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On the indirect relationship between protein dynamics and enzyme activity

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ABSTRACT

The behaviors of simple thermal systems have been well studied in physical chemistry and the principles obtained from such studies have been applied to complex thermal systems, such as proteins and enzymes. But the simple application of such principles is questionable and may lead to mistakes under some circumstances. In enzymology, the transition state theory of chemical reactions has been accepted as a fundamental theory, but the role of protein dynamics in enzyme catalysis is controversial in the context of transition state theory. By studying behaviors of complex thermal systems, we have revised the Arrhenius equation and transition state theory and our model is validated in enzymology. Formally speaking, the revised Arrhenius equation is apparently similar to a conventional Arrhenius equation, but the physical meanings of its parameters differ from that of traditional forms in principle. Within this model, the role of protein dynamics in enzyme catalysis is well defined and quantified.

Contents

Conventional thermodynamics was developed in the study of behaviors of simple thermal systems such as the ideal gas (Schroeder, 2000). When it was fully established, the principles have been gradually applied to fields of complex thermal systems. Although great achievements have been made over time, we must bear in mind that the validation of the principles of conventional thermodynamics in the field of complex thermal systems has not been fully tested. When it occurs, different conclusions of science may be obtained by applying different research methods. As chemical studies now target the behaviors of complex thermal systems such as biological systems and Nano-scale materials, the case becomes more and more serious (Dill and Bromberg, 2010).

Clearly, the behaviors of complex thermal systems differ from that of simple thermal systems. Currently, there are two different ways to handle these differences. Some scientists, particularly experimentalists, prefer to revise principles of conventional thermodynamics and believe that it can account for all behaviors of complex thermal systems. In another way, some believe that the behaviors of complex thermal systems differ from that of simple thermal systems in principle and new principles of physics and chemistry should be proposed. By the second way, the dynamic

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system and dissipative structure theory have been proposed (Kondepudi and Prigogine, 2014). Inspired by such approaches, we have proposed new principle of irreversible thermodynamics and protein thermodynamic structure theory (Zhao, 2009, 2013).

A fundamental task of science for both biology and physical chemistry is to reveal thermodynamic mechanisms for biological processes and expose the logical relationship between protein motion and function (Frauenfelder and McMahon, 1998; Berendsen and Hayward, 2000). In order to resolve this, one problem of science should firstly be addressed. As biological macromolecules, including proteins, enzymes, DNA and RNA, are all complex thermal systems, we should ascertain whether the thermodynamical principles for simple thermal systems are still suitable or validated in the field of complex thermal systems. If the answer is no, we should endeavor to find the right formulation of thermodynamics for complex thermal systems.

At present, studies on the relationship between protein dynamics and protein function are focusing on the relationship between protein dynamics and enzyme activity. This is currently under fierce debate. One opinion, which is held by many experimental scientists, is that there is a general relationship between protein dynamics and enzyme activity (Bhabha et al., 2011; Henzler-Wildman et al., 2007; Klinman and Kohen, 2013; Garcia-Viloca et al., 2004). Another opinion is that if there is such relationship, the effects of protein dynamics on enzyme activity should be expressed in parameters of enzyme kinetics, particularly activation energy of enzymatic reaction. There is no experimental report that the activation energy of enzymatic reaction could be influenced by a change of protein dynamics, and thus the relationship between protein dynamics and enzyme activity is often disputed (Kamerlin and Warshel, 2010a; Warshel and Ram, 2016).

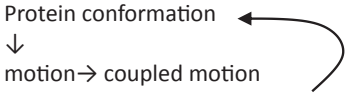
Here we show that the Arrhenius equation and transition state theory, which works well for simple chemical reactions, should be revised in the field of complex thermal systems or enzymatic-catalyzed reactions. This revised Arrhenius equation makes clear the effect of protein dynamics on enzyme activity and changes the parameters of enzymatic kinetics. In addition, our conclusion is that protein dynamics takes its role in enzyme activity by influencing thermodynamic states of protein conformation; or in other words, there is indirect relation between protein dynamics and enzyme activity.

1. Behaviors of simple and complex thermal system

Conventional thermodynamics was developed from studies of the behavior of simple thermal systems. For simple thermal systems, the motions of all the components are completely independent of each other; there are no coupled motions among the components and there is no infrastructure within the system.

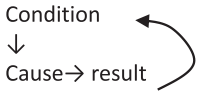
However, the coupled motion or cooperativity is a common phenomenon in protein conformational change. In view of conventional theory, the behaviors of a protein show complexity (Frauenfelder, 2002; Karplus, 2000). In studies of protein thermodynamic structure theory, we have proposed the scientific definition of complex and stable thermal systems (Zhao, 2011a, 2012).

Within a protein, a logical cyclic relationship can be found.



The protein conformation modulates the range and amplitude of internal motion of components of a protein. The change of internal motion can also influence the coupled motion within a protein. The nature of the coupled motions of a protein determines protein conformation.

A more general definition of a system could be expressed.



In this definition, a complex thermal system acts an indivisible whole in thermodynamics and represents one degree of freedom of thermodynamics.

For simple thermal system

$$E = \text{constant}$$

$$E_W = E_1 + E_2 + E_3 + \ldots$$

$$N_p = \sum_i e^{\frac{-E_i}{kT}}$$

where E is energy of its components. E_i is the energy of *I*th component of the system. E_w is the total energy of thermal system. Np is partition function of its components.

For complex thermal system

$$\Delta G = \Delta G^0 - F(s)$$

$$\Delta G = \Delta G^0 - B(s - s_0) \text{ (in linear area)}$$

$$E_W \leq E_1 + E_2 + E_3 + \ldots$$

$$N_p = \sum_i e^{\frac{-[\Delta G^0{}_i - F_i(s)]}{kT}}$$

where ΔG is the energetic level of complex system, F is a function, b is revision efficiency, s is the quantification of an environmental factor, Ew is the total energy of thermal system. N_{ps} is the partition function of the state of a complex thermal system.

Within a complex thermal system, the energy of the total system comes from coupled motion of its components; the uncoupled motion makes no contribution to the total energy of the system, and so the energy of the system is not the sum of energy of its components. The concept of the partition function of a complex thermal system makes it possible to calculate the distribution curve of thermodynamic states of complex thermal systems (Zhao, 2012, 2015).

From the equations above, we can deduce the differences between the behaviors of complex thermal systems when compared to simple thermal systems.

One major difference is that the thermodynamic state of a complex thermal system is sensitive to a change of temperature (or other types of environmental factors) and the abundance of one state can dramatically change over a small temperature range (i.e. the abundance of one state can reach close to 100%). In contrast, the abundance of one quantum state of a molecule at a high energetic level can only reach up to 50% in a broad range of temperatures. See Fig. 1 for detail.

There is a special phenomenon in the behavior of a complex thermal system, or abundance curve shift along an environmental factor (e.g. temperature). When a complex thermal system is changed, for example by a change in sequences, its properties will change also. Compared with the original system, the distribution curve of a new state of the system will appear at a different position along coordinates of an environmental factor (temperature in this

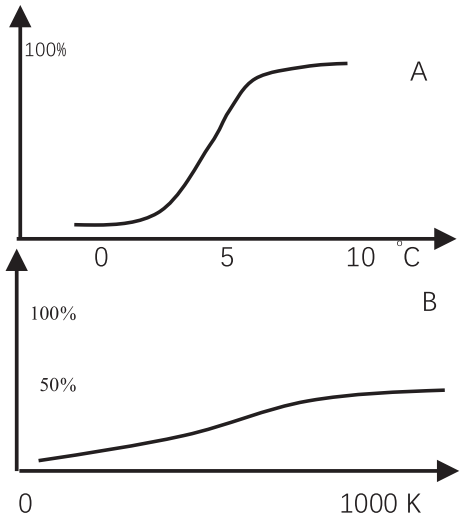


Fig. 1. Distribution curves of a state of the complex thermal system and a quantum state of a molecule.
Fig. 1A is the distribution curve of a state of the complex thermal system. Fig. 1B is a distribution curve of a quantum state of a molecule at a high level of energy.

figure), but the general shape of it may be unaltered (Zhao, 2012, 2015, 2016). Three typical examples of this phenomenon are shown in Fig. 2.

In Fig. 2, three typical distribution curves and shifts are shown. In practice, we can judge the existence of complex thermal systems by the distribution curve shift phenomenon (Zhao, 2012, 2015, 2016). In the field of biochemistry, there is no report about distribution curve shifts of active conformations of an enzyme, but the shift of enzyme activity curve along temperature gradients is a common phenomenon in biological evolution or biological adaptation of microorganisms (Feller and Gerday, 2003; Katava et al., 2016). According to our model, the underlying phenomenon of it is distribution curve shift of active conformations of an enzyme.

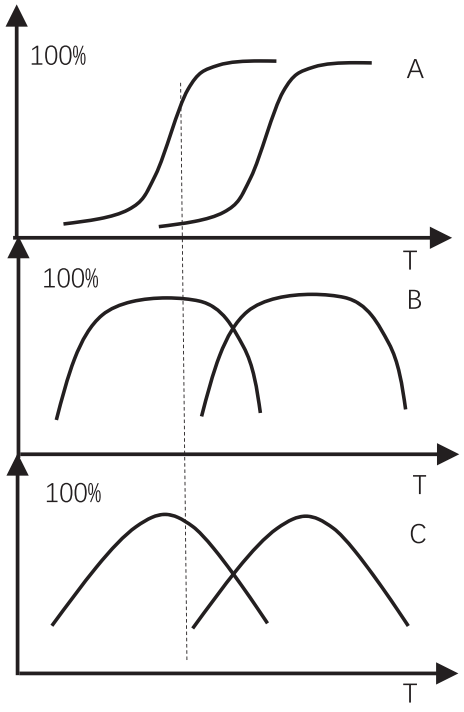


Fig. 2. Three typical distribution curve shifts of the state of a complex thermal system.
Fig. 2A describes general profile of distribution curve shift of two-states transition of a system. Fig. 2B pictures shift of stability curve of the mutated and the original protein with high thermal stability. Fig. 2C is the shift of protein conformation with low thermal stability.

According to protein thermodynamic structure theory, a protein is composed of many complex thermal sub-systems, which are called potherses (Zhao, 2013). The protein conformational states, the foldons, are special potherses, although they were proposed within different models of science (Frauenfelder et al., 1988; Panchenko et al., 1996, 1997). In addition, in view of protein dynamics, a potherse represents an ensemble of protein conformations at the atom level. Theoretically speaking, the exciton in a nanoscale system is a special complex thermal system (Scholes and Rumbles, 2006).

2. Transition state theory and the Arrhenius equation for chemical reactions of complex thermal systems

The Arrhenius equation was first proposed as an experimental equation of rate for chemical reactions. Currently, there are many revisions for it (Laidler, 1984; Hänggi et al., 1990). The underlying principles of it was provided by collision and transition state theory. The collision theory can handle the rates of elementary reactions and transition state theory can handle rather complex chemical reactions (Laidler and King, 1983; Truhlar et al., 1996). Now the transition state theory acts as a standard theory for chemical reactions. Transition state theory has been part of enzymology for a long time (Pauling, 1948).

Although the correctness of transition state theory in the field of simple chemical reactions is beyond doubt, its correctness in the field of complex thermal systems has not been fully tested. In view of systems theory, the so called complex reactions in chemistry are rather simple when compared to reactions of complex thermal systems, such as enzyme-catalyzed reactions (Karplus, 2000).

Several aspects of chemical reactions have been ignored in conventional theory of transition state theory and we discuss these as follows.

Firstly, it cannot give precise description for complex chemical reactions with multiple steps except for where there is only one rate-limiting step. Secondly, the rate limiting steps of a complex reaction may change under different conditions (see following discussion). Thirdly, the relationship between intermediates and the transition state of a chemical reaction cannot be analyzed by this theory. For example, the relationship between enzyme-substrate complex and the transition state of enzyme catalyzed reaction cannot be theoretically answered within this theory. Thus the role of the stable enzyme substrate complex has been ignored and the essential relationship between protein dynamics and enzyme activity cannot be resolved. Fourthly, the conventional transition state theory focuses on the breaking and formation of chemical bonds at the transition state. This is not true for some reactions, for example, the activation energy of protein inactivation (denaturation) certainly comes from the protein conformational change. Sixthly, the nonlinear Arrhenius or Eyring plots can be observed under some circumstances but there is no consensus on this (Truhlar and Kohen, 2001).

Thus, in order to improve the validity of transition state theory and the Arrhenius equation, these should be revised under specific cases, particularly in the field of complex thermal systems. Taking this into consideration, we have revised these equations in our studies of the working mechanism of enzyme action and regulation (Zhao, 2011a, 2012, 2011b). The main points are as follows.

1) The enzyme-substrate complex undergoes several conformational changes along the reaction coordinate and the transition state of an enzyme catalyzed reaction represents a special conformational state of the enzyme-substrate complex.
2) Although the enzyme substrate binding lowers the total energy of the enzyme substrate complex, the energetic level of the enzyme substrate complex in the reaction coordinate is elevated. The transition state of enzyme-reactants represents one type of thermal system and its state can be precisely described by the partition function of the thermal system (Zhao, 2012).

According to our model, the enzyme catalyzed reaction can be expressed

$$\begin{array}{c} (ES)^{IN} \\ \uparrow\downarrow \\ E+S \rightleftharpoons ES \rightleftharpoons (ES)^{*} \rightarrow EP \rightarrow E+P \\ \uparrow\downarrow \\ (ES)^{IN} \end{array}$$

where E is enzyme, S is substrate, $(ES)^{IN}$ represents inactive conformation of enzyme substrate complex, $(ES)^{*}$ is transition state of enzyme substrate complex.

By applying partition function of complex thermal system, the revised Arrhenius equation of enzymatic reactions can be obtained (Zhao, 2012, 2015).

$$K = APexp\left\{\frac{-\Delta G_0 - b[T - T_0]}{k_B T}\right\} = AD_T Pexp\left(-\frac{\Delta G_a}{k_B T}\right)$$

where K is rate constant of enzymatic reaction, k_B is Boltzmann constant, A is traditional pre-exponential factor of Arrhenius equation and its meanings is same as that for simple chemical reaction. ΔG_0 is activation energy at reference temperature and ΔG_a is apparent activation energy of enzymatic reaction. The $(\Delta G_0 - b[T - T_0])$ represents activation energy at a temperature. AD_T is apparent pre-exponential factor of Arrhenius equation for enzymatic reaction. P is abundance of active conformation of enzyme.

The equation can give a precise description of the enzymatic kinetics, in both linear and nonlinear ranges of Arrhenius plot of enzymatic activity (Arcus et al., 2016; Daniel and Danson, 2010).

Within our model, the activation energy of an enzyme catalyzed reaction is not constant. This prediction is supported by the following observations:

1. The activation energies of enzymatic reactions differ from each other when different substrates of the enzyme are utilized (Fersht, 1999).
2. The activation energy of enzymatic reactions is changeable when mutations in the enzyme amino-acid sequence occur (Fields and Houseman, 2004).
3. The change of activation energy is related to the deviation of transition state from normal state. The KIE (Kinetic Isotope Effects) can be utilized to monitor the deviation of the transition state of enzymatic catalyzed reactions. The research shows that the transition state of the enzyme reactions may be different under different conditions (Klinman, 1981; Otzen et al., 1999)
4. The pre-exponential factor of an enzymatic reaction is environmentally dependent (Fenimore et al., 2002).

According to our model, the energetic level of diversified intermediates of an enzyme catalyzed reaction is pictured in Fig. 3.

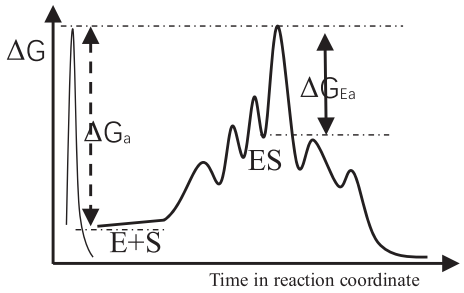


Fig. 3. Diagram of enzymatic catalyzed reaction (cited from Zhao, 2011b with minimal revision).

On the left in Fig. 3 is the energetic level of un-catalyzed chemical reaction, whereas on the right is the energetic level of the enzyme catalyzed reaction. ΔG_a is activation energy of un-catalyzed reaction. ΔG_{Ea} is activation energy of enzyme catalyzed reaction. Then $\Delta G_a - \Delta G_{Ea} \leq \Delta G_B$ where ΔG_B is the binding affinity of enzyme for substrate.

We conclude that the high efficiency of enzymes to catalyze the chemical reaction comes from the binding affinity between enzyme and substrate. Although binding between enzyme and substrate lowers the energy of enzyme substrate complex, it enhances the energetic level of enzyme substrate complex in the direction of chemical reaction.

This statement does not imply that an increase of affinity between enzyme and substrate will improve the catalytic power of the enzyme. Only interactions between enzyme and substrate, which appear both in the equilibrium state and the transition state of enzyme substrate complex, could lower the activation energy of the enzyme-catalyzed reaction. Some types interactions may be harmful to enzyme activity for if they impair the protein conformational change from ground state to transition state of enzyme substrate complex.

For alcohol dehydrogenase, the rate of enzyme-catalyzed reaction is increased as the molecular weight of substrates is increased from alcohol to hexanol; the rate will be lowered when the molecular weight of the substrate is increased further (e.g. Raia et al., 2001; Inoue et al., 2005). This phenomenon can be easily explained by our theory.

3. Alcohol dehydrogenase

Here we take alcohol dehydrogenase as an example to show that the revision of the Arrhenius equation can precisely describe the properties of enzyme kinetics for this enzyme (Zhao, 2013; Nagel et al., 2011; Plapp, 2010). Diverse aspects of the enzyme properties and kinetics are shown in Fig. 4.

At low and high temperatures, alcohol dehydrogenase is inactivated due to protein denaturation and the presence of the active conformation of enzyme depends on the temperature, so the nonlinear Arrhenius plot can be observed. In the linear range of the Arrhenius plot, the active conformation of enzyme is 100% present and is not sensitive to temperature (as well as protein flexibility, protein dynamics). The enzymatic kinetics has been well studied by many scientists (e.g. Lee et al., 2007). Within this figure, we can see that our model can explain all properties of the enzymatic kinetics.

4. Protein folding kinetics

The physics and chemistry of protein folding is complex (Zhao, 2013; Kim and Baldwin, 1990; Chan and Dill, 1998). Small proteins are typically used in studying of study protein folding kinetics.

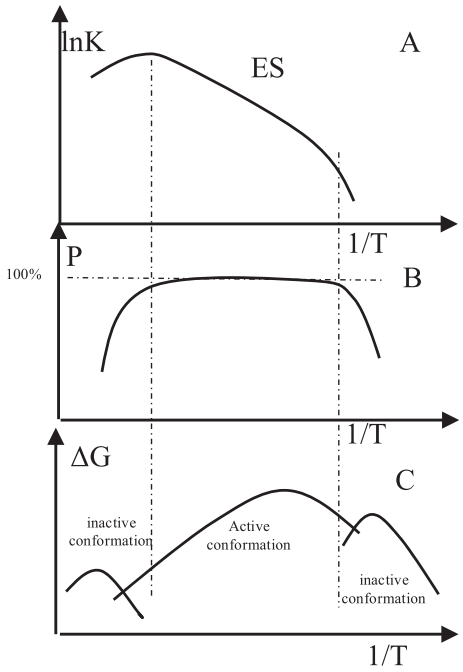
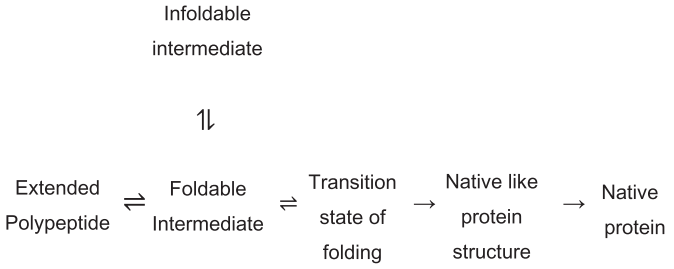


Fig. 4. Diagram of alcohol dehydrogenase. Fig. 4A is an Arrhenius plot for the enzyme kinetics. Fig. 4B is the abundance curve of the active conformation of enzyme substrate complex. Fig. 4C is the protein conformational stability curve of the enzyme substrate complex; three conformations are drawn in this figure. The Fig. 4C was drawn according to protein stability curve and properties of the enzyme (Zhao, 2013; Becktel and Schellman, 1987).

Many reports have been published, but there is no consensus about kinetics (Dimitriadis et al., 2004; Scalley and Baker, 1997; Bunagan et al., 2006; Nguyen et al., 2003; Oliveberg et al., 1995).

According to the irreversible thermodynamic structure theory and revised transition state model, the protein folding can be expressed



The energetic level of diversified intermediates of protein folding is shown as follows (See Fig. 5 for detail).

Briefly speaking, the extended protein conformation (or nascent polypeptide) first collapses into many conformations due to hydrophobic interaction among its components and it will likely sample an ensemble of collapsed conformations; if it by chance is converted into a foldable conformation (it is one type of collapsed conformation), it can fold further and finally native protein can be formed. A highlighted feature of protein folding is that the activation energy does not arise from strong chemical bond breaking or formation, but weak interactions involved in protein conformational change.

The rate of protein folding can be expressed

$$K = \mathrm{AD}_T P_{FI} \exp(-\Delta \mathrm{G}_a / k_B \mathrm{T})$$

In conventional theory for simple thermal system, the energy of the foldable intermediate and transition state is constant and this equation can be expressed as

$$K = \mathrm{AD}_T P_{FI} \exp\left(-\frac{\Delta \mathrm{G}_a}{k_B \mathrm{T}}\right) = \mathrm{AD}_T \exp\left(-\frac{[\Delta \mathrm{G}_a + \Delta G_{FI}]}{k_B \mathrm{T}}\right)$$

where ΔG_{FI} is the stability of foldable intermediate. However, the case is totally different for complex thermal systems. In this case, the energy of both foldable intermediate and the transition state are not constant and they are changeable under different conditions. The P_{FI} can only act as an independent variable and it cannot be merged into exponential term of the Arrhenius equation. It marks the fundamental difference between simple and complex thermal systems.

According to this model, the kinetics of protein folding can be pictured in Fig. 6.

Within this model, at both low and high temperature the abundance of foldable intermediate is low, which results in a decrease of folding rate. The abundance of foldable intermediate is precisely controlled by laws of equilibrium thermodynamics, thus the nonlinear Arrhenius plot is caused by a process of equilibrium thermodynamics, and not the process of chemical kinetics.

This model is supported by many experimental results.

1) In the presence of protein denaturant, the stability of a protein conformation is decreased, and the optimum temperature for protein folding is decreased also (Scalley and Baker, 1997).
2) Salt and other substances, if they have an impact on protein conformation stability, can influence the protein folding (Cao and Li, 2008; Song et al., 2007).

This analysis indicated that protein kinetics can be fully described by the revised Arrhenius equation.

5. Relation among protein dynamics and enzyme activity

As we have discussed, there is an indirect relation between protein dynamics and enzyme activity. The logical relationship of protein dynamics to many concepts in enzyme kinetics and regulation are summarized in Fig. 7. All these relationships have been

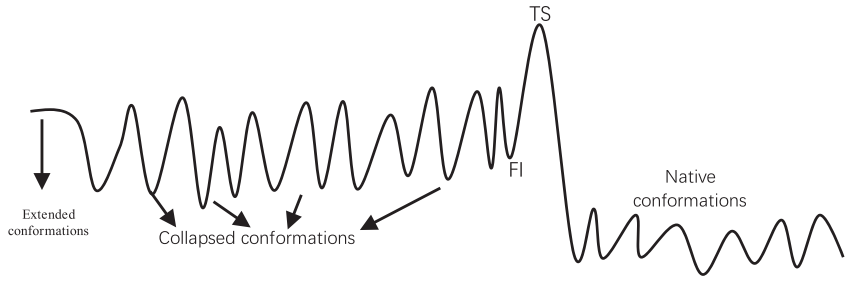


Fig. 5. Diagram of protein folding process, where FI is foldable intermediate, TS is transition state of protein folding.

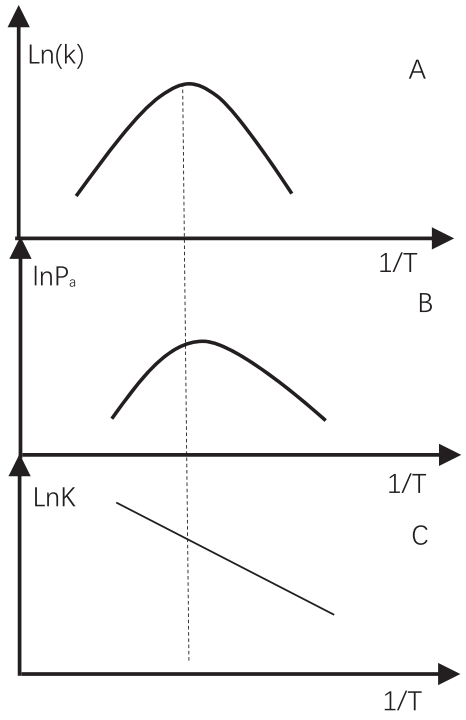


Fig. 6. Diagram of folding kinetics of small protein.
Fig. 6A is Arrhenius lot of rate of protein folding. Fig. 6B is the abundance of foldable intermediate (lnP_a). Fig. 6C is Arrhenius lot of rate of protein folding when the abundance of foldable intermediate is constant by adjusting the protein conformation stability (Scalley and Baker, 1997).

demonstrated and confirmed experimentally.

As shown in Fig. 7, protein dynamics can influence enzyme activity in several ways. Firstly, it can influence the stability of a protein conformation, and then alter the abundance of the active conformation of the enzyme; or in other words, it can inactivate or activate an enzyme (Ostermann et al., 2000; Csermely et al., 2010; Giordano et al., 2005). This represents a thermodynamic model for enzyme regulation (Zhao, 2015). Secondly, it can regulate the detailed state of the active conformation of the enzyme and the binding affinity between enzyme and substrates can be influenced. In this way the K_m of the enzymatic reaction is changed. This binding process between protein and ligand has been well studied (Zou et al., 2002; Yancey and Somero, 1979). Thirdly, it can modulate the fine structure and thermal stability of the transition state of the enzyme substrate complex, thus the activation energy and other parameters of enzyme kinetics can be altered. Thus, all effects

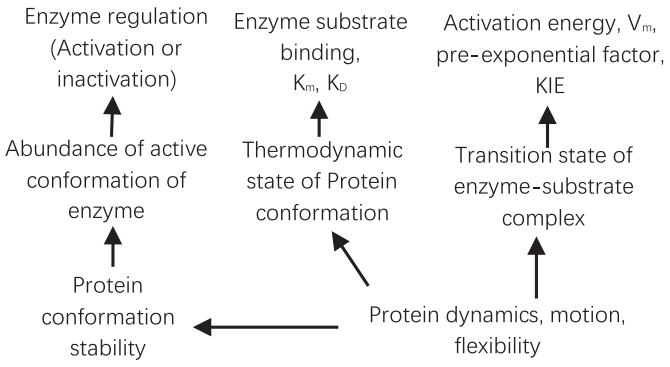


Fig. 7. Logic relationships of different concepts in enzymology.

of protein dynamics on enzyme activity can be expressed in the parameters of enzymatic kinetics and protein conformation.

Within our model, the activation energy of the enzymatic reaction is not constant and changeable with temperature or other environmental factors. The D_T represents the contribution of protein dynamics to enzyme activity.

At present, it is impossible to measure D_T alone experimentally. But the apparent pre-exponential factor of Arrhenius equation, or AD_T, could be easily measurable. So we can define and quantify the dynamic contribution of enzyme activity by monitoring the change of AD_T under varying conditions. We predict that AD_T is changeable with components of solvent or buffer, such as protein denaturant.

The pre-exponential factor of the Arrhenius equation is ignored by most experimental scientists and there are few studies of this. The abnormal value of the pre-exponential factor of the Arrhenius equation for enzyme reactions has been reported. Nagel et al. have done so, but he fail to give explanation (Nagel et al., 2011). It is a prediction of our theory.

As there is an indirect relation between protein dynamics and enzyme activity, an increase of protein flexibility may not affect enzyme activity if it does not alter the abundance of the active conformation of the enzyme. Many experimental results indicate that enzyme activity remains unchanged when the protein flexibility is enhanced by urea at low concentration at which protein denaturation does not occur (e.g. Aceto et al., 1992; Rangarajan et al., 1992).

6. Protein dynamics and abundance curve of active conformation of enzyme

Much confusion in protein science and enzymology has been caused by misunderstanding of the indirect relation between protein dynamics and the abundance curve of protein conformation (or protein stability). The principle is pictured in Fig. 8.

We can see in Fig. 8, a conformational distribution curve can be generated from different protein conformation stability curves. Or in other words, the probability of the active conformation is not determined by protein dynamics directly. This principle provides a reasonable answer to the debate on the protein conformational rigidity and protein dynamics (Jaenicke, 2000; Hernández et al., 2000; Myers et al., 1995). In view of biochemistry, the change of abundance of the active conformation responding to a stimulus represents structural rigidity of the protein conformation and it can be analyzed in Fig. 8A; thus, the protein conformation rigidity (flexibility) differs from thermal stability (flexibility) of it in principle.

In studying the thermal adaptation of proteins, it has been observed that a change of protein flexibility or dynamics induced by amino-acid mutation has accompanied the alteration of the protein properties. It is evidence for the relation between protein dynamics and enzyme activity (ZAvodszky et al., 1998; Georlette et al., 2004). Our view is that a change of protein dynamics is a necessary but not sufficient condition. Within our model, adjustment of conformation rigidity, rather than protein dynamics (thermal stability), is a key event in the thermal adaptation of proteins (ZAvodszky et al., 1998). Compared to mesophillic enzymes, the distribution curve of active conformation of the enzyme of thermophiles has been shifted into range of high temperature (Fig. 2B).

Henzler-Wildman et al. utilize NMR techniques to study protein dynamics, and found a correlation of protein motion and enzyme activity (Henzler-Wildman et al., 2007). They have concluded that the larger-scale motions in substrate-free adenylate kinase are not random, but preferentially follow the pathways that create the configuration capable of proficient chemistry. Such preferred

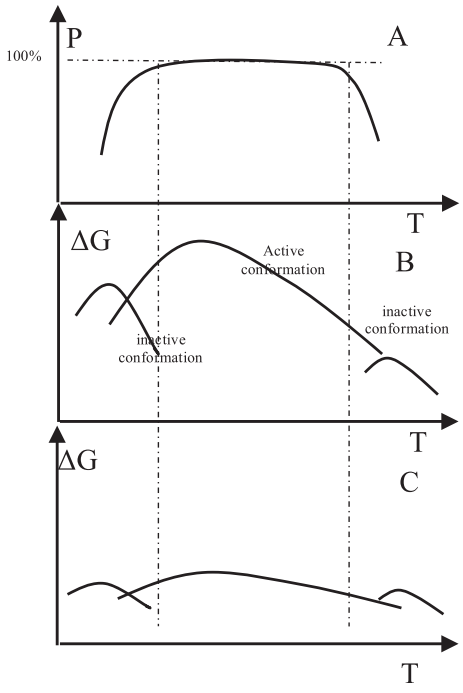


Fig. 8. Diagram of relation between conformation curve and protein conformation stability. Fig. 8A is the distribution curve of active conformation of enzyme. Fig. 8B and C is the protein conformation stability curves. Both Figs. 8B and C can give same distribution curve of active conformation of enzyme (Fig. 8A).

directionality, encoded in the fold, may contribute to catalysis in many enzymes. According to our model, the larger-scale motions in active conformations of enzymes are not totally random, but preferentially follow the pathways that create the favorable configuration in transition state of enzymatic reaction.

7. The dispute about scientific concept and criteria

Some scientists have noticed that substitution of amino acid residues distant from the active site of an enzyme have great impact on enzyme activity (Benkovic et al., 1988; Wang et al., 2006; Benkovic and Hammes-Schiffer, 2003; Caratzoulas et al., 2002). So the concept of promoting motion or vibration of enzyme activity has been proposed. The revision of this concept is coupled motion network (Hammes-Schiffer and Benkovic, 2006; Fu et al., 2012). This view goes too far. In my opinion, some motions of dynamic sites of a protein can influence the pathway of protein conformational change, modulate the fine structure or thermodynamic parameters of conformation of enzyme substrate complex, but most motions have nothing to do it. Their contribution to accelerate the rate of enzymatic reaction comes from fine configuration of transition state of enzyme substrate complex, and energy of such motion cannot lower the activation energy of enzymatic reaction directly.

The KIE (kinetic isotope effect) of enzymatic reaction has been utilized to monitor the transition state of enzymatic reaction and compression concept in enzymatic reaction has been proposed; it is said that distance between donor and acceptor of transferring hydrogen is compressed within enzyme (Klinman and Kohen, 2013; Klinman, 1981, 2009; Knapp et al., 2002; Hay and Scrutton, 2012; Hay et al., 2010). Within our model, there is an indirect relationship between the distance of atoms and protein dynamics. The change of protein dynamics is firstly influenced by the volume of a protein and which finally determines the distance between different atoms (Chalikian, 2003; Heremans, 1982; Gekko and Hasegawa, 1989; Liu and Warshel, 2007).

Overall, the advocators for the relation between protein dynamics and enzyme activity have found many interesting phenomena and relationships between protein dynamics and enzyme activity can be confirmed. However, the underlying principle for it, which is a prerequisite to persuade others, has not been proposed within such studies.

Some scientists deny completely such a relation (Kamerlin and Warshel, 2010a; Warshel and Ram, 2016; Glowacki et al., 2012; Olsson et al., 2006; Kamerlin and Warshel, 2010b). The reason is rather simple and clear. If protein dynamics play a role in enzyme activity, we should find its contribution to enzyme activity in the parameters of enzymatic kinetics. Or in other words, the effect of protein dynamics in enzyme catalysis should be expressed in parameters of enzyme kinetics. Theoretically speaking, this scientific criterion is quite right. However, the theoretical foundation of their argument is conventional transition state theory of enzymatic kinetics, the role of protein conformation in enzyme activity cannot be analyzed by it, thus their opinion is questionable. Another reason to rebut the direct relation between protein dynamics and enzyme activity is that the energy of motion or vibration is a concept of mechanics and the activation energy is a concept of thermodynamics, two types of energy cannot be transformed with each other freely and directly.

8. Conclusion

Our analysis has shown that the validation of chemical laws in the field of complex thermal systems, particularly for biophysics and biochemistry, should be tested carefully. A revision of transition state theory and the Arrhenius equation allows enzymatic kinetics and the working mechanism of enzyme action to be interpreted theoretically. Our analysis also shows that the dispute regarding the role of protein dynamics in enzyme activity arises from an incorrect understanding of transition state theory in enzymology. As the indirect relation between protein dynamics and parameters of enzymatic kinetics has been established theoretically, the dispute should be ended.

Finally, we would like to thank all scientists who have been involved in the dispute for their contribution to the science although most of their work has not been cited in this paper. Surely, the dispute has inspired us to reshape our thoughts and to revise the theory of science, and thus the progress of science can be made.

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