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# A THEORETICAL STUDY ON THE POSSIBILITY OF OCCURENCE OF DAMPED OSCILATIONS IN FIRST ORDER ENZYME REACTION SYSTEMS WITH DISSOCIABLE MODIFIERS

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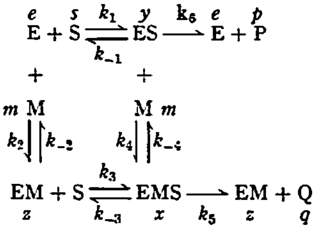
The rate equations for the so-called lag-times of some first order enzyme reaction systems with dissociable activator or inhibitor has been analyzed theoretically as well as experimentally. The experimental calculation to find in what condition the damped oscilation takes place in the system has been carried out by an electronic digital computor according to the Monte Carlo method. It was found that there are some possibilities that the transient state of enzyme reactions modified by the presence of a dissociable activator or inhibitor may be accompanied with a damped oscilation.

Many researchers have been interested in the problem as to whether we can see damped oscilations in enzyme reactions1). This is one of the most interesting problems in the biophysical field. If damped oscilation would be observed in some enzyme reactions experimentally or if it might be expected to occur theoretically, we could have a hope to explain the origin of some oscilatory behaviors experienced in living organisms in the frame of the theory of reaction rates. The author analyzed the so-called lag-times of some first order enzyme reactions theoretically as well as experimentally, and found the possibility that the transient state of the enzyme reactions modified by the presence of a dissociable activator or inhibitor may be accompanied with a damped oscilation.

The calculation was generally carried out by the KDC–I electronic digital computer. The number of effective figures of the computor is 9 in the case of floating point.



Scheme I

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1) J. A. Christiansen, *Advances in Enzymol.*, **23**, 83 (1961)

## Characteristic Equation

A first order enzyme reaction, the rate of which is regulated by a certain modifier, is shown in Scheme I. In the case of a reaction with an activator we must make the rate constant $k_6$ equal to zero, while in the case with an inhibitor, the rate constant $k_5$ should be put equal to zero. For the sake of convenience both cases have been illustrated in one scheme. We shall make the assumption that the total enzyme concentration in the system considered is very small as compared with the substrate concentration.

The rate equations are given by

$$\left.\begin{aligned}
&\frac{dx}{dt}=k_3sz+k_4my-x(k_{-3}+k_{-4}+k_5)\,,\\
&\frac{dy}{dt}=k_1se+k_{-4}x-y(k_{-1}+k_4m+k_6)\,,\\
&\frac{dz}{dt}=k_2me+x(k_{-3}+k_5)-z(k_{-2}+k_3s)\,,\\
&\frac{dp}{dt}=k_6y \qquad \text{or} \qquad \frac{dq}{dt}=k_5x\,,\\
&e_0=e+x+y+z\,,
\end{aligned}\right\} \tag{1}$$

where $e$, $x$, $y$, $z$, $s$ and $m$ are the concentrations of free enzyme, three kinds of enzyme complexes, substrate and modifier, respectively and the rate constants are referred to Scheme I. The terms which involve the reverse rate constants of $k_5$ and $k_6$ have been omitted, because they are small quantities of the second order in the initial stage of the reaction upon which our main concern will be focussed.

The Laplace transforms of the first three equations of (1) are,

$$\left.\begin{aligned}
&(r+k_{-3}+k_{-4}+k_5)X(r)-k_4mY(r)-k_3sZ(r)=x(0+)\,,\\
&(k_1s-k_{-4})X(r)+(r+k_1s+k_{-1}+k_4m+k_6)Y(r)+k_1sZ(r)=\frac{k_1se_0}{r}+y(0+)\,,\\
&(k_2m-k_{-3}-k_5)X(r)+k_2mY(r)+(r+k_2m+k_{-2}+k_3s)Z(r)=\frac{k_2me_0}{r}+z(0+)\,,
\end{aligned}\right\} \tag{2}$$

where $X(r)$ denotes the Laplace transform of $x(t)$, and so on, and where $0+$ means to take the limit at $t=0$ from the right on the time-axis.

Furthermore, we have in the case of activation

$$Q(r)=k_5\frac{X(r)}{r}\,, \tag{3}$$

and in the case of inhibition

$$P(r)=k_6\frac{Y(r)}{r}\,. \tag{4}$$

From Eqs. (2), (3) and (4) we obtain

$$Q(r)=\frac{k_5 g}{r^2(r^3+ar^2+br+c)}, \tag{5}$$

$$P(r)=\frac{k_6 g}{r^2(r^3+ar^2+br+c)}. \tag{6}$$

where

$$a=k_1 s+k_{-1}+k_2 m+k_{-2}+k_3 s+k_{-3}+k_4 m+k_{-4}+k_5+k_6, \tag{7}$$

$$\begin{aligned} b=&\begin{vmatrix} k_{-3}+k_{-4}+k_5 & -k_4 m \\ k_1 s-k_{-4} & k_1 s+k_{-1}+k_4 m+k_6 \end{vmatrix} \\ &+\begin{vmatrix} k_{-3}+k_{-4}+k_5 & -k_3 s \\ k_2 m-k_{-3}-k_5 & k_2 m+k_{-2}+k_3 s \end{vmatrix} \\ &+\begin{vmatrix} k_1 s+k_{-1}+k_4 m+k_6 & k_1 s \\ k_2 m & k_2 m+k_{-2}+k_3 s \end{vmatrix}, \end{aligned} \tag{8}$$

$$c=\begin{vmatrix} k_{-3}+k_{-4}+k_5 & -k_4 m & -k_3 s \\ k_1 s-k_{-4} & k_1 s+k_{-1}+k_4 m+k_6 & k_1 s \\ k_2 m-k_{-3}-k_5 & k_2 m & k_2 m+k_{-2}+k_3 s \end{vmatrix}, \tag{9}$$

and $g$ is either a constant or a function of $r$.

Now the characteristic equation is given from (5) or (6):

$$r^3+ar^2+br+c=0. \tag{10}$$

If this equation has three real roots, the concentration $p(t)$ or $q(t)$ must take the following form so long as the early stage of the reaction is concerned:

$$p(t) \text{ or } q(t)=A_0+A_1 t+A_2\exp[-B_2 t]+A_3\exp[-B_3 t]+A_4\exp[-B_4 t]. \tag{11}$$

On the other hand, if Eq. (10) has one real root and a pair of conjugate roots, the concentration must be a form of damped oscilation.

$$p(t) \text{ or } q(t)=A_0+A_1 t+A_2\exp[-B_2 t]+\exp[-B_3 t](A_3'\cos\omega t-A_3''\sin\omega t). \tag{12}$$

## Does the Damped Oscilation occur ?
## The Monte Carlo Method

In order to solve the present problem, we only have to see in what conditions the discriminant of (10) has a negative sign. It is an algebraic function of the sixth order with respect to the rate constants. Furthermore, the following relation should be taken into consideration:

$$\frac{k_{-3}}{k_3}\Big/\frac{k_{-1}}{k_1}=\frac{k_{-4}}{k_4}\Big/\frac{k_{-2}}{k_2}=\alpha, \tag{13}$$

which is easily derived from the equilibrium consideration. It is by no means an easy task to solve the problem analytically, except a particularly simple case which will be described below.

Under these circumstances we may assume a large number of sets of plausible values for $k$'s and evaluate the discriminant for each of them. In order to reach any meaningful conclusions in this way, we employed the Monte Carlo method, all the calculations having been carried out by the computer, referred to above.

Before entering into the subject, we may make a digression: a particularly simple case:

$$k_1 = k_3, \quad k_{-1} = k_{-3}, \quad k_2 = k_4, \quad k_{-2} = k_{-4}, \quad k_5 \ll k_{-3}, \quad k_6 \ll k_{-1} \tag{14}$$

is considered. This is one of the typical cases usually met with. The characteristic equation is given by

$$(r + k_1 s + k_{-1})(r + k_2 m + k_{-2})(r + k_1 s + k_{-1} + k_2 m + k_{-2}) = 0 . \tag{15}$$

It clearly has three real roots and thus no damped oscillation occurs in this case.

The following notations are introduced:

$$\begin{gathered} X_0 = X_8 = k_1 s , \quad X_1 = X_9 = k_{-1} + k_6 , \quad X_2 = X_{10} = k_{-2} , \quad X_3 = X_{11} = k_2 m , \\ X_4 = X_{12} = k_{-3} + k_5 , \quad X_5 = k_3 s , \quad X_6 = k_4 m \quad \text{and} \quad X_7 = k_{-4} . \end{gathered} \tag{16}$$

Then $a$, $b$ and $c$ of Eq. (10) will be expressed as follows:

$$\begin{aligned} a &= \sum_{i=0}^{7} X_i , \\ b &= \sum_{i=0}^{7} X_i (X_{i+2} + X_{i+3}) + \sum_{i=0}^{3} X_i X_{i+4} , \\ c &= \sum_{i=0}^{7} X_i X_{i+2} (X_{i+4} + X_{i+5}) . \end{aligned} \tag{17}$$

We will make calculations in two step. In the first step we neglect $k_5$ and $k_6$, both being assumed very small compared with $k_{-3}$ and $k_{-1}$, respectively. In the second step we abandon this restriction.

*Calculation 1* $k_5 \ll k_{-3}$ and $k_6 \ll k_{-1}$.

The following procedure is employed.

i) A set of values for $X_{0\sim6}$ is prepared which is a logarithmic sequence.

ii) One of the values is picked up at random and put into the array of $X_i$.

iii) After the arrays of $X_{0\sim6}$ and $X_{8\sim12}$ are filled up, $X_7$ is calculated from $X_{0\sim6}$ according to the equation,

$$X_7 = \frac{X_0 X_2 X_4 X_6}{X_1 X_3 X_5} , \tag{18}$$

which is derived from (13).

iv) $a$, $b$ and $c$ are calculated by (17).

v) The ratio,

$$h = \frac{a^2b^2 + 18abc}{4a^3c + 4b^3 + 27c^2} \tag{19}$$

is calculated instead of the discriminant,

$$d = (a^2b^2 + 18abc) - (4a^3c + 4b^3 + 27c^2), \tag{20}$$

because the former is more convenient than the latter for the systematic study by the computer.

$h < 1$, $h = 1$ and $h > 1$ correspond respectively to $d < 0$, $d = 0$ and $d > 0$. If $h < 1$, we can expect that the damped oscilation will occur.

To save time only the case of $h < 1$ is printed, because the speed of printing is very slow compared with that of calculation by the computer. Since we have a considerable error in calculation in the least valuable figure or one more, we must compare $h$ with a number slightly larger than 1.

vi) $h$ is compared with an appropriate reference, $+1.00000100$ in this study.

If $h < +1.00000100$, the number of calculation and the values of $a$, $b$, $c$ and $h$ are printed.

If $h \geqslant +1.00000100$, nothing is printed.

vii) Then the values put in $X$'s array are renewed and the above process is repeated.

The following sets of values were selected.

Set a.
$+1.00000000 \times 10^1$
$+1.00000000 \times 10^0$
$+1.00000000 \times 10^{-1}$
$+1.00000000 \times 10^{-2}$

Set b.
$+1.00000000 \times 10^1$
$+3.16227766 \times 10^0$
$+1.00000000 \times 10^0$
$+3.16227766 \times 10^{-1}$
$+1.00000000 \times 10^{-1}$
$+3.16227766 \times 10^{-2}$
$+1.00000000 + 10^{-2}$

*Calculation 2* $k_5$ or $k_6$ takes any value.

To simplify the calculation, the whole arrays of $X_i$ are filled up by the values, picked up at random.

Three modifications were applied to the processes of the Calculation 1 in Calculation 2, as follows:

i) A set of values for $X_{0\sim7}$ is prepared which is a logarithmic sequence.

ii) One of the values is picked up at random and put into the array of $X_i$.

iii) After the arrays of $X_{0\sim7}$ and $X_{8\sim12}$ are filled up, $a$, $b$ and $c$ are calculated by (17).

iv) The ratio, $h$, is calculated.

v) $h$ is compared with the same reference as Calculation 1. If $h < +1.00000100$, the number of calculation and the values of $a$, $b$, $c$ and $h$ are printed.

If $h \geqslant +1.00000100$, nothing is printed.

vi) Then the values put in $X$'s array are renewed and the above process is repeated.

The Set a. in the Calculation 1 was used.

## Results and Discussion

*Calculation 1*

(a) The calulations were repeated about 370 times. Only 19 calculations among them were found to have the ratio, $h$, smaller than the reference and all $h$ of the 19 calculations were between 0.999999992 and 1.00000027.

(b) The calculations were repeated about 1,400 times. Only in 14 calculations the ratio, $h$, was found to be smaller than the reference and all $h$'s of the 14 calculations were between 1.00000000 and 1.00000077.

From these results of calculation by the Monte Carlo method, it was presumed that the chance for the discriminant to take negative values is extremely rare and that no damped oscilation can practically be expected to occur in the first order enzyme reaction systems considered.

*Calculation 2*

The calculations were repeated about 1200 times. 140 calculations among them were found to have the ratio, $h$, smaller than the reference. 44 $h$'s of the 140 calculations were between 0.940 and 0.999. Consequently it is expected that damped oscilation occurs in the first order enzyme reaction systems when $k_5$ or $k_6$ takes appreciable values. (In this Calculation 2 all the values filled in the $X$'s array were not contrary to the relation (13).)

For example, let $X_i$'s take plausible values as follows:

$$X_0 = k_1 s = 10/\text{sec}, \qquad X_1 = k_{-1} = 1/\text{sec}, \qquad X_2 = k_{-2} = 10/\text{sec},$$

$$X_3 = k_2 a = 1/\text{sec}, \qquad X_4 = k_{-3} + k_5 = 10/\text{sec}, \qquad X_5 = k_3 s = 1/\text{sec},$$

$$X_6 = k_4 a = 10/\text{sec} \qquad \text{and} \qquad X_7 = k_{-4} = 1/\text{sec}.$$

Then the concentration $p(t)$ becomes,

$$p(t) = -0.1587 + 2.5t - 0.1135 \exp[-22t] + 0.0248 \exp[-11t](11 \cos 9t + 9 \sin 9t). \quad (21)$$

(21) shows that such a system is acompanied with the oscilation whose period is $2\pi/9$ sec and which is damped according to the term exp[$-11$t].

## Summary

The characteristic equation for the first order enzyme reaction systems modified by some dissociable modifier, Scheme I, was introduced and its algebraic properties were studied.

It was found theoretically that damped oscilation should not occur in a special case where $k_3 = k_1$, $k_{-3} = k_{-1}$, $k_4 = k_2$ and $k_{-4} = k_{-2}$ and when $k_5$ and $k_6$ and are negligibly small compared with $k_{-3}$ and $k_{-1}$ respectively, and it was presumed by the Monte Carlo method that damped oscilation might not occur in more general cases where $k_1$, $k_{-1}$, $k_2$, $k_{-2}$, $k_3$, $k_{-3}$, $k_4$ and $k_{-4}$ take arbitrary values but where $k_5$ and $k_6$ are negligibly small compared with $k_{-3}$ and $k_{-1}$ respectively.

However it was concluded by the Monte Carlo mothod that damped oscilation is possible in

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the system where $k_5$ or $k_6$ takes appreciable values.

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