enzymatic cycle (i.e., to reach final state 3) for the first time at time t if at $t = 0$ the system is started in state n. Computing explicitly these functions will provide a *full dynamic description* of the catalysis

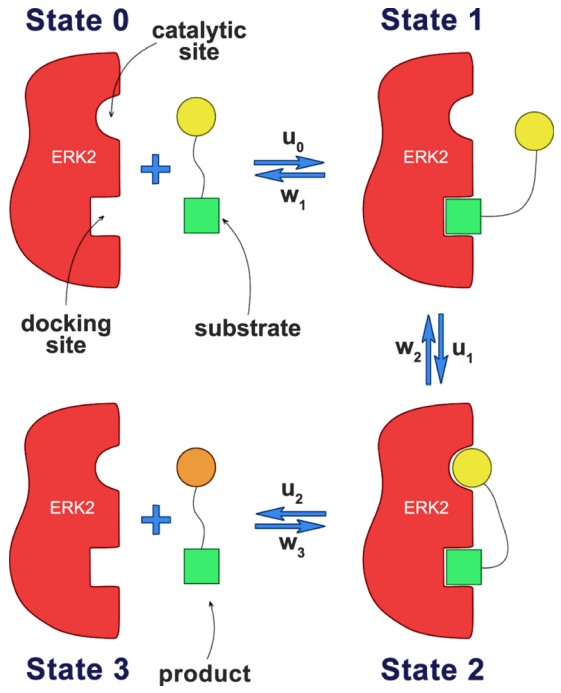

Figure 2. Schematic view of the three-state enzymatic catalysis by ERK2 proteins. The enzyme has two special regions: the catalytic site and the docking site. The substrate is viewed as consisting of two parts: green domain can bind to the docking site, and the yellow domain can bind to the catalytic site. These domains sequentially bind to the enzyme.

process. This is an important advantage of the first-passage method over conventional chemical-kinetic approaches that can calculate only the mean times for transitions. The temporal evolution of first-passage probabilities is given by the set of backward master equations,[14,15] which are also related to ordinary kinetic equations

$$\frac{dF_0(t)}{dt} = u_0F_1(t) - u_0F_0(t) \tag{1}$$

$$\frac{dF_1(t)}{dt} = u_1F_2(t) + w_1F_0(t) - (u_1 + w_1)F_1(t) \tag{2}$$

$$\frac{dF_2(t)}{dt} = u_2F_3(t) + w_2F_1(t) - (u_2 + w_2)F_2(t) \tag{3}$$

In addition, we have the initial condition, $F_3(t) = \delta(t)$, which has the following physical meaning. If the enzyme starts at $t = 0$ in state 3, then our product is already formed, so the reaction is immediately completed.

It is convenient to calculate the first-passage probabilities using the Laplace transformations, $\int_0^\infty e^{-st}F_n(t)\,dt = \widetilde{F}_n(s)$. Then eqs 1−3 can be written as the following algebraic expressions

$$(s + u_0)\widetilde{F}_0(s) = u_0\widetilde{F}_1(s) \tag{4}$$

$$(s + u_1 + w_1)\widetilde{F}_1(s) = u_1\widetilde{F}_2(s) + w_1\widetilde{F}_0(s) \tag{5}$$

$$(s + u_2 + w_2)\widetilde{F}_2(s) = u_2 + w_2\widetilde{F}_1(s) \tag{6}$$

In eq 6, we explicitly used the initial condition in the form $\widetilde{F}_3(s) = 1$. These equations can be exactly solved, and we are interested in only the first-passage probability starting from

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state 0, which describes the full enzymatic process. It can be easily shown that

$$\widetilde{F_0}(s) = \frac{u_0u_1u_2}{s(s+u_1+w_1)(s+u_2+w_2)+u_0(s+u_1)(s+u_2)+sw_2(u_0-u_1)} \tag{7}$$

One can check this result in the limiting case of $s = 0$ because in this case, it should give the probability for the reaction to take place if one can wait infinite amount of time. As expected, we obtain $\widetilde{F_0}(s=0) = 1$.

From the expression for the first-passage probability, one can find the average time for the catalytic reaction (turnover time)[14,15]

$$T_0 = -\frac{d\widetilde{F_0}}{ds}(s=0) \tag{8}$$

It yields

$$T_0 = \frac{u_0u_1 + u_0u_2 + u_1u_2 + u_0w_2 + u_2w_1 + w_1w_2}{u_0u_1u_2} \tag{9}$$

The first transition rate, u_0, must be proportional to the concentration of the substrate S, that is, $u_0 = k_0S$. Then, the expression for the turnover time can be written as

$$T_0 = \frac{u_1u_2 + u_2w_1 + w_1w_2}{k_0u_1u_2}\frac{1}{S} + \frac{u_1 + u_2 + w_2}{u_1u_2} \tag{10}$$

One can see that eq 10 has an expected Michaelis−Menten form, that is, the enzymatic rate (defined as the inverse turnover time) is proportional to S for low concentration of substrates, and it saturates for large concentration of substrates. Now we can explicitly determine the relevant kinetic parameters in terms of the microscopic transition rates from our model

$$K_M = \frac{u_1u_2 + u_2w_1 + w_1w_2}{k_0(u_1 + u_2 + w_2)} \tag{11}$$

$$k_{cat} = \frac{u_1u_2}{u_1 + u_2 + w_2} \tag{12}$$

and the specificity constant is given by

$$\frac{k_{cat}}{K_M} = \frac{k_0}{1 + \frac{w_1}{u_1} + \frac{w_1w_2}{u_1u_2}} \tag{13}$$

From eq 13, it can be immediately seen that the maximum value of the specificity constant is limited by k_0, which is the rate constant for the substrate binding to the docking site. This is consistent with the views that docking interactions govern enzyme specificity.

To better understand the microscopic picture of the enzymatic process, we assume that the substrate interacts with the enzyme at the catalytic site with energy ε_c, whereas at the docking site, the interaction strength is equal to ε_d. Then, the detailed balance-like arguments suggest that the transition rates are related via

$$\frac{u_0}{w_1} = \frac{u_0^{(0)}}{w_1^{(0)}}e^{-\beta\varepsilon_d} \tag{14}$$

$$\frac{u_1}{w_2} = \frac{u_1^{(0)}}{w_2^{(0)}}e^{-\beta\varepsilon_c} \tag{15}$$

$$\frac{u_2}{w_3} = \frac{u_2^{(0)}}{w_3^{(0)}}e^{\beta(\varepsilon_d+\varepsilon_c)} \tag{16}$$

In these expressions, the rates with superscript (0) correspond to transitions rates when the corresponding interaction energies are equal to zero.

More explicitly, we can write the transition rates in the following form[15]

$$u_0 = u_0^{(0)}e^{-\beta\theta\varepsilon_d}, \quad w_1 = w_1^{(0)}e^{\beta(1-\theta)\varepsilon_d} \tag{17}$$

$$u_1 = u_1^{(0)}e^{-\beta\theta\varepsilon_c}, \quad w_2 = w_2^{(0)}e^{\beta(1-\theta)\varepsilon_c} \tag{18}$$

$$u_2 = u_2^{(0)}e^{\beta\theta(\varepsilon_d+\varepsilon_c)} \tag{19}$$

Here, a parameter $0 < \theta < 1$ specifies how the interaction energy is distributed between the forward and backward transitions.[15] Generally, these parameters should be different for each transition. However, we explicitly checked that allowing different values for the distribution parameter, θ, does not change the physical picture of the process. Thus, to simplify the calculations, it was assumed that all distribution parameters for all transitions are the same. In addition, we will drop superscript (0) in the equations below.

This theoretical method allows us to analyze the turnover times as a function of interaction energies at the catalytic and docking sites. The final expression for T_0 yields

$$T_0 = \frac{u_1u_2e^{\beta\theta\varepsilon_d} + u_2w_1e^{\beta(\theta\varepsilon_c+\varepsilon_d)} + w_1w_2e^{\beta(1-\theta)(\varepsilon_d+\varepsilon_c)}}{k_0u_1u_2e^{\beta\theta\varepsilon_d}}\frac{1}{S} + \frac{u_1e^{-\beta\theta\varepsilon_c} + u_2e^{\beta\theta(\varepsilon_d+\varepsilon_c)} + w_2e^{\beta(1-\theta)\varepsilon_c}}{u_1u_2e^{\beta\theta\varepsilon_d}} \tag{20}$$

In addition, the effect of mutations on the enzymatic catalysis can be discussed using a similar approach.[14,15] The expressions for the Michaelis−Menten parameters in terms of interaction energies at the docking and catalytic sites are given by

$$K_M = \frac{u_1u_2e^{\beta\theta\varepsilon_d} + u_2w_1e^{\beta(\theta\varepsilon_c+\varepsilon_d)} + w_1w_2e^{\beta(1-\theta)(\varepsilon_d+\varepsilon_c)}}{k_0(u_1e^{-\beta\theta\varepsilon_c} + u_2e^{\beta\theta(\varepsilon_d+\varepsilon_c)} + w_2e^{\beta(1-\theta)\varepsilon_c})} \tag{21}$$

$$k_{cat} = \frac{u_1u_2e^{\beta\theta\varepsilon_d}}{u_1e^{-\beta\theta\varepsilon_c} + u_2e^{\beta\theta(\varepsilon_d+\varepsilon_c)} + w_2e^{\beta(1-\theta)\varepsilon_c}} \tag{22}$$

and the specificity constant is

$$\frac{k_{cat}}{K_M} = \frac{k_0}{1 + \frac{w_1}{u_1}e^{\beta((1-\theta)\varepsilon_d+\theta\varepsilon_c)}\left(1 + \frac{w_2}{u_2}e^{\beta((1-\theta)\varepsilon_c-\theta(\varepsilon_c+\varepsilon_d))}\right)} \tag{23}$$

Then, mutations at the catalytic and docking sites can be viewed as corresponding to different interaction energies that deviate from the corresponding energies for reactions with the wild-type (WT) substrate.

RESULTS AND DISCUSSION

Let us consider first the effect of interactions in the docking site. Our model predicts that because mutations in the docking

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site are associated with different values of ε_D, the chemical-kinetic parameters of the system will also vary. The dependence of the kinetic parameters on the binding energy, ε_d, is presented in Figures 3–6. For these calculations, we vary the binding

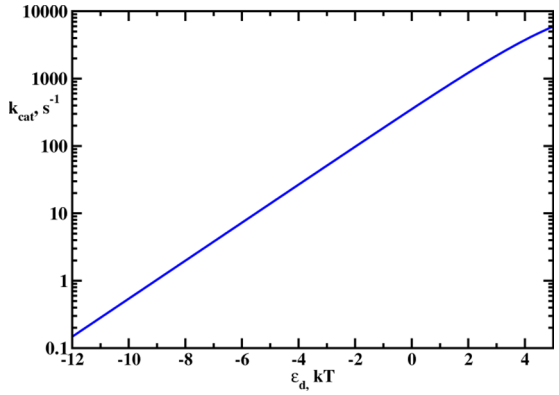

Figure 3. Dependence of k_{cat} on the binding energy in the docking region (ε_d). For calculations, the following model parameters were used: $u_1 = 2500\ s^{-1}$, $u_2 = 2500\ s^{-1}$, $w_1 = 2500\ s^{-1}$, $w_2 = 2500\ s^{-1}$, $k_0 = 10^6\ s^{-1}\ M^{-1}$, $\theta = 0.6$, $\varepsilon_c = -2.9k_BT$.

energy in the docking site, ε_d, keeping all other microscopic parameters fixed. Because the individual transition rates are not yet determined in experiments, we choose some arbitrary, but reasonable, parameters. Thus, the results presented in these figures are mostly semiquantitative. They illustrate the general trends on how the docking interactions affect the enzymatic cycle.

As shown in Figures 3 and 4, both kinetic parameters, K_M and k_{cat}, depend almost exponentially on the docking binding

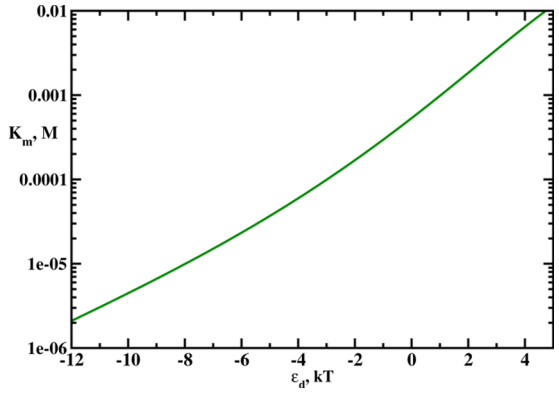

Figure 4. Dependence of K_M on the binding energy in the docking region (ε_d). For calculations, the following model parameters were used: $u_1 = 2500\ s^{-1}$, $u_2 = 2500\ s^{-1}$, $w_1 = 2500\ s^{-1}$, $w_2 = 2500\ s^{-1}$, $k_0 = 10^6\ s^{-1}M^{-1}$, $\theta = 0.6$, $\varepsilon_c = -2.9k_BT$.

energy. Making the interaction energy stronger (more negative) in the docking region leads to a decrease in both catalytic and Michaelis constants. This suggests that very strong attractive attractions between the substrate and the enzyme in the docking domain would actually significantly slow down the catalytic rate of the process ($k_{cat} \to 0$). In this case, the enzymatic rate will be very low because the substrate will have a low probability to detach from the enzyme molecule. The ratio of catalytic and Michaelis constants is also an important quantity, which determines the specificity of the enzymatic reaction, which is plotted in Figure 5. As one can see, the specificity parameter is a nonmonotonic function of the binding

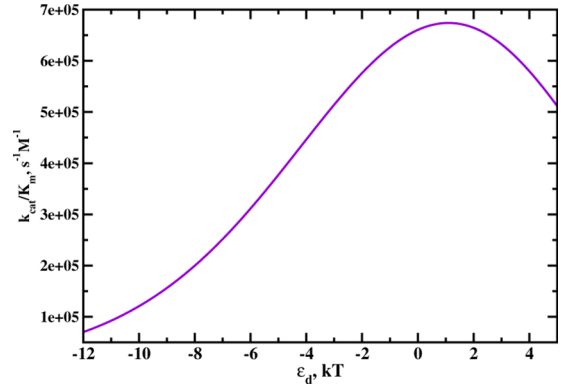

Figure 5. Dependence of the specificity parameter, k_{cat}/K_M, on the binding energy in the docking region (ε_d). For calculations, the following model parameters were used: $u_1 = 2500\ s^{-1}$, $u_2 = 2500\ s^{-1}$, $w_1 = 2500\ s^{-1}$, $w_2 = 2500\ s^{-1}$, $k_0 = 10^6\ s^{-1}\ M^{-1}$, $\theta = 0.6$, $\varepsilon_c = -2.9k_BT$.

energy in the docking domain, supporting the idea that the additional interactions outside the catalytic site are important for increasing the specificity of the enzymatic processes. But our theory also predicts a nonmonotonic behavior for the turnover time as a function of the binding energy at the docking site, as indicated in Figure 6. This is an important result because it suggests that another function of the docking interactions is to optimize also the dynamics of the enzymatic reactions.

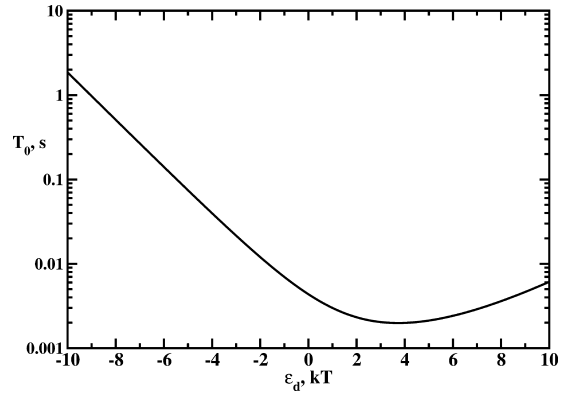

Figure 6. Dependence of the turnover time, T_0, on the binding energy in the docking region (ε_d). For calculations, the following model parameters were used: $u_1 = 2500\ s^{-1}$, $u_2 = 2500\ s^{-1}$, $w_1 = 2500\ s^{-1}$, $w_2 = 2500\ s^{-1}$, $k_0 = 10^6\ s^{-1}\ M^{-1}$, $\theta = 0.6$, $\varepsilon_c = -2.9k_BT$.

A similar analysis can be done on the role of the interactions at the catalytic site. The results are presented in Figures 7–10. Varying the strength of the interactions in the enzymatic pocket produces a nonmonotonic dependence for k_{cat}, which is different from the docking interactions (see Figure 7). But physically these results can be easily explained. For very strong attractions between the substrate and catalytic site, product formation will be difficult because the substrate will not leave the enzyme pocket. For very weak attractions, the substrate shows a very low tendency to enter the enzymatic pocket, and this is also not beneficial for product formation. The Michaelis constant, K_M, for attractive interactions depends exponentially on ε_c, but it saturates for repulsions (Figure 8). As shown in

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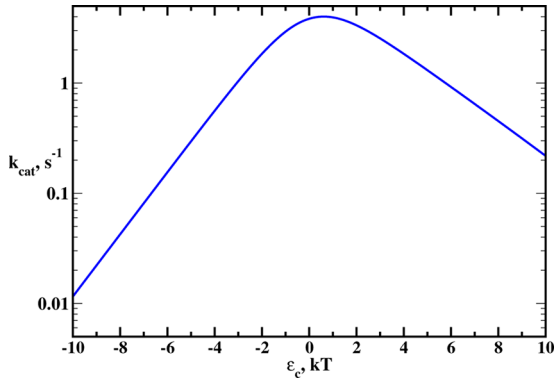

Figure 7. Dependence of k_{cat} on the binding energy in the catalytic region (ε_c). For calculations, the following model parameters were used: $u_1 = 2500\ s^{-1}$, $u_2 = 2500\ s^{-1}$, $w_1 = 2500\ s^{-1}$, $w_2 = 2500\ s^{-1}$, $k_0 = 10^6\ s^{-1}\ M^{-1}$, $\theta = 0.6$, $\varepsilon_d = -8.9k_BT$.

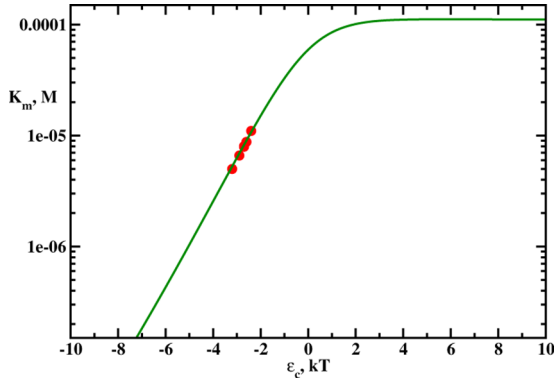

Figure 8. Dependence of K_M on the binding energy in the catalytic region (ε_c). Experimental data are shown by red dots. For calculations, the following model parameters were used: $u_1 = 2500\ s^{-1}$, $u_2 = 2500\ s^{-1}$, $w_1 = 2500\ s^{-1}$, $w_2 = 2500\ s^{-1}$, $k_0 = 10^6\ s^{-1}\ M^{-1}$, $\theta = 0.6$, $\varepsilon_d = -8.9k_BT$.

Figure 9, the specificity decreases as a function of binding energy ε_c, and it is largest for strong attractions. This behavior is different from the docking interaction effect. But the turnover time (Figure 10) again shows a nonmonotonic behavior.

To test our theoretical method, it is important to compare our predictions with available experimental data, which are

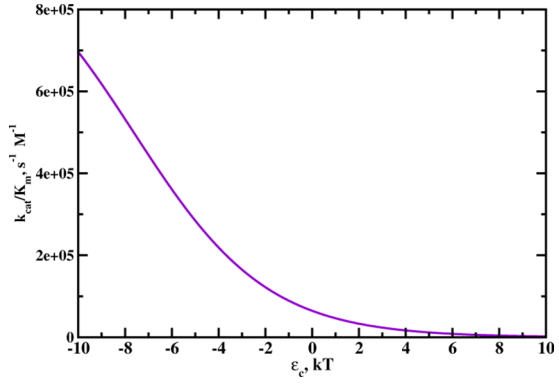

Figure 9. Dependence of specificity parameter k_{cat}/K_M on the binding energy in the catalytic region (ε_c). For calculations, the following model parameters were used: $u_1 = 2500\ s^{-1}$, $u_2 = 2500\ s^{-1}$, $w_1 = 2500\ s^{-1}$, $w_2 = 2500\ s^{-1}$, $k_0 = 10^6\ s^{-1}\ M^{-1}$, $\theta = 0.6$, $\varepsilon_d = -8.9k_BT$.

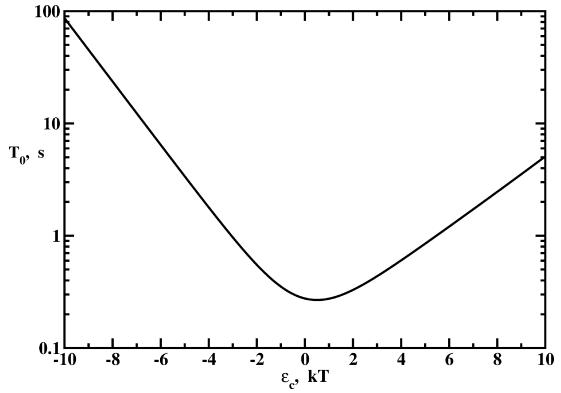

Figure 10. Dependence of the turnover time on the binding energy in the catalytic region (ε_c). For calculations, the following model parameters were used: $u_1 = 2500\ s^{-1}$, $u_2 = 2500\ s^{-1}$, $w_1 = 2500\ s^{-1}$, $w_2 = 2500\ s^{-1}$, $k_0 = 10^6\ s^{-1}\ M^{-1}$, $\theta = 0.6$, $\varepsilon_d = -8.9k_BT$.

given in Tables 1 and 2. For this, we need to estimate the values of ε_d and ε_c from the experiments. There are measurements by

Table 1. Estimates of the Binding Energies for the WT EtsΔ138 Substrate and its Mutants in the Catalytic (ε_c) and Docking (ε_d) Regions of the ERK2 Enzyme[a]

S	K_d, μM	K_1, M^{-1}	K_2	ε_D, k_BT	ε_C, k_BT
WT	6.6	7.7×10^3	18.7	−8.9	−2.9
Mutations in the Catalytic Region					
TA	8.0	7.7×10^3	15.2	−8.9	−2.7
TV	8.8	7.7×10^3	13.8	−8.9	−2.6
TG	7.9	7.7×10^3	15.4	−8.9	−2.7
TR	4.9	7.7×10^3	25.5	−8.9	−3.2
TD	8.0	7.7×10^3	15.2	−8.9	−2.7
TE	11.0	7.7×10^3	10.8	−8.9	−2.4
Mutations in the Docking Region					
F120A	68.0	7.5×10^2	18.7	−6.6	−2.9

[a]The values for K_d are obtained in experiments.[17]

Table 2. Experimentally Measured Catalytic Constants for Phosphorylation of EtsΔ138 (WT) and Its Mutants by ERK2[a]

S	k_{cat}, s^{-1}
WT	19.6
TA	3.8
TV	1.0
TG	0.6
TR	n/d
TD	n/d
TE	n/a
F120A	19.8

[a]Symbol "n/d" means no activity is detected, whereas "n/a" corresponds to no available data.

Dalby et al. on the dissociation constants of the complexes of ERK2 and WT protein *Ets*Δ138 (with only first 138 amino acid resides) and its mutants that can be used to obtain such estimates.[16,17] To do this, let us consider the following kinetic scheme that describes these experiments[16,17]

$$E + S \leftrightarrow ES \leftrightarrow ES' \leftrightarrow ES'' \leftrightarrow E + P \quad (24)$$

with the equilibrium constants for the first and second stages given by

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$$K_1 = \frac{[ES]}{[E][S]}, \quad K_2 = \frac{[ES']}{[ES]} \tag{25}$$

In addition, we assume that

$$\frac{d[ES'']}{dt} = 0 \tag{26}$$

which means that we solve our kinetic scheme using a quasistationary assumption on the concentration of ES″ compound. The following equations can be derived for determining the experimental dissociation constant

$$K_d = \frac{[E][S]}{[ES] + [ES'] + [ES'']} \tag{27}$$

$$\frac{1}{K_d} = \frac{[ES]}{[E][S]} + \frac{[ES']}{[E][S]} + \frac{[ES'']}{[E][S]} \tag{28}$$

$$\frac{1}{K_d} = K_1\left(1 + K_2 + K_2\frac{k_3}{k_{-3} + k_4}\right) \tag{29}$$

If the catalytic stage, which corresponds to the transition between ES′ and ES″, is a rate-limiting step, then $k_3 \ll k_4$, k_{-3}, and we obtain

$$K_d \approx \frac{1}{K_1(1 + K_2)} \tag{30}$$

It should also be noted that for this kinetic scheme one can show that $K_M \approx K_d$.

In experiments,[16,17] a dissociation constant for molecule EtsΔ51−138, which has only the docking domain, has been measured. Then, using eq 30, we obtain a value $K_1 = 1/K_d = 7.7 \times 10^3$ M^{-1}. From this, we estimate the docking binding energy as $\varepsilon_d = -k_BT \ln K_1 \simeq -8.9k_BT$. Now we can compute the values of K_1 and K_2 for the WT EtsΔ138 protein and its mutants, assuming that the mutations in the docking domain do not change K_2 and that the mutations in the catalytic domain do not change K_1. This corresponds to an assumption that the two binding sites (catalytic and docking) are independent of each other, which might be reasonable because they are separated by a relatively large distance. Considering first the mutations in the catalytic region, this means that K_1 is the same for all mutated species and the corresponding parameters K_2 are computed from the experimental values of K_d using eq 30. Then, the binding energy in the catalytic region is calculated as $\varepsilon_c = -k_BT \ln K_2$. Similarly, for the mutations in the docking region, we assume that K_2 is the same as in the WT case, and from eq 30, it leads to the corresponding value of K_1. The results of our calculations are presented in Table 1.

Using our approximate calculations, the effect of mutations can now be analyzed. We estimated that the binding energy in the docking site for the WT substrate is $\varepsilon_D \simeq -8.9k_BT$, whereas mutation F120A in the docking region leads to a slightly weaker interaction, $\varepsilon_D \simeq -6.6k_BT$ (see Table 1). This change does not affect much the catalytic constant, k_{cat}, as indicated below in Table 2, but the Michaelis constant ($K_M \approx K_d$) increases by an order of magnitude. From this, we conclude that the specificity (k_{cat}/K_M) of the substrate mutated in the region responsible for the docking interactions (F120A) also decreases. This is in excellent agreement with experimental measurements.[16] This also emphasizes the importance of docking interactions for the specificity of the system

Now, let us consider the effect of mutations in the catalytic region. For the WT substrate, we estimate the catalytic binding energy as $\varepsilon_c = -2.9k_BT$ (see Table 1). It is interesting that the substrate−enzyme interaction at the catalytic site is relatively weak. This might be the reason for ERK2 to involve the additional interactions to sustain its high specificity. One should also note that some mutations (TA,TV,TG, TD, and TE) lead to weaker interactions comparing with the WT substrate. At the same time, mutation TR makes the binding at the catalytic site stronger. In all cases, these deviations from the WT energies are rather small. But this leads to some changes in the Michaelis constant ($K_M \approx K_d$), which are consistent with our qualitative predictions given in Figure 8. However, our theoretical predictions on qualitative trends for k_{cat} as shown in Figure 7 only partially agree with experimental measurements given in Table 2. Most likely, this is due to the fact that all mutations are introduced right next to the place where the threonine residue is being phosphorylated. This changes the orientation of OH groups in the active site of the protein. In case of TR and TD mutations, strong Coulomb interactions may also lead to binding of the threonine outside the active site, thus preventing its phosphorylation. All these additional processes are not accounted in our simple theoretical model. To test our theory properly, it is probably reasonable to introduce mutations further away from the threonine residue. This way the mutations will lead to a change in the energy of binding without affecting much the orientation of the threonine at the active site.

CONCLUSIONS

We developed a simple theoretical model to analyze the mechanisms of enzymatic catalysis by proteins that utilize additional docking interactions outside of the catalytic site. The advantage of our method is its quantitative nature that allows us to explicitly evaluate all relevant kinetic parameters. This theoretical approach is applied then for describing the enzymatic processes catalyzed by ERK2 proteins. It is argued that different mutations correspond to variations in the binding energies at the docking and catalytic regions, which modify the dynamics of the enzymatic processes. Our theoretical calculations suggest that there are two main functions of the additional docking interactions outside of the enzymatic pocket. First, it helps the enzyme to increase its affinity, and this is important for effective functioning in the complex cellular medium in which many chemically similar species coexist. But another important finding is that the docking interactions optimize the dynamics of the enzymatic process by minimizing the turnover time. However, it is not clear which of these two phenomena, the optimization of specificity or dynamics, dominate the overall behavior of ERK2 enzymes.

Although our theoretical method is able to capture some properties of enzymatic processes in systems with additional interactions outside of the catalytic site, it is important to note that the presented theoretical model is rather oversimplified and many important features are not taken into account. There are additional recruitment interactions in ERK2 enzyme that we do not take into account. Our method also ignores the processes that are taking place while the substrate is in the enzyme pocket. In addition, the model assumes that the processes taking place in the docking and catalytic regions are independent of each other, but this might not be the case always because of possible allosteric phenomena. However, on the positive side, our approach gives specific quantitative

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experimentally testable predictions. It will be important to validate them in experimental studies as well as applying more advanced theoretical and computational methods. This strategy will help in elucidating the complex mechanisms of enzymatic processes.

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Notes

The authors declare no competing financial interest.

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