한국농화학회지 Vol. 19, No. 3. Sept. 1976
J. Korean Agricultural Chemical Society

Kinetic Analysis of Rate Limiting Step in the Metabolic Process.

[Part I.] A Modified Emzymatic Method

Kui-Nam, Shin and Ik-Bai, Koh
Dept. of Agricultural Chemistry, College of Agriculture, Chonnam National University
(Received December 29, 1975)

代謝過程에 있어서 律速段階의 速度論的 解析

〔第一報〕 수정된 효소적 방법에 대하여

申 貴 男 · 高 翊 培
全南大學校 農科大學 農化學科
(1975년 12월 29일 수리)

SUMMARY

The determination of rate limiting step in the metabolic process is of great importance to understand the metabolic properties. In this paper the authors propose a modified enzymatic method instead of thermodynamic method. This method is based on the assumption that the over-all rate increment would be larger than by any other steps to which the individual enzyme are added respectively, provided that the enzyme participated in the rate limiting step is added to the reactions composed of n steps of metabolic process with which n kind of enymes are concerned. The present paper deals with analysis and discussion about some factors having influence on the proposed process, mainly about the metabolic process constituted with homogeneous steps. The results show that the determination of rate limiting step by a modified enzymatic method is feasible, provided that some restrictions are added in any type of mechanisms.

INTRODUCTION

In the metabolic process of living cells, there must always be a rate limiting step which controls the metabolic process. According to the thermodyamic definition this step is defined as the minimal change in Gibbs' free energy (ΔG) of a characterizd reaction and the following equation is usually employed to determine the step;

$$\Delta G = -RT\ ln\ K_{eq} = \Delta H - T\Delta S = nF\Delta E$$

However the application of above equation to such objective is rather complicate, since each value which contains in each step of metabolic process must be determined respectively. In order to reduce such complication the authors present a modified enzymatic method which appears to be no report in the literature.

ANALYSIS

To determine a rate limiting step the authors consider three factors, i.e., the mechanism types

of the rate limitien step and of each step in the metabolic process, the influence on the over-all rate increment by adding the individual enzyme to each step and the influence of feedback inhibition which may be occured ay adding the enzyme.

As we shall show in the subsequent sections, various mechanism types of enzymatic reaction are given as follows.

1, Mechanism types involving one substrate and one product.[1)-5)]

1*a*. $A+E \underset{k2}{\overset{k1}{\rightleftharpoons}} \times \xrightarrow{k3} E+P$

1*b*. $A+E \underset{k2}{\overset{k1}{\rightleftharpoons}} \times \underset{k4}{\overset{k3}{\rightleftharpoons}} E+P$

1*c*. $A+E \underset{k2}{\overset{k1}{\rightleftharpoons}} \times \underset{k4}{\overset{k3}{\rightleftharpoons}} Y \underset{k6}{\overset{k5}{\rightleftharpoons}} E+P$

1*d*. $A+E \underset{k2}{\overset{k1}{\rightleftharpoons}} \times \underset{k4}{\overset{k3}{\rightleftharpoons}} X'+P,\ X' \underset{k6}{\overset{k5}{\rightleftharpoons}} EQ \underset{k8}{\overset{k7}{\rightleftharpoons}} E+Q$

where k_1, k_2, k_3,···are rate constant.

II, Mechanism types involving two substrates and two products.[1)6)7)]

2a. Ping-pong mechanism.

$$A+E \underset{k2}{\overset{k1}{\rightleftharpoons}} (AE \rightleftharpoons PE) \underset{k4}{\overset{k3}{\rightleftharpoons}} E'+P$$

$$B+E' \underset{k6}{\overset{k5}{\rightleftharpoons}} (BE \rightleftharpoons QE') \underset{k8}{\overset{k7}{\rightleftharpoons}} E+Q$$

2b. Ordered mechanism.[1)8)-10)]

$$A+E \underset{k2}{\overset{k1}{\rightleftharpoons}} AE$$

$$AE+B \underset{k4}{\overset{k3}{\rightleftharpoons}} (AEB \rightleftharpoons QEP)$$

$$\underset{k6}{\overset{k5}{\rightleftharpoons}} QE+P$$

$$QE \underset{k8}{\overset{k7}{\rightleftharpoons}} E+Q$$

2c. Random mechanism.[1)6)7)]

$$A+E \underset{}{\overset{K1}{\rightleftharpoons}} AE \qquad PE \underset{}{\overset{K2}{\rightleftharpoons}} E+P$$

$$AEB \underset{k2}{\overset{k1}{\rightleftharpoons}} PEQ$$

$$B+E \underset{}{\overset{K5}{\rightleftharpoons}} BE \qquad QE \underset{}{\overset{K6}{\rightleftharpoons}} E+Q$$

where k_1, k_3,···are rate constant and K_1, K_2, K_3,···are equilibrium constant.

I. Mechanism types involving one substrate and one product.

Mechanism 1 a: This mechanism represents an intermediate complex retaining all the atoms originally present in both substrate A and enzyme. E· Hence the kinetic equations are as follows.

$$-\frac{da}{dt}=k_1ae-k_2x \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots (1a-1)$$

$$\frac{dx}{dt}=k,ae-(k_2+k_3)x \cdots\cdots\cdots\cdots\cdots (1a-2)$$

$$\frac{de}{dt}=(k_2+k_3)x-k_1ae \cdots\cdots\cdots\cdots\cdots (1a-3)$$

$$\frac{dp}{dt}=k_3x \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots (1a-4)$$

$$e_o=e+x \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots (1a-5)$$

where a, x and p are concentrations of substrate A, intermediate complex X and P, respectively; e_o and e are those of enzymes which present initially and after time t, respectively.

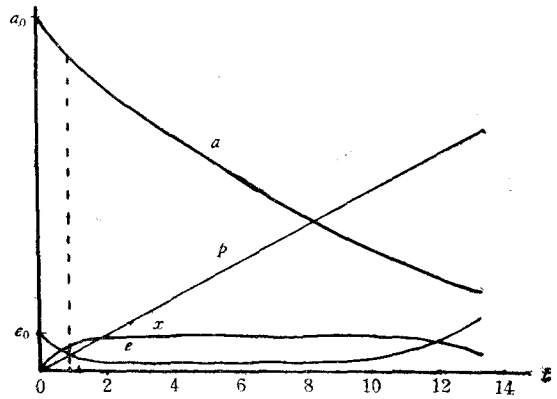


Fig. 1. Progress curve for $A+E \underset{k2}{\overset{k1}{\rightleftharpoons}} \times \xrightarrow{k3} E+P$, with $k_1 \simeq k_2 \simeq k_3$; $a_o \simeq e_o$—adopted from w.w. Cleland.[11)]

In such a case, the explicit solution is not possible without some restrictions and they can only be integrated numerically, preferably with help of computer.

A typical solution provided by prof. W,W. Cleland[1)11)] is given in Fig. 1., in which we observe that the slopes of the progress curve for

both X and e versus time are vitually equal to zero over the considerable portion of the plot. Thus the steady state approximation is valid for all times (t) beyond the dotted line, i.e., throughout the whole period except for a very brief period prior to the establishment of the steady state, where dx/dt=0 and de/dt=0 The larger the ratio a./e. the smaller the initial period during which the steady state approximation does not hold. For this reason we obtain the following equations.

$$dx/dt \simeq 0 \simeq k,ae-(k_2+k_3)x \text{ or } k_1ae \simeq (k_2+k_3)x$$

Substituting in equation (1a-5),

$$\text{gives } x=\frac{k_1ae_o}{k_1a+k_2+k_3}\left(e_0=\frac{(k_2+k_3)x}{k_1a}+x\right.$$

$$\left.=\frac{(k_1a+k_2+k_3)x}{k_1a}\right)$$

and substituting this in equation (1a-4);

$$v=-\frac{da}{dt}=\frac{dp}{dt}=k_3x=\frac{k_1k_3ae_o}{k_1a+k_2+k_3}$$

$$=\frac{k_3ae_o}{\frac{k_2+k_3}{k_1}+a}=\frac{V\cdot a}{K_a+a} \quad \cdots\cdots\cdots\cdots (1a-6)$$

where $V=k_3e_o$ $K_a=\frac{k_2+k_3}{k_1}$

In the integrated form, this becomes

$$V\cdot t=K_a l_n\frac{a_o}{a}+(a_o-a) \quad \cdots\cdots\cdots\cdots (1a-7)$$

where V. and K_a represent maximum velocity of enzymatic reaction and Michaelis constant, respectively.

Equation (1a-6) is called Michaelis-Menten equation and it contains two parameters which are both necessary and sufficient to define the rate law for these reactions, and the equation can be rewritten for any initial substrate concentration;

$$(V_o)_{a=ao}=\frac{k_3a_oe_o}{K_a+a_o}=ke_o\left(k=\frac{k_3a_o}{K_a+a_o}\right)$$

$$\therefore\left(\frac{dv_o}{de}\right)_{a=const}=k$$

Where V_o is initial velocity ($t=0$) of the reaction.

Therefor this means that the velocity (v_o) is proportional to enzyme concentration (l_o) and this has been used as a basis for the method of enzyme assays. We transformed the equation (la-6) into the "rate constant equation" other than Lineweaver and Burk, Eadie and Hofstee[1] as shown in Fig. 2.

$$v=k_3x=\frac{k_1k_3ae_o}{k_1a+k_2+k_3} \quad \therefore x=\frac{k_1ae_o}{k_1a+k_2+k_3}$$

$$=\frac{v}{k_3};\ \frac{e_o}{v}=\frac{k_1a+k_2+k_3}{k_3k_1a}=\frac{1}{k_3}+\frac{1}{a}$$

$$\left(\frac{k_2+k_3}{k_1k_3}\right) \quad \cdots\cdots\cdots\cdots (1a-8)$$

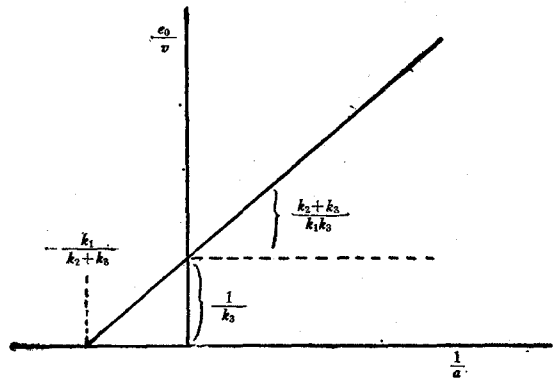


Fig. 2. Reciprocal plot of the "rate constant equation" for enzyme catalyzed reaction.

According to above equation provided that pure enzyme is added during the reaction process, k_3 and k_2/k_1 can be calculated.

Mechanism 1b;

This is analogous to the mechanism 1a except for reversible formation of the enzyme substrate complex; this kinetic equations are as follows,

$$-\frac{da}{dt}=k_1ae-k_2x \quad \cdots\cdots\cdots\cdots (1b-1)$$

$$\frac{dx}{dt}=(k_1a+k_4p)e-(k_2+k_3)x \quad \cdots\cdots (1b-2)$$

$$\frac{de}{dt}=(k_2+k_3)\ x-(k_1a-k_4p)e \quad \cdots\cdots (1b-3)$$

$$\frac{dp}{dt}=k_2x-k_4pe \quad \cdots\cdots\cdots\cdots (1b-4)$$

$$e_o=e+x \quad \cdots\cdots\cdots\cdots (1b-5)$$

In the steady state, $e_o \ll a_o$ and/or dx/dt=dp/dt =0 and $(k_1a+k_4p)e=(k_2+k_2)x$. Thus as in the case of mechanism la. we obtain the following "rae constant equation."

$$-\frac{da}{dt}=\frac{dp}{dt}=\frac{(k_1k_3a-k_2k_4p)e_0}{(k_2+k_3)+k_1a+k_4p}$$

$$=\frac{(N_1a-N_2P)e_0}{C_0+C_1a+C_2P} \quad \cdots\cdots\cdots\cdots (1b-6)$$

were $C_0=k_2+k_3$ $C_1=k_1$ $C_2=k_4$ $N_1=k_1k_3$ $N_2=k_2k_4$

This equation (1b-6) reduces to the Michaelis-

Menten equation in terms of the initial velocity v_1 for the forward reaction if we set $p=0$, and does to that for the backward reaction with initial velocity v_2 if we set a=0;

$$(v_1)_{p=0}=\frac{k_1k_3a}{k_2+k_3+k_1a}=\frac{V_1\cdot a}{Ka+a};\quad \frac{e_0}{v_1}=\frac{k_2+k_3}{k_1k_3}\times\frac{1}{a}+\frac{1}{k_3} \qquad \cdots\cdots(1b-7)$$

$$(v_2)_{a=0}=\frac{k_2k_4P}{k_2+k_3+k_4P}=\frac{V_2P}{K_p+P};\quad \frac{e_0}{v_2}=\frac{k_2+k_3}{k_2k_4}\times\frac{1}{p}+\frac{1}{k_2} \qquad \cdots\cdots(1b-8)$$

Where K_a and K_p indicated the Michaelis constants of substrate and of product, respectively. In this instance k_1, k_2, k_3 and k_4 can calculate from the experimentally determined parameters K_a, K_p, V_1, V_2 provided that e_0 is known.

Where $V_1=k_3\ e_0$, $V_2=k_2e_0$, $K_a=\dfrac{V_1+V_2}{k_1e_0}$

and $K_p=\dfrac{V_1+V_2}{k_4e_0}$ and at V=0, Keq=

$$\frac{k_1k_3}{k_2k_4}=\frac{V_1K_p}{V_2K_a}=\frac{P}{a}$$

Where Keq is equilibrium constant.

This relationship is derived by Haldane,[1] who states that the kinetic parameters of a reversible reaction catalyzed by enzyme are not independent of one another and are limited by the thermodynamic equilibrium constants for the over-all reaction. Thus by substituting above e_0 or Keq in the equation (1b-6) we obtain slightly different form of equation (1b-6)

$$v_1=\frac{V_1a}{K_a+a+\dfrac{K_aP}{KP}}=\frac{k_1k_3ae_0}{k_2+k_3+k_{1a}+k_4p} \qquad \cdots\cdots(1b-9)$$

$$v_2=\frac{V_2P}{K_p+P+\dfrac{K_pa}{Ka}}=\frac{-k_2k_4Pe_0}{k_2+k_3+k_1a+k_4p} \qquad \cdots\cdots(1b-10)$$

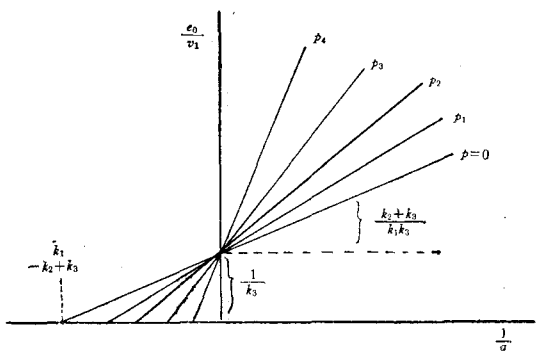
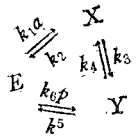


Fig. 3. The "rate constant equation" in the presence of product.

From above results we find a completely symmetrical set of equation, which now states that an enzymatic reaction slows down as equlbrium is approached, not only by virtue of thermodynamic backward reaction but also of the reason that an increasing proportion of the enzyme is immobilized as an EP complex as p increases. Such a product inhibition is an intrinsic property of many reversible mechanism of enzymatic reactions. In this case as we have shown in Fig. 3, the term $\dfrac{k_2+k_3+k_4P}{k_1k_3}$ must be minimum in order to increase the over-all reaction rate, accordingly the concentration of product (p) must be zero. This indicates that to eliminate the influence of product on the reaction velocity it is desirable to remove the product as the substrate of the just next step reaction of the limiting step or to measure the velocity at the initial state prior to form the product.

Mechanism 1c :



The pertinent steady state and conservation equations are as follows.

$$-\frac{da}{dt}=k_1ae-k_2x \qquad \cdots\cdots(1c-1)$$

$$\frac{dx}{dt}=k_1ae-(k_2+k_3)x+k_4y \qquad \cdots\cdots(1c-2)$$

$$\frac{dy}{dt}=k_3x+k_6ep-(k_4+k_5)y \qquad \cdots\cdots(1c-3)$$

$$\frac{de}{dt}=k_2x+k_5y-(k_1a-k_6p)e \qquad \cdots\cdots(1c-4)$$

$$\frac{dp}{dt}=k_5y-k_6ep \qquad \cdots\cdots(1c-5)$$

$$e_0=e+x+y \qquad \cdots\cdots(1c-6)$$

In the steady state $dx/dt=dy/dt=de/dt=0$,

this yields three linear equations to be solved for three unknowns and they are substituted into the velocity equation (1c—1). This can be done by solving a third order determinant or direct graphic method due to King and Altman;[9]

$$\frac{e}{e_0}=\frac{k_2k_5+k_2k_4+k_3k_5}{\sum} \quad \cdots\cdots\cdots\cdots (1c-7)$$

$$\frac{x}{e_0}=\frac{k_1k_5a+k_1k_4a+k_4k_6p}{\sum} \quad \cdots\cdots\cdots (1c-8)$$

$$\frac{y}{e_0}=\frac{k_2k_6p+k_3k_6p+k_1k_3a}{\sum} \quad \cdots\cdots\cdots (1c-9)$$

$\sum$=sum of all numerater terms

Then this partition equations are substituted into the "rate constant equation" (1c—1) as in the case 1b.

$$v=\frac{(k_1k_3k_5a-k_2k_4k_6p)eo}{k_2k_5+k_2k_4+k_3k_5+k_1(k_3+k_4+k_5)a+k_6(k_2+k_3+k_4)p^9}\cdot\frac{(N_1a-N_2p)e_0}{c_0+c_1a+c_2p} \quad \cdots\cdots\cdots\cdots (1c-10)$$

$$;\frac{e_0}{(v_1)_{p=0}}=\frac{(k_2k_5+k_2k_4+k_3k_5)}{(k_1k_3k_5)}\ \frac{1}{a}+\frac{k_3+k_4+k_5}{k_3k_5} \quad \cdots\cdots\cdots\cdots (1c-11)$$

$$\frac{e_0}{(v_2)_{a=0}}=\frac{(k_2k_3+k_2k_4+k_3k_5)}{(k_2k_4k_6)}\ \frac{1}{p}+\frac{k_2+k_3+k_4}{k_2k_4} \quad \cdots\cdots\cdots\cdots (1c-12)$$

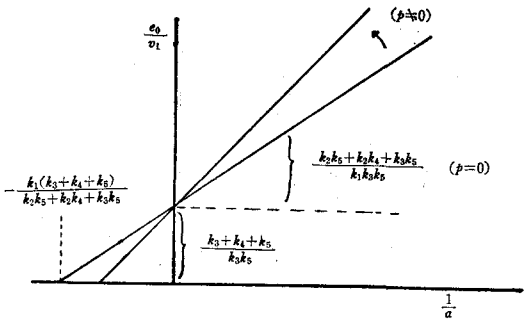


Fig. 4. Reciprocal plot of the "rate constant equation" for mechanism 1c.

Hence the case of mechanism 1c is resemble to that of mechanism 1b except for complexity. Mechanism 1d;

$$\begin{array}{ccc} E & \overset{k_1a}{\rightleftharpoons} & X \\ {\scriptstyle k_7q}\uparrow\downarrow{\scriptstyle k_8} & \overset{k^2}{\underset{k_6}{\rightleftharpoons}} & {\scriptstyle k_4}\uparrow\downarrow{\scriptstyle k_3p} \\ Y & \underset{k_5}{\rightleftharpoons} & X' \end{array}$$

$$V'=\frac{k_3k_5k_7e_0}{k_5k_7+k_3k_7+k_3k_5+k_4k_7p}$$

$$K'=\frac{k_2^2k_7(k_2+k_3)(k_8+k_7q)+k_2k_4k_7^2p(k_2k_8+(k_2+k_6)q)}{k_1k_2k_3(k_3k_5+k_3k_7+k_5k_7+k_4k_7p)}$$

In such case only certain restricted mechanisms permit the complete evaluation of all individuall rate constants in terms of the kinetic parameters. If we assume $k_5 \gg k_6$ and $k_5 \gg k_7$ and also that P and Q are not inhibitory each other, then the above equation is simplified as follows.

$$V'=\frac{k_2k_3e_0}{k_3+k_7} \qquad K'=\frac{(k_2+k_3)k_7}{k_1(k_3+k_7)}$$

The pertinent steady state and conservation equations are as follows.

$$-\frac{da}{dt}=k_1a-k_2x \quad \cdots\cdots\cdots\cdots (1d-1)$$

$$\frac{dx}{dt}=k_1ae-k_4py-(k_2+k_3)x \quad \cdots\cdots (1d-2)$$

$$\frac{dy}{dt}=k_3x-k_4py+k_6e-k_5y \quad \cdots\cdots (1d-3)$$

$$\frac{dq}{dt}=k_5y+k_8e-k_6e-k_7eq \quad \cdots\cdots (1d-4)$$

$$\frac{de}{dt}=k_3x-k_1ae+k_7e-k_8e\cdot q \quad \cdots\cdots (1d-5)$$

$$e_0=e+x+y+x' \quad \cdots\cdots\cdots\cdots (1d-6)$$

When we assumed that the third step is irreversible $k_5 \gg k_6$ the "rate constant equation" is as follows.

$$v=\frac{V'a}{a-K'}$$

where

$$\therefore\ v=\frac{V'a}{K'+a}=\frac{ae_0k_3k_7}{k_2+k_7}\div\frac{ak_1(k_3+k_7)+k_7(k_2+k_3)}{k_1(k_3+k_3)}$$

$$=\frac{k_1k_3k_7ae_0}{ak_1(k_3+k_7)+k_7(k_2+k_3)}$$

$$\therefore\frac{e_0}{v}=\frac{k_3+k_7}{k_3k_7}+\frac{(k_2+k_3)}{k_1k_3}\cdot\frac{1}{a}$$

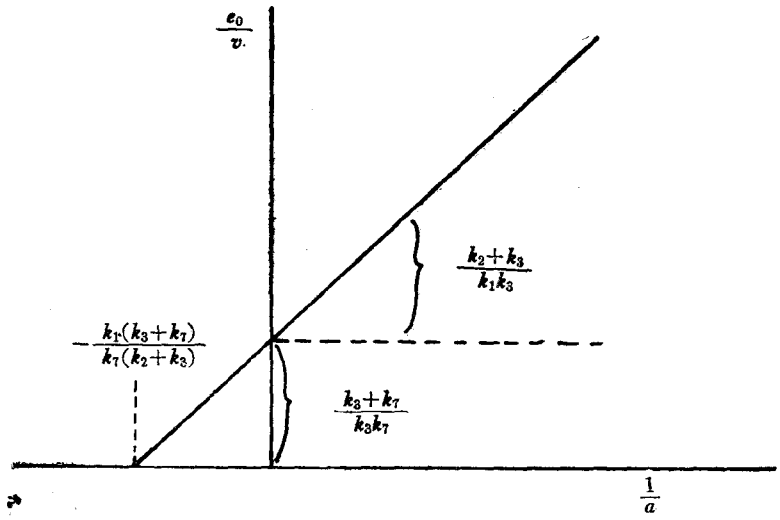


Fig. 5. Reciprocal plot of the "rate constant equation" of mechanism 1d under some restricted conditions.

II. Mechanism types involving two substrates and two products.

The kinetic theory for these mechanisms have been due mainly to the works of Alberty[3)~8)] and Cleland.[11)] According to their works most enzymatic reactions involve more than one substrate, and any accurate description must be taken into account of this fact. In this case general equation for a reversible reaction is given as follows.

$$A+B \rightleftarrows P+Q$$

and the experimental facts on which much of this theory is based are quite analogous to those for one substrate reactions; the variation of the initial velocity as a function of the variation of the concentration of one (e.g.,a, the variable substrate) of two substrates is given by a Michaelis-Menten equation (Ia-6), provided that (1) the same restrictions are added as have been discussed on one substrate reaction especially with respect to the absence of products, and (2)

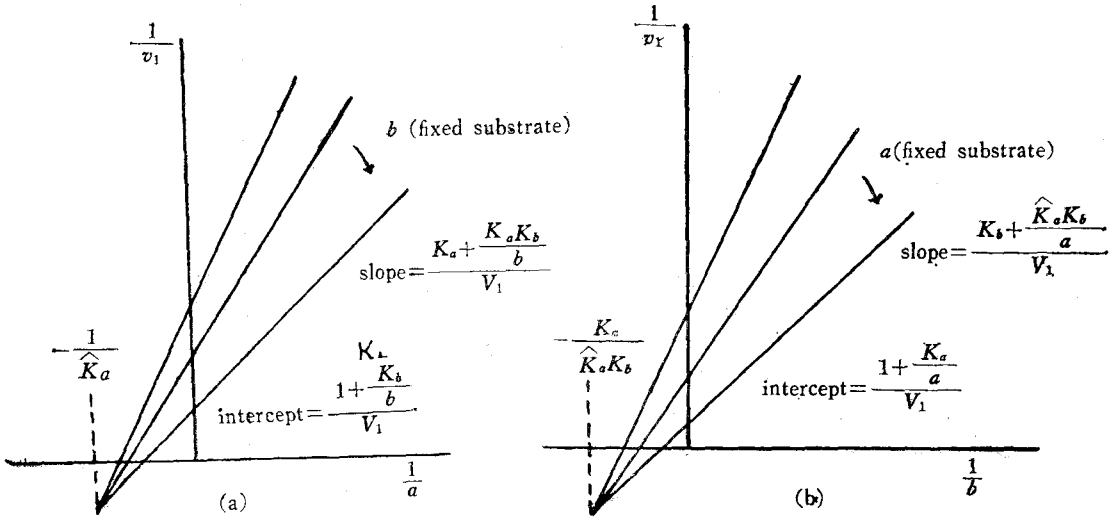


Fig.6. Reciprocal plot of general rate equation for two substrates reaction especially for the ordered mechanism.

that the second substrate (here b, the fixed substrate) is held constant at a concentration $b_1 \gg e_0$. If a similar set of measurments is then carried out at a different concentration $b_2 \gg e_0$, again tho variation of the initial velocity as a function of the concentration of substrate A follows the Michaelis-Menten equation, but in this case kinetic equation requires a total of four parameters; as shown in the equation (2a-1),

A,B; a,b; K_a, K_b; $\tilde{K}_a$, $\tilde{K}_b$; k_{2n-1};; V_1, K_1 (forward reaction)

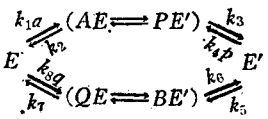
P,Q; p,q; K_p,K_q; $\tilde{K}_p$, $\tilde{K}_q$; k_{2n}; V_2, K_2 (reverse reaction)

Where a and b indicate the concentration of substrate A and B, p and q of product P and Q, respectively.

and also, K_a, K_b, K_p, K_q; Michaelis constant

$\tilde{K}_a$, $\tilde{K}_b$, $\tilde{K}_p$, $\tilde{K}_q$; dissociation constant

2a. Ping-pong mechanism:



Kinetic analysis shows that for a mechanism of this kind the last term (the one in a, b) in the equation (2a-1) or (2a-2) is missing and the rate equation is given as follows;

$$\frac{V_1}{v_1}=1+\frac{K_a}{a}+\frac{K_b}{b}\ (\tilde{K}_aK_b=0)\cdots\cdots(2a\text{-}1)$$

The reciprocal plot of above equation (2a-1) exhibits an unusual feature. Concideration of the complete rate equation for the reversible over-all

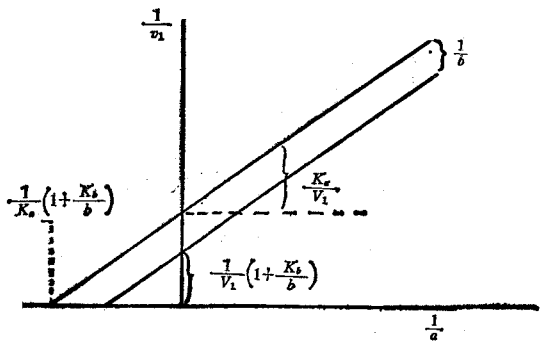


Fig. 7. Reciprocal plot for the ping-pong mechanism.

(refering to (1b-6))

k_{2n-1}, k_{2n}: individual rate constant

V_1, V_2: maximal velocity

K_1, K_2: equilibrium constant for the over-all reaction. Thus we obtain the following two general kinetic equations.

$$v_1=\frac{v_1ab}{\tilde{K}_aK_b+K_ba+K_ab+ab};\quad \frac{V_1}{v_1}$$
$$=1+\frac{K_a}{a}+\frac{K_b}{b}+\frac{\tilde{K}_aK_b}{ab}\cdots\cdots\cdots(2a\text{-}1)$$

$$v_2=\frac{V_2pq}{\tilde{K}_qK_p+K_qp+K_pq+pq};\quad \frac{V_2}{v_2}$$
$$=1+\frac{K_p}{p}+\frac{K_q}{q}+\frac{K_p\tilde{K}_q}{pq}\cdots\cdots\cdots(2a\text{-}2)$$

Thus the reciprocal plots for two substrate reactions are shown in Fig. 6.

In general the rate equations in two substrates reaction fall into above categories. (To simplify we used parameter instead of rate constants)

Ex. $\underset{(A)}{RH_2}+\underset{(E)}{E-F} \rightleftharpoons EA \rightleftharpoons \underset{E'}{E'P} \rightleftharpoons \underset{P}{E-FH_2}+R$

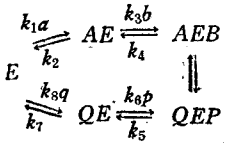
$\underset{(B)}{OX}+\underset{(E_1)}{E-FH_2} \rightleftharpoons E'B \rightleftharpoons EQ \rightleftharpoons \underset{(E)}{E-F}+\underset{Q}{OXH_2}$

reaction shows another characteristic of this mechanism; Q(not the product P) is a competitive inhibitor for A and P (not Q) also for B, but A is an uncompetitive inhibitor of B and vice versa.

Several different Haldane relations are obtained;

$$K_{eq}=\frac{\tilde{K}_p\tilde{K}_q}{\tilde{K}_a\tilde{K}_b}=\frac{V_1\tilde{K}_pK_q}{V_2\tilde{K}_aK_b}=\frac{V_1K_p\tilde{K}_q}{V_2K_a\tilde{K}_b}$$
$$=\frac{V_1^2K_pK_q}{V_2^2K_a\tilde{K}_b}\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots(2a\text{-}2)$$

2b. Orderd mechanism:



Ex. $\underset{A}{NAD^+}+\underset{EA}{E} \rightleftharpoons \underset{EA}{NAP^+E}$

$\underset{EA}{NAD^+E}+\underset{B}{R_1R_2CHOH} \rightleftharpoons \underset{EAB}{(NAD^+ECHOH}$

$R_1R_2) \rightleftharpoons$

$\underset{QEP}{NADH\cdot E\cdot COR_1R_2} \rightleftharpoons \underset{QE}{NADH\cdot E}+\underset{P}{R_1R_2CO}$

$\underset{QE}{NADH\cdot E} \rightleftharpoons \underset{Q}{E}+NADH$

This mechanism also gives rise to the rate law of equation (2a-1) and (2a-2), but because of their symmetry it is not obvious which substrate is to be identified as substrate A and which one is obligated to interact with the enzyme before the reaction can proceed. This identification can usually be accomplished by means of binding studies; if the coenzymes are participated in the reaction, they are much more tightly bound than their respective substrates.

Furthermore only A (not B) is competitively inhibited by its own product Q, especially in this instance the mechanism type with one central complex contains only eight rate constants and this expression involvs both rate constants and kinetic parameters;

$$k_1=\frac{V_1}{e_oK_a} \quad k_2=\frac{V_1\tilde{K}_a}{e_oK_a} \quad k_3=\frac{V_1}{e_oK_b}\left(1+\frac{k_4}{k_5}\right)$$

$$k_4=\frac{V_2k_2}{k_2e_o-V_2} \quad k_5=\frac{V_1k_7}{k_7e_o-V_1} \quad k_6=\frac{V_2}{e_oK_p}$$

$$\left(1+\frac{k_5}{k_4}\right) \quad k_7=\frac{\tilde{K}_qV_2}{K_qe_o} \quad k_8=\frac{V_2}{e_oK_q} \quad \cdots(2b\text{-}1)$$

Thus its reciprocal plots are shown in Fig.6.

2c. Random mechanism:

Ex. Many kinases; Phosphocreatine+ADP→ Creatine+ATP In this mechanism all possible binary enzyme substrate complexes are formed rapidly and reversibly, the magnitude of any dissociation constant for a reactant is unaffected prior to attachment of any other reactant to the enzyme, and the only slow step is the interconversion of the two ternary complexes, at $K_1=K_7$ and $K_5=K_3$ and for the reverse direction $K_2=K_8$ and $K_6=K_4$.

The rate law is given by equation (2a-2) with

$$V_1=k_1e_o \quad V_2=k_2e_o$$
$$\tilde{K}_a=K_a=K_1=K_7$$
$$\tilde{K}_p=K_p=K_2=K_8 \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots(2c\text{-}1)$$
$$\tilde{K}_b=K_b=K_3=K_5$$
$$\tilde{K}_q=K_q=K_4=K_6$$

The distinguished experimental feature of this mechanism is that the intersection point of 1/V versus 1/a or 1/b plots are all met on the abscissa; this point is equal to $-\frac{1}{\tilde{K}_a}$ in the 1/a plot and $-\frac{1}{\tilde{K}_b}$ in the 1/b plot, because the rate law may be written as follows.

$$v_1=\frac{V_1ab}{\tilde{K}_a\tilde{K}_b+\tilde{K}_ba+\tilde{K}_ab+ab}$$

$$\text{and}\ \frac{V_1}{v_1}=\left(1+\frac{\tilde{K}_a}{a}\right)\left(1+\frac{\tilde{K}_b}{b}\right) \cdots\cdots\cdots\cdots(2c\text{-}2)$$

If we put b=constant, above the rate equation

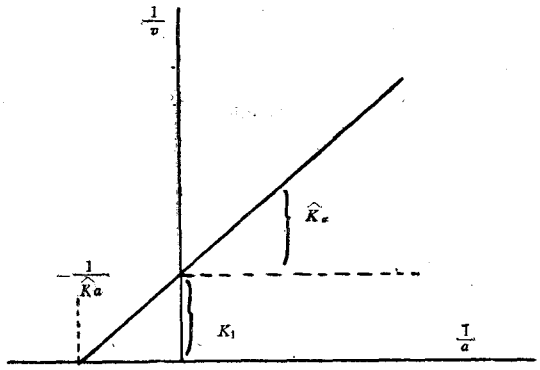


Fig. 8. Reciprocal plot for the random mechanism.

may be written as follows;

$$\frac{V_1}{v_1}=1+\tilde{K}_a\cdot\frac{1}{a}; \quad \frac{e_o}{v_1}=k_1+\tilde{K}_a\frac{1}{a}$$

Thus the reciprocal plot for the above equation is shown in Fig. 8.

RESULTS AND DISCUSSION

As we mentioned above, the mechanism of enzymatic reaction are mainly classified into following seven sections, 1a, 1b, 1c, 1d, 2a, 2b, and 2c

I. Mechanism types involving one substrate and one product.

1a. The equation $A+E\underset{k2}{\overset{k1}{\rightleftharpoons}}\times\xrightarrow{k3}E+P$ represents an irreversible mechanism. Although such a mechanism is practically not involved in the rate limiting step by general definition, it is convenient to explain the other more complex mechanism with this form under some restricted conditions; in the case of $a\rangle\rangle K_a$ in the Michaelis Menten equation (1a-6) the reaction becomes zero order, because of $v=V(v=k_3x$ and $V=k_3e_o$, accordingly $e_o=x)$

This indicates that when the e_0 is completely converted to complex with substrate the reaction

rate is equal to limiting maximum velocity. From the above results, we can elicit that e_0 is directly proportional to v and V. Hence we assumed that in the metabolic process composed of n steps in which the rth step is limiting step, if the e_1 moles of enyme individual to the first step is added to the same step which contains e_0 moles of enzyme initially, the total concentration may be increased to e_0+e_1. Consequently we obtain the following equation; at initial state $e_0=\frac{V_o}{k_3}$ and in the second state it can put $e=\frac{V}{k_3}$ where $e=e_0+e_1$, and the amount of product corresponding to e_0 may be used for the second steps of this process. Thus the product increment can caluculate by the equation (1a−7).

$$et=\frac{V_t}{k_3}=\frac{K_a}{k_3}ln\frac{a_0}{a}=\frac{K_a}{k_3}ln\frac{a_0}{a_0-p}$$

$$=-\frac{K_a}{k_3}ln\frac{a_0-_0}{a_0}=\frac{K_a}{k_3}ln\frac{p}{a_0}$$

$$\therefore\ P_{(1)}=\frac{K_aV}{k^2_3t}\sqrt{e}\cdot a_{0(1)}=F_{(1)}\sqrt{e}\cdot a_{0(1)},$$

$$\left(F_{(1)}=\frac{K_aV}{K_3^2t}\right)$$

Hence the rate equation of the second step may be given as follows,

$$V_{(2)}=\frac{V^{(2)}\left(F_{(1)}\sqrt{e}+a_{(2)}\right)}{K_{a(2)}+\left(F_{(1)}\sqrt{e}+a_{(2)}\right)}=\frac{V_{(2)}a'_{(2)}}{K_{a(2)}+a'_{(2)}}$$

$$(a'_{(2)}=F_{(1)}\sqrt{e}+a_{(2)}$$

where the numbers in parenthesis, i.e., (1), (2), ……(r) etc. indicate the order of step:

When it continues from the first step to the rth one, the following rase equation may be obtained;

$$v'_{(r)}=\frac{V_{(r)}a'_{(r)}}{K_{a(r)}+a'_{(r)}}$$

On the other hand when the enzyme is added directly to the rth step, the rate equation is written as follows,

$$v''_{(r)}=\frac{V'_{(r)}a''_{(r)}}{K_{a(r)}+a''_{(r)}}$$

while $a'_{(r)}\fallingdotseq a''_{(r)}$ $V_{(r)}\ll V'_{(r)}$ $\therefore v'_{(r)}\ll v''_{(r)}$

This result proves that the addition of enzyme to the rth step in the metabolic process promotes the reaction more rapidly than to any one of other steps except rh one to which same amount of enzyme is added seperately.

When the feedback inibition is existed in the metabolic process, the final product always inhibits is existed in the metabolic process, the final product always inhibits the first step enzyme of that process and the more final product increases the more feedback inhibition, consequentlly the addition of the enzyme to the rth step may increase more feedback inhibition, compared with that of any other steps in the corresponding situations.

Thus the result shows contrast to the case above mentioned. However provided that the final product is removed by measuring the rate at the (n-1) th step or by any other method, we obtain the results like that of above case.

1b. In this mechanism the EAcomplex (x) is reversible state;

$$A+E\underset{k2}{\overset{k1}{\rightleftharpoons}}\times\underset{k4}{\overset{k3}{\rightleftharpoons}}E+P$$

However provided that the rate constant k_4 of product decomposition is eliminated the results are as same as that of case la. For this purpose we can take several methods;

1) Measuring the reaction rate at the initial state before forming the final product may prevent the interfering of the product.

2) Removing the product as the substrate for just next step when the both respective enzymes are added to the rth and(r+1)th steps makes the reaction rate greater than at any other two steps which are adjacent. When the feedback inhibition present in this process the rate measurement may be carried out at the (n-1) th step which is just before the final step as in the case of la in order to remove the final product inhibition.

1c. There are six rate constants which are more complicate than the case of 1b. However the equation of coefficient form is very similar to that of 1b;

$$\frac{(N_1a-N_2P)eo}{C_o+C_1a+C_2P}$$

where $N_1=k_1k_3k_5$ $N_2=k_2k_4k_6$

$$C_0=k_2k_5+k_2k_4+k_3k_5$$
$$C_1=k_1k_3+k_1k_4+k_1k_5$$
$$C_2=k_2k_6+k_3k_6+k_4k_6$$

Thus the reciprocal plot of 1c is so similar to that of 1b that the rate measurment also may be made as same as that of 1b.

1d. This mechanism is much more complicate than any other one substrate mechanism. In such a case the mechanism can not be analyzed without some restrictions. However under some restricted conditions like the above case 1b and 1c the rate limiting step can be determined.

II. Mechanism types involving two substrates and two products.

2a. A swe mentioned above, the variation of the initial velocity as a function of the variation of the one substrate concentration of the two substrates (the concentration of other one maintains constant) is given by a Michaelis-Menten equation. However when the both substrates are "cooperative compounds" each other (A and B), we must remove the final products (P and Q) by early cited methods to determine the rate limiting step

2b Among the most important reactions, the reactions which catalyzed by a variety of NAD- and NADP-requiring dehydrogenases are obeyed to this mechanism. In such a case both coenzyme and substrateare called in terms of "cooperative substrate", by the authors, therefore the final products (P and Q) must be removed to determine the rate limiting step.

2c. This mechanism is entirely similar to one substrate reversible mechanism, because of $V_1=k_1e_0$ and $V_2=k_2e_0$. Hence provided that the products (P and Q) are removed, the result may be $V_1 \gg V_2$, then V is directly proportional to the amount of e_0.

要 約

代謝過程에 있어서 律速段階의 결정은 그 代謝의 성질을 이해하는 데 대단히 중요한 것이다. 저자들은 이것의 결정을 위해서 熱力學的 方法 대신에 수정된 酵素的 方法을 제시 하였다. 이 방법은 단계로 된 代謝過程에서 律速段階에 고유한 酵素를 加했을 때는 다른 어떤 段階에 고유한 酵素를 加했을 때보다도 전체적인 代謝 反應速度가 가장 증가한다는 假定에 기초를 둔 것이다. 본 연구에서는 수정된 酵素的 方法에 의한 反應速度 測定에 있어서, 이에 영향을 미치는 數種의 因子에 대하여 解析하였으며 주로 동일한 反應機作 형태로 구성된 代謝過程에 대하여 解析하였다. 이 結果, 본 방법에 의한 律速段階의 決定은 약간의 制限이 加해질 경우 임의 형태의 機作으로 구성된 代謝過程에서도 測定이 가능하다는 것을 보여 주고 있다.

REFERENCES

1. Mahler H.R. and EH. Cordes: Biological Chemistry", 227, Harper & Row, New York (1968)
2. Volkenstein M. V. and B. N. Goldstein: Biochim. Biophys. Acta, **115**, 471 (1966)
3. Alberty R.A.: J. Am. Chem. Soc., **75**, 1928 (1953)
4. Smith R. M. and R. A. Alberty: J. Am. Chem. Soc., **78**, 2376 (1955)
5. Alberty R. A.: J. Am. Chem. Soc., 80, 1777 (1958)
6. Miller W. G. and R. A. Alberty: J. Am. Chem. Soc., **80**, 5146 (1958)
7. Peller L. and R. A. Alberty: J. Am. Chem. Soc., **81**, 5907 (1959)
8. Bloomfield V., Leonard Peller and R. A. Alberty: J. Am. Chem. Soc., **84**, 4367 (1962)
9. King E. L. and C. Altman: J. Phys. Chem., **60**, 1375 (1956)
10. Frieden C.: J. Am. Chem. Soc., **79**, 1894 (1956)
11. Cleland W. W.: Biochim. Biophys. Acta, **67**, 104 (1963)