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ORIGINAL PAPER

A novel approach to measure all rate constants in the simplest enzyme kinetics model

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Abstract Enzymes play vital roles in life processes. Almost all biochemical reactions are mediated by enzymes. The rate constants of enzyme kinetics are the most important parameters for the reactions catalyzed by enzymes. In 1902, Adrian Brown proposed a simple single-substrate-single-product model which contains only three rate constants k_1, k_{-1} and k_2. So far, biologists can measure the Michaelis constant K_M and the catalytic constant k_{cat}, which actually is equal to k_2, according to Michaelis–Menten equation. Using temperature jump method or transient state kinetics, k_1, k_{-1} and k_2 can be determined. However, these methods are complicated. In this article, we design a novel simple method that could determine the rate constants k_1 and k_{-1} based on knowing k_{cat} and K_M. Our numerical experiments show that the three rate constants can be calculated rather precisely. Hence, we believe that biochemists could design experiments to measure the rate constants based on our method.

Keywords Michaelis constant · Catalytic constant · Michaelis–Menten equation

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1 Introduction

Enzymes play important roles in life processes [6]. They are involved in almost all chemical reactions concerning metabolism. The rate of reactions and the change of rate of reactions under different conditions contain information on functions and structures of enzymes. Thus, a major problem of enzyme kinetics is to determine the enzymatic reaction rates, which is stated in many famous text books such as [27].

Brown [4], as the first one to study enzyme kinetics, proposed the basic model for enzyme kinetics. In his model, there is only one substrate S, which is catalyzed by enzyme E into product P. This reaction consists of two successive elementary reactions. The substrate and enzyme form a complex called enzyme-substrate complex denoted by C in the first reaction, and the complex breaks down into product and enzyme in the second one:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \overset{k_2}{\to} P + E, \tag{1}$$

Here k_1 and k_{-1} denote, respectively the forward and reverse rate constants of the formation of the enzyme-substrate complex, and k_2 denotes the rate constant of the decomposition of the complex into product and enzyme.

This reaction process can then be described by the following system of differential equations [24]:

$$dS(t)/dt = -k_1 S(t)E(t) + k_{-1}C(t) \tag{2}$$
$$dE(t)/dt = -k_1 S(t)E(t) + (k_{-1} + k_2)C(t) \tag{3}$$
$$dC(t)/dt = k_1 S(t)E(t) - (k_{-1} + k_2)C(t) \tag{4}$$
$$dP(t)/dt = k_2 C(t) \tag{5}$$

with the initial condition

$$(S(0), E(0), C(0), P(0)) = (S_0, E_0, 0, 0), \tag{6}$$

where $E(t)$, $S(t)$, $C(t)$ and $P(t)$ denote the concentrations of enzyme, substrate, enzyme-substrate complex and product at time t during the process, respectively. Obviously, we have the following equalities:

$$E(t) + C(t) = E_0 \tag{7}$$
$$S(t) + C(t) + P(t) = S_0. \tag{8}$$

Owing to these equalities, the reaction can be described by $(S(t), E(t))$, $(S(t), P(t))$ or $(P(t), E(t))$. Furthermore, the (2–5) are equivalent to Eqs. 10 and 11 below.

As these systems of differential equations can not be explicitly integrated, many enzymologists added more conditions or assumptions on these systems to simplify this problem. Michaelis and Menten [16] added the condition that $k_{-1} \gg k_2$. However, this condition is usually unrealistic and has little usage. Briggs and Haldane [3] proposed

the famous assumption in enzyme kinetics, i.e. the Quasi-Steady-State Assumption (QSSA) under a more realistic condition $S_0 \gg E_0$. Recently, [11] have proved that QSSA is always true if $S_0 \gg E_0$, and hence call it Quasi-Steady-State Law (QSSL).

By QSSL, the Eqs. (2–5) can be approximately solved explicitly, and some relations between rate constants can be gotten. Especially, Briggs and Haldane got the famous Michaelis–Menten equation:

$$v_0 = V_{\max} S/(K_M + S), \tag{9}$$

where K_M is the Michaelis constant defined as $K_M = (k_{-1} + k_2)/k_1$, v_0 is the initial velocity of the reaction and $V_{\max} = k_2 E_0$ is called the maximal velocity, which is indeed the supremum of the velocity but is never reached. In fact, the Michaelis–Menten equation holds not only at assemble level of enzyme molecules, but also at single-molecule level by the statistical analysis of the stochastic behave of single-molecule enzyme catalysis [1, 6]. Lineweaver and Burk [12] found that the reciprocal form of the Michaelis–Menten equation can be used to calculate K_M and $V_{\max}$. Then k_2 follows by the equality $V_{\max} = k_2 E_0$.

There are many books discussing the estimations of k_2 and K_M based on the reciprocal form of Michaelis–Menten equation such as [22, 23, 27]. Besides this method, some more effective methods are also considered [8, 19–21]. Linearizations like the one proposed by Lineweaver and Burk are of relative poor accuracy [5], although they gave nice approximation in an era when we had no computers. Nowadays, kinetic data are commonly treated by computers using complicated statistical methods such as nonlinear regression [27]. In a word, k_2 and K_M have already been possible to be measured.

By using temperature jump method or transient state kinetics, all the three rate constants k_1, k_2 and k_{-1} can be determined. However, these methods are somewhat complicated, and require particular equipments. So far, no paper gives methods to calculate all the three rate constants like Lineweaver–Burk's method, i.e. one needs only to measure concentrations of reactants at a few different times.

Since K_M can be determined, it yields a relation between k_1 and k_{-1}. In order to measure all the three rate constants, we must find another relation between k_1 and k_{-1}. In this paper, we find that all the trajectories $(S(t), E(t))$ started from different initial concentrations of substrate have the same tangent at the end. With such an additional relation, k_1 and k_{-1} can be completely calculated. Numerical experiments show that our method is effective. Hence, biochemists can design experiments based on our method to measure all rate constants.

Our method is derived by rigorous mathematics. Mathematics has been applied to biology in many directions, such as the application of game theory to evolution by [25, 26] and chaos to ecology by [15]. Interesting applications of mathematics to biology can be found in many fundamental books on mathematical biology, such as [17, 18] and [7]. The qualitative theory of dynamical systems has more than 100 years' history [2], and is applied to many disciplines of sciences including biology [9, 13, 14]. In this paper, we utilize this theory to analyze enzyme kinetics data, and it seems very effective. Perhaps, the most surprising thing is that the result we got here can be checked by biochemical experiments in principle.

Section 2 gives the details of our method. Section 3 illustrates some numerical experiments to test our method. Moreover, some discussions about our method and some suggestions to biologists for designing experiments are proposed. Section 4 is the conclusion section. The mathematical foundations of the method are given in Appendix.

2 Method to measure all the rate constants

In the single-substrate-single-product enzyme reaction (1), k_1, k_2 and k_{-1} are three rate constants. There are many methods for determining the parameters of the Michaelis–Menten equation (i.e. $V_{\max}$ and K_M). By knowing $V_{\max}$ and K_M, we already know k_2 and a relation between k_1 and k_{-1}. So, what we need to evaluate k_1 and k_{-1} is another relation between k_1 and k_{-1}.

The reaction can also be described by equations

$$dS/dt = -k_1 SE + k_{-1}(E_0 - E), \tag{10}$$

$$dE/dt = -k_1 SE + (k_{-1} + k_2)(E_0 - E), \tag{11}$$

with the initial condition $(S(0), E(0)) = (S_0, E_0)$, which is equivalent to system (2–8).

Now, a new relation between k_1 and k_{-1} is given by

$$T_1 = -\left(k_1 E_0 - (k_{-1} + k_2) + \sqrt{(k_1 E_0 + k_{-1} + k_2)^2 - 4k_1 k_2 E_0}\right) \Big/ 2k_{-1}, \tag{12}$$

where $T_1 = \lim_{t \to +\infty} (E(t) - E_0)/S(t)$. (The detailed deduction of this relation is in Appendix.) For convenience, we denote the concentrations of substrate, enzyme and product at the moment $\hat{t}$ near the end of the reaction by $\hat{S}$, $\hat{E}$ and $\hat{P}$, respectively. Hence, T_1 can be approximated by $\hat{T}_1 = (\hat{E} - E_0)/\hat{S}$ (see Figs. 1 and 2). Moreover, as $(\hat{S}, \hat{E})$ approaches the end point $(0, E_0)$ of the reaction, $\hat{T}_1 = (\hat{E} - E_0)/\hat{S}$ approaches T_1.

Then, by (12) and the following two equations

$$V_{\max} = k_2 E_0, \tag{13}$$

$$K_M = (k_{-1} + k_2)/k_1, \tag{14}$$

k_1, k_2 and k_{-1} are solved as,

$$k_2 = V_{\max}/E_0, \tag{15}$$

$$k_1 = (V_{\max}/E_0)T_1^2 \Big/ \left(K_M(-T_1 + T_1^2) - E_0(-T_1 + 1)\right), \tag{16}$$

$$k_{-1} = K_M \left((V_{\max}/E_0)T_1^2 \Big/ \left(K_M(-T_1 + T_1^2) - E_0(-T_1 + 1)\right)\right) - V_{\max}/E_0. \tag{17}$$

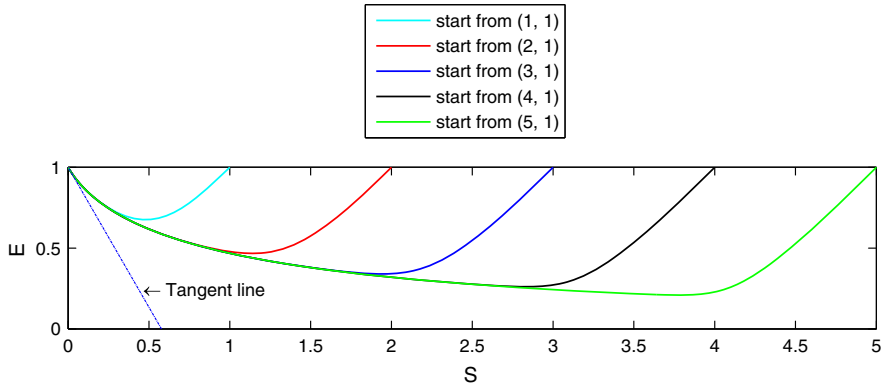

Fig. 1 Trajectories of different reactions: each curve indicates the trajectory of the concentrations of enzyme and substrate from different initial conditions. All of them have a common tangent line at the end point (0, 1). In other words, the ratio of $(E - E_0)/S$ on each trajectory approximates the slope of the tangent line when S is sufficiently small

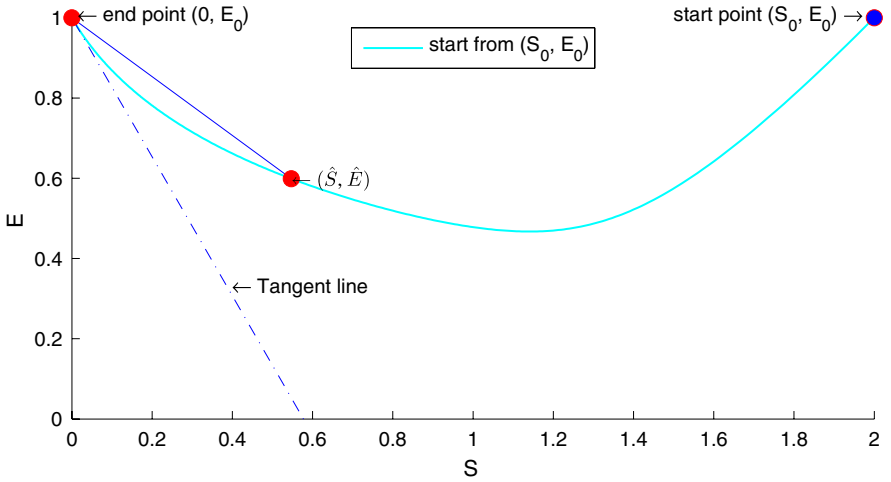

Fig. 2 Secant line versus tangent line: Slope of the secant line between two red points is $(\hat{E} - E_0)/\hat{S}$, which approximates the slope of the tangent line, when $\hat{S}$ is sufficiently small

Since $V_{\max}$ and K_M are assumed known, estimates of k_1, k_2 and k_{-1} are obtained from Eqs. 15–17, if T_1 is replaced by $\hat{T}_1 = (\hat{E} - E_0)/\hat{S}$.

Define

$$\hat{k}_1(\hat{T}_1) = (V_{\max}/E_0)\hat{T}_1^2 \Big/ \Big(K_M(-\hat{T}_1 + \hat{T}_1^2) - E_0(-\hat{T}_1 + 1)\Big) . \tag{18}$$

Then $k_1 = \hat{k}_1(T_1)$.

The Taylor expansion of $\hat{k}_1(\hat{T}_1)$ at T_1 is

$$\hat{k}_1(\hat{T}_1) = \hat{k}_1(T_1) - M(\hat{T}_1 - T_1) + o(|\hat{T}_1 - T_1|^2), \tag{19}$$

where M is a constant equal to

$$(V_{\max}/E_0)T_1\,(K_M T_1 - E_0 T_1 + 2\,E_0)\,\Big/\Big(-K_M T_1 + K_M {T_1}^2 + E_0 T_1 - E_0\Big)^2$$

When one uses $\hat{T}_1$ instead of T_1, the error between k_1 and $\hat{k}_1$ is $|k_1 - \hat{k}_1| = |M||\hat{T}_1 - T_1| + o(|\hat{T}_1 - T_1|^2)$, which shows that $\hat{k}_1$ approximates k_1 better, when $\hat{T}_1$ is more approximate to T_1.

Use the same method, we could have $|k_{-1} - \hat{k}_{-1}| = K_M|M||\hat{T}_1 - T_1| + o(|\hat{T}_1 - T_1|^2)$, which shows the same result that $\hat{k}_{-1}$ approximates k_{-1} better, when $\hat{T}_1$ is more approximate to T_1.

3 Results and discussions

3.1 Numerical simulations

In the above section, we mentioned that T_1 could be approximated by $(\hat{E} - E_0)/\hat{S}$, and then, more importantly, the rate constants k_1, k_2 and k_{-1} could be calculated.

So, in this section, we simulate the reaction processes by computers to show how to evaluate the three rate constants and how well our method works. In the following simulations, we use fourth order Runge-Kutta method to calculate the reaction processes, and the step-length is set as 0.00002.

T_1 can be approximated by $(\hat{E} - E_0)/\hat{S}$ if $\hat{S}$ is small enough. At first, we do some numerical experiments to show that T_1 can be well estimated if a proper $\hat{S}$ and its corresponding $\hat{E}$ are measured.

As stated above, the process of the reaction (1) can be described by Eqs. 10 and 11 with the initial condition $(S(0), E(0)) = (S_0, E_0)$. After setting the rate constants $k_1 = 0.3$, $k_2 = 0.2$, $k_{-1} = 0.1$ and the initial condition $(S(0), E(0)) = (20, 0.5)$, the time evolutions of $E(t)$ and $S(t)$ can be calculated by numerical integration. The trajectory of $(S(t), E(t))$ is plotted in Fig. 3.

In the above reaction,

$$\begin{aligned} T_1 &= -\left(k_1 E_0 - (k_{-1} + k_2) + \sqrt{(k_1 E_0 + k_{-1} + k_2)^2 - 4k_1 k_2 E_0}\right)\Big/ 2k_{-1} \\ &= -0.6861. \end{aligned} \tag{20}$$

Several pairs of $(\hat{S}, \hat{E})$ are chosen, and the corresponding values of $\hat{T}_1$ are calculated. The results are listed in Table 1. From the results, we observe as expected that T_1 is well approximated by $\hat{T}_1$ as $(\hat{S}, \hat{E})$ approaches the point $(0, E_0)$.

Since we have had the approximation of T_1, that is $\hat{T}_1$, the three rate constants k_1, k_2 and k_{-1} can be solved by Eqs. 15–17. Assuming K_M and $V_{\max}$ are exact, we can see clearly the error caused by our method.

In this numerical experiment, we have $K_M = (k_{-1} + k_2)/k_1 = 1$ and $V_{\max} = k_2 E_0 = 0.1$. So $\hat{k}_2 = V_{\max}/E_0 = 0.2$. For different values of $\hat{T}_1$, we have different $\hat{k}_1$

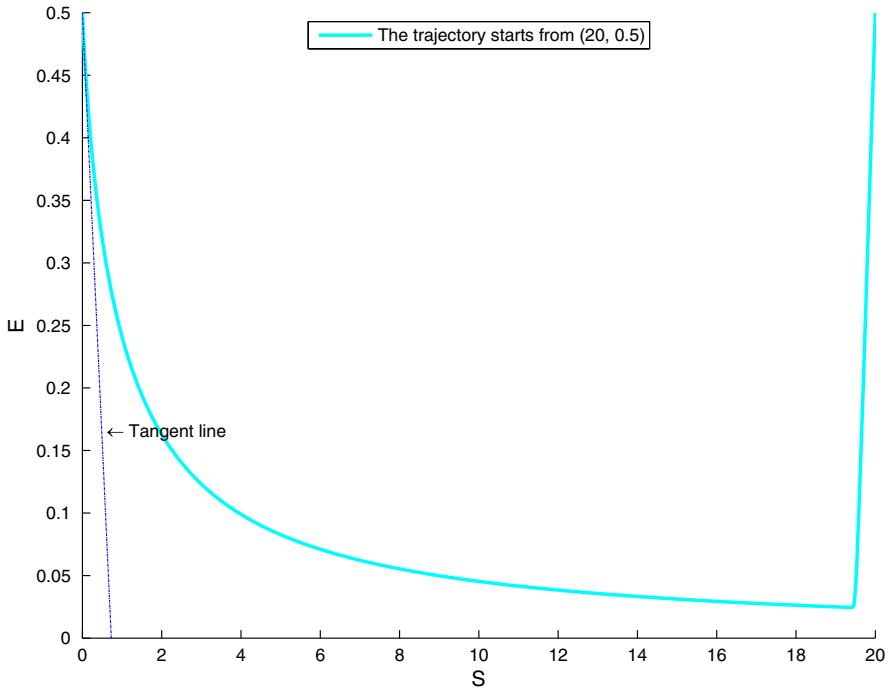

Fig. 3 Trajectory of a reaction (1): the rate constants are $k_1 = 0.3, k_2 = 0.2$ and $k_{-1} = 0.1$, and the initial condition is $(S(0), E(0)) = (20, 0.5)$

Table 1 The first two columns of this table list the concentrations of $\hat{S}$ and $\hat{E}$, respectively

$\hat{S}$	$\hat{E}$	$\hat{T}_1$	$\hat{k}_1$	$\hat{k}_2$	$\hat{k}_{-1}$
0.0400	0.4749	−0.6283	0.3779	0.2	0.1779
0.0360	0.4772	−0.6334	0.3682	0.2	0.1682
0.0320	0.4796	−0.6386	0.3591	0.2	0.1591
0.0280	0.4820	−0.6440	0.3504	0.2	0.1504
0.0240	0.4844	−0.6495	0.3422	0.2	0.1422
0.0200	0.4869	−0.6551	0.3343	0.2	0.1343
0.0160	0.4894	−0.6609	0.3268	0.2	0.1268
0.0120	0.4920	−0.6669	0.3197	0.2	0.1197
0.0080	0.4946	−0.6731	0.3129	0.2	0.1129
0.0040	0.4973	−0.6795	0.3063	0.2	0.1063
0.0020	0.4986	−0.6828	0.3031	0.2	0.1031
0.0010	0.4993	−0.6845	0.3016	0.2	0.1016
0.0005	0.4997	−0.6853	0.3008	0.2	0.1008
0.0001	0.4999	−0.6860	0.3002	0.2	0.1002

The third column lists the estimated values of T_1, which are denoted by $\hat{T}_1$. And the last three columns give the corresponding estimations of $\hat{k}_1, \hat{k}_2$ and $\hat{k}_{-1}$

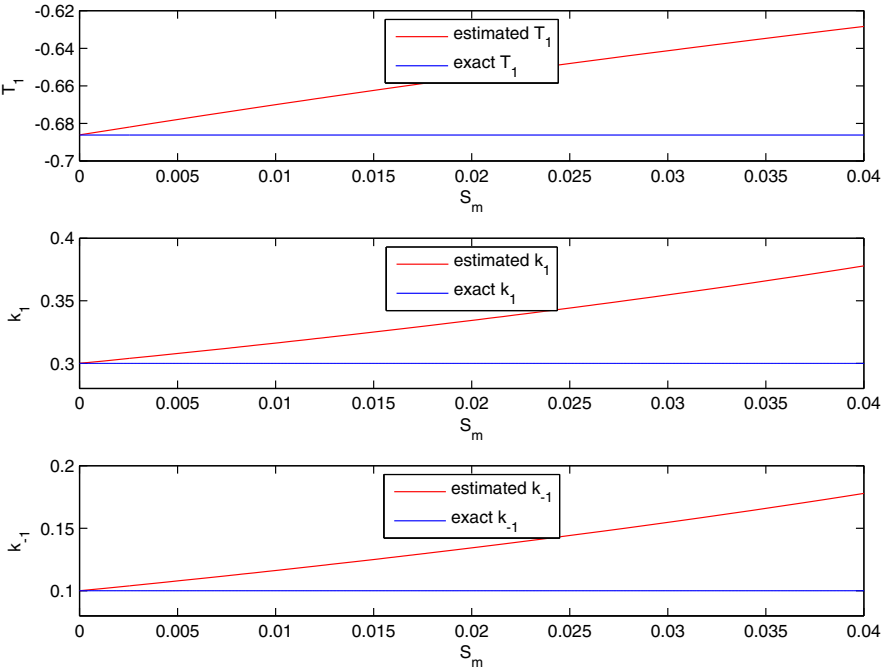

Fig. 4 Estimated values: From top to bottom, they indicate the estimations of T_1, k_1 and k_{-1} with respect to different values of $\hat{S}$ from 0.04 to 0, respectively

and $\hat{k}_{-1}$. Calculating results show that our method is a better approximate when $\hat{T}_1$ is more approximate to T_1.

To give a more detailed and straightforward description of our experimental results, we plot them in a graph (see Fig. 4). From this graph, we can obviously see that T_1, k_1 and k_{-1} can be well calculated as long as sufficiently small $\hat{S}$ and its corresponding $\hat{E}$ were measured.

3.2 Discussion

So far, we have introduced a method to evaluate the rate constants, and the results of our numerical experiments show the effectiveness of the method. For the equalities (7) and (8), we claim that if biochemists can measure any of the pairs $(\hat{S}, \hat{E})$, $(\hat{S}, \hat{P})$ and $(\hat{P}, \hat{E})$ in a single-substrate-single-product enzyme catalyzed reaction, the three rate constants can be calculated rather precisely. And theoretically, the nearer the end of the reaction the measurements are done, the more accurate the results are. This is also supported by the simulations in the last section. As listed in Table 1, when $\hat{S}$ approaches 0, the approximations $\hat{k}_1$, $\hat{k}_2$ and $\hat{k}_{-1}$ approach their exact values.

However, we do not suggest biologists to do measurements too close to the end of the reaction in experiments. Because if it is done too close to the end, any small measurement error will lead to large error in T_1, and hence to large errors in k_1 and

Table 2 The first two columns of this table list the concentrations of $\hat{S}$ and $\hat{E}$, respectively

$\hat{S}$	$\hat{E}$	$\hat{T}_1$	$\hat{k}_1$	$\hat{k}_{-1}$
0.22017669	0.39493330	−0.4772	−1.3518	−1.5518
0.09902899	0.44402015	−0.5653	0.6254	0.4254
0.00472997	0.49679151	−0.6783	0.3075	0.1075
0.00005564	0.49996183	−0.6860	0.3001	0.1001
0.00001376	0.49999056	−0.6861	0.3000	0.1000
0.00000322	0.49999779	−0.6861	0.3000	0.1000
0.00000039	0.49999973	−0.6865	0.2997	0.0997
0.00000009	0.49999994	−0.7155	0.2769	0.0769
0.00000003	0.49999999	−0.4413	−0.4602	−0.6602

The third column lists the estimated values of T_1, which are denoted by $\hat{T}_1$. And the last two columns give the corresponding estimations of $\hat{k}_1$ and $\hat{k}_{-1}$. In each cases, $\hat{k}_2 = 0.2$, so we do not list them in this table explicitly

k_{-1}. Such kind of cases occur as well as in numerical simulations, if the precision of the simulation is low. Table 2 gives an example showing such cases.

For the numerical experiment in Table 2, the reaction processes of (10) and (11) are simulated by the Runge-Kutta-Fehlberg method in Matlab by setting $k_1 = 0.3$, $k_2 = 0.1$, $k_{-1} = 0.1$, $S_0 = 20$ and $E_0 = 0.5$.

In this simulation we see that, if some properly small $\hat{S}$ and its correspondence $\hat{E}$ were chosen, T_1 and then k_1, k_2 and k_{-1} are calculated with small deviations from their exact values (see Row 3 to 7 in Table 2). However, when $\hat{S}$ was too small, for the unavoidable errors of numerical integration, the errors of $\hat{S}$ would lead to large errors in T_1, k_1 and k_{-1} (see the last two rows in Table 2). The details are shown in Table 2. Here we do not discuss the accuracy about k_2, because in this article we assume K_M and $V_{\max}$ have been given.

In the view point of theoretical analysis, the measurement should be done near the end of the reactions for the accuracy. But considering the unavoidable measurement errors, it should not be done too close to the end. So, some tradeoff should be considered in biochemical experiments.

Actually, to evaluate K_M and $V_{\max}$ accurately is still a challenge, which attracts many talented scientists to study. The good performance of our methods depends partially on evaluating K_M and $V_{\max}$ accurately. So, further study to evaluate the rate constants k_1, k_2 and k_{-1} together by measuring only concentrations, without the assumption that K_M and $V_{\max}$ are know, is expected.

4 Conclusion

Enzyme kinetics, as an important branch of enzymology, is to study the rates of chemical reactions catalyzed by enzymes. So, how to measure the rate constants in enzyme catalyzed reactions naturally becomes a fundamental problem in enzyme kinetics. Now biochemists can determine these constants by the temperature jump method or

transient state kinetics. But the necessary equipments and techniques limit the use of these methods.

In this article, we proposed a novel method to calculate all the three rate constants in (1), in which only one pair of concentrations of the reactants are needed. This method is based on carefully analyzing the structure of integral curves near the singularity point in a dynamical system.

To test our method we did some numerical analysis, and the results showed its effectiveness.

By this method, all the three rate constants can be calculated. Knowing all the rate constants should be helpful for deeper insights to the function and structure of enzymes. Furthermore, it gives more information about the reaction mechanism.

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Appendix

It will be seen in this appendix that the system of differential equations we encountered is an autonomous system on the plane having only one singularity, that is a stable nodal point. Moreover, the solutions, i.e. the integral curves of the system under initial conditions which have biological meaning will approach the singularity when time goes to infinity. And these integral curves must enter the singularity in the direction of the eigenvector with the smaller absolute value of the eigenvalue [10, pp. 90–94].

Consider the system

$$\begin{cases} dS/dt = -k_1 SE + k_{-1}(E_0 - E), \\ dE/dt = -k_1 SE + (k_{-1} + k_2)(E_0 - E). \end{cases} \tag{21}$$

We have studied this system in our former paper [11].

The singularity of this system is $(S, E) = (0, E_0)$. The linearization of this system around this singularity is

$$\begin{cases} dS/dt = -k_1 E_0 S - k_{-1}(E - E_0), \\ dE/dt = -k_1 E_0 S - (k_{-1} + k_2)(E_0 - E). \end{cases} \tag{22}$$

And its characteristic equation is

$$\lambda^2 + (k_1 E_0 + k_{-1} + k_2)\lambda + k_1 k_2 E_0 = 0, \tag{23}$$

where λ are the eigenvalues and can be solved as

$$\lambda = \left(-(k_1 E_0 + k_{-1} + k_2) \pm \sqrt{(k_1 E_0 + k_{-1} + k_2)^2 - 4k_1 k_2 E_0}\right) \Big/ 2. \tag{24}$$

As both eigenvalues are negative, this singularity is a stable nodal point. The eigenvectors are

$$V_{\mp} = \left(k_{-1}, (-k_1E_0 + k_{-1} + k_2 \mp \sqrt{(k_1E_0 + k_{-1} + k_2)^2 - 4k_1k_2E_0})\Big/2\right). \quad (25)$$

We have proved that the solution of (21) with initial condition $(S(0), E(0)) = (S_0, E_0)$ denoted by $(S(t), E(t))$ will approach the singularity as Lemma 3 in [11]. Therefore, the solution will enter the singularity $(0, E_0)$ in the direction of an eigenvector. Since $E(t) < E(0)$ and $S(t) > 0$ imply $(E(t) - E_0)/S(t) < 0$, we see that $(S(t), E(t))$ must enter $(0, E_0)$ in the direction of V_-. Hence,

$$\lim_{t\to+\infty}(E(t) - E_0)/S(t) = -\left(k_1E_0 - (k_{-1} + k_2) + \sqrt{(k_1E_0 + k_{-1} + k_2)^2 - 4k_1k_2E_0}\right)\Big/2k_{-1} \quad (26)$$

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