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KINETICS OF SUICIDE SUBSTRATES

STEADY-STATE TREATMENTS AND COMPUTER-AIDED EXACT SOLUTIONS

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A steady-state differential equation that describes the kinetics of suicide substrate was derived for a scheme presented by Walsh et al. (Walsh, C., Cromartie, T., Marcotte, P. and Spencer, R. (1978) Methods Enzymol. 53, 437–448). Using its analytical solutions, the progress curves of substrate disappearance, product formation and enzyme inactivation were calculated for a hypothetical model system, and were compared with the exact solutions which were obtained by the numerical computation on a set of rate equations. The results obtained with the present analytical solutions were much more consistent with the exact solutions than those obtained using Waley's solutions (Waley, S.G. (1980) Biochem. J. 185, 771–773). The most important factor for a system of suicide substrates was found to be the term $(1 + r)\mu$ instead of $r\mu$ as proposed by Waley, where r is the ratio of the rate constant of product formation to that of enzyme inactivation and μ is the ratio of initial concentration of enzyme to that of suicide substrate. In cases where this term has a value greater than unity, all the molecules of suicide substrate are used up leaving some enzyme molecules still active. To the contrary, in cases where the term has a value smaller than unity, all the enzyme molecules are inactivated with some molecules of suicide substrate being left unreacted. When the term is equal to unity, then all the enzyme molecules are inactivated and all the molecules of the suicide substrate are converted. Practical methods for estimating kinetic parameters are described.

Introduction

Representative kinetics for a suicide substrate have recently been worked out for the first time by Waley [1] on the basis of the steady-state kinetics of catalytic amounts of enzyme. The scheme solved by Waley was that originally proposed by Walsh et al. [2] as illustrated by Eqn. 1,

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} X \xrightarrow{k_{+2}} Y \xrightarrow{k_{+3}} E + P \qquad (1)$$
$$\downarrow k_{+4}$$
$$E_i$$

where E, S, P, X, Y, and E_i stand for enzyme, suicide substrate, product, first and second enzyme-suicide substrate complexes, and inactivated enzyme, respectively. Waley's theory has several important features in describing the kinetics of suicide substrates as partly illustrated by the progress curves of enzyme inactivation which Waley computed for his model system.

However, a close look at the logical construction of Waley's theory revealed that the balance sheet of the suicide substrate invariably involves an inconsistency which reaches no longer negligible levels with increasing ratio of initial concentration of enzyme to that of suicide substrate. In other words, if the concentration of suicide substrate which remained

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unreacted, that which remained bound to the inactivated enzyme, and that which was converted into products were summed up on the basis of Waley's equation at times after initiation of the enzyme reaction, then the total concentration deviates from the initial one. We found that the major cause for this inconsistency lies not in the steady-state method per se, but in somewhat rough approximations incorporated into Waley's theory.

To overcome this theoretical difficulty and to reconstruct the kinetics of suicide substrates, we derived an improved steady-state differential equation, and solved it analytically. In the present paper, we will describe derivation of theoretical equations, their properties and their applications to a hypothetical model system. We have thus extended Waley's theory, and explored in detail the kinetics of suicide substrate.

Theoretical

Rate equations and conservation relations

For the sake of convenience, we will use the same notations for Eqn. 1 as those defined by Waley [1]. The concentrations of species involved in Eqn. 1 are denoted by italic lower-case symbols. Then, Eqn. 1 is described by the following six rate equations,

$$\frac{de}{dt} = -k_{+1}es + k_{-1}x + k_{+3}y \tag{2}$$

$$\frac{ds}{dt} = -k_{+1}es + k_{-1}x \tag{3}$$

$$\frac{dx}{dt} = k_{+1}es - (k_{-1} + k_{+2})\,x \tag{4}$$

$$\frac{dy}{dt} = k_{+2}x - (k_{+3} + k_{+4})\,y \tag{5}$$

$$\frac{dp}{dt} = k_{+3}y \tag{6}$$

and

$$\frac{de_i}{dt} = k_{+4}y \tag{7}$$

By summing up Eqns. 3 to 7, we have

$$\frac{d}{dt}(s + x + y + p + e_i) = 0 \tag{8}$$

If we integrate Eqn. 8 under the initial conditions of $s_{t=0} = s_0$, $x_{t=0} = 0$, $y_{t=0} = 0$, $p_{t=0} = 0$ and $e_{i_{t=0}} = 0$, we obtain

$$s + x + y + p + e_i = s_0 \tag{9}$$

Similarly, we have the conservation relation with regard to enzyme by summing up Eqns. 2, 4, 5 and 7,

$$\frac{d}{dt}(e + x + y + e_i) = 0$$

which yields after integration under the initial conditions of

$$e_{t=0} = e_0,\ x_{t=0} = 0,\ y_{t=0} = 0 \text{ and } e_{i_{t=0}} = 0,$$

$$e + x + y + e_i = e_0 \tag{10}$$

We may obtain exact solutions of Eqn. 1 by assigning to the rate constants as well as to e_0 and s_0 appropriate values for a hypothetical model or experimentally determined ones for an actual system, followed by numerically computing the simultaneous rate equations with the aid of the conservation relations.

Derivation of steady-state differential equation

According to the steady-state method in the enzyme kinetics, we may equate the time derivatives of x and y to zero, and obtain the following set of simultaneous equations,

$$k_{+1}es - (k_{-1} + k_{+2})\,x = 0 \tag{11}$$

$$k_{+2}x - (k_{+3} + k_{+4})\,y = 0 \tag{12}$$

From eqn. 11,

$$x = \frac{k_{+1}es}{k_{-1} + k_{+2}}$$

and substituting the conservation relation on enzyme of Eqn. 10 into the term es in the dividend of this equation, we obtain

$$x = \frac{k_{+1}(e_0 - x - y - e_i)\,s}{k_{-1} + k_{+2}}$$

which may be rewritten in Eqn. 13,

$$x = \frac{k_{+1}(e_0 - e_i - y)\,s}{k_{-1} + k_{+2} + k_{+1}s} \tag{13}$$

With respect to y, we obtain Eqn. 14 from Eqn. 12,

$$y = \frac{k_{+2}}{k_{+3} + k_{+4}} x \tag{14}$$

Substituting Eqn. 13 into Eqn. 14, we obtain

$$y = \left(\frac{k_{+2}}{k_{+3} + k_{+4}}\right)\left(\frac{k_{+1}(e_0 - e_i - y)\, s}{k_{-1} + k_{+2} + k_{+1}s}\right)$$

which may be rewritten in Eqn. 15,

$$y = \frac{k_{+1}k_{+2}as}{k_{+1}(k_{+2} + k_{+3} + k_{+4})\, s + (k_{-1} + k_{+2})(k_{+3} + k_{+4})} \tag{15}$$

where

$$a = e_0 - e_i \tag{16}$$

From Eqns. 14 and 15, we obtain

$$x = \frac{k_{+1}(k_{+3} + k_{+4})\, as}{k_{+1}(k_{+2} + k_{+3} + k_{+4})\, s + (k_{-1} + k_{+2})(k_{+3} + k_{+4})} \tag{17}$$

By substituting Eqns. 10, 15 and 17 into Eqn. 3, we obtain

$$\frac{ds}{dt} = -k_{+1}(a - x - y)\, s + k_{-1}x$$

$$= -k_{+1}\{a - [k_{+1}(k_{+3} + k_{+4})\, as]\, [k_{+1}(k_{+2} + k_{+3} + k_{+4})\, s + (k_{-1} + k_{+2})(k_{+3} + k_{+4})]^{-1} - \frac{k_{+1}k_{+2}as}{k_{+1}(k_{+2} + k_{+3} + k_{+4})\, s + (k_{-1} + k_{+2})(k_{+3} + k_{+4})}\}\, s + \frac{k_{-1}k_{+1}(k_{+3} + k_{+4})\, as}{k_{+1}(k_{+2} + k_{+3} + k_{+4})\, s + (k_{-1} + k_{+2})(k_{+3} + k_{+4})}$$

$$= \frac{(k_{+3} + k_{+4})\{k_{+1}k_{-1} - k_{+1}(k_{-1} + k_{+2})\}\, as}{k_{+1}(k_{+2} + k_{+3} + k_{+4})\, s + (k_{-1} + k_{+2})(k_{+3} + k_{+4})}$$

$$= -\frac{as}{\frac{(k_{+2} + k_{+3} + k_{+4})}{k_{+2}(k_{+3} + k_{+4})}\, s + \frac{(k_{-1} + k_{+2})}{k_{+1}k_{+2}}}$$

Therefore,

$$\frac{ds}{dt} = -C\left(1 + \frac{1}{r}\right)\frac{as}{B + s} \tag{18}$$

where

$$B = \left(\frac{k_{-1} + k_{+2}}{k_{+1}}\right)\left(\frac{k_{+3} + k_{+4}}{k_{+2} + k_{+3} + k_{+4}}\right) \tag{19}$$

$$C = \frac{k_{+2}k_{+3}}{k_{+2} + k_{+3} + k_{+4}} \tag{20}$$

and

$$r = \frac{k_{+3}}{k_{+4}} \tag{21}$$

As will be shown later in Discussion, Eqn. 18 provides with several aspects of improvements on Waley's theory. The constants B and C may be regarded as having been extended from Michaelis constant and maximum velocity in the Michaelis-Menten type kinetics, respectively. The ratio r has been termed the partition ratio by Walsh et al. [2] and its reciprocal the inhibitory constant by Rando [3].

Utilizing the partition ratio r together with rate Eqns. 6 and 7, we may obtain the relationship between product and inactivated enzyme as follows,

$$p = re_i \tag{22}$$

Analytical solutions of the improved steady-state differential equation

Eqn. 18 may not analytically be solved because the variable a has not been expressed in terms of the variable s alone. However, we may obtain an expression of a in terms of s by using the steady-state method. As we mentioned earlier for derivation of Eqns. 11 and 12, we may equate the time derivatives of x and y to zero. Thus we obtain from Eqn. 8,

$$\frac{d}{dt}(s + p + e_i) = 0$$

Integrating this equation under the initial conditions described above, we have,

$$s + p + e_i = s_0 \tag{23}$$

Therefore, we can express a in terms of s as follows,

$$a = e_0 - \frac{s_0 - s}{1 + r} \tag{24}$$

Substituting Eqn. 24 into Eqn. 18, we can integrate the latter with the following result,

$$t = \frac{1}{C}\left[\frac{r\frac{B}{s_0}}{1-(1+r)\mu}\ln\left((1+r)\mu\frac{\frac{s}{s_0}}{\frac{s}{s_0}-1+(1+r)\mu}\right)\right.$$
$$\left. - r\ln\frac{\frac{s}{s_0}-1+(1+r)\mu}{(1+r)\mu}\right] \quad (25)$$

where

$$\mu = \frac{e_0}{s_0} \quad (26)$$

Using Eqns. 22 and 23, we may further obtain the steady-state solutions for p and e_i as follows;
with regard to p,

$$t = \frac{1}{C}\left[\frac{r\frac{B}{s_0}}{1-(1+r)\mu}\ln\left(\frac{1-\frac{1+r}{r}\frac{p}{s_0}}{1-\frac{1}{r\mu}\frac{p}{s_0}}\right)\right.$$
$$\left. - r\ln\left(1-\frac{1}{r\mu}\frac{p}{s_0}\right)\right] \quad (27)$$

and with regard to e_i,

$$t = \frac{1}{C}\left[\frac{r\frac{B}{s_0}}{1-(1+r)\mu}\ln\left(\frac{1-(1+r)\mu\frac{e_i}{e_0}}{1-\frac{e_i}{e_0}}\right)\right.$$
$$\left. - r\ln\left(1-\frac{e_i}{e_0}\right)\right] \quad (28)$$

As will be shown later, we may test under various parametric conditions the validities of the present analytical solutions by comparing their progress curves with those obtained by the numerical computation on the simultaneous differential equations of Eqns. 3, 4, 5 and 7.

Relationship between the present analytical solutions and Waley's theory

The steady-state differential equation derived by Waley [1] for the rate of decrease in substrate concentration has the following form,

$$\frac{ds}{dt} = -\frac{Aras}{B+s} \quad (29)$$

where

$$A = \frac{k_{+2}k_{+4}}{k_{+2}+k_{+3}+k_{+4}}$$

Our Eqn. 18 becomes identical with Eqn. 29 under a particular condition that the partition ratio r is sufficiently greater than unity as

$$r \gg 1$$

Under this condition, Eqn. 23 can be expressed as follows,

$$s_0 = s + p$$
$$= s + re_i$$

and accordingly Eqn. 24 is rewritten as

$$a = e_0 - \frac{s_0 - s}{r}$$

With this relation, we can integrate Eqn. 29, and we obtain immediately all analytical solutions derived by Waley [1].

There is another way to derive Waley's solutions; because the condition of $r \gg 1$ may be expressed as

$$1 + r \simeq r$$

we may derive all the analytical solutions of Waley's theory by substituting this relation into our Eqns. 25 to 27. It is thus clear that Waley's theory represents one of the particular aspects of the present steady-state differential equation.

Theoretical validity test

Theoretical concentrations of the species in Eqn. 1 in their final states

Theoretically, final states of suicide substrate in Eqn. 1 are classified a priori into the following three cases; first, all the enzyme molecules are inactivated leaving some of the suicide substrate unreacted;

second, all the enzyme molecules are inactivated without substrate molecules left; and third, some of the enzyme molecules are left still active with no substrate molecules left. Throughout these three cases, we have noticed that the two intermediary complexes x and y decrease to inifinitesimal levels. Hence at the final state the conservation relation with regard to substrate, i.e., Eqn. 9 can be rewritten precisely as

$$\begin{aligned} s_0 &= s_f + p_f + e_{if} \\ &= s_f + (1+r)e_{if} \end{aligned} \tag{30}$$

where suffix f indicates the final state.

The first case may be described by the following two relations,

$$\begin{cases} e_{if} = e_0 \\ s_f > 0 \end{cases}$$

Then immediately, we obtain,

$$\begin{aligned} \frac{p_f}{s_0} &= \frac{1}{s_0} r e_{if} \\ &= r\mu \end{aligned} \tag{31}$$

and

$$\frac{s_f}{s_0} = 1 - (1+r)\mu \tag{32}$$

Because $s_f > 0$, we may identify the parametric condition for the first case as

$$(1+r)\mu < 1 \tag{33}$$

The second case is expressed by the following two relations as

$$\begin{cases} e_{if} = e_0 \\ s_f = 0 \end{cases}$$

Then,

$$\begin{aligned} \frac{p_f}{s_0} &= \frac{1}{s_0} r e_{if} \\ &= r\mu \end{aligned} \tag{34}$$

and by substitution of two relations into Eqn. 30, we have

$$(1+r)\mu = 1 \tag{35}$$

which is the parametric condition for the second case.

The third case may be expressed by the following two relations,

$$\begin{cases} s_f = 0 \\ e_{if} < e_0 \end{cases}$$

Then, from Eqn. 30,

$$e_{if} = \frac{s_0}{1+r}$$

that is,

$$\frac{e_{if}}{e_0} = \frac{1}{(1+r)\mu} \tag{36}$$

or

$$\frac{p_f}{s_0} = \frac{1}{1+r} \tag{37}$$

Because $e_{if} < e_0$, the parametric condition for the third case is obtained as

$$(1+r)\mu > 1 \tag{38}$$

Final concentrations of the species in Eqn. 1 predicted by the analytical solutions

Through simple calculations, we may determine for each reacting species their final concentrations using the analytical solutions. If we pay attention to the logarithmic terms of Eqn. 25, it is clear that the following conditions must be satisfied in the final state.

$$\frac{s_f}{s_0} - 1 + (1+r)\mu > 0$$

Rearranging, we have

$$\frac{s_f}{s_0} > 1 - (1+r)\mu \tag{39}$$

The right-hand side of Eqn. 39 is allowed any values,

TABLE I

COMPARISON BETWEEN FINAL VALUES OF REACTING SPECIES IN EQN. 1 PREDICTED BY PRESENT ANALYTICAL SOLUTIONS AND WALEY'S SOLUTIONS

The present analytical solutions gave exactly the same final values as those predicted by the conservation relations and were summarized under Method A. Results obtained by Waley's solutions were summarized under Method B. Recoveries were calculated as the summation of s_f/s_0, p_f/s_0 and $\mu e_{if}/e_0$ and presented in this table as normalized recovery (cf. Eqn. 30).

Normalized concentrations	Methods	Parametric conditions				
		$r\mu <$ $(1+r)\,\mu < 1$	$r\mu <$ $(1+r)\,\mu = 1$	$r\mu < 1 <$ $(1+r)\,\mu$	$1 = r\mu <$ $(1+r)\,\mu$	$1 < r\mu <$ $(1+r)\,\mu$
s_f/s_0	A	$1-(1+r)\,\mu$	0	0	0	0
	B	$1-r\mu$	$1-r\mu$	$1-r\mu$	0	0
p_f/s_0	A	$r\mu$	$r\mu$	$r/(1+r)$	$r/(1+r)$	$r/(1+r)$
	B	$r\mu$	$r\mu$	$r\mu$	1	1
e_{if}/e_0	A	1	1	$1/(1+r)\,\mu$	$1/(1+r)\,\mu$	$1/(1+r)\,\mu$
	B	1	1	1	1	$1/r\mu$
Normalized recovery	A	1	1	1	1	1
	B	$1+\mu$	$1+\mu$	$1+\mu$	$1+\mu$	$1+1/r$

positive, zero or negative. Therefore, it is possible to have the following three cases;

$$\frac{s_f}{s_0} = 1-(1+r)\,\mu \qquad \text{when } 1 > (1+r)\,\mu$$

$$\frac{s_f}{s_0} = 0 \qquad \text{when } 1 = (1+r)\,\mu$$

and

$$\frac{s_f}{s_0} = 0 \qquad \text{when } 1 < (1+r)\,\mu$$

Through calculations similar to those described above, we may obtain the results summarized in Table I.

The final values of the species in Eqn. 1 predicted by the present analytical solutions were all consistent with those theoretically calculated on the basis of the conservation relation of Eqn. 30 under all the parametric conditions. On the other hand, the values obtained by Waley's solutions were not only partially consistent with the theoretical ones, but also inconsistent with the normalized recovery under any conditions.

Computer-aided validity test

Methods

To deduce the kinetic behavior of the suicide substrate in Eqn. 1 and to test under various parametric conditions the validities of the present analytical solutions, we performed computations on a hypothetical model system that was assigned the following values for its parameters; s_0, 0.5; k_{+1}, 16; k_{-1}, 4; k_{+2}, 12; k_{+3}, 10; k_{+4}, 2; and accordingly, r, 5; B, 0.5 and C, 5. The e_0-value was varied between 0.01 and 0.5, thus yielding the μ-value range between 0.005 and 1.0.

To obtain exact solutions of the simultaneous differential equations of Eqns. 3, 4, 5 and 7, the Runge-Kutta-Gill's method compiled into a FORTRAN program (Code SRKGS, Science Subroutine Library, Fujitsu Ltd., Tokyo) was used with the aid of Eqn. 10. The self-consistency of these exact solutions was tested by computing the recovery of suicide substrate using Eqns. 9 and 22.

Numerical solutions were computed until the differences between these and their preceding values became less than or equal to 10^{-6}.

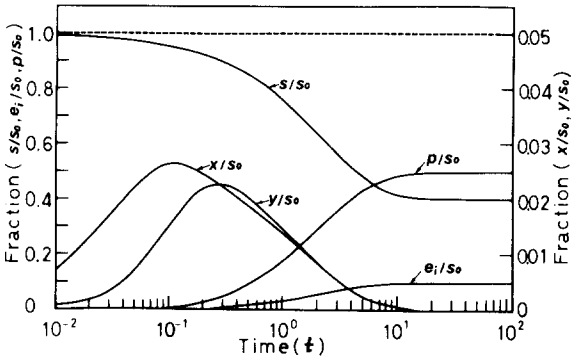

Fig. 1. Progress curves of the reacting species in Eqn. 1 for a hypothetical model system of the suicide substrate. Curves were computed by the numerical method with the initial substrate concentration normalized to 1.0. The μ-value for these particular computations was set at 0.1, other parametric values being set as described in Methods. The dotted line represents the perfect recovery of the suicide substrate throughout the reaction period.

Exact solutions of the simultaneous differential equations

Fig. 1 summarizes one of the typical numerical solutions of the simultaneous differential equations computed by the Runge-Kutta-Gill's method with the μ-value of 0.1. The self-consistency of the solutions is reflected by the perfect recovery of suicide substrate at all times during the course of reaction. That the rate constant k_{+3} was set at a value 5-times greater than k_{+4} showed its effect on the progress of reaction in such a manner that the increase in product concentration preceeded that of the enzyme inactivation. Thus, about 60% of the suicide substrate was converted into the product before the enzyme was completely inactivated.

Progress curves computed by the analytical solutions

Progress curves computed by the present analytical solutions were compared with those by the numerical method. As far as the μ-values were kept relatively small, the progress curves using the present analytical solutions followed those as given by the numerical method with high fidelity (Fig. 2).

However, with the increasing value of μ, the progress curve for the decrease in substrate concentration computed by present analytical solutions and Waley's solutions deviated gradually from the corresponding ones obtained by the numerical method. Fig. 3 shows one such case where μ was set at 1.0. It could be seen that the progress curve for p/s_0 computed by Waley's solution increased at a much higher rate than that obtained by the numerical method attaining its equilibrium at a significantly higher level than the curve by the numerical method. This error seems to be the major cause that the recovery of suicide substrate computed by Waley's solutions was about 120% which may not be negligible. In this context, the computations based on the present analytical solutions showed the progress curves for s/s_0, p/s_0 and e_i/e_0 all converging to the same equilibria as those attained by the exact solutions.

Our typical results shown in Figs. 2 and 3 thus indicated that the present analytical solutions yield the progress curves that are consistent with those obtained by the numerical method in a much wider

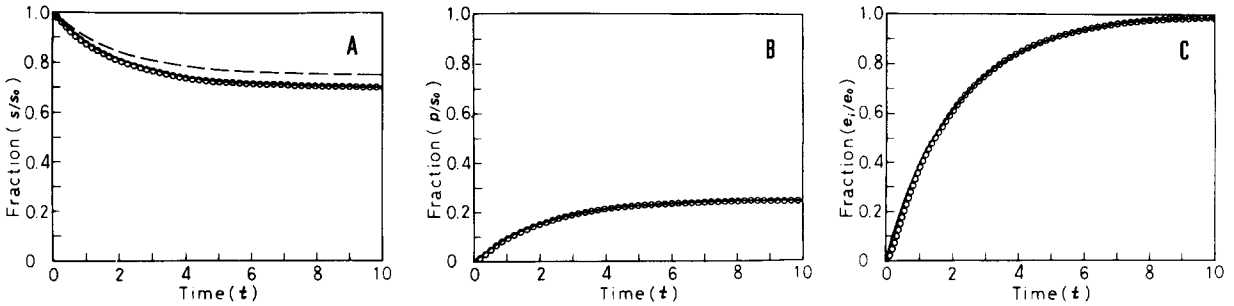

Fig. 2. Comparison between the progress curves computed by numerical method (○), present analytical solutions (———) and Waley's solutions (- - - - - -). The μ-value for these particular computations was set at 0.05, other parametric values being described in Methods. In both (B) and (C), Waley's solutions gave the same results as those obtained by the present analytical solutions.

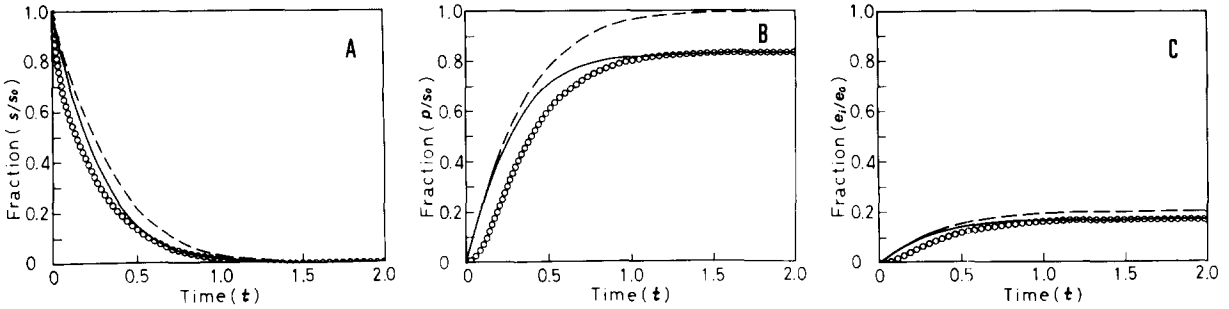

Fig. 3. Comparison between the progress curves computed by numerical method, present analytical solutions and Waley's solutions. The μ-value for these computations was 1.0. Other conditions and symbols are the same as in Fig. 2.

range of parametric conditions than Waley's solutions.

Discussion

Aspects of the improved steady-state differential equation

Waley's differential equation cited as Eqn. 29 in this paper is essentially the same as that of the usual Michaelis-Menten formula. Although the constants A and B in Waley's equation contain the rate constant k_{+4} for the enzyme inactivation process, it only serves to modify the rate of product formation by Michaelis-Menten type reaction. On the other hand, the differential equation that we derived in the present study, i.e., Eqn. 18, contains explicitly both processes of product formation and enzyme inactivation in the term $C(1 + 1/r)$ and we have thus called it the improved steady-state differential equation. Theoretical as well as computer-aided validity tests revealed that the present analytical solutions derived from the improved differential equation yield much better results than Waley's solutions.

Derivation of the Michaelis-Menten type kinetics from the present analytical solutions

It is clear from Eqn. 1 that in the case of $k_{+4} \to 0$, the suicide substrate kinetics degenerate into one of the ordinary Michaelis-Menten type kinetics which had been described by Dixon and Webb [4] as an enzyme reaction that involves two consecutive intermediates (cf. the equations of IV. 33 through 40 given by Dixon and Webb [4]). Therefore, we studied the properties of Eqn. 25 in the case of $k_{+4} \to 0$.

Under this condition, we immediately obtain Eqn. 40,

$$t = \frac{1}{C'}\left[-\frac{B'}{e_0}\ln\frac{s}{s_0} + \frac{s_0 - s}{e_0}\right] \tag{40}$$

where

$$C' = \lim_{k_{+4}\to 0} C = \frac{k_{+2}k_{+3}}{k_{+2} + k_{+3}}$$

and

$$B' = \lim_{k_{+4}\to 0} B = \frac{k_{-1} + k_{+2}}{k_{+1}} \cdot \frac{k_{+3}}{k_{+2} + k_{+3}}$$

Now, the time derivative of Eqn. 40 takes the following form,

$$\frac{ds}{dt} = -C' \frac{e_0 s}{s + B'} \tag{41}$$

In Eqn. 41, we may replace B' and $C'e_0$ with K_{mf} and V_f, respectively, and we obtain the equations which have been described by Dixon and Webb in their equations IV. 39 to 40 [4].

Estimation of parameters

Direct consequences of the present study involve

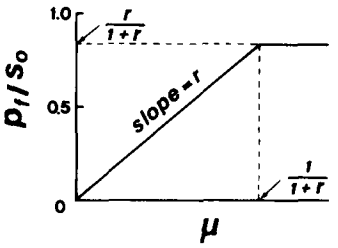

Fig. 4. An idealized plot of p_f/s_0 against various μ-values.

the exploration of the methods for obtaining the kinetic parameters in Eqn. 1. The first candidate of the parameters to be determined would be the partition ratio r. By constructing a plot of p_f/s_0 against various μ-values according to Eqns. 31, 34 and 37, we may obtain a figure such as idealized in Fig. 4. The initial slope of the line gives the value of r, and the portion of the line that is parallel to the abscissa, if extended to the ordinate, gives the value of $r/(1+r)$. Where the determination of e_f may easily be made, the relationship between e_f/e_0 and μ may be useful (cf. Table I).

Once the partition ratio r is determined, then the following method will be available for determining the other parameters B and C. In the cases where initial reaction rate defined as $v_0 = (ds/dt)_{t=0}$ can be determined before any appreciable amounts of enzyme are inactivated, a double-reciprocal plot of Lineweaver-Burk plot of Eqn. 18 will give the estimates of B and C according to the following equation,

$$\frac{1}{v_0} = \frac{1}{C\left(1+\frac{1}{r}\right)e_0} + \frac{B}{C\left(1+\frac{1}{r}\right)e_0}\,\frac{1}{s_0}$$

Hence, the vertical intercept is the reciprocal of $C(1+1/r)e_0$ and the slope $B/((1+1/r)e_0)$, respectively.

However, in practice, the determination of initial reaction rates by measuring the rate of decrease in substrate concentration often accompanies some difficulties. For such cases, one would have to estimate v_0 by determining the rate of product formation followed by correcting it according to the relation between s and p as

$$s_0 = s + \left(1+\frac{1}{r}\right)p$$

which may be rearranged as

$$s_0 - s = \left(1+\frac{1}{r}\right)p$$

Thus, the determination of p will lead to the determination of initial reaction rates for the decrease in substrate concentration. In these cases, however, before utilizing the Lineweaver-Burk type plot, it would be necessary to calculate the concentration of active enzyme at the time of determination of p according to the following relation,

$$a = e_0 - e_i$$

$$= e_0 - \frac{p}{r}$$

Limitation of steady-state method

Comments are necessary on the fact that the results obtained by the present improved analytical solutions still contain some deviations from those obtained by the numerical computation based on the simultaneous differential Eqns. 3–7. If a comparison is made between the results shown in Figs. 2 and 3, then it will be easily seen that the deviations are dependent on μ-values.

In relation to this particular property of the deviation, Walter [5] has clearly demonstrated that the steady-state method is responsible for errors in enzyme kinetics. For example, if the method is applied to such enzyme kinetics that involve two intermediates, it will result in larger errors with increasing μ-values. In fact, the present assumption that the time derivatives of x and y are zero has been found to be invalid except for only one time point each for x and y (Fig. 1). The present study thus suggests that the argument put forward by Walter holds true in the kinetics of suicide substrates of Eqn. 1.

Therefore, when experiments on suicide substrate are designed to include determination with larger μ-values, caution should be used in view of the fact that even the improved analytical solutions give only rough estimates of reaction rates. However, it is hopefully expected that in later phases of the reaction, when most of the usual laboratory determinations are performed, the improved analytical solutions will give satisfactory estimates and will be useful as well in deducing the principal kinetic properties of the enzyme that interacts with suicide substrates.

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