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On Catalysis by Biological Macromolecular Enzymes

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Abstract

Classical enzyme kinetics are interpreted from a new angle here, and biological macromolecular enzyme catalysis is viewed and explored at the molecular level. The time course of sequential catalytic events is analyzed, the relationships between catalytic efficiency, catalytic rate/velocity and the amount of time consumed are established. This writing tries to connect the microscopic molecular behavior of enzymes to kinetic data obtained in experiment, and the equations proposed here can be testified and examined by future experiments.

Key words: catalysis, kinetics, time, biological macromolecular enzyme, large biological macro-substrate, catalytic step, catalytic efficiency, turnover number

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Introduction

Some of the basic theories of biochemistry come from chemistry[1, 2], which is dealing with small molecules most of the time. Although enzyme kinetic principles transplanted from chemistry have changed a lot to adapt biological specificity, some of the description can be improved to better reveal molecular events of biochemical reactions.

A suitable way to describe biochemical reactions in detail is needed. Comprehensive biochemistry specific explanation is required to precisely describe the nature of biological macromolecular enzyme catalysis and kinetics. This is important for the advance of not only science, but also biochemical engineering, drug discovery and other applications or technologies as well. In fact, enzyme kinetics can be viewed from another angle.

Catalytic rate/velocity depends on the amount of time the enzyme consumed to successfully convert certain amount of substrates into products[3-6]. The catalytic process actually includes not only the traditionally defined chemical transformation step (which may include multiple chemical sub-steps itself), but also other related physical/biophysical/biochemical catalytic steps; for instance, diffusion, enzyme-substrate recognition/binding step and product release step are involved as well. This is the case for chemical

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reactions as well as biochemical reactions catalyzed by biological macromolecular enzymes. If any of these 'trivial' steps takes time to accomplish, it will affect the overall catalytic rate and cannot be ignored if accuracy is required. Here, this writing tries to discuss the complete catalytic cycle as a whole.

Turnover, catalytic step and catalytic cycle in the following discussion mean to refer to those related to biochemical reactions catalyzed by biological macromolecular enzymes, unless stated otherwise. This writing will concentrate on catalysis in aqueous solution by biological macromolecular enzymes, although some of the contents can be applied to other systems, reactions or catalysis as well.

Hemoglobin diffuses at a rate of about 5μm·s^{-1} in cells[7]. It takes roughly 10^{-7} second on average for one molecule to meet with another in aqueous solution, with a concentration of 10mM, and if the concentration is 1μM, the time is ~10^{-3} second. Tumbling of proteins in aqueous solution is at nano second (10^{-9}s) time range. Local motions of an enzyme, like the motions of side chains of surface residues, take roughly 10^{-12}-10^{-9} second; it takes longer time when residues with large bulky side chains are involved. Medium scale conformational change up to several Angstroms like loop motion, hinge bending motion and some domain movement takes usually

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about 10^{-9}-10^{-4} second to accomplish[8-11]. The further the movement and the larger the moving portion, the longer time it will take. Large-scale conformational change takes roughly about 10^{-4}-10^{0} second to accomplish, and some large-scale conformational change can take seconds or even longer time. The amount of time it takes for the substrate-to-product chemical conversion step by different enzymes vary a lot, from 10^{-7}-10^{0}s to considerably long time[12, 13]. Huge enzyme machinery catalyzed complex biosynthesis will be discussed separately later. Traditionally, the substrate-to-product chemical conversion step is regarded as the rate limiting step[3-6], and the diffusion process, reorientation, recognition/tethering and the conformational change step will be largely ignored in practice. This is also a fundamental presumption of classical kinetic theory and theories on biochemical catalysis and enzymology. But from the numbers listed above, it is obvious that other steps can happen at similar time scale as the chemical step[8-12, 14, 15], thus possibly affecting the catalytic rate as such.

Turnover number k_{cat} of the H_2O_2 to water plus dioxygen reaction catalyzed by catalase is around 4×10^{7} s^{-1}[12]. This value means that the catalytic rate can be partially limited by diffusion rate as well. In several other cases, binding, conformational change or product release are the rate limiting steps, respectively, and these facts have

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been supported by numerous experiments by different technologies [16-27]. Enzyme catalyzed biochemical reactions have a lot of steps involved; theoretically, any step can be rate limiting. In short, besides the chemical step, other steps could be rate limiting as well, both in theory and in reality.

The discussion here is statistics, probability and frequency based analysis, concerning the events of the whole overall biochemical process catalyzed by enzyme ensembles in a given aqueous solution system, rather than the behavior of an isolated single individual enzyme molecule, although sometimes certain catalytic event of a single enzyme will be highlighted to explain what may be happening to the molecules in a batch. This is because a single catalytic cycle of a single individual enzyme may be random and be affected by a lot of occasional factors.

The first assumption

All the biochemical reactions catalyzed by any free biological macromolecular enzymes in homogenous aqueous solution systems should follow the same unified general principle; there should be no exceptions. This is the first assumption.

'Homogeneous aqueous solution system' means that all the participants of the catalysis are homogeneously distributed within the solution system and are freely diffusible in the solution. There shall be

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no denaturants in the system, so that the enzyme is properly folded and active, and all the way through the catalytic process, it's kept under such mild conditions. If the system goes so far away from normal physiological condition that the enzyme gets denatured, the discussion here may not stand valid for the case anymore.

If membrane protein is solubilized by detergents or lipids and is freely diffusible, and homogeneous aqueous solution system is also formed; both the substrate and the product is water soluble, and the catalytic center locates at the solvent exposing surface; everything behaves very much alike water-soluble enzyme and aqueous solution system, then this first assumption shall still apply.

Membrane enzymes restrained in two-dimensional lipid bilayer system are different circumstances. First case, membrane protein enzyme is in lipid-bilayer systems; both the substrate and product are water soluble, and the catalytic center locates at the solvent exposing site. It's like the enzyme is immobilized within the two-dimensional space; the diffusion process/kinetics are different in that only substrate and product diffuse in aqueous solution, but not the enzyme. Conformational change of enzyme can be different in that lipid molecules are involved in the movement as well, so the conformational change kinetics can be different. Second case, both membrane protein enzyme and substrate are hydrophobic and

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restrained in lipid-bilayer systems; the membrane has a two-dimensional space[28]; the diffusion of both enzyme and substrate in lipid bilayer will be constrained in this two-dimensional space. The diffusion and the conformational change rates would be distinct from those in aqueous solution.

Basic assumption one of previous kinetic theories is about rate limiting issue.

Scientists used to believe that there were two distinct kinds of reactions in solutions, diffusion-controlled reaction and activation-controlled reaction[29-31]; the rate constants were also expressed in distinct equations. Activation energy is required for the chemical conversion step[1], and usually this step is regarded as rate determining (activation-controlled reaction); if the chemical conversion is so fast that the diffusion step becomes rate limiting, then it's diffusion-controlled reaction. These two cases reveal two important rate limiting sources or origins in solution. Actually, as discussed above, other steps like conformational change step have been shown nowadays sometimes to be rate limiting as well.

Basic assumption two of previous theories is about the relative amount of reactants.

Traditionally, the amount of substrates is assumed to be much greater than the amount of enzymes. And the active site of the

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enzyme is supposed to be saturated by substrate, and this is a basic assumption forming the foundation of classical kinetic theories for initial catalytic rate analysis[3-6]. In practice, the rate constant and catalytic velocity are obtained experimentally by using kinetic equations based on this assumption. Usually, this may be the case immediately after enzyme is mixed with high concentrations of small-sized low-molecular-weight substrate (SMS) in vitro, whether this assumption stands true or not for SMS or large biological macro-substrates (LBMS) under normal physiological conditions, in vitro or in vivo, will be left as an open question here. But if the concentrations of both enzyme and substrate are taken into consideration, the description about the reaction shall be more precise. And the author believes the concentration of enzyme is not always so negligible as supposed.

The concentration of large biological macro-substrates (LBMS) is usually lower than that of small-sized low-molecular-weight substrate (SMS) in the cell[32, 33]; the enzyme catalytic center is readily accessible to the SMS. LBMS diffuses slower, rotates slower than SMS, which all makes LBMS take additional and longer time to diffuse, to meet with the enzyme and to take the right orientation and accommodate specific parts into the catalytic pocket, both in vivo and in vitro. Much more importantly, it's always a mutual process of

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recognition between the enzyme and the large biological macro-substrate[22, 24, 34, 35]. In this case, the time consumed by recognition and binding process, etc, can no longer be neglected; it has to be taken into consideration. In other words, the experimentally obtained catalytic turnover number and velocity actually include the contributions of other steps (sometimes significant) besides the chemical step, although the researcher may not realize it. This is the case for both LBMS and SMS, and for the reasons discussed above, it is more important and serious for LBMS. For physiologically relevant enzyme catalysis, the concentration of LBMS is usually at similar order of magnitude with that of the enzyme. The presumption that the concentration of the enzyme is negligible if compared to that of the substrate will not typically stand true anymore for LBMS. Therefore, it's quite a different scenario for LBMS involved catalysis.

As discussed above, these two basic assumptions sometimes do not stand true for certain catalysis. Enzyme kinetic theories, including Michaelis–Menten Kinetics, Briggs-Haldane's theory, Quadratic Velocity Equation (tight-binding equation or the Morrison equation), and those theories on enzymatic rate enhancement, etc, play important roles in the research of catalysis and enzymology[1, 3-6]. Aside from these principles, some other universally suitable kinetic principles may be extracted from innumerable available examples now.

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Master equation

Consider the whole picture of a single turnover (or single catalytic cycle) of a biological macromolecular enzyme catalyzed multiple turnover reaction, the enzyme and the substrate have to firstly diffuse to meet with each other; the two reactants need to rotate to the right orientation to tether, to recognize and start to bind, and the enzyme performs conformational change; then the substrate is converted to the product through the chemical conversion step; and then product is released and the enzyme enters another catalytic cycle. The sequential catalytic events in the whole catalytic cycle is like a pipeline; although there might be bottle necks, each of every component step, if the catalytic cycle can be divided into discrete elementary steps, takes time to accomplish and contributes to the catalytic efficiency and velocity. Actually, as the biochemical catalysis proceeds, each cycle of it will have to get through every single step and cannot skip any one.

The amount of time that one turnover spends comes from the combination of each single step of the catalytic cycle. All of the time-consuming steps limit the overall turnover number of catalysis. If any step takes such short time on average that it is negligible in comparison with other steps, then it can be omitted for simplicity, and which step to ignore depends on the situation, and it can be different from case to case.

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These tethering/recognition, conformational change, chemical/biochemical conversion process, and product release step happen in ordered sequence; let the coherent process be carefully divided so that each step simply do not overlap in time axis with one another; the amount of time consumed by these sequential steps are addable. In very viscous systems or cases of LMWS or diluted reactants, diffusion takes considerable amount of time; diffusion can be an independent step without overlap in time course with other steps, in the same catalytic cycle or from nearby cycle, and the time consumed by diffusion will be addable to that of other steps. When the enzyme is saturated by the SMS, the chances are there is substrate immediately available near the catalytic centre, the higher the concentration of the substrates in the more inviscid/frictionless system and the slower the other steps, the greater the probability of this.

Let there be n steps in a biological macromolecular enzyme catalyzed multiple turnover reaction; within a single turnover, each step i takes time t_i on average to accomplish. Then the total amount of time t consumed by a whole single turnover is the sum of the time consumed by all the steps. Time is addable.

$$t=\sum_{1\rightarrow n} t_i$$

Catalytic coefficient (turnover number) k is equivalent to the number of substrate molecules converted to product per unit time by

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a single enzyme molecule (or per single enzyme active site). And t is still the same, defined as the total time a single turnover takes by a single enzyme on average. Then,

$kt=1$

$k\cdot(t_1+t_2+t_3+\cdots+t_i+\cdots+t_n)=1$

t_i is the amount of time step i takes on average within a single turnover. And catalytic coefficient k_i of step i is defined as the turnover number of a step-i-dedicated single enzyme per unit time.

$t_ik_i=1$

The catalytic coefficient k_i is the fastest possible catalytic coefficient of step i. Imagine the enzyme is dedicated to step i and doing nothing else, and substrate of step i or product of step i-1 is immediately available in excess. Then, k_i of any step i will be larger in value than k; this means if the enzyme catalyzes only that single step, it will result in more turnover numbers. Because normally the enzyme is busy with other catalytic steps during time $t-t_i$, the overall output of the whole catalytic cycle will decrease to a level below the throughput capacity (or flux) of any single step i. Both k and k_i reveal the average catalytic efficiency of enzyme, the overall efficiency or efficiency of step i, respectively.

If any one step i is the only rate limiting step, and $t\approx t_i$, then $k\approx k_i$. And this step i can be the diffusion step (a diffusion-controlled

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reaction), or the enzyme conformational change step, or the substrate-to-product chemical conversion step (an activation-controlled reaction), or the product release step, or another step. There are times when the second most time-consuming step j also takes considerable amount of time, for instance, $t_j/t>20\%$. Then the two steps i and j are both rate limiting; the throughput of other steps are so big that they are all waiting for these two steps; the $t-t_i-t_j\approx0$, then $k\approx k_ik_j/(k_i+k_j)$. There are cases when the third most time consuming step x also takes considerable amount of time, for instance, $t_x/t>10\%$, the $t-t_i-t_j-t_x\approx0$, then $k\approx k_ik_jk_x/(k_ik_x + k_xk_j + k_ik_j)$. Similar equations could also be deduced, and so on.

For simplicity, four major steps will be discussed here. First, the diffusion step, a process the enzyme and the substrate diffuse in aqueous solution to reach each other. Second, the reactants conformational change step, the enzyme and sometimes both the enzyme and the substrate perform conformational change. Third, the substrate to product chemical conversion step. Fourth, the product release step. Each step of the four takes time t_{difu}, t_{conf}, t_{chem} and t_{prod} on average within a single turnover, respectively.

t_{difu}, diffusion time, is the time consumed on average for an effective enzyme substrate encounter within the aqueous solution system. This parameter is dependent on concentration, viscosity,

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temperature, pressure, electrostatic attraction, electromagnetic effect, etc. With the help of diffusion, the enzyme substrate solution system is virtually a uniformly distributed system, even for the many turnover cycles of the whole catalytic reaction. $k_{difu}=1/t_{difu}$.

t_{chem}, the total time spent on average by the chemical conversion step within a single turnover of a catalysis. t_{chem} is independent of free substrate concentration or free enzyme concentration. For this chemical conversion part of a catalytic cycle, many classical biochemical principles still apply, like the transition state theory and Arrhenius equation, etc. For multistep chemical conversions, there could be t_{chem1}, t_{chem2}, ..., and $t_{chem}= t_{chem1}+ t_{chem2}+....$

t_{conf}, structure conformational change time within a single catalytic cycle; t_{conf} is a parameter dependent mainly on the molecules' intrinsic structure and character, temperature and pulling forces from other macromolecules, etc. Sometimes, the enzyme-substrate complex (or enzyme-substrate-modulator complex) performs conformational change as a whole. $k_{conf}=1/t_{conf}$. t_{prod} is the amount of time consumed on average by product release step within a single catalytic cycle, $k_{prod}=1/t_{prod}$.

For a certain catalysis, if all other steps could be ignored, then for the whole single turnover, time $t\approx t_{difu}+t_{conf}+t_{chem}+t_{prod}$. Turnover number within one-unit time $k=1/t$, and $k_{difu}=1/t_{difu}$, $k_{conf}=1/t_{conf}$,

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$k_{chem}=1/t_{chem}$, $k_{prod}=1/t_{prod}$, then

$$k=k_{difu}k_{conf}k_{chem}k_{prod}/(k_{difu}k_{conf}k_{prod}+k_{conf}k_{chem}k_{prod}+k_{difu}k_{chem}k_{prod}+k_{difu}k_{conf}k_{chem})$$

The four major steps are present in all biological macromolecular catalysis, although for some enzymes/catalysis, one or more of these steps take time that are negligible; for some others, there may be additional major steps involved.

t_{phys}, the total time consumed on average by the physical or biophysical steps within a single turnover of a specific enzyme catalysis, including the time required for enzyme and substrate diffusion, rotation, collision/tethering/binding, conformational change and product release process, etc. Although protonation/deprotonation can also take some time and is neither always part of the substrate-to-product conversion process nor always the physical process, it's usually short and too complex to be scrutinized here. In one word, t_{phys} is the sum-time of all the physical steps before and after the chemical conversion; t_{phys} is the preparation time for chemical conversions to occur. If only diffusion, conformational change, and product release steps account for the majority of physical process, then $t_{phys}\approx t_{difu}+t_{conf}+t_{prod}$. Chemical conversion steps are quite different from physical or biophysical steps in at least the following aspects, ①strong covalent bond change,

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②driving forces, ③rate limiting origins, ④activation energy involved or not.

Without disturbance, normally the enzyme would visit each of every unit step periodically. The catalytic process is like many enzymes action in parallel, each one conducts tandem repeats of catalytic cycles, only that the enzymes are usually not synchronized. As for the starting point of a single turnover from the many continuous catalytic cycles, it's up to the situation.

The master equations indicate at least the following.

1. The step which is the most time consuming will be the 'rate-limiting' or efficiency-limiting step. Traditionally, the step which requires the most activation energy is regarded as the rate-limiting step, this may still stand correct for the sub-steps of the substrate to product chemical conversion step, but can't be applied beyond. Although 'rate-limiting step' can be the one to optimize to greatly improve the overall catalytic efficiency and catalytic rate, it's not the sole factor that dictates the catalytic efficiency, but all the steps combined together.

2. Time is addable, the above discussion has explained how. But catalytic velocity (concentration per unit time measured in like $\mu M \cdot min^{-1}$ or $mM \cdot s^{-1}$) of each step or catalytic efficiency (catalytic coefficient or turnover number k_i in s^{-1}) of each step are not addable.

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This writing will definitely be applicable for unidirectional irreversible catalysis; the general concept here shall be useful for studying other catalysis as well. In this writing, velocity of each step is the throughput of the step within the fixed-volume system, and velocity of the whole catalysis is the net velocity.

3. Experimentally obtained single turnover time t_{exp} ($=1/k_{exp}$) is the sum of the time actually consumed on average by all the steps within the single catalytic cycle. The experimentally obtained turnover number k_{exp} actually equals to the k from the master equation discussed above, rather than the coefficient of any single step k_i. Therefore, t_{exp} consists of the time spent by all the steps of the enzyme catalyzed reaction.

$$1/k_{exp}=t_{exp}=\sum_{1\rightarrow n} t_i$$

t_{exp}, experimentally obtained single turnover time of a specific biological macromolecular enzyme catalysis. Again, if only diffusion, conformational change, chemical conversion, product release and step j account for the majority of catalytic time,

$$t_{exp}\approx t_{difu}+t_{conf}+t_{chem}+t_{prod}+t_j.$$

The second assumption

The catalytic coefficients k_{chem}, k_{conf}, k_{prod}, and $k_{1\text{-}difu}=1/(t\text{-}t_{difu})$ of any biological macromolecular enzyme are parameters that correlate with and only with the intrinsic character of the enzyme (or the

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enzyme-substrate complex), the temperature T, the pressure P, the viscosity η, the density ρ and other biophysical/biochemical properties of the system. Except k_{difu} and thus k, catalytic coefficient of all other steps is totally independent of free substrate concentration. This is the second assumption.

Intrinsic characters of the enzyme include all those factors that affect the activity of the enzyme used in the experiment, like the primary sequence, three dimensional structure or conformation, the modification state of the enzyme; whether the enzyme is apo or holo (with cofactors incorporated or not), with modulators or effectors or inhibitor or activator bound or not, the presence or absence of other attached regulatory molecules, etc[20, 36-40].

The properties of the solution system include physical (like temperature, pressure, viscosity, density, etc)[41-43] and chemical conditions. The latter includes the pH, the ion strength, types and concentration of solute or electrolyte, the presence and concentration of certain chemicals or loose interactors like effectors, regulators, substrate analogues that can function as antagonist, etc. Chemical conditions would affect the catalysis differently from case to case. The most suitable chemical condition for catalysis is different from enzyme to enzyme. Therefore, the effect of certain chemical conditions on catalysis or more specifically on catalytic coefficient needs to be

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evaluated and examined case by case.

If we'd like a catalytic coefficient (turnover number) to reveal the properties of the enzyme and the physical & chemical conditions of the system, like the pressure, the temperature, the viscosity, density, etc, then it should have nothing to do with substrate concentration, enzyme concentration, or enzyme-substrate complex concentration. The catalytic coefficients k_{chem}, k_{conf}, k_{prod} are parameters of this kind.

The third assumption

The k_{difu}, diffusional collision/encounter rate $V_{difu}(=k_{collision}[E][S])$, thus the overall catalytic coefficient k and overall catalytic rate/velocity $V_{overall}$ should be correlated with substrate concentration. The rate or velocity of conformational change and chemical conversion step combined depends on enzyme-substrate complex concentration [ES], $V_{conf\text{-}chem}=k_{conf\text{-}chem}[ES]$, $1/k_{conf\text{-}chem} = t_{conf} + t_{chem}$; the rate or velocity of the three steps combined (conformational change, chemical conversion and product release steps) shall approximately depend on enzyme-substrate complex concentration as well, $V_{conf\text{-}chem\text{-}prod}\approx k_{conf\text{-}chem\text{-}prod}\cdot[ES]$, $1/k_{conf\text{-}chem\text{-}prod}\approx t_{conf}+t_{chem}+t_{prod}$. This is the third assumption.

Only velocity/rate of the diffusion step depends on the concentration of free substrate[29-31], not the velocity of other steps. Velocities of conformational change, chemical conversion or product

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release steps are unlinked with free substrate concentration. Michaelis-Menten equation describes and only describes relationship between initial velocity V_0 (concentration per unit time in like $\mu M \cdot min^{-1}$ or $mM \cdot s^{-1}$) and substrate concentration[3, 4], and it is very appropriate for the steady state initial velocity analysis when the product is generated at a linear velocity and catalytic rate shows linear dependence on active enzyme concentration [E]. What K_m means down to the bottom? $K_m/V_{max} \cdot P_{arameter}$ is the linear dependence index of $1/V_0$ on $1/[S]$, $P_{arameter}$ is an adjustment parameter. For catalysis not obeying Michaelis-Menten kinetics, there could be alternative equations describing the relationship between V_0 and [S]. Michaelis-Menten equation especially the Lineweaver-Burk form is one of the functions describing the relationship between initial catalytic velocity V_0 and substrate concentration [S], and the application of this function is only appropriate within certain low [S] ranges.

Experimentally obtained one probable relationship between substrate concentration and initial velocity is like this (Fig.1)[44]. With the increase of substrate concentration [S], the initial velocity V_0(concentration per unit time) is ever growing until it reaches a plateau near V_{max}(concentration per unit time in like $\mu M \cdot min^{-1}$), as the substrate concentration gets to near saturation.

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Interpretation to this phenomenon. When [S] is small, $k_{collision}$·[S]·[E] is small, diffusion step is rate limiting. As [S] increases to the middle part of the curve, maybe both diffusion and other steps like chemical conversion step are rate limiting. When [S] is near saturation, other steps like chemical conversion step are the major rate-limiting step, even with the increase of [S], t_{difu} will not become noticeably shorter and diffusion will not improve the overall throughput of the catalysis significantly. As discussed before, [S] will not affect the values of t_{conf}, t_{chem}, t_{prod}, V_{conf}, V_{chem}, or V_{prod} either. Michaelis-Menten equation is describing the $1/V_0$ dependence on 1/[S], and as usually practiced, actually only when [S] is small (the left bottom corner of the curve) can Michaelis-Menten equation be used to obtain the linear dependence index of $1/V_0$ on 1/[S]. Michaelis-Menten equation is only appropriate for the left bottom corner part of the curve. This is because when [S] increases to the point where other steps other than the diffusion step like chemical conversion step start to become rate-limiting, the obtained 'dependence index'(actually not anymore) of $1/V_0$ on 1/[S] becomes a parameter with mixed contributions from both the diffusion step and other steps like the chemical conversion step, and the researcher simply can not tell how much each one contributes. A relationship between rate/velocity of conformational change, chemical conversion

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or product release step and free substrate concentration [S] is meaningless. Therefore, the 'dependence index', if obtained by using results including high [S], is no longer appropriate to use, because the parameter loses its original denotation.

Then comes another question, when [S] gets to near saturation, is the experimentally obtained turnover number $k_{exp\text{-}cat\text{-}sat}$ standing for that of the chemical conversion step? Not really. Is there any direct relationship between this $k_{exp\text{-}cat\text{-}sat}$ and the activation energy E_a? No necessary direct correlation. When [S] gets to near saturation, we can only say that only diffusion time t_{difu} is definitely negligible, this means that $1/k_{exp\text{-}cat\text{-}sat} \approx t_{conf} + t_{chem1} + t_{chem2} + t_{prod}$, if other steps are negligible as well. From the curve, it is obvious that, like any other trivial steps, the amount of time spent by diffusion is always there, with the increase of [S] it can be ignored, but it never really disappears.

Turnover number (in s^{-1}), rate/velocity (concentration per unit time in like $\mu M \cdot min^{-1}$ or $mM \cdot s^{-1}$), and another parameter $Conv_{speed}$, if defined as the amount of substrate molecules converted to product per unit time by all the committed active enzyme molecules in the system, measured in moles per unit time (like $mol \cdot s^{-1}$), have something in common in essence: they all indicate the throughput of the catalysis in a given unit of time, although they are representing in different ways. $Conv_{speed}$ correlates with both turnover number and enzyme

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amount. Velocity/rate correlates with turnover number, the volume of the system and the amount of reactants.

Diffusion process

Brownian motions of substrate and enzyme take place and contribute to the homogeneous distribution of the system. Diffusion process is different from all other steps in that usually at least two participants are involved, and one complex is formed in the end. Diffusional-movement velocity of molecule depends on the molecular weight, viscosity, temperature and density of the system, etc[45-49].

$$\tfrac{1}{2}mV_{difuMotil}^2=3k_BT/2$$

$$mV_{difuMotil}^2=3k_BT$$

$$V_{rms\text{-}difuMotil}^2= 3k_BT/m=3RT/M_W$$

$V_{difuMotil}$ is diffusional-movement velocity, m is molecular mass in gram, M_W is molar mass in Dalton, k_B is the Boltzmann constant, T is the Kelvin Temperature, R is the gas constant, and $V_{rms\text{-}difuMotil}$ is root mean square (rms) molecular-diffusional-movement velocity.

An enzyme catalyzed reaction will only occur if the reactant molecules/particles come within a distance R* from each other. Then the rate of the encounter would be dependent on the frequency of molecular collisions[29-31]. Problems of complex diffusion process of multiple reactants could always be dissected into the diffusion and collision of two, first between reactant1 and reactant2, then between

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reactant1-2 complex and reactant3, etc. One instance, a biological macromolecular enzyme catalyzes the modification of a LBMS using compound1 in the presence of ATP. Although multiple routes are possible, the formation of enzyme-LBMS-compound1-ATP quaternary complex via diffusion can usually be roughly studied through the investigation of diffusion and encounter of enzyme and LBMS. Here, the case of one enzyme and one substrate will be taken as an example for the following (and above) discussion.

First circumstance, no distant attraction or repulsion between the enzyme and substrate; the two reactants only come to each other by chance.

In aqueous solution,

collision/encounter rate=$4\pi R^*(D_E+D_S)N_A$ [E][S]

Collision rate constant $k_{collision}=4\pi R^* N_A(D_E+ D_S)$,

$k_{collision}=4\pi R^* N_A[k_BT/(c_E\pi\eta R_E)+k_BT/(c_s\pi\eta R_S)]$,

π is a constant with a value ~3.14159265, D_E and D_S are the diffusion coefficients of the two reactants Enzyme and Substrate in solution, N_A being Avogadro's number with a value of $6.0222\times10^{23}mol^{-1}$, [E],[S] are the concentrations of the two reactant molecules Enzyme and Substrate, respectively. R_E, R_S are the effective radius (or gyration radius) of Enzyme and Substrate, respectively, T is the absolute temperature, k_B is Boltzmann constant with a value of

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$1.3806\times10^{-23}JK^{-1}$, η is the viscosity. The value of constants c_E, c_s depend on the shape and property of the molecules, and can be obtained from experiment.

Since Boltzmann constant $k_B = R/N_A$, R is the gas constant,

$k_{collision}=(4RT/\eta)\cdot R^{*}\cdot[1/(c_E R_E)+1/(c_s R_S)]$,

Collision/encounter rate$=(4RT/\eta)\cdot R^{*}[1/(c_E R_E)+1/(c_s R_S)]\cdot[E][S]$

If R is used in units of $J\cdot mol^{-1}\cdot K^{-1}$, T in Kelvin, η in poise (P, 1 P = 0.1 $kg\cdot m^{-1}\cdot s^{-1}$), $k_{collision}$ will have units of $m^3\cdot mol^{-1}\cdot sec^{-1}$.

$k_{difu}=1000\cdot k_{collision}\cdot[S]$, [S] in $mol\cdot L^{-1}$, k_{difu} has unit of s^{-1}, collision/encounter rate/velocity has unit of $M\cdot s^{-1}$. $k_{collision}$ is much more important a parameter than k_{difu}, and diffusion step of many catalysis can be studied by examining $k_{collision}$, [E] and [S].

The encounter rate constant of diffusion in aqueous solution is $\sim7.4\times10^{9}$ $M^{-1}s^{-1}$ ($mol^{-1}\cdot L\cdot s^{-1}$) for two molecules with molecular weight of 190g/mol (approximately 1nm in size), with diffusion coefficient D_{1nm} of $4.9\times10^{-6}cm^2s^{-1}$. For protein molecules with molecular weight of 41 kilodalton (approximately 5nm in size) in aqueous solution, encounter rate constant is $\sim6.3\times10^{9}$ $M^{-1}s^{-1}$, and diffusion coefficient D_{5nm} is $8.3\times10^{-7}cm^2s^{-1}$. When the concentration of the molecules falls within milli molar (mM, 10^{-3}M) range, the time it takes on average for an encounter is about 10^{-6} second; when the concentration of the molecules falls within micro molar (μM, 10^{-6}M) range, the time it takes

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for an encounter is about 10^{-4}~10^{-3} second.

The effects of pH, certain ion and certain chemical on these parameters or functions need to be obtained separately for each case[48, 50]: examine the rates at each of the pH, or each of the ion concentration, and obtain a case specific factor, for example pH specific value of c_E, c_S, or R*.

Second circumstance, there is coulomb interaction (attraction /repulsion) between the reactants (for example between the enzyme and substrate),

$$U=(e^2/4\pi\varepsilon_0)\cdot(Z_EZ_S/\varepsilon_R R^*)$$

$$f(u)=(U/k_BT)/(e^{U/k_BT}-1)$$

Z_E, Z_S are reactant charge numbers, $e^2/4\pi\varepsilon_0=2.307\times10^{-28}$Jm, ε_R is relative permittivity. If $Z_E\cdot Z_S=0$, then f(u)=1.

$$k_{collision\text{-}coulomb}=4\pi R^* N_A[k_BT/(c_E\pi\eta R_E)+k_BT/(c_S\pi\eta R_S)]\cdot f(u)$$

$$k_{collision\text{-}coulomb}=(4RT/\eta)\cdot R^*\cdot[1/(c_ER_E)+1/(c_S R_S)]\cdot f(u),$$

When the enzyme is saturated by the SMS, the required effectual Brownian motion distance of substrate is very short. The chances are there is substrate immediately available near the catalytic centre[4-6], the higher the concentration of the substrates in the more inviscid system, the shorter the efficacious diffusion distance required. If the enzyme catalysed biochemical reaction is the conversion from a SMS to a small molecular weight product, the diffusion process will

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scarcely affect the catalytic rate/velocity significantly unless ①the combined process of conformational change, chemical conversion and product release steps are very fast, much faster than diffusion step, ②the reactant concentration is very low, ③ the system is very viscous, ④there is repulsion between reactants.

After diffusion, the reactants are physically close to each other; the substrate may rotate, roll, crawl or hop on the surface of the enzyme for a successful in-catalytic-pocket binding. The reorientation and tethering process is dependent on the surface property of the enzyme and the substrate, like electrostatic property, the shape and hydrophobicity of the enzyme and substrate, etc.

Similarly, another parameter $k_{difuSub}=1000\cdot k_{collision}\cdot[E]$ may be defined, with unit of s^{-1}, which reveals the number of enzyme molecules one substrate molecule will meet with on average in the solution system within one second.

Conformational change and structural re-organization/rearrangement

Conformational change prepares both the enzyme and the substrate with correct geometry and electrostatics ready for substrate to product conversion. Conformational change can be induced by enzyme substrate binding or can be a spontaneous process, the enzyme or substrate may sample a broad distribution of

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conformations and visit the chemical-conversion ready conformation with variable frequencies. For LBMS, the enzyme-LBMS complex frequently performs conformational change as an integrated unity. Induced fit can have a very amplified scale and a different meaning when comes to biological macromolecular enzyme and macro-substrate. Conformational change is independent of and not coupled to the diffusion step.

Catalysis relevant conformational change can be classified into three different categories, first, the minor-scale conformational change of the active site that happens in parallel with the chemical step; second, the large-scale conformational change that is separable in time course from the substrate to product chemical conversion step; third, large-scale conformational change that happens in parallel with the chemical conversion step.

For some enzymes, the catalytic process is related to local motion of surface residues only. Some tiny-scale conformational change can be happening in parallel with substrate to product chemical conversion step, and the two events cannot be separated in time course from each other. Sometimes, only local minor-scale motion of surface residues is required to achieve catalysis. Some conformational change and chemical steps may be coupled; for example, the bound substrate can be converted to product through the

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action (side chain vibration, rotation or flipping) of the residues at the catalytic centre[51]. Local motion can be quick and fast, taking little time. This is a case where conformational change shows little observable additional constrain, aside from chemical conversion, on the catalytic rate.

Large-scale conformational change and chemical conversion steps, just like the previous case, may also happen in parallel. For some enzymes/catalysis, both minor-scale conformational change and large-scale conformational change take place, either can overlap to some extent to the substrate to product conversion process. On the other hand, large-scale conformational change step can be independent of and separable from the chemical conversion step[23, 26, 27, 41], especially when large-scale conformational change is correlated with molecular recognition or product release. In either case, large-scale conformational change may significantly affect the overall rate of catalysis.

For the three categories discussed above, the tiny-scale dynamics of the enzyme does not form an independent step. The large-scale conformational change does form an independent unique step, and it will be talked about here. Large-scale conformational change accounts for the majority part of the dynamics of the enzyme, both from the time and space point of view; it takes the majority amount of time,

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and covers the majority scale of distance.

The amount of time consumed by conformational change is mainly arisen from large-scale conformational change. There are real cases where conformational-change efficiency affects the catalytic rate/velocity of biochemical reactions[23, 26, 27]; this happens since large proportions of the enzyme involve in large-scale conformational change. A couple of different factors can be given here about what may affect conformational-change efficiency and rate/velocity.

Intrinsic factors of the enzyme that affect the conformational-change efficiency. One, the rigidity, stability and flexibility of the enzyme would affect the conformational-change efficiency. Thermal stable enzymes tend to have enhanced hydrogen bonding network, hydrophobic interaction and other weak interactions; these interactions collectively make the enzyme stable, and contribute to the rigidity and reduced flexibility at ambient temperatures. The presence of linkers, hinges or long chains may contribute to the flexibility of the enzyme. Two, steric hindrance. Certain residues of the enzyme may hinder the conformational change of the enzyme through steric effect, thus reducing the conformational-change efficiency. Enzyme-substrate interaction may be decelerated by steric frustration as well. Three, residues of the enzyme or certain factors do not affect the conformational change of

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the free enzyme, but affect the enzyme-substrate mutual recognition and binding, either accelerating or decelerating. In other words, the conformational-change efficiency of the enzyme-substrate complex as a whole can be affected by certain residues or certain factors. Modification of the enzyme by other enzymes will sometimes affect the conformational-change efficiency through the second or the third mechanism.

Environmental factors that could affect the conformational-change efficiency. The presence of certain chemicals, certain cofactors, ligands, modulators or certain regulatory molecules and ion strength, etc, may affect the conformational-change efficiency. The pH of the solution system would influence the protonation state of both the enzyme and the substrate, may thus affecting efficiency of conformational change. Lots of the electrostatic interaction within the enzyme or the enzyme-substrate complex could be affected by protonation state or pH. The enzyme may be hindered from or promoted to efficient conformational change because of electrostatic repulsion or attraction between different residues or domains caused by protonation state change, respectively. The affinity between the enzyme and the substrate may be affected by the protonation state or pH as well.

Concerning how conformational change affects catalytic

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efficiency and rate, there can be three distinct pathways. First, conformational change affects the enzyme-substrate recognition/binding. The second, conformational change affects the substrate-to-product chemical conversion; without the conformational change, the biochemical environment of the active site will not be prepared ready for the chemical conversion to be catalysed. The third, conformational change affects the product release step.

Temperature, pressure, viscosity and density could all affect conformational-change efficiency[15, 23, 42, 43, 52-60]. Relationship between conformational-change coefficient and physical conditions of the solution system is proposed here.

$$k_{conf}=c_{adjust}Tp/(\sigma+\eta)$$

T is the absolute temperature measured in Kelvin, p is the pressure in pascal (1Pa=1 $kg\cdot m^{-1}\cdot s^{-2}$), c_{adjust} is an adjustment constant, η is the viscosity in poise (P, 1 P = 0.1 $kg\cdot m^{-1}\cdot s^{-1}$), σ is another adjustment parameter with unit the same as viscosity. k_{conf} is measured in s^{-1}.

This equation supposes no melt of the enzyme or enzyme-substrate complex, especially no melt of the interdomain linker region if the conformational change happens between the two domains. Near the melting temperature, this function may not apply.

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As discussed above, there are chemical parameters that can affect the conformational-change efficiency. The effect of pH, certain ion, certain chemicals, certain cofactors or modulators, etc, on conformational-change efficiency or on certain parameters need to be obtained separately for each case. For instance, the rates at each of the pH or each of the ion concentration could be examined, and a case specific factor could be obtained, for example pH specific value of c_{adjust}.

Long-range effect on catalysis is one of the revealing phenomena that conformational-change step does affect catalytic rate seriously[35, 61, 62]. Residues far away from the catalytic center show significant impact on both conformational-change efficiency and catalytic rate; those residues do not affect the catalytic center or the substrate to product chemical conversion step but the conformational change step; conformational-change-efficiency alteration is the primary cause of catalytic-rate change.

For cases like enzyme-LBMS-ATP-compound1, enzyme and LBMS are major players of both diffusion and conformational-change steps, what if enzyme and LBMS form a binary complex and then perform conformational change, while at the same time ATP and compound1 diffuse to bind to the binary complex? Is there overlap between the diffusion and conformational change steps? How could

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this be explained? Actually, let this complicated process be simplified to these steps or similar may be a feasible and credible approximation, first, diffusion and encounter of enzyme and LBMS, then conformational change of enzyme-LBMS binary complex, then diffusion and encounter of enzyme-LBMS binary complex with ATP and compound1, the process of which is swift.

Overall rate/velocity of three steps combined (conformational change, chemical conversion and product release) approximately depends on the concentration of enzyme-substrate complex,

$V_{conf\text{-}chem\text{-}prod} \approx k_{conf\text{-}chem\text{-}prod} \cdot [ES]$ or

$V_{conf\text{-}chem\text{-}prod} \approx k_{conf\text{-}chem\text{-}prod} \cdot [ES_1S_2S_3\cdots]$.

$1/k_{conf\text{-}chem\text{-}prod} = 1/k_{conf} + 1/k_{chem} + 1/k_{prod}$

$V_{conf\text{-}chem\text{-}prod}$ is the maximum throughput or flux of the process measured in molar per unit time (in like Ms^{-1}, $mol \cdot L^{-1} \cdot s^{-1}$).

Substrate-to-product chemical conversion step

The classical kinetic theories actually explain the chemical conversion step explicitly, and these theories illustrate why and how chemical steps are accelerated by the enzymes. Equilibrium constant K_{eq} and Gibbs standard free energy change ΔG° describe the direction, favorability and the final state of the chemical conversion step, like the relationship between final concentrations of the product and the reactants (K_{eq}), and the total energy released or absorbed during the

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reaction(ΔG°). Equilibrium constant has a direct relationship with standard free energy change. From transition state theory and Arrhenius Equation[1], the relationship between the activation energy $\Delta G^{\ddagger}$, temperature and the rate constant $k_{Gibchem}$ of chemical steps are established[63-65], $-\Delta G^{\ddagger}=RT\cdot\ln(k_{Gibchem}h/k_BT)$. Two adjustment factors A_1, A_2 are introduced into the equation to estimate a relationship between chemical conversion coefficient or turnover number k_{chem} and temperature,

$$k_{chem} = A_1 (k_BT/h)e^{-A_2\cdot\Delta G\ddagger/RT} = A_1(k_BT/h)e^{-A_2(\Delta H\ddagger - T\Delta S\ddagger)/RT}.$$

R is the ideal gas constant (8.314 $JK\cdot mol^{-1}$), k_B is Boltzmann constant with a value of $1.3806\times10^{-23}JK^{-1}$, and T is the absolute temperature, frequency factor A_1k_BT/h can be obtained experimentally. Enzymes lowers the activation energy, increases the possibility of substrate reaching the required state, thus speeding up the chemical step.

This equation works fine particularly for the chemical conversion step of a catalysis, but does not always work for the catalytic process as a whole. It is true within the chemical conversion step that, the sub-step which requires the highest activation energy is the rate limiting sub-step. If the whole catalytic cycle is concerned, certain step like the physical/biophysical process can be rate limiting but can have nothing to do with activation energy of chemical step at all. Some

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unidirectional and irreversible reactions do not follow equilibrium thermodynamics[66, 67], but the master equation will still apply.

$$\overset{t_{chem1}}{\underset{}{}}\quad\overset{t_{chem2}}{\underset{}{}}$$

$$A + B + E \rightleftharpoons EAB \rightleftharpoons ECD \rightleftharpoons E\text{-products} \rightleftharpoons E + products$$

or

$$\overset{k_{chem1}}{\underset{}{}}\quad\overset{k_{chem2}}{\underset{}{}}$$

$$A + B + E \rightleftharpoons EAB \longrightarrow ECD \longrightarrow E\text{-products} \rightleftharpoons E + products$$

Consistent with the master equation, t_{chem1}, t_{chem2}, k_{chem1}, k_{chem2} are defined similarly. And the equation stands the same, $t_{chem} = t_{chem1} + t_{chem2}$, $t_{chem1}k_{chem1}=1$, $t_{chem2}k_{chem2}=1$, $t_{chem}k_{chem}=1$, this is in agreement with previous theory on catalysis as well.

The overall rate of substrate to product chemical conversion step is mainly constrained by the rate limiting sub-step which requires the highest activation energy. The rate limiting sub-step of chemical process may follow the Arrhenius equation, then the whole chemical process roughly follows Arrhenius equation, and k_{chem} roughly equals to the rate limiting chemical sub-step k_{chemi}. If there are two rate-limiting chemical sub-steps, i and j, both may follow Arrhenius equation,

$$k_{chemi} = A_{i1}\,(k_B T/h)e^{-A_{i2}\cdot \Delta G‡/RT},$$

$$k_{chemj} = A_{j1}\,(k_B T/h)e^{-A_{j2}\cdot \Delta G‡/RT},$$

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let $1/k_{chem}=1/k_{chemi}+1/k_{chemj}$, then the Arrhenius equation for the whole chemical conversion step will be different from that of step i or j.

Product-release step

Sometimes, the product-release step could be the rate-limiting step[16, 26, 41]. On one occasion, the product-release process means huge conformational change, and product-dispensing-conformational-change step can be time consuming. Another situation, the product may exhibit strong affinity to the enzyme, resulting in slow release.

Can product-release step overlap in time course with substrate-binding step? Can product release happen the same time as substrate binds to the enzyme? It's possible but not always. Sometimes, the presence of substrate facilitates the release of product, because the substrate has higher affinity to the enzyme than the product. The product-release step and conformational-change step before chemical conversion are not consecutive steps but separated by the chemical step. Will things change in essence if the start of a catalytic cycle is defined alternatively? Probably not. If another catalytic cycle is defined to start immediately after substrate is converted to product, and all the things the enzyme do after this moment means to prepare the enzyme ready for the next catalytic

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cycle, they are probably still two separate events, probably interrupted by the diffusion step in between.

If product release means large-scale conformational change, there is a function describing the product-release kinetics.

$k_{prod}=c_{adjust\text{-}prod}Tp/(\sigma+\eta)$

Could this function be combined with that of the conformational-change step before the substrate-to-product chemical conversion? Is it possible that the two functions merge into one, and a different value for factor c_{adjust} and σ are obtained after the combination? Maybe this is plausible in certain circumstances.

The rate or velocity of product-release step depends on enzyme-product concentration [EP], $V_{prod}=k_{prod}[EP]$. Product dissociation kinetics need to be studied experimentally for further systematic analysis, especially for cases like rate-limiting product release caused by strong binding.

The catalytic cycle as a whole

The master equation elucidates catalytic kinetics of macromolecular biological enzyme in aqueous solution at molecular level. Although for many different enzyme molecules in a system, the amount of time each takes to accomplish a single turnover may be different; the amount of time may be distributed in a certain manner. Although for even the same enzyme molecule conducting multiple

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turnovers (catalytic cycles), the time span of each turnover may be different, which may follow a certain distribution. Let the averaged typical single catalytic cycle by a single enzyme be analyzed, all other enzymes will be copies of this one; let time flows, catalytic cycles will be periodical tandem repeats of this single catalytic cycle. Then the behavior of bulk enzyme could be deduced from the analysis of single enzyme. And these functions here could be connected to and be applied to experimental study.

Three different situations will be analyzed. First, diffusion step is rate limiting, and all the three steps (conformational change, chemical conversion, or product release) combined are not rate limiting, then $k_{conf\text{-}chem\text{-}prod}$ probably need not to be considered; [ES] is changing as [S] decreases. Second, diffusion step is fast, the three steps combined is rate limiting. [ES] is virtually constant, so that steady state approximation can be applied. Then $k \approx k_{conf\text{-}chem\text{-}prod}$, and actual $k_{conf\text{-}chem\text{-}prod}$ is slightly larger than the experimentally obtained k_{exp}. Third, both diffusion and the three steps combined are rate limiting. The overall catalytic rate/velocity $V_{overall}$ and turnover number k of the catalysis could be examined and obtained experimentally.

$$1/k = t_{difu} + t_{conf\text{-}chem\text{-}prod}$$

$$1/V_{overall} \approx [E][S]/t_{difu} + [ES]/t_{conf\text{-}chem\text{-}prod}$$

From these two functions, both t_{difu} and $t_{conf\text{-}chem\text{-}prod}$ could be

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resolved.

From this discussion, a much more systematic and balanced analysis of catalytic process is possible. For instance, the catalytic rate is affected by temperature, this is not only because temperature affects the activation of chemical-conversion step, but also because temperature affects the physical steps of each catalytic cycle as well.

Binding energy contributes to reaction specificity and catalysis, this is the classical expression about the relationship between binding energy and catalysis. But previously, relationship between binding efficiency and catalytic efficiency (or catalytic rate) is not clear. Now, correlation between enzyme-macro-substrate recognition/binding efficiency, conformational change efficiency and catalytic rate, between chemical conversion efficiency and catalytic rate, and between product release efficiency and catalytic rate, can be obtained experimentally based on related functions by using kinetic, biophysical and biochemical technologies.

Efficient binding, by itself, definitely contributes to catalysis, by increasing turnover numbers. The binding energy can reduce activation energy of chemical conversion step, thus accelerating the chemical step, but strong binding may slow down the product release or enzyme-product-complex dissociation. Here an atypical example is discussed. For LBMS involved multiple reactant catalysis, the binding

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energy between enzyme and LBMS does not necessarily contribute to the velocity of the catalysis directly, although it accounts for the majority of the binding energy. Binding energy is not always the driving force sometimes not the only driving force for catalysis, for instance, nucleotide triphosphate involved catalysis. Usually the catalysis comes to a halt in the absence of nucleotide triphosphate. Reaction coupling or the step-by-step release of covalent bond energy as one sequential reaction can explain the driving effect of NTPs. A detailed mechanism-based explanation of relationship between binding efficiency, binding energy and catalytic rate requires further investigation.

Huge enzyme machinery catalyzed complex biosynthesis includes multiple rounds of conformational change, chemical conversion etc to manufacture a single macromolecular product. For these complex biochemical reactions, chemical conversion process, conformational change and other steps may be interspersed with one another. Biosynthesis can repeat to generate required amount of macromolecular copies. DNA replication[68, 69], protein synthesis by ribosome[70, 71], and transcription[72-75] and some other biosynthetic events are very sophisticated long-lasting processes, the incorporation of deoxynucleotides, amino acids, or nucleotides are similar repeat with unidentical substrates for the multiple subunit

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enzyme machine, respectively. Catalytic step of these complex catalysis may be different from above. The heterogeneous repeat may be regarded as sub turnover cycle, and statistical information about the GATC/GAUC contents of the DNA/RNA product, the amino acid composition of the polypeptide chain, respectively, may be included to analyze the catalytic process in detail.

Summary and perspectives

This writing tries to explain catalytic kinetics from molecular level point of view. Inspected microscopic molecular events from this detailed kinetic study will provide fresh insight into the catalytic mechanism of enzyme. The relationship between catalytic rate and substrate concentration, biophysical, biochemical conditions etc can explain various experimental phenomena in general. Classical theories about enzyme catalysis also utilize statistical concept to describe the kinetics, and multiple enzymes/possibilities are concerned at any given unit of time[4-6]. With the advancement of science and technology, especially with the development of single molecular manipulation and detection techniques[76-78], study in detail and in depth of the catalytic behavior of singular enzyme will become feasible, which will provide fresh insight into the catalytic mechanism of biological macromolecular enzymes. Extensive further experimental research is required to combine this writing, classical kinetic theories

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and experimentally obtained single molecular actual behavior.

Now that enzyme catalytic efficiency and catalytic rate/velocity can be affected by so many factors at so many steps, a lot of different strategies and approaches can be utilized for enzyme engineering, drug discovery[79, 80], signaling pathway manipulation or metabolic pathway modulation and so on.

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Figure Legend

Fig.1. Typical curve of a function $1/y=(a/x)+b$, the shape of the curve resembles some of the relationship between the initial velocity V_0(as Y axis) and the substrate concentration [S] (as X axis).

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