Relationship between preexponent and distribution over activation barrier energies for enzymatic reactions

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Abstract

A relationship between the preexponent of the rate constant and the distribution over activation barrier energies for enzymatic/protein reactions is revealed. We consider an enzyme solution is an ensemble of individual molecules with different values of the activation barrier energy described by the distribution. From solvent viscosity effect on the preexponent we derive the integral equation for the distribution and find its approximate solution. Our approach enables us to attain a twofold goal. Firstly it yields a simple interpretation of solvent viscosity dependence for enzymatic/protein reactions that requires neither a modification of the Kramers’ theory nor that of the Stokes law. Secondly our approach enables us to deduce the form of the distribution over activation barrier energies. The obtained function has a familiar bell-shaped form and is in qualitative agreement with results of single enzyme kinetics measurements. General formalism is exemplified by the analysis of literature experimental data.

Key words: enzyme catalysis, solvent viscosity, Kramers’ theory, single enzyme kinetics.

1 Introduction

The idea of conformational heterogeneity (multiple conformational substates) [1], [2], [3], [4] received a new impetus in the last fifteen years with introducing the concepts of static [5], quasistatic [6] and dynamic disorder [7], [8], [9], [10]. The results of these studies suggest that there is a broad distribution over the values of the catalytic reaction rate constant $k_2$ for an ensemble...
of enzymes and the rate of a single enzyme strongly fluctuates in time [5, 7, 6, 8, 9, 10]. The reason is that in different conformational substates that an enzyme can probe it has different catalytic efficiency. However it is widely believed that both static and dynamic disorder of reaction rates are essentially indistinguishable in ensemble averaged experiments [7], i.e., the distribution can not manifest itself in ensemble averaged kinetic measurements and is noticeable only in single molecule kinetics [5, 6, 8, 9, 10]. The main reason for the appearance of the distribution is believed to be the fluctuations of the value of the electric field strength in the active sites of the enzymes from the ensemble [11]. The constant electric fields in enzyme active sites created be preorganized dipoles of protein structure are considered as the key factor for the stabilization of the transition state and as a result for the values of the activation barrier energies [12, 13, 14, 15, 16]. In other words they determine enzyme catalytic efficiency [12, 13, 14, 15, 16]. That is why the variability of the electric field strength in enzyme active sites has to lead to a broad distribution over the values of activation barrier energy, i.e., to that of the catalytic reaction rate constant.

In our opinion the point of view that the distribution over the values of activation barrier energy can not manifest itself in ensemble averaged kinetic measurements is inconsistent. Indeed ensemble studies by Frauenfelder and coworkers on rebinding of CO to hemeproteins upon photodissociation long ago revealed dispersed kinetics resulted from heterogeneity [1, 17]. The fluctuations of rate constants (for which recently the term "dynamic disorder" was coined [7, 8, 9, 11]) were also inferred from ensemble studies [1]. One can conclude that if the distribution over activation barrier energies exists it must affect ensemble kinetics somehow. The distribution acquires a status of a unique characteristic of the system and is of primary interest. The aim of the present paper is to show that the existence of the distribution is closely related with the effective preexponent of the enzymatic reaction rate constant. The main premise of the paper is that the existence of the distribution is sufficient for the appearance of the experimentally observable dependence of the enzymatic reaction rate constant on solvent viscosity that manifests itself in the preexponent. Thus we persist to attain a twofold goal. On the one hand we show that the existence of the distribution enables us to interpret experimental data for the effect of solvent viscosity on enzymatic reactions. On the other hand these data enable us to deduce the form of the distribution that can be in principle compared with that obtained from single enzyme kinetic measurements. Regrettfully this comparison can be only indirect and qualitative because for explored by now enzymes exhibiting the solvent viscosity dependence any single enzyme kinetics measurements are impossible. The latter are feasible only for enzymes possessing a unique fluorescent active group such as, e.g., famous flavin adenine dinucleotide in the cholesterol oxidase [7]. However for such enzymes data on solvent viscosity dependence of the reaction rate constant are not available.
Viscosity dependence of enzymatic and protein (ligand binding/rebinding) reactions is known for a long time \[18\], \[19\], \[20\], \[21\], \[22\], \[23\], \[24\], \[25\], \[26\], \[27\], \[28\], \[29\], \[30\], \[31\], \[32\], \[33\], \[17\], \[34\], \[35\], \[36\]. For such reactions the functional dependence of the reaction rate constant for the rate limiting stage \(k\) on solvent viscosity \(\eta\) has the form

\[ k \propto \frac{1}{(\eta/\eta_0)^\beta} \]

where \(\eta_0\) is the viscosity of pure solvent (for water \(\eta_0 = 1 \text{ cP}\) at room temperature) and \(0 < \beta < 1\) (usually \(\beta \approx 0.4 \div 0.8\)). This dependence is experimentally verified in the range of variation of solvent viscosity by two orders of magnitude \(\eta < 100 \text{ cP}\). Similar dependence also takes place for folding of proteins (see \[37\], \[38\], \[39\] and refs. therein) and at formation of protein structure \[40\]. However we will not touch upon these processes in the present paper.

The famous transition state theory in application to rate constants of enzymatic reaction deals predominantly with their free energies (see extensive review of Truhlar and coauthors \[41\] and refs. therein) but fails to shed light on the solvent viscosity dependence of their preexponents. The main tool to describe the solvent viscosity effect on a reaction rate constant is the high friction limit (also called strong damping or overdamped regime) of the Kramers’ theory \[42\] or its modifications \[43\], \[44\] combined with the Stokes law for the friction coefficient. There are many approaches to interpret the effect of solvent viscosity on enzymatic rate constant \[19\], \[22\], \[25\], \[30\], \[31\], \[33\], \[46\], \[47\]. A brief survey of these approaches was presented in our previous paper \[47\]. The model developed there required neither modification of the Kramers’ model nor that of the Stokes law. The main premise of our approach was in the fact that a realistic enzyme solution is actually an ensemble of individual molecules with different characteristics (conditions for the movement of the system along the reaction coordinate). It was shown that the experimentally observed dependence \[11\] can be obtained if we take into account heterogeneity of conditions in the ensemble. The effective reaction rate constant was obtained by averaging of individual Kramers’ rate constants over the distribution. The aim of \[47\] was to show that the idea of heterogeneity enables one to resolve the problem of solvent viscosity effect on enzymatic reactions in a conceptually much more simple way than modification of either the Kramers’ theory or that of the Stokes law employed previously for interpretation of the effect under consideration.

In that regard we stress that the idea about the importance of nonhomogeneity of protein solution for all aspects concerning viscosity dependent effects was introduced earlier in the papers \[31\], \[34\], \[36\]. In these papers a useful notion of local microscopic viscosity was shown to provide quantitative description of experimental data on translational and rotational diffusion of proteins. At
this approach the role of hydrodynamic interactions is highlighted. Making use of the notion of hydrodynamic radius the authors of [34], [36] succeeded in taking into account not only the molecular weight of cosolvent molecules but also their shape and size and obtained quantitative description of translational and rotational protein dynamics in the presence of polymeric cosolvent. However taking into account the nonhomogeneity of the bulk solution is not sufficient for interpretation of experimental data on solvent viscosity dependence for enzymatic and protein reactions because for the latter the protein structural fluctuations are of utmost importance [34]. The analysis similar to that performed for rotational diffusion is not possible in this case because the size of that part of the protein responsible for the fluctuating motion is not known [34]. For enzymatic and protein reactions only experimental data for the dependence of fractional exponents on cosolvent molecular weight are available [30].

In the present paper we retain the main idea of the model [47] but suggest its different technical realization. It seems regretful but inevitable that the way by which the distribution can be introduced in the ensemble of enzymes is not unique. In [47] we made use of the distribution over the weight with which the contribution from solvent viscosity enters into the viscosity for the movement of the system along the reaction coordinate. Thus in fact we carried out the averaging of the preexponent in the effective reaction rate constant over the preexponents of Kramers’ rates for individual samples from the ensemble of enzymes. In the present paper we explore another option and show that the peculiarities of enzyme kinetics enable us to make use of the distribution over activation barrier energies. As was mentioned above the existence of the latter is convincingly confirmed by experimental data from single enzyme kinetics measurements. Thus in the present case we carry out averaging of the preexponent in the effective reaction rate constant over the exponents of Kramers’ rates for individual samples from the ensemble of enzymes. The merit of this approach is that the concept of the distribution over activation barrier energies causes no doubts in the community of the researches. That is why its application to the problem of solvent viscosity effect on enzymatic reactions seems to provide intuitively more simple and acceptable physical picture. The cost for this physical simplicity is somewhat larger mathematical complexity of the present model compared with the previous one. Of course the final choice between these options will be done on the basis of their theoretical non-contradictability and capacity for description of experimental data.

In in the present model as well as in our previous model [47] the experimental dependence of the fractional exponent $\beta$ on the characteristics of cosolvent molecules is incorporated as input information. As was mentioned above for enzymatic and protein reactions only experimental data for the dependence of fractional exponents on cosolvent molecular weight are available [30]. This fact imposes a natural limitation on both models. The present model likewise
that of [47] is able to take into account only cosolvent molecular weight but not the shape and size of cosolvent molecules.

The paper is organized as follows. In Sec. 2 the discrete averaging of the rate constant for the ensemble of enzymes is discussed. In Sec. 3 the continuous version of such averaging within the framework of Kramers’ theory is considered. In Sec. 4 the obtained integral equation for the distribution over activation barrier energies is analyzed. In Sec. 5 the general formalism is applied to the analysis of experimental data. In Sec. 6 the results are discussed and the conclusions are summarized.

2 Discrete averaging of the rate constant for enzymatic reactions

Our primary aim is to derive the averaging procedure for the reaction rate constant. First we consider the value of the reaction rate constant obtainable in the experiment. The initial reaction rate for the Michaelis-Menten scheme (MM) \( E + S \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} P + E \) is given by the well known expression [48]

\[
V \equiv \frac{d[P]}{dt} = \frac{k_2 [E_T][S]}{K_M + [S]}
\]

where \([E_T]\) is the total substrate concentration and the Michaelis constant is

\[
K_M = \frac{k_{-1} + k_2}{k_1}
\]

The reaction rate constant is measured at the excess of the substrate

\[
[S] \gg K_M
\]

Thus one obtains

\[
V \approx k_2 [E_T]
\]

Here \(k_2\) is the effective reaction rate constant that is the average over the ensemble of enzymes. Thus in the experiment one actually measures the value

\[
k_2^{eff} \approx \frac{V}{[E_T]}
\]
Now let us derive the explicit expression for the value of \( k_{eff}^2 \). For the sake of convenience of notation in this Sec. we assume that the ensemble of enzymes consists of a discrete set of pools with different values of the reaction rate constants \( k_i^2 \). That is we consider the kinetic scheme \( E + S \xrightarrow{k_{i}} ES^i \xrightarrow{k_{i}^{-1}} P + E \) where \( i = 1, 2, 3, \ldots \). For the quasi-stationary concentration of the \( i \)-th enzyme-substrate complex \((d[ES^i]/dt = 0)\) we have the usual expression

\[
[ES^i] = \frac{k_1 [E] [S]}{k_{-1} + k_i^2}
\]  

(6)

The total enzyme concentration is given by the expression

\[
[E_T] = [E] + \sum_i [ES^i]
\]  

(7)

From (6) and (7) we obtain the quasi-stationary concentration of the \( i \)-th enzyme-substrate complex

\[
[ES^i] = \frac{k_1 [S] [E_T]}{(k_{-1} + k_i^2) \left\{1 + k_1 [S] \sum_j 1/\left(k_{-1} + k_j^2\right)\right\}}
\]  

(8)

Substituting (8) into the expression for the initial reaction rate

\[
V \equiv \frac{d[P]}{dt} = \sum_i k_i^2 [ES^i]
\]  

(9)

we obtain

\[
V = k_1 [S] [E_T] \sum_i \frac{k_i^2}{(k_{-1} + k_i^2) \left\{1 + k_1 [S] \sum_j 1/\left(k_{-1} + k_j^2\right)\right\}}
\]  

(10)

At the excess of the substrate we have

\[
k_1 [S] \sum_j 1/\left(k_{-1} + k_j^2\right) \gg 1
\]  

(11)

Under this requirement we obtain

\[
V \approx [E_T] \sum_i k_i^2 \delta_i
\]  

(12)
where we have denoted
\[ \delta_i = \frac{1}{(k_{-1} + k_i^2) \sum_j 1/\left(k_{-1} + k_j^2\right)} \] (13)

It is obvious that the values \( \delta_i \) represent normalized fractions, i.e.,
\[ \sum_i \delta_i = 1 \] (14)

Comparing (12) with (5) we finally obtain
\[ k_{2\text{eff}} = \sum_i k_2^i \delta_i \] (15)

The latter is the discrete version of the averaging of the reaction rate constant for enzymatic reactions.

3 Continuous averaging for enzymatic reactions within the framework of Kramers’ theory

The main tool to describe the viscosity dependence of a reaction rate constant is the high friction limit (also called strong damping or overdamped regime) of the Kramers’ theory [42]. In it the reaction is conceived as a diffusion process of a particle with some effective mass along a reaction coordinate over some potential surface. The friction coefficient for the particle \( \mu \) is supposed to obey the Stokes law \( \mu = 6\pi l \eta \) where \( l \) is the characteristic linear size of the particle and \( \eta \) is the viscosity for the movement of the system along the reaction coordinate. The famous Kramers’ formula for the high friction limit is
\[ k_K = \frac{\omega_a \omega_b}{2\pi \mu} \exp \left[ -\frac{\epsilon}{k_B T} \right] \] (16)

where \( \omega_a \) and \( \omega_b \) are characteristic frequencies of the potential surface at the bottom and at the top of the well respectively, \( \epsilon \) is the activation barrier height, \( k_B \) is the Boltzman constant and \( T \) is the temperature.

We denote
\[ a = \frac{\omega_a \omega_b}{12\pi^2 l \eta_0} \] (17)
\[ \alpha = \frac{1}{k_B T} \] (18)
and rewrite the Kramers’ formula as follows

\[ k_K = \frac{a}{\eta/\eta_0} \exp (-\alpha \epsilon) \]  

(19)

On the other hand as was discussed in Introduction for enzymatic reactions the experiment yields

\[ k_2^{eff} = \frac{b}{(\eta/\eta_0)^\beta} \exp (-\alpha E) \]  

(20)

Here \(0 < \beta < 1\) is the empirical parameter while \(b\) and \(E\) are effective values for corresponding parameters. As was stated in Introduction the main premise of the present paper is to relate effective reaction rate constant \(k_2^{eff}\) with the existence of some distribution \(\rho(\epsilon)\) over the barrier heights \(\epsilon\) in the ensemble of enzymes. In other words we assume that for an individual enzyme (sample) from the ensemble the Kramers’ formula (19) with a definite value of \(\epsilon\) takes place. Then the effective rate constant (20) results from the averaging over the ensemble with the distribution \(\rho(\epsilon)\). Thus the ensemble is characterized by the distribution \(\rho(\epsilon)\) over the values of the energy \(\epsilon\).

The continuous version of the discrete averaging considered in the previous Sec. is obtained as follows:

\[ k^i_2 \rightarrow k_2(\epsilon) = \frac{a}{\eta/\eta_0} \exp (-\alpha \epsilon) \]  

(21)

\[ \sum_j 1/ (k_{-1}^j + k^j_2) \rightarrow \int_0^\infty d\epsilon \frac{\rho(\epsilon)}{k_{-1}^j + a/ (\eta/\eta_0) \exp (-\alpha \epsilon)} \]  

(22)

\[ \sum_i \delta_i = 1 \rightarrow \int_0^\infty d\epsilon \rho(\epsilon) = 1 \]  

(23)

\[ E = \sum_i \epsilon_i \delta_i \rightarrow E = \int_0^\infty d\epsilon \rho(\epsilon) \epsilon \]  

(24)

\[ k_2^{eff} = \sum_i k^i_2 \delta_i \rightarrow k_2^{eff} = \int_0^\infty d\epsilon \rho(\epsilon) k_2(\epsilon) \]  

(25)

Taking into account (20) and (13) we obtain from the latter expression the equation for the unknown function of the distribution \(\rho(\epsilon)\)

\[ \frac{b}{(\eta/\eta_0)^\beta} \exp (-\alpha E) = \]
\[
\int_0^\infty d\epsilon \frac{\rho(\epsilon)}{1 + (k_{-1}/a) (\eta/\eta_0) \exp(\alpha\epsilon)} \left[ \int_0^\infty d\epsilon \frac{\rho(\epsilon)}{k_{-1} + a/ (\eta/\eta_0) \exp(-\alpha\epsilon)} \right]^{-1}
\]  

(26)

We denote
\[
c = \frac{k_{-1} \eta}{a \eta_0}
\]

(27)

Then (26) can be cast in the form
\[
\frac{b}{a} \left( \frac{\eta}{\eta_0} \right)^{1-\beta} \int_0^\infty d\epsilon \frac{\rho(\epsilon) \exp(\alpha\epsilon)}{1 + c \exp(\alpha\epsilon)} = \int_0^\infty d\epsilon \frac{\rho(\epsilon)}{1 + c \exp(\alpha\epsilon)}
\]

(28)

Taking into account the obvious identity
\[
\frac{\exp(\alpha\epsilon)}{1 + c \exp(\alpha\epsilon)} = \frac{1}{c} \left( 1 - \frac{1}{1 + c \exp(\alpha\epsilon)} \right)
\]

(29)

and the righthand side of (23) we obtain the integral equation for the distribution \(\rho(\epsilon)\)
\[
\int_0^\infty d\epsilon \frac{\rho(\epsilon)}{1 + (k_{-1}/a) (\eta/\eta_0) \exp(\alpha\epsilon)} = \frac{1}{1 + (k_{-1}/b) (\eta/\eta_0)^{1-\beta} \exp(\alpha E)}
\]

(30)

The righthand sides of (23) and (24) yield two equations
\[
\int_0^\infty d\epsilon \rho(\epsilon) = 1
\]

(31)

\[
E = \int_0^\infty d\epsilon \rho(\epsilon) \epsilon
\]

(32)

for finding two effective parameters \(b\) and \(E\) that can be directly compared with experimentally observable values (note that the parameter \(a\) is expressed via molecular parameters by (17) and is considered as a given one). Thus the equations (30), (31) and (32) represent a closed system of equations for our problem. It is worthy to stress that the equation (30) is strict and exact as long as the requirement of the excess of the substrate
\[
k_1 [S] \int_0^\infty d\epsilon \frac{\rho(\epsilon)}{k_{-1} + a/ (\eta/\eta_0) \exp(-\alpha\epsilon)} >> 1
\]

(33)
4 Analysis of the integral equation (30)

We denote

\[ q = \frac{k_{-1} \exp (\alpha E)}{b} \left( \frac{a}{k_{-1}} \right)^\beta \] \tag{34}

As we will see later in practice the requirement

\[ q >> 1 \] \tag{35}

is satisfied. In this range the following solution of the integral equation (30) can be guessed

\[
\rho(\epsilon) = \frac{\alpha \sin (\pi \beta)}{2\pi} \left[ \frac{1}{\cos (\pi \beta) + \cosh [\alpha \beta \epsilon - \ln q]} + \right.
\]

\[
\frac{\pi}{q} \sum_{n=0}^{[1/\beta-1]} \left( -\frac{1}{q} \right)^n \exp \left\{ - \left[ 1 - (1 + n)\beta \right] \alpha \epsilon \right\} \right]
\] \tag{36}

where \([x]\) in \(\sum_{x}\) means the integer part of \(x\). The latter distribution can be verified by direct substitution into (30) and numerical integration. It yields excellent coincidence of the lefthand side of the equation with the function in its righthand side for a wide range of physically reasonable parameters defined by the requirement (35). In Fig. 1 and Fig. 2 the distribution (36) is depicted at different combinations between the values of the parameters \(\beta\) and \(q\).

Within the range of validity of (36) outlined by the requirement (35) we obtain from (31)

\[ \csc \left[ \pi \beta \left( 1 - \frac{\sin (\pi \beta)}{2q(1 - \beta)} \right) + O \left( \frac{1}{q^2} \right) \right] = \frac{1 + q \cos (\pi \beta)}{q \sin (\pi \beta)} \] \tag{37}

From (32) we obtain

\[
\pi \alpha |E|^2 = \ln (q) \arctan \left( \frac{\sin (\pi \beta)}{1 + \cos (\pi \beta)} \right) + 2\pi \beta \ln 2 - 4L \left( \frac{\pi \beta}{2} \right) +
\]

\[
L \left( \theta - \frac{\pi \beta}{2} \right) - L \left( \theta + \frac{\pi \beta}{2} \right) + 2L \left( \frac{\pi \beta}{2} \right) + \frac{\ln (q)}{2} \times
\]
\[
\left\{ \frac{\pi}{2} - \arcsin \left[ \frac{1 + \cosh (\ln(q)) \cos(\pi \beta)}{\cos(\pi \beta) + \cosh (\ln(q))} \right] \right\} + \frac{\pi \beta \sin(\pi \beta)}{2q(1 - \beta)} + O \left( \frac{1}{q^2} \right) \quad (38)
\]

where

\[
\theta = \arctan \left[ \tanh \left( \frac{\ln(q)}{2} \right) \tan \left( \frac{\pi \beta}{2} \right) \right] \quad (39)
\]

and \(L(x)\) is the Lobachevskii function \(L(x) = -\int_0^x dt \ln(\cos t)\). Recalling that \(q = q(b, E)\) (see (34)) we conclude that equations (37) and (38) are a system of two equations for two unknown parameters \(b\) and \(E\).

There are two ways to analyze the equations obtained. The first way can be called direct (or purely theoretical) one. At this approach we assume the parameter \(a\) given by (17) as a known one (i.e., we assume \(\omega_a, \omega_b\) and \(l\) to be known values), calculate theoretical parameters \(b\) and \(E\) from (37) and (38), identify these parameters with effective values of corresponding parameters known from the experiment and finally compare the theoretical values obtained with experimental values. Parameter \(q = q(b, E)\) is eliminated as an independent value at such approach. In practice this way is hampered by the fact that the values \(\omega_a, \omega_b\) and \(l\) are generally unknown and besides it is rather difficult to solve the system of equations (37) and (38). The second way can be called inverse (or experimentally motivated) one. At this approach we identify parameters \(b\) and \(E\) with effective values extracted from the experiment, consider them as given values while consider the parameters \(a\) and \(q\) as unknown ones. Equation (37) at large values of \(q\) transforms into approximate identity. Thus we conclude that within the range of the validity of our approach outlined by the requirement (35) the normalization of the distribution is always approximately satisfied. Then equation (38) gives the relationship between the parameters \(q\) and \(E\) (direct dependence \(E = E(q)\) or implicit dependence \(q = q(E)\)). In Fig.3 this relationship is depicted at different values of the parameter \(\beta\). At given values of \(E\) and \(\beta\) we can define from (38) the value of the parameter \(q\). From (34) we obtain that the parameter \(a\) is unambiguously determined by \(q\) at given values of \(\beta, b\) and \(E\)

\[
a = k_{-1} \left[ \frac{bq}{k_{-1} \exp(\alpha E)} \right]^{1/\beta} \quad (40)
\]

In practice it is more convenient to proceed along the second way. Thus the following strategy is suggested by the present approach. We consider the empirical fractional exponent \(\beta\), the effective preexponent parameter \(b\), the effective activation energy \(E\) and the reaction rate constant \(k_{-1}\) as given experimental
values. Then we solve the implicit equation (38) to find the value of the parameter \( q \) as a function of the values \( \beta \) and \( E \). If the obtained value of \( q \) satisfies the requirement (35) then our distribution (36) satisfies the normalization requirement (31). Knowing \( \beta \), \( b \), \( E \), \( q \) and \( k_{-1} \) we obtain from (40) the value of the unknown parameter \( a \). The latter is directly related with molecular parameters of the system via relationship (17) and thus provides information of considerable interest. From consideration of (19) and (20) we intuitively anticipate that the value of the parameter \( a \) should not differ significantly from the value of the parameter \( b \). Then the approximate coincidence of the obtained value of \( a \) with the value of \( b \) would mean that our model works satisfactorily well and our approach yields reasonable interpretation experimental data. In the next Sec. we exemplify this strategy by the analysis of data for oxygen escape from hemerythin presented in [30].

5 Analysis of the experimental data from [30]

There are very few enzymatic/protein reactions for which sufficiently complete set of experimental data can be found in the literature. In fact oxygen escape from hemerythin explored in [30] seems to be a unique example. Even for this protein "glycerol is the only cosolvent for which extensive data are available" [30]. For oxygen escape from hemerythin with viscosity varied by glycerol \( k_{esc} (s^{-1}) = 4 \times 10^9 (\eta/\eta_0)^{-0.54} \exp \left[-H_{esc}/(RT) \right] \) where \( H_{esc} = 13 \text{kJ} \cdot \text{mol}^{-1} \) at the temperature of 278 K [30]. Thus we have the following values of the parameters: \( b = 4 \times 10^9 \), \( \beta = 0.54 \) and \( E = 13 \text{kJ} \cdot \text{mol}^{-1} \) at the temperature of 278 K [30]. The latter means that \( \alpha E = E/(k_B T) \approx 5.63 \). From Fig. 3 we obtain for such values of \( \alpha E \) and \( \beta \) that \( \log q \approx 1.2 \), i.e., \( q \approx 15.85 \). This value of the parameter \( q \) satisfies the requirement (35) though with some extension (see Discussion), i.e., our distribution (36) satisfies the normalization requirement (31). The distribution (36) for these values of the parameters is depicted in Fig. 4. In Fig.5 both hands of the integral equation (30) are plotted with the distribution (36) and the parameters obtained.

For oxygen escape from hemerythin the role of \( k_{-1} \) is played by the rate constant \( k_{int} (s^{-1}) = 10^8 \exp \left[-H_{int}/(RT) \right] \) where \( H_{int} = 4 \text{kJ} \cdot \text{mol}^{-1} \) at the temperature of 278 K [30]. Thus we have \( k_{-1} \approx 1.77 \cdot 10^7 \text{ s}^{-1} \). Substituting all parameters into (40) we finally obtain \( a \approx 2.0 \cdot 10^9 \). This value is sufficiently close to the value of the parameter \( b = 4 \times 10^9 \) that provides strong evidence in favor of our approach. We conclude that for oxygen escape from hemerythin the combination of molecular parameters is

\[
a = \frac{\omega_a \omega_b}{12 \pi^2 \eta_0} \approx 2.0 \cdot 10^9
\]
The latter estimate enables one to make assumptions about the shape of the potential energy along the reaction coordinate (characterized by the frequencies $\omega_a$ and $\omega_b$) or the linear size $l$ of the the effective particle used in the Kramers' theory.

6 Discussion

We reveal the relationship between the preexponent of the effective reaction rate constant and the distribution over activation barrier energies for enzymatic/protein reactions. This relationship arises from the averaging of the reaction rate constant in enzyme solution over the ensemble of individual enzyme molecules described in Sec. 3. In the mathematical form it is formalized in the integral equation (30) that possess considerable generality and strictness as long as the requirement of the excess of the substrate (33) is satisfied. Solution of this integral equation yields the normalized distribution over the values of activation barrier energy (36). The typical behavior of the obtained distribution over activation barrier energies is depicted in Fig. 1 and Fig. 2. They show that depending on the combinations of the parameters $\beta$ and $q$ two cases are possible within the range of validity of our approach $q >> 1$: 1. the distribution becomes wider with the decrease of the parameter $\beta$ while the position of its maximum retains its value unchanged (see Fig. 1) and 2. the distribution becomes wider with the decrease of the parameter $\beta$ while the position of its maximum is shifted to higher values (see Fig. 2).

The analysis of experimental data within the framework of the present approach requires combined information on both viscosity and temperature dependence of the reaction rate constant. That is from the experimentally observable value of the effective reaction rate constant

$$k_{2}^{eff} = \frac{b}{(\eta/\eta_0)^{\beta}} \exp \left( -\frac{E}{k_BT} \right)$$

we need to extract three parameters: $b$, $\beta$ and $E$. Besides the value of the reaction rate constant $k_{-1}$ for enzymatic reaction or its analog $k_{int}$ for protein reactions (see previous Sec.) is necessary for the analysis. All these parameters are considered as input information for the present model. The theoretically calculated value of the parameter $a$ must approximately coincide (or at least be commensurable) with the given value $b$ extracted from experimental data. The latter requirement is the criterion for the validity of the present approach. The example of experimental data for oxygen escape from hemerythin with viscosity varied by glycerol from the paper [30] analyzed in the previous Sec. testifies that this criterion can in fact be satisfied for realistic situations. Regretfully these data seem to be unique in regard of their completeness and sufficiency.
for our approach. New experimental measurements are highly desirable in this field of science especially concerning enzymatic rather than protein reactions.

The present approach retains the main idea of our previous model [47] and its capacity for interpretation of experimental data on solvent viscosity dependence for enzymatic reactions. However technical implementation of the present model is different than that of [47]. Here we make use of the distribution over activation barrier energies that has considerable conceptual benefits. The latter distribution is not merely an abstract notion. The progress in single enzyme kinetics measurements has done this distribution to be actually a directly observable experimental function. The distribution over activation barrier energies obtained in the present paper has a familiar bell-shaped form. In contrast the distribution over the weight with which the contribution from solvent viscosity enters into the viscosity for the movement of the system along the reaction coordinate employed in [47] looks somewhat unusual. The latter has divergencies (although integrable, i.e., the distribution is still normalized) at both ends of the range. Thus the distribution obtained in the present paper intuitively seems to be more simple and comprehensible. For this simplification in physical picture we have to pay by increased mathematical complexity of the present model compared with that from [47]. The integral equation obtained in the latter paper had exact solution. For the integral equation derived in the present paper we have been able to obtain only an approximate solution. However this solution yields excellent accuracy within the physically interesting range of the parameters outlined by the requirement (35) as has been verified by numerical integration. We can formulate the range of the validity of our distribution (36) as follows: the latter is the more satisfactory the better the requirement (35) \( q \gg 1 \) is fulfilled. In the case of oxygen escape from hemerythin for which we have rather small value \( q \approx 15.85 \) and the requirement (35) is satisfied with some extension. However from Fig.5 we see that even in this case we obtain satisfactorily good approximation for the solution of the integral equation (30). The small value of \( q \) for this protein reaction is a result of rather low effective activation barrier energy \( H_{esc} = 13 \text{kJ \cdot mol}^{-1} \) at the temperature of 278 K [30] that yields \( E/(k_BT) \approx 5.63 \). For most enzymatic reaction effective activation barrier energies are much higher so that the ratio of \( E/(k_BT) \) attains typical values 15 ÷ 20 at room temperature. From Fig. 3 we see that for such cases the parameter \( q \) attains very large values up to \( 10^3 \div 10^4 \). Thus we conclude that for these reactions the requirement (35) is fulfilled very well. As a result we conclude that for most enzymatic reactions our distribution (36) provides approximation to the solution of the integral equation (30) with excellent accuracy. We anticipate that for such reactions our formalism yields very good quantitative description of experimental data.

The obtained bell-shaped form of the distribution over activation barrier energies is in qualitative agreement with that of the distribution over the reaction rate constant obtained in single enzyme experiments [10]. However the search
of any quantitative correspondence seems to be meaningless and premature. The direct comparison of the distribution obtained from our analysis based on solvent viscosity dependence for enzymatic reactions with those extracted from single enzyme kinetics measurements is hampered by the fact that these two fields have been developed independently and no objects of mutual interest have been explored by now. It seems highly desirable to explore an enzyme for which on the one hand single enzyme kinetics measurements would be feasible and on the other hand sufficiently extensive experimental data for solvent viscosity effect on its reaction rate constant would be available. Data on such object would provide the possibility for the direct and crucial experimental verification of the predictions of the present model. In that regard the investigations of solvent viscosity dependence for cholesterol oxidase (which is the work horse for single enzyme kinetics measurements [7]) and that for \( \beta \)-galactosidase (for which in [10] the distribution over the reaction rate constant is obtained) seem to be of primary interest. The experimental data on solvent viscosity dependence for these enzymes of the same completeness as those for oxygen escape from hemerythin would be invaluable for quantitative verification of the present model.

We conclude that there is the inherent relationship between the distribution over activation barrier energies and the preexponent of the effective reaction rate constant for the solution of enzymes. Our model yields simple interpretation and the quantitative description of the available experimental data on solvent viscosity dependence for enzymatic/protein reactions. The approach is in conceptual coherence with the modern trend stimulated by single enzyme kinetics.

Acknowledgements. The author is grateful to Dr. Yu.F. Zuev for helpful discussions. The work was supported by the grant from RFBR and the programme "Molecular and Cellular Biology" of RAS.
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Fig. 1. Distribution $\rho(\epsilon)$ over the activation barrier energies $\epsilon$ (eq. (36)) at increasing values of the fractional power exponent $\beta$ and different values of the parameter $q$: $\beta = 0.5$, $q = 6$ (thick line); $\beta = 0.6$, $q = 8$; $\beta = 0.7$, $q = 11$; $\beta = 0.8$, $q = 16$; $\beta = 0.9$, $q = 23$ (thin line).
Fig. 2. Distribution $\rho(\epsilon)$ over the activation barrier energies $\epsilon$ (eq. (36)) at increasing values of the fractional power exponent $\beta$ and different values of the parameter $q$: $\beta = 0.5$, $q = 29$ (thick line); $\beta = 0.6$, $q = 41$; $\beta = 0.7$, $q = 58$; $\beta = 0.8$, $q = 82$; $\beta = 0.9$, $q = 117$ (thin line).
Fig. 3. Relationship between the parameters \( q \) and \( E \) (eq. (38)) at increasing values of the fractional power exponent \( \beta \): \( \beta = 0.4 \) (thick line); \( \beta = 0.5 \); \( \beta = 0.6 \); \( \beta = 0.7 \); \( \beta = 0.8 \); \( \beta = 0.9 \) (thin line).
Fig. 4. Distribution $\rho(\epsilon)$ over the activation barrier energies $\epsilon$ (eq. (36)) for the realistic case of experimental data on oxygen escape from hemerythin from the paper (30). The values of the parameters are: $\beta = 0.54; T = 278 \, K \, (\alpha = 1/(k_B T) \approx 2.6 \cdot 10^{13}); q = 15.85$. 


left and right hands of eq. (30)

Fig. 5. Left (thin line) and right (thick line) hands of eq. (30) with the distribution $\rho(\epsilon)$ over the activation barrier energies $\epsilon$ (eq. (36)) for the realistic case of experimental data on oxygen escape from hemerythin from the paper (30). The values of the parameters are: $\beta = 0.54$; $T = 278$ $K$ ($\alpha = 1/(k_B T) \approx 2.6 \cdot 10^{13}$); $q = 15.85$. 