stability of the metal-enzyme complex is predominantly due to the metal-sulfur bond.

ACKNOWLEDGMENTS

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A Stochastic Approach to Enzyme-Substrate Reactions*

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This paper presents a stochastic approach to the study of enzyme-substrate reactions. Stochastic models are constructed for various enzyme-substrate systems including the Michaelis-Menten scheme. The stochastic models provide a framework from which the fluctuations in the number of reactant species during the early stages of the reaction may be obtained. Solutions of the stochastic equations for the second moment are obtained by assuming that the second moment of the concentration variable may be expressed in terms of the first moment. This technique reduces the calculation of the variance to the determination of the first moment. It is also shown that under certain conditions (e.g., $\langle s \rangle \gg \langle e \rangle$) the stochastic rate equations correspond to the deterministic equations. Rigorous solutions of these rate equations cannot be found; however, by invoking the stationary-state hypothesis, approximate expressions for the first moments are obtained.

It has been shown by several authors (Delbruck, 1940; Singer, 1953; Renyi, 1954; Bartholomay, 1958, 1959, 1962a,b; Ishida, 1960; McQuarrie, 1963; McQuarrie et al., 1964) that the course of a chemical reaction can be treated as a stochastic process. The conventional approach to chemical kinetics may be called deterministic, since once the initial number of reactant molecules is known the value at subsequent times is predicted by mathematical expressions. Furthermore, the conventional approach predicts no fluctuations for the number of reactant molecules and any fluctuations are usually attributed to experimental error. On the other hand, the stochastic approach treats the number of reactant molecules as a time-varying discrete random variable and is concerned with determining the probability that the reaction system is in a certain state. The stochastic approach also provides a well-defined method of determining the extent of the fluctuations of the number of reactant species. Such fluctuations are inherent to some extent in all chemical reactions and in some reactions these fluctuations may be relatively large, e.g., systems containing a small number of interacting species. The stochastic model also provides a method of determining the extent to which the conventional rate expressions are applicable to small systems.

This paper discusses stochastic models for various enzyme-substrate mechanisms. Bartholomay (1962a, b) considered a stochastic model for enzyme-substrate reactions in terms of the Michaelis-Menten mechanism. Bartholomay showed that the stochastic model corresponds "in the mean" with the deterministic model, but no mathematical expression for the variance of the reactant species was found. The inability to determine the fluctuations is owing to the appearance of nonlinear stochastic equations. In a previous paper (McQuarrie et al., 1964) we proposed an approximation that permits the determination of the second moment with relative ease. We shall illustrate the application of this approximate method to stochastic models of various enzyme-substrate mechanisms including the Michaelis-Menten scheme.
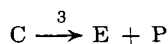
EXAMPLES

Michaelis-Menten Scheme.—
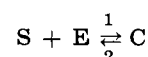
$$S + E \underset{2}{\overset{1}{\rightleftharpoons}} C$$

$$C \xrightarrow{3} E + P$$

Letting $E(t)$, $S(t)$, $C(t)$, $P(t)$, represent the number of E, S, C, and P molecules, respectively, at time t the stochastic model is then completely defined by the following assumptions:

(1) The probability of the transition $(s + 1, e + 1, c - 1, p) \rightarrow (s, e, c, p)$ in the interval $(t, t + \Delta t)$ is $k_1(s + 1)(e + 1)\Delta t + o(\Delta t)$, where k_1 is a constant and $o(\Delta t)$ is defined so that $o(\Delta t)/\Delta t \rightarrow 0$ as $\Delta t \rightarrow 0$.

(2) The probability of the transition $(s - 1, e - 1, c + 1, p) \rightarrow (s, e, c, p)$ in the interval $(t, t + \Delta t)$ is $k_2 (c + 1) \Delta t + o(\Delta t)$, where k_2 is a constant.

(3) The probability of the transition $(s, e - 1, c + 1, p - 1) \rightarrow (s, e, c, p)$ in the interval $(t, t + \Delta t)$ is $k_3 (c + 1) \Delta t + o(\Delta t)$, where k_3 is a constant.

(4) The probability of the transition $(s, e, c, p) \rightarrow (s, e, c, p)$ in the interval $(t, t + \Delta t)$ is $1 - \{k_1(s)(e) - k_2(c) - k_3(c)\} \Delta t + o(\Delta t)$.

In view of these assumptions the following relation is obtained:

$$
\begin{aligned}
P(s, &e, c, p, t + \Delta t) \\
&= k_1 (s + 1) (e + 1) \Delta t\, P(s + 1, e + 1, c - 1, p, t) \\
&+ k_2 (c + 1) \Delta t\, P(s - 1, e - 1, c + 1, p, t) \\
&+ k_3 (c + 1) \Delta t\, P(s, e - 1, c + 1, p - 1, t) \\
&+ [1 - \{k_1 (s) (e) + k_2 (c) \\
&+ k_3 (c)\} \Delta t]\, P(s, e, c, p, t) + o(\Delta t)
\end{aligned}
$$

(1)

where $P(s, e, c, p, t) = \text{Prob}\{S(t) = s, E(t) = e, C(t) = c, P(t) = p\}$. By transposing the term $P(s, e, c, p, t)$, dividing by Δt, and passing to the limit one easily gets the differential difference equation:

$$
\begin{aligned}
\frac{dP(s, e, c, p, t)}{dt} \\
= k_1 (s + 1) &(e + 1)\, P(s + 1, e + 1, c - 1, p, t) \\
+ k_2 (c + 1)\, &P(s - 1, e - 1, c + 1, p, t) \\
+ k_3 (c + 1)\, &P(s, e - 1, c + 1, p - 1, t) \\
- \{k_1 (s) (e) &+ k_2 (c) + k_3 (c)\}\, P(s, e, c, p, t)
\end{aligned}
$$

(2)

Since the quantities of main interest in the stochastic approach are only the first and second moments of the variables, much effort can be saved by applying methods which produce these lower moments. A set of equations involving the time derivatives of the moments of the variables can be generated from the differential difference equation for the process under consideration. The moments of a variable $X(t)$ are defined by

$$\langle x^n \rangle = \sum_{x=0}^{x_0} x^n\, P(x, t)$$

(3)

Therefore, a differential equation involving the time derivative of the nth moment can be obtained by multiplying the differential difference equation by x^n and summing over x. This technique is equivalent to the use of the cumulant generating function which generates the cumulants of the process (Bharucha-Reid, 1960).

Multiplication of (2) by e and e^2, respectively, followed by summation over e gives

$$\frac{d \langle e \rangle}{dt} = -k_1 \langle (e) (s) \rangle + (k_2 + k_3) \langle c \rangle$$

(4)

$$
\begin{aligned}
\frac{d \langle e^2 \rangle}{dt} &= -2k_1 \langle (e)^2 (s) \rangle + k_1 \langle (e) (s) \rangle + 2k_2 \langle (e) (c) \rangle \\
&+ (k_2 + k_3) \langle c \rangle + 2k_3 \langle (e) (c) \rangle
\end{aligned}
$$

(5)

The variation of a variable relative to the first moment or mean of that variable is expressed by the coefficient of variation $CV(t)$ where

$$[CV(t)]^2 = \frac{\langle x^2 \rangle - \langle x \rangle^2}{\langle x \rangle^2}$$

(6)

Previous studies of stochastic models for first- and second-order reactions (Bartholomay, 1958, 1959; McQuarrie *et al.*, 1964) showed that the coefficient of variation appears to increase exponentially with time. We found (McQuarrie *et al.*, 1964) that during the early stages of the reaction a good approximation for $CV(t)$ can be made if it is assumed that

$$[CV(t)]^2 = [\exp (\alpha t) - 1]$$

(7)

where α is a constant. From (6) and (7) we get

$$\langle x^2 \rangle = \langle x \rangle^2 \exp (\alpha t)$$

(8)

The constant α can be determined from the equation involving the time derivative of the second moment if it is assumed that $\langle x^n \rangle = x_0^n$ at time $t = 0$.

Even though the above approximation has been applied to reactions of the irreversible type (McQuarrie *et al.*, 1964) it also provides a good approximation for reversible reactions (see Appendix).

If we let

$$\langle e^2 \rangle = \langle e \rangle^2 \exp (\alpha_1 t)$$

(9)

differentiating with respect to t gives

$$\frac{d \langle e^2 \rangle}{dt} = \alpha_1 \langle e \rangle^2 \exp (\alpha_1 t) + 2 \langle e \rangle \exp (\alpha_1 t) \frac{d \langle e \rangle}{dt}$$

(10)

By equating (10) and (5), setting $t = 0$, and assuming $E(0) = e_0$, $S(0) = s_0$, $C(0) = 0$, $P(0) = 0$, it can be easily shown that $\alpha_1 = (s_0/e_0)k_1$.

Multiplication of (2) by s and s^2 respectively followed by summation over s gives

$$\frac{d \langle s \rangle}{dt} = k_2 \langle c \rangle - k_1 \langle (e) (s) \rangle$$

(11)

$$
\begin{aligned}
\frac{d \langle s^2 \rangle}{dt} &= -2k_1 \langle (e) (s)^2 \rangle + k_1 \langle (e) (s) \rangle \\
&+ 2k_1 \langle (c) (s) \rangle + k_2 \langle c \rangle
\end{aligned}
$$

(12)

Again if we let $\langle s^2 \rangle = \langle s \rangle^2 \exp (\alpha_2 t)$, equation (12) is satisfied if $\alpha_2 = (e_0/s_0)k_1$.

The calculation of the variance depends on the knowledge of the first moment or mean of the concentration variables. The rate equations (4) and (11) cannot be solved unless some further relation between $\langle (e) (s) \rangle$ and $\langle e \rangle \langle s \rangle$ is found. It can be shown that if $s >> e$, $\langle (e) (s) \rangle$ can be approximated by the product $\langle e \rangle \langle s \rangle$.

It can be shown (Parzen, 1960) that if two variables x_1 and x_2 have finite variances then

$$
2 \text{ cov } (x_1, x_2) = \\
\text{var } (x_1 + x_2) - \text{var } (x_1) - \text{var } (x_2)
$$

(13)

where $\text{cov } (x_1, x_2) = \langle (x_1) (x_2) \rangle - \langle (x_1) \rangle \langle x_2 \rangle$; $\text{var } (x_1) = \langle x_1^2 \rangle - \langle x_1 \rangle^2$. Putting $x_1 = e$, $x_2 = s$,

$$2 \text{ cov } (e, s) = \text{var } (e + s) - \text{var } (e) - \text{var } (s)$$

(14)

In the early stages of the reaction $s >> e$, s is essentially constant and $\text{cov } (e, s) = 0$ or $\langle (e) (s) \rangle = \langle e \rangle \langle s \rangle$. Equations (4) and (11) become identical with the deterministic rate expressions

$$\frac{d [e]}{dt} = -k_1 [e] [s] + (k_2 + k_3) [c]$$

(15)

$$\frac{d [s]}{dt} = k_2 [c] - k_1 [e] [s]$$

(16)

and for early stages in the reaction good approximations to $\langle e \rangle$ and $\langle s \rangle$ can be found.

Equations (15) and (16) cannot be solved unless some approximation is made. If it is assumed that the concentration of the complex reaches a stationary state very quickly, a solution of these equations can be found (Benson, 1960).

The stationary-state concentration of the complex

is given by

$$\langle c \rangle = \frac{\langle s \rangle \langle e \rangle}{K_1}, \qquad K_1 = (k_2 + k_3)/k_1 \qquad (17)$$

By substituting (17) into (16) and using the conservation condition $\langle e \rangle = \langle e_0 \rangle - \langle c \rangle$ the stationary state rate of reaction is given by

$$\frac{-d\langle s \rangle}{dt} = \frac{k_3 \langle e_0 \rangle \langle s \rangle}{\langle s \rangle + K_1} \qquad (18)$$

Equation (18) can be integrated to give

$$\langle s_0 \rangle - \langle s \rangle + K_1 \ln \frac{\langle s_0 \rangle}{\langle s \rangle} = k_3 \langle e_0 \rangle t \qquad (19)$$

An approximate form of this last equation, valid during the early stages of the reaction, that is when $(\langle s_0 \rangle - \langle s \rangle)/\langle s \rangle << 1$, is

$$\ln \frac{\langle s_0 \rangle}{\langle s \rangle} \approx \frac{k_3 \langle e_0 \rangle t}{\langle s_0 \rangle + K_1} \qquad (20)$$

where the substitution $\langle s \rangle / \langle s_0 \rangle \approx 1 + \ln \langle s \rangle / \langle s_0 \rangle$ has been made.

The enzyme-substrate systems usually studied are examples of closed systems; matter neither leaves nor enters the system and the total mass of the system remains constant while the composition of the system changes as a result of the reactions taking place. This conservation condition is usually implicit in the reaction scheme and can be used to determine a relation between the variances of the species taking part in the reaction process. For the Michaelis-Menten mechanism we have the following conservation conditions:

$$P(t) = s_0 - S(t) - C(t) \qquad (21)$$

$$E(t) = e_0 - C(t) \qquad (22)$$

From (21) it can be easily shown that $\mathrm{var}(p) = \mathrm{var}(s) + \mathrm{var}(c) + 2\,\mathrm{cov}(s, c)$ or

$$\mathrm{var}(p) = \mathrm{var}(s + c) \qquad (23)$$

Similarly from (22) we get

$$\mathrm{var}(e) = \mathrm{var}(c) \qquad (24)$$

Multiplication of (25) by e and e^2, respectively, followed by summation over e, gives

$$\frac{d\langle e \rangle}{dt} = -k_1 \langle (e)(s) \rangle + k_2 \langle c_1 \rangle + k_{2n+1} \langle c_n \rangle \qquad (26)$$

$$\frac{d\langle e^2 \rangle}{dt} = -2k_1 \langle (e)(s)^2 \rangle + k_1 \langle (e)(s) \rangle + 2k_2 \langle (c_1)(e) \rangle + k_2 \langle c_1 \rangle + 2k_{2n+1} \langle (c_n)(e) \rangle + k_{2n+1} \langle c_n \rangle \qquad (27)$$

Again, if we put $\langle e^2 \rangle = \langle e \rangle^2 \exp(\alpha, t)$ in (27) we get $\alpha_1 = (s_0/e_0)k_1$. Similarly, multiplication of (25) by s and s^2, respectively, followed by summation over s gives

$$\frac{d\langle s \rangle}{dt} = -k_1 \langle (e)(s) \rangle + k_2 \langle c_1 \rangle \qquad (28)$$

$$\frac{d\langle s^2 \rangle}{dt} = -2k_1 \langle (e)(s^2) \rangle + k_1 \langle (e)(s) \rangle + 2k_2 \langle (c_1)(s) \rangle + k_2 \langle c_1 \rangle \qquad (29)$$

Putting $\langle s^2 \rangle = \langle s \rangle^2 \exp(\alpha_2 t)$ in (29) gives $\alpha_2 = (e_0/s_0)k_1$.

The rate equations obtained for this enzyme-substrate system also approach the corresponding deterministic rate expressions when the ratio of enzyme to substrate becomes small. Equations (26) and (28) correspond to the deterministic equations when the term $\langle (e)(s) \rangle$ is replaced by the product $\langle e \rangle \langle s \rangle$. Such a substitution would be valid during the early stages of the reaction when $\langle s \rangle >> \langle e \rangle$. Then by assuming $\langle (e)(s) \rangle = \langle e \rangle \langle s \rangle$ equations (26) and (28) become

$$\frac{d\langle e \rangle}{dt} = -k_1 \langle e \rangle \langle s \rangle + k_2 \langle c_1 \rangle + k_{2n+1} \langle c_n \rangle \qquad (30)$$

$$\frac{d\langle s \rangle}{dt} = -k_1 \langle e \rangle \langle s \rangle + k_2 \langle c_1 \rangle \qquad (31)$$

Solutions to these equations can be found by assuming that the complexes reach a steady state (Dixon and Webb, 1964). In general, then, for n complexes the stationary reaction rate is given by

$$\frac{-d\langle s \rangle}{dt} = \frac{K_1 \langle e_0 \rangle \langle s \rangle}{\langle s \rangle + K_2} \qquad (32)$$

where

$$K_1 = \frac{\text{product of all odd-numbered constants except } k_1}{\text{sum of all permissible combinations of } (n-1) \text{ constants not containing } k_1 \text{ or } k_2}$$

$$K_2 = \frac{\text{sum of all permissible combinations of } n \text{ constants not containing } k_1}{\text{sum of all permissible combinations of } n \text{ constants containing } k_1}$$

The Michaelis-Menten Mechanism Assumes that Only One Intermediate is Involved in Reaction Process.—In this section we consider a process in which n intermediates are involved.

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} C_1 \underset{k_4}{\overset{k_3}{\rightleftarrows}} C_2 \underset{k_6}{\overset{k_5}{\rightleftarrows}}$$

$$C_3 \underset{k_8}{\overset{k_7}{\rightleftarrows}} \ldots \underset{k_{2n}}{\overset{k_{2n-1}}{\rightleftarrows}} C_n \overset{k_{2n+1}}{\longrightarrow} E + P$$
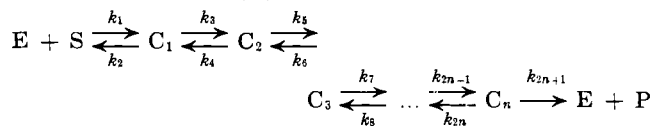
If we again let $E(t), S(t), C_1(t), \ldots, C_n(t), P(t)$ be the number of E, S, $C_1, \ldots, C_n$, and P molecules, respectively, at time t, then we get the stochastic differential-difference equation

The term "permissible combinations" means combinations excluding consecutive subscripts. For example, the combination $k_2 k_5 k_6$ is not permissible because of the consecutive subscripts 5 and 6. For a system where $n = 2$ we would have

$$K_1 = \frac{k_3 k_5}{k_3 + k_4 + k_5}, \qquad K_2 = \frac{k_2 k_4 + k_2 k_5 + k_3 k_5}{k_1(k_3 + k_4 + k_5)}$$

The general solution of (32) is given by $\langle s_0 \rangle - \langle s \rangle + K_2 \ln \langle s_0 \rangle / \langle s \rangle = K_1 \langle e_0 \rangle t$, with the approximate solution as

$$\ln \frac{\langle s_0 \rangle}{\langle s \rangle} \approx \frac{K_1 \langle e_0 \rangle t}{\langle s_0 \rangle + K_2} \qquad (33)$$

$$\frac{dP(e, s, c_1, \ldots, c_n, p, t)}{dt} = k_1(e + 1)(s + 1) P(e + 1, s + 1, c_1 - 1, \ldots, c_n, p, t)$$

$$+ k_2(c_1 + 1) P(e - 1, s - 1, c_1 + 1, \ldots, c_n, p, t)$$

$$+ \sum_{j=1}^{n-1} k_{2j+1} (c_j + 1) P(e, s, c_1, \ldots, c_j + 1, c_{j+1} - 1, \ldots, p, t)$$

$$+ \sum_{j=2}^{n} k_{2j} (c_j + 1) P(e, s, c_1, \ldots, c_{j-1} - 1, c_j + 1, \ldots, c_n, p, t)$$

$$+ k_{2n+1} (c_n + 1) P(e - 1, s, c_1, \ldots, c_n + 1, p - 1, t)$$

$$- \{k_1(e)(s) + k_2(c_1) + \sum_{j=1}^{n-1} k_{2j+1} (c_j) + \sum_{j=2}^{n} k_{2j} (c_j)$$

$$+ k_{2n+1} (c_n)\} P(e, s, c_1, \ldots, c_n, p, t) \qquad (25)$$

Presence of an Inhibitor (Dixon and Webb, 1964).—

$$E + S \underset{2}{\overset{1}{\rightleftarrows}} C_1 \overset{3}{\longrightarrow} E + P$$

$$E + I \underset{5}{\overset{4}{\rightleftarrows}} C_2$$
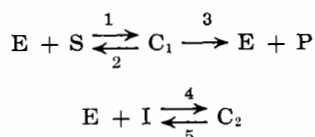
Let $E(t)$, $S(t)$, $I(t)$, $C_1(t)$, $C_2(t)$, $P(t)$ be the number of E, S, I, C_1, C_2 and P molecules in the system at time t. The stochastic differential-difference equation for this process is

$$
\begin{aligned}
\frac{dP(e, s, i, c_1, c_2, p, t)}{dt} \\
= k_1 (e + 1) (s + 1) P(e + 1, s + 1, i, c_1 - 1, c_2, p, t) \\
+ k_2 (c_1 + 1) P(e - 1, s - 1, i, c_1 + 1, c_2, p, t) \\
+ k_3 (c_1 + 1) P(e - 1, s, c_1 - 1, c_2, p - 1, t) \\
+ k_4 (e + 1) (i + 1) P(e + 1, s, i + 1, c_1, c_2 - 1, p, t) \\
+ k_5 (c_2 + 1) P(e - 1, s, i - 1, c_1, c_2 + 1, p, t) \\
- \{k_1 (e) (s) + k_2 (c_1) + k_3 (c_1) + k_4 (e) (i) \\
+ k_5 (c_2)\} P(e, s, i, c_1, c_2, p, t)
\end{aligned}
\tag{34}
$$

Proceeding as before it can be shown that

$$\langle e^2 \rangle = \langle e \rangle^2 \exp [(k_1 s_0 + k_4 i_0)t/e_0] \tag{35}$$

$$\langle s \rangle^2 = \langle s \rangle^2 \exp [(e_0/s_0)k_1 t] \tag{36}$$

Using the conservation conditions

$$e_0 = E(t) + C_1(t) + C_2(t) \tag{37}$$

$$s_0 = S(t) + C_1(t) + P(t)$$

$$i_0 = I(t) + C_2(t)$$

we get

$$\text{var } (e) = \text{var } (c_1 + c_2)$$

$$\text{var } (s) = \text{var } (p + c_1) \tag{38}$$

$$\text{var } (i) = \text{var } (c_2)$$

The stochastic equations for the rate of change of enzyme, substrate, and complex concentrations in this system are

$$\frac{d \langle e \rangle}{dt} = -k_1 \langle (e) (s) \rangle + (k_2 + k_3) \langle c_1 \rangle - k_4 \langle (e) (i) \rangle + k_5 \langle c_2 \rangle \tag{39}$$

$$\frac{d \langle s \rangle}{dt} = -k_1 \langle (e) (s) \rangle + k_2 \langle c_1 \rangle \tag{40}$$

$$\frac{d \langle c_1 \rangle}{dt} = k_1 \langle (s) (e) \rangle - (k_2 + k_3) \langle c_1 \rangle \tag{41}$$

$$\frac{d \langle c_2 \rangle}{dt} = k_4 \langle (e) (i) \rangle - k_5 \langle c_2 \rangle \tag{42}$$

These equations become identical with the deterministic rate equations under the conditions that $\langle (e)(s) \rangle \approx \langle e \rangle \langle s \rangle$ and $\langle (e)(i) \rangle \approx \langle e \rangle \langle i \rangle$. In this system we assume that $\langle i \rangle >> \langle e \rangle$ so that the latter approximation is essentially valid.

The application of the stationary-state hypothesis to this system leads to the following stationary-state concentrations of $\langle c_1 \rangle$ and $\langle c_2 \rangle$:

$$\langle c_1 \rangle = \frac{\langle s \rangle \langle e \rangle}{K_1}, \qquad K_1 = \frac{k_2 + k_3}{k_1} \tag{43}$$

$$\langle c_2 \rangle = K_2 \langle e \rangle \langle i \rangle, \qquad K_2 = k_4/k_5 \tag{44}$$

Using the conservation condition $\langle e \rangle = \langle e_0 \rangle - \langle c_1 \rangle - \langle c_2 \rangle$, equations (43) and (44) can be combined to give

$$\langle c_1 \rangle = \frac{\langle e_0 \rangle \langle s \rangle}{K_1 + K_1 K_2 \langle i \rangle + \langle s \rangle} \tag{45}$$

From equation (40) and the assumption $\langle (e)(s) \rangle = \langle e \rangle \langle s \rangle$ we have

$$\frac{d \langle s \rangle}{dt} = -k_1 \langle s \rangle \langle e \rangle + k_2 \langle c_1 \rangle$$

or

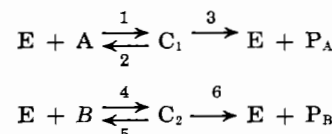$$\frac{d \langle s \rangle}{dt} = -k_3 \langle c_1 \rangle \tag{46}$$

Combining (45) and (46) we get

$$\frac{d \langle s \rangle}{dt} = -\frac{k_3 \langle e_0 \rangle \langle s \rangle}{K_1 + K_1 K_2 \langle i \rangle + \langle s \rangle} \tag{47}$$

By assuming that during the early stages $\langle i \rangle \approx \langle i_0 \rangle$ we get the approximate solution

$$\ln \frac{\langle s_0 \rangle}{\langle s \rangle} \approx \frac{k_3 \langle e_0 \rangle t}{\langle s_0 \rangle + K_1 + K_1 K_2 \langle i_0 \rangle} \tag{48}$$

Enzyme Catalyzing Two Reactions Simultaneously (Dixon and Webb, 1964).—

$$E + A \underset{2}{\overset{1}{\rightleftarrows}} C_1 \overset{3}{\longrightarrow} E + P_A$$

$$E + B \underset{5}{\overset{4}{\rightleftarrows}} C_2 \overset{6}{\longrightarrow} E + P_B$$

The stochastic differential difference equation for this process is

$$
\begin{aligned}
\frac{dP(e, a, b, c_1, c_2, p_A, p_B, t)}{dt} = k_1 (e + 1) (a + 1) P(e + 1, a + 1, b, c_1 - 1, c_2, p_A, p_B, t) \\
+ k_2 (c_1 + 1) P(e - 1, a - 1, b, c_1 + 1, c_2, p_A, p_B, t) \\
+ k_3 (c_1 + 1) P(e - 1, a, b, c_1 + 1, c_2, p_A - 1, p_B, t) \\
+ k_4 (e + 1) (b + 1) P(e + 1, a, b + 1, c_1, c_2 - 1, p_A, p_B, t) \\
+ k_5 (c_2 + 1) P(e - 1, a, b - 1, c_1, c_2 + 1, p_A, p_B, t) \\
+ k_6 (c_2 + 1) P(e - 1, a, b, c_1, c_2 + 1, p_A, p_B - 1, t) \\
- \{k_1 (e) (a) + k_2 (c_1) + k_3 (c_1) + k_4 (e) (b) + k_5 (c_2) + k_6 (c_2)\} P(e, a, b, c_1, c_2, p_A, p_B, t)
\end{aligned}
\tag{49}
$$

If we again proceed as before, the second moments of the variables $A(t)$, $B(t)$, and $E(t)$ are

$$\langle a^2 \rangle = \langle a \rangle^2 \exp [(e_0/a_0) k_1 t] \tag{50}$$

$$\langle b^2 \rangle = \langle b \rangle^2 \exp [(e_0/b_0) k_4 t] \tag{51}$$

$$\langle e^2 \rangle = \langle e \rangle^2 \exp [(k_1 a_0 + k_4 b_0)t/e_0] \tag{52}$$

From the conservation conditions

$$a_0 = A(t) + C_1(t) + P_A(t)$$

$$b_0 = B(t) + C_2(t) + P_B(t) \tag{53}$$

$$e_0 = E(t) + C_1(t) + C_2(t)$$

we get

$$\text{var } (p_A) = \text{var } (a + c_1)$$

$$\text{var } (p_B) = \text{var } (b + c_2) \tag{54}$$

$$\text{var } (e) = \text{var } (c_1 + c_2)$$

The stochastic rate equations for this particular reaction scheme are

$$\frac{d \langle a \rangle}{dt} = -k_1 \langle (e) (a) \rangle + k_2 \langle c_1 \rangle \tag{55}$$

$$\frac{d \langle b \rangle}{dt} = -k_4 \langle (e) (b) \rangle + k_5 \langle c_2 \rangle \tag{56}$$

$$\frac{d \langle c_1 \rangle}{dt} = k_1 \langle (e) (a) \rangle - (k_2 + k_3) \langle c_1 \rangle \tag{57}$$

$$\frac{d \langle c_2 \rangle}{dt} = k_4 \langle (e) (b) \rangle - (k_5 + k_6) \langle c_2 \rangle \tag{58}$$

By assuming that $\langle (e) (b) \rangle \approx \langle e \rangle \langle b \rangle$ and $\langle (e) (a) \rangle \approx \langle e \rangle \langle a \rangle$, the stochastic rate equations become identical with the deterministic equations.

Expressions for $\langle a \rangle$ and $\langle b \rangle$ were found by applying

the stationary-state hypothesis. The stationary-state concentrations of $\langle c_1 \rangle$ and $\langle c_2 \rangle$ are

$$\langle c_1 \rangle = \frac{\langle e \rangle \langle a \rangle}{K_a}, \qquad K_a = \frac{k_2 + k_3}{k_1}$$

$$\langle c_2 \rangle = \frac{\langle e \rangle \langle b \rangle}{K_b}, \qquad K_b = \frac{k_5 + k_6}{k_4}$$

Using the conservation condition $\langle e \rangle = \langle e_0 \rangle - \langle c_1 \rangle - \langle c_2 \rangle$ these equations can be rearranged to give

$$\langle c_1 \rangle = \frac{K_b \langle e_0 \rangle \langle a \rangle}{K_a K_b + K_a \langle b \rangle + K_b \langle a \rangle} \qquad (59)$$

$$\langle c_2 \rangle = \frac{K_a \langle e_0 \rangle \langle b \rangle}{K_a K_b + K_a \langle b \rangle + K_b \langle a \rangle} \qquad (60)$$

From (55) and (56) and the assumptions $\langle (e) \ (a) \rangle \approx \langle e \rangle \langle a \rangle$, $\langle (e) \ (b) \rangle \approx \langle e \rangle \langle b \rangle$ we have

$$\frac{d \langle a \rangle}{dt} = -k_1 \langle e \rangle \langle a \rangle + k_2 \langle c_1 \rangle$$

$$\frac{d \langle b \rangle}{dt} = -k_4 \langle e \rangle \langle b \rangle + k_5 \langle c_2 \rangle$$

or

$$\frac{d \langle a \rangle}{dt} = -k_3 \langle c_1 \rangle \qquad (61)$$

$$\frac{d \langle b \rangle}{dt} = -k_6 \langle c_2 \rangle \qquad (62)$$

Dividing (61) by (62) gives

$$\frac{d \langle a \rangle}{dt} \bigg/ \frac{d \langle b \rangle}{dt} = \frac{k_3 \langle c_1 \rangle}{k_6 \langle c_2 \rangle} = g\frac{\langle a \rangle}{\langle b \rangle}, \ g = k_3 K_b / k_6 K_a \qquad (63)$$

Integration gives

$$\frac{\langle a_0 \rangle}{\langle a \rangle} = \left\{ \frac{\langle b_0 \rangle}{\langle b \rangle} \right\}^g \qquad (64)$$

This relation between $\langle a \rangle$ and $\langle b \rangle$ permits one to obtain solutions to (61) and (62). Eliminating the term $\langle b \rangle$ in (59), then substituting the result in (61) and integrating gives

$$K_a \ln \frac{\langle a_0 \rangle}{\langle a \rangle} + \left\{ \frac{g K_a \langle b_0 \rangle}{K_b} \right\} \left\{ 1 - \left(\frac{\langle a \rangle}{\langle a_0 \rangle} \right)^{1/g} \right\}$$

$$+ \langle a_0 \rangle \left(1 - \frac{\langle a \rangle}{\langle a_0 \rangle} \right) = -k_3 \langle e_0 \rangle t \qquad (65)$$

During the early stages of the reaction we assume that

$$\left\{ \frac{\langle a \rangle}{\langle a_0 \rangle} \right\}^{1/g} \approx 1 + \frac{1}{g} \ln \frac{\langle a \rangle}{\langle a_0 \rangle}$$

and (65) becomes

$$\left\{ K_a + \langle b_0 \rangle \frac{K_a}{K_b} + \langle a_0 \rangle \right\} \ln \frac{\langle a_0 \rangle}{\langle a \rangle} = -k_3 \langle e_0 \rangle t$$

It should be pointed out that the relation between $\langle a \rangle$ and $\langle b \rangle$ given by equation (64) is valid only during those stages of the reaction when the substrates A and B are both present.

APPENDIX

McQuarrie (1963) has shown that the mean and the variance of A for the reversible first order reaction $A \underset{2}{\overset{1}{\rightleftarrows}} B$ are

$$\langle a \rangle = \frac{x_0}{k_1 + k_2} \{ k_1 e^{-(k_1 + k_2)t} + k_2 \} \qquad (I)$$

$$\text{var } (a) = \frac{x_0 w}{1 + \lambda} \left\{ 1 - \frac{w}{1 + \lambda} \right\} \qquad (II)$$

where x_0 is the initial number of A molecules, $\lambda = k_1/k_2$, and $w = \lambda e^{-(k_1 + k_2)t} + 1$. From (I) and (II) it can be shown that

$$\text{CV } (a, t) = \frac{1}{\langle a \rangle} - \frac{1}{x_0} \qquad (III)$$

During the early stages of the reaction we assume

$$e^{-(k_1 + k_2)t} \approx 1 - (k_1 + k_2)t \qquad (IV)$$

From (I) and (IV) equation (III) becomes

$$\text{CV } (a, t) = \frac{1}{x_0} \left\{ \frac{k_1 t}{1 - k_1 t} \right\} \qquad (V)$$

when it is assumed that $\langle a^2 \rangle = \langle a \rangle^2 \ e^{\alpha t}$ it can be shown that $\alpha = k_1/x_0$ or that

$$\text{CV } (a, t) = e^{k_1 t / x_0} - 1 \qquad (VI)$$

Equation (VI) is in good agreement with (V) during the early stages of the reaction. The application of the approximation $\langle x^2 \rangle = \langle x \rangle^2 e^{\alpha t}$ to reversible second-order reactions does not seem unreasonable in light of the results obtained for the reversible first-order reaction even though the validity of such an extension cannot be proved.

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