A Stochastic Model for a Closed Biochemical System at Equilibrium†

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This paper presents a stochastic model, a theoretical multi-variate probability function describing concentrations of reactants in a closed biochemical system at equilibrium. The theory applies to the complete range of biochemical systems from single enzyme reactions to combinations of reactions to complete pathways. Prior to examining the general system, probability functions are derived for the following systems as examples: a reaction with a competitive inhibitor, a bisubstrate reaction using the ping-pong mechanism and a series of two mono-substrate reactions. The theory of Markov processes is used to derive the probability functions for each of the example systems and then for the general system which includes the example systems as special cases. The probability function for any appropriate biochemical system proves to be the product of independent Poisson probabilities conditioned on the conservation equations. Finally, the implications of the theory are briefly discussed and possible extensions proposed.

1. Introduction

Previous stochastic treatments of closed biochemical systems consider only one type of enzyme reaction, the reversible Michaelis–Menten mechanism defined by the model:

\[ S + E \xrightleftharpoons[k_2]{k_1} C \xrightleftharpoons[k_4]{k_3} P + E. \]

\( S, E, C \) and \( P \) represent free substrate, free enzyme, enzyme-substrate (or enzyme-product) transition complex and free product, respectively. Each of the earlier considerations of this reaction concerns deriving a probability generating function (pgf) in which the random variables represent the

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concentrations of these reactants and \( k_1, k_2, k_3 \) and \( k_4 \), the rate constants for
the individual steps of the reaction, serve as the parameters. The derivations
employ the theory of Markov processes (Feller, 1968).

The initial (unsuccessful) attempts (Bartholomay, 1962a,b, 1964; Jachimowski, McQuarrie & Russell, 1964) to derive a pgf consider the complete
time course of the reaction when \( k_4 = 0 \). Later investigators are more successful
as a result of restricting the phase of the reaction. Specifically, the cases
considered are the initial phase when \( k_4 = 0 \) (Heyde & Heyde, 1969), the
equilibrium phase when \( k_2 = k_4 \) (Darvey & Staff, 1967) and the equilibrium
phase without any restrictions on \( k_4 \) (Staff, 1970). A similar model, but for
chemical reactions, is successfully derived to describe the number of con-
versions in a reaction (Orriss, 1969).

This consideration differs from previous ones in not being limited to the
Michaelis–Menten mechanism. It treats a wide range of additional bio-
chemical systems in the equilibrium phase, to extend Staff’s results. Staff,
however, solves a differential equation for the pgf, whereas here a system of
linear equations is solved to find the probability function. The derivation of
the linear equations and their solution for each of three example systems,
chosen to demonstrate various features found in biochemical systems, is
presented in the next three sections. These example systems are:

(i) A reaction with a competitive inhibitor.

(ii) A bisubstrate reaction using the ping-pong mechanism.

(iii) A series of two mono-substrate reactions. (Mahler & Cordes (1971)
list and describe the mechanisms of these and other systems.) With the
insight gained from these example systems’ derivations, the general case
equations are solved.

2. A Reaction with a Competitive Inhibitor

Consider a reversible Michaelis–Menten mechanism with a competitive
inhibitor defined by the model:

\[
S + E \xrightarrow{k_1} C \xrightarrow{k_2} P + E \quad I + E \xrightarrow{k_5} D.
\]

\( S, E, C, P, I \) and \( D \) represent free substrate, free enzyme, enzyme-substrate
(or enzyme-product) transition complex, free product, inhibitor and enzy-
me-inhibitor complex, respectively. The rate constants for the individual steps
of the reaction are given by \( k_j: j = 1,2,\ldots,6 \). The conservation equations,

\[
\begin{align*}
S + P + C &= S_0, \\
E + C + D &= E_0, \\
i + d &= I_0,
\end{align*}
\]
complete the definition of the system. \( s, e, c, p, i \) and \( d \) symbolize concentrations of reactants designated by the corresponding large letters: \( S_0, E_0 \) and \( I_0 \) are initial concentrations of substrate, enzyme and inhibitor (assuming the initial concentrations of product and the two complexes are zero).

In order to derive the probability function for the reactants in system (1) the following assumptions are made.

(i) \( s, e, c, p, i \) and \( d \) take on integer values only (reactant concentrations are expressed in molecules).

(ii) The future state of the system depends only on the present state and not on the history of the system (the Markov property).

(iii) The probability of a conversion from one set of reactants to another is proportional to the concentrations of the reactants making the conversion.

(iv) The probability of more than one conversion in a time unit \( \Delta t \) is \( o(\Delta t) \) where:

\[
o(\Delta t)/\Delta t \to 0,
\]

as

\[
\Delta t \to 0.
\]

Each arrow in system (1) represents a conversion from one set of reactants to another and therefore from one state \((s, e, c, p, i, d)\) to another \((s', e', c', p', i', d')\). Using the assumptions made above, all possible conversions between states and their respective probabilities can be expressed as follows:

<table>
<thead>
<tr>
<th>Conversions</th>
<th>Probabilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>((s, e, c, p, i, d) \to (s-1, e-1, c+1, p, i, d))</td>
<td>(k_{1}se\Delta t + o(\Delta t))</td>
</tr>
<tr>
<td>((s, e, c, p, i, d) \to (s+1, e+1, c-1, p, i, d))</td>
<td>(k_{2}ce\Delta t + o(\Delta t))</td>
</tr>
<tr>
<td>((s, e, c, p, i, d) \to (s, e+1, c-1, p+1, i, d))</td>
<td>(k_{3}ce\Delta t + o(\Delta t))</td>
</tr>
<tr>
<td>((s, e, c, p, i, d) \to (s, e-1, c+1, p-1, i, d))</td>
<td>(k_{4}pe\Delta t + o(\Delta t))</td>
</tr>
<tr>
<td>((s, e, c, p, i, d) \to (s, e-1, c, p, i-1, d+1))</td>
<td>(k_{5}c\Delta t + o(\Delta t))</td>
</tr>
<tr>
<td>((s, e, c, p, i, d) \to (s, e+1, c, p, i+1, d-1))</td>
<td>(k_{6}d\Delta t + o(\Delta t))</td>
</tr>
<tr>
<td>((s, e, c, p, i, d) \to (s, e, c, p, i, d))</td>
<td>(1-(k_{1}se+k_{2}ce+k_{3}ce+k_{4}pe+k_{5}c+k_{6}d)\Delta t + o(\Delta t))</td>
</tr>
</tbody>
</table>

From the conservation equations (2).

\[
c = S_0 - s - p.
\]

\[
d = I_0 - i.
\]

\[
e = E_0 - S_0 - I_0 + s + p + i.
\]
Therefore only $s$, $p$ and $i$ are needed to describe the state of the system. Rewriting expression (3) by making each conversion to the state $(s, p, i)$ and replacing $c$, $d$ and $e$ from equations (4) yields:

| Conversions          | Probabilities                                                      |
|----------------------|===================================================================|
| $(s+1, p, i) \rightarrow (s, p, i)$ | $k_4(s+1)(E_0-S_0-I_0+s+p+i+1)\Delta t + o(\Delta t)$ |
| $(s-1, p, i) \rightarrow (s, p, i)$ | $k_2(S_0-S-p+1)\Delta t + o(\Delta t)$ |
| $(s, p-1, i) \rightarrow (s, p, i)$ | $k_3(S_0-S-p+1)\Delta t + o(\Delta t)$ |
| $(s, p+1, i) \rightarrow (s, p, i)$ | $k_5(p+1)(E_0-S_0-I_0+s+p+i+1)\Delta t + o(\Delta t)$ |
| $(s, p, i+1) \rightarrow (s, p, i)$ | $k_6(i+1)(E_0-S_0-I_0+s+p+i+1)\Delta t + o(\Delta t)$ |
| $(s, p, i-1) \rightarrow (s, p, i)$ | $k_6(I_0-i+1)\Delta t + o(\Delta t)$ |

This list includes all possible ways of attaining the state $(s, p, i)$ in a time unit $\Delta t$. Therefore it is possible to write the probability of a state $(s, p, i)$ at time $t+\Delta t$ in terms of the probabilities of all the origin states at time $t$ as:

$$P(s,p,i; t+\Delta t) = \frac{1}{\Delta t} \left[ (k_1 s+k_4 p+k_5 i)(E_0-S_0-I_0+s+p+i) + (k_2+k_3)(S_0-S-p)+k_6(I_0-i) \right] P(s,p,i; t)$$

where:

$$\Omega_1 = \{(s, p, i): s = 0, 1, \ldots, S_0, p = 0, 1, \ldots, S_0, i = 0, 1, \ldots, I_0, S_0-F_0 \leq s+p \leq S_0, I_0-E_0 \leq i, S_0+I_0-E_0 \leq s+p+i\}$$

and $\delta_{m,n}$ is the Kronecker delta,

$$\delta_{m,n} = \begin{cases} 1 & \text{if } m = n, \\ 0 & \text{if } m \neq n. \end{cases}$$

Subtracting $P(s,p,i; t)$ from both sides, dividing by $\Delta t$ and taking the limit
as $\Delta t \to 0$ yields the forward Kolmogorov equations:

$$
\lim_{\Delta t \to 0} \frac{P(s, p, i; t + \Delta t) - P(s, p, i; t)}{\Delta t} = \frac{\partial P(s, p, i; t)}{\partial t} = -\left[ (k_1 s + k_4 p + k_5 i)(E_0 - S_0 - I_0 + s + p + i) + (k_2 + k_3)(S_0 - s - p) + k_6(i_0 - i) \right] P(s, p, i; t) \\
+ (1 - \delta_{s,S_0})k_1(s + 1)(E_0 - S_0 - I_0 + s + p + i + 1) P(s + 1, p, i; t) \\
+ (1 - \delta_{s,S_0})k_2(S_0 - s + p + 1) P(s - 1, p, i; t) \\
+ (1 - \delta_{p,S_0})k_3(S_0 - s - p + 1) P(s, p - 1, i; t) \\
+ (1 - \delta_{p,S_0})k_4(p + 1)(E_0 - S_0 - I_0 + s + p + i + 1) P(s, p + 1, i; t) \\
+ (1 - \delta_{p,S_0})k_5(i + 1)(E_0 - S_0 - I_0 + s + p + i + 1) P(s, p, i + 1; t) \\
+ (1 - \delta_{i,S_0})k_6(i_0 + 1)(E_0 - S_0 - I_0 + s + p + i + 1) P(s, p, i + 1; t) \\
+ (1 - \delta_{i,S_0})k_7(i_0 - i + 1) P(s, p, i - 1; t), \quad (s, p, i) \in \Omega_1.
$$

(9)

To this point the standard Markov procedure used by other investigators has been followed. Their next step would be to transform the system of forward Kolmogorov equations into a differential equation in the probability generating function. In making this transformation, probabilities containing a product of two concentrations, which always occur in biochemical systems, cause this partial differential equation to be second order. Therefore, solution is difficult and an alternative method is used here.

The equilibrium phase of the reaction is being considered ($t = \infty$), so the derivative of the probability with respect to $t$ is zero. Letting $\frac{\partial P(s, p, i; t)}{\partial t} = 0$ in expression (9) leaves a system of linear equations in the unknown probabilities:

$$
[(k_1 s + k_4 p + k_5 i)(E_0 - S_0 - I_0 + s + p + i) + (k_2 + k_3)(S_0 - s - p) \\
+ k_6(i_0 - i)] P(s, p, i) \\
+ (1 - \delta_{s,S_0})k_1(s + 1)(E_0 - S_0 - I_0 + s + p + i + 1) P(s + 1, p, i) \\
+ (1 - \delta_{s,S_0})k_2(S_0 - s + p + 1) P(s - 1, p, i) \\
+ (1 - \delta_{p,S_0})k_3(S_0 - s - p + 1) P(s, p - 1, i) \\
+ (1 - \delta_{p,S_0})k_4(p + 1)(E_0 - S_0 - I_0 + s + p + i + 1) P(s, p + 1, i) \\
+ (1 - \delta_{i,S_0})k_5(i + 1)(E_0 - S_0 - I_0 + s + p + i + 1) P(s, p, i + 1) \\
+ (1 - \delta_{i,S_0})k_6(i_0 + 1)(E_0 - S_0 - I_0 + s + p + i + 1) P(s, p, i + 1) \\
+ (1 - \delta_{i,S_0})k_7(i_0 - i + 1) P(s, p, i - 1), \quad (s, p, i) \in \Omega_1.
$$

(10)

where $\Omega_1$ is given in expression (7) and $t$ no longer enters into the probabilities.

The solution to the system of linear equations in equation (10) is a probability function for which the random variables are the concentrations of the reactants in system (1). Since there is an equation for each probability, there are the same number of equations as unknowns; there is one dependency in the system of equations but the requirement that the probabilities sum to
one compensates for it to yield a unique solution given by:

$$P(s, p, i) = \frac{1}{\alpha s!p!(I_0 - i)!}(k_2/k_1)^i(k_3/k_4)^j(k_6/k_5)^j$$

$$s + p + i!(S_0 - s - p)!(E_0 - S_0 - I_0 + s + p + i)!$$

(11)

where $\Omega_1$ is given in expression (7) and $\alpha$ is a normalizing constant.

3. A Bisubstrate Reaction using the Ping-pong Mechanism

Now consider a bisubstrate reaction operating by the ping-pong mechanism defined by the model:

$$S + E \xrightarrow{k_1} C_1 \xrightarrow{k_2} P + E'$$

$$T + E' \xrightarrow{k_3} C_2 \xrightarrow{k_4} Q + E.$$  

(12)

Two products, $P$ and $Q$, are formed from two substrates, $S$ and $T$. The obligatory first substrate, $S$, combines with the enzyme, $E$, to form a complex, $C_1$; then product, $P$, and an altered enzyme, $E'$, are released. The second substrate, $T$, combines with $E'$ to form complex $C_2$; then product, $Q$, and the original enzyme, $E$, are released. Representing the concentrations of the reactants by the corresponding small letters, the conservation equations are:

$$s + c_1 + p = S_0, \quad t + c_2 + q = T_0,$$

$$e + e' + c_1 + c_2 = E_0, \quad s + q - e = S_0 - E_0.$$  

(13)

where $S_0$, $T_0$ and $E_0$ are initial concentrations of $S$, $T$ and $E$, respectively (assuming the initial concentrations of $P$, $Q$, $E'$, $C_1$ and $C_2$ equal zero). With the addition of these four equations to the definition of system (12), only four random variables are needed to describe the state of the system: $s$, $p$, $t$ and $q$ are used.

Making the same assumptions as for the inhibited reaction already discussed, the conversions between states and the probabilities of each occurring are:

<table>
<thead>
<tr>
<th>Conversion</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>$(s+1, p, t, q)$ $\rightarrow$ $(s, p, t, q)$</td>
<td>$k_1(s+1)(e+1)\Delta t + o(\Delta t)$</td>
</tr>
<tr>
<td>$(s-1, p, t, q)$ $\rightarrow$ $(s, p, t, q)$</td>
<td>$k_2(e+1)\Delta t + o(\Delta t)$</td>
</tr>
<tr>
<td>$(s, p-1, t, q)$ $\rightarrow$ $(s, p, t, q)$</td>
<td>$k_3(c_1+1)\Delta t + o(\Delta t)$</td>
</tr>
<tr>
<td>$(s, p+1, t, q)$ $\rightarrow$ $(s, p, t, q)$</td>
<td>$k_4(p+1)(e'+1)\Delta t + o(\Delta t)$</td>
</tr>
<tr>
<td>$(s, p, t+1, q)$ $\rightarrow$ $(s, p, t, q)$</td>
<td>$k_5(t+1)(e'+1)\Delta t + o(\Delta t)$</td>
</tr>
<tr>
<td>$(s, p, t-1, q)$ $\rightarrow$ $(s, p, t, q)$</td>
<td>$k_6(c_2+1)\Delta t + o(\Delta t)$</td>
</tr>
<tr>
<td>$(s, p, t, q-1)$ $\rightarrow$ $(s, p, t, q)$</td>
<td>$k_7(c_2+1)\Delta t + o(\Delta t)$</td>
</tr>
<tr>
<td>$(s, p, t, q+1)$ $\rightarrow$ $(s, p, t, q)$</td>
<td>$k_8(q+1)(e+1)\Delta t + o(\Delta t)$</td>
</tr>
<tr>
<td>$(s, p, t, q)$ $\rightarrow$ $(s, p, t, q)$</td>
<td>$1 - (k_1 se + k_2 s' + k_3 c_1 + k_4 p' + k_5 t e' + k_6 c_2 + k_7 c_3 + k_8 q e)\Delta t + o(\Delta t)$</td>
</tr>
</tbody>
</table>
where $e$, $e'$, $c_1$ and $c_2$ are expressed in terms of the random variables, $s$, $p$, $t$ and $q$, from equations (13) as:

$$
e = E_0 - S_0 + s + q, \quad e' = p + t - T_0,$$

$$c_1 = S_0 - s - p, \quad c_2 = T_0 - t - q.$$  \hspace{1cm} (15)

Following the same procedure as before, the system of linear equations arrived at is:

$$
\begin{align*}
(k_1 e + k_2 c_1 + k_3 c_1 + k_4 p e' + k_5 c_2 + k_7 c_2 + k_8 q e) P(s, p, t, q) \\
= (1 - \delta_{s, s_0}) k_1 (s + 1)(e + 1) P(s + 1, p, t, q) \\
+ (1 - \delta_{s, s_0}) k_2 (c_2 + 1) P(s - 1, p, t, q) \\
+ (1 - \delta_{p, p_0}) k_3 (c_1 + 1) P(s, p - 1, t, q) \\
+ (1 - \delta_{p, p_0}) k_4 (p + 1)(e' + 1) P(s, p + 1, t, q) \\
+ (1 - \delta_{t, T_0}) k_5 (c_2 + 1) P(s + 1, p, t, q - 1) \\
+ (1 - \delta_{t, T_0}) k_6 (p + 1)(e' + 1) P(s, p + 1, t, q - 1).
\end{align*}

(16)

where:

$$\Omega_2 = \{(s, p, t, q); s = 0, 1, \ldots, S_0, p = 0, 1, \ldots, S_0, \\
t = 0, 1, \ldots, T_0, q = 0, 1, \ldots, T_0, \quad s + p + t, \quad s + p + t, \quad T_0 \leq p + t, \\
S_0 - E_0 \leq s + p \leq S_0, \quad T_0 - E_0 \leq t + q \leq T_0, \quad S_0 - E_0 \leq s + q\}$$  \hspace{1cm} (17)

and $\delta_{m, n}$ is given by expression (8).

The solution to this system of equations can be shown to be:

$$P(s, p, t, q) = \frac{1}{\alpha} \frac{(k_2/k_1)(k_3/k_4)^p(k_6/k_2)^q(k_5/k_2)^q}{s!p!t!q!e!e'!c_1!c_2!},$$  \hspace{1cm} (18)

where $\Omega_2$ is given in expression (17), $\alpha$ is a normalizing constant and $e$, $e'$, $c_1$ and $c_2$ are given in equations (15).

4. A Series of Two Enzyme Reactions

The third example biochemical system considered here is a series of two reversible Michaelis–Menten mechanisms defined by the model:

$$S + E_1 \xrightleftharpoons[k_{-1}^{k_1}]{k_1^{k_2}} C_1 \xrightleftharpoons[k_{-3}^{k_3}]{k_3^{k_4}} P + E_1, \quad P + E_2 \xrightleftharpoons[k_{-5}^{k_5}]{k_5^{k_6}} C_2 \xrightleftharpoons[k_{-7}^{k_7}]{k_7^{k_8}} Q + E_2.$$  \hspace{1cm} (19)

The product of the first reaction serves as the substrate of the second reaction to form a short pathway. $S$, $P$ and $Q$ represent the intermediates in the
pathway; $E_1$ and $E_2$ symbolize enzymes for the two reactions and $C_1$ and $C_2$ are complexes containing $E_1$ and $E_2$, respectively. Letting the corresponding small letters represent concentrations of the reactants in equations (19), the conservation equations are:

\[ s + p + q + c_1 + c_2 = S_0, \quad e_1 + c_1 = E_{01}, \quad e_2 + c_2 = E_{02}, \]  

where $S_0$, $E_{01}$ and $E_{02}$ are initial concentrations of $S$, $E_1$ and $E_2$ (assuming the initial concentrations of $P$, $Q$, $C_1$ and $C_2$ are zero). These equations reduce the number of variables needed to describe the state of the system to four, $s$, $p$, $q$ and $e_1$ are used.

Making the same assumptions and following the same procedure as before, the system of linear equations becomes:

\[
k_1 s e_1 + k_2 c_1 + k_3 c_1 + k_4 p e_1 + k_5 p e_2 + k_6 c_2 + k_7 c_2 + k_9 q e_2 = P(s, p, q, e_1) \\
= (1 - \delta_{s,0})(1 - \delta_{e_1,0})k_1(s+1)(e_1+1) P(s+1, p, q, e_1+1) \\
+ (1 - \delta_{s,0})(1 - \delta_{e_1,0})k_2(c_1+1) P(s-1, p, q, e_1-1) \\
+ (1 - \delta_{p,0})(1 - \delta_{e_1,0})k_3(c_1+1) P(s, p-1, q, e_1-1) \\
+ (1 - \delta_{p,0})(1 - \delta_{e_1,0})k_4(p+1)(e_1+1) P(s, p+1, q, e_1+1) \\
+ (1 - \delta_{p,0})(1 - \delta_{e_1,0})k_5(p+1)(e_2+1) P(s, p+1, q, e_1) \\
+ (1 - \delta_{q,0})(1 - \delta_{e_1,0})k_6(c_2+1) P(s, p-1, q, e_1) \\
+ (1 - \delta_{q,0})(1 - \delta_{e_1,0})k_7(c_2+1) P(s, p-1, q, e_1) \\
+ (1 - \delta_{q,0})(1 - \delta_{e_1,0})k_8(q+1)(e_2+1) P(s, p, q+1, e_1), \quad (s, p, q, e_1) \in \Omega_3.
\]

where:

\[
\Omega_3 = \{(s, p, q, e_1): s = 0, 1, \ldots, s = 0, 1, \ldots, S_0, p = 0, 1, \ldots, S_0, q = 0, 1, \ldots, S_0, e_1 = 0, 1, \ldots, E_{01}, S_0 - E_{01} - E_{02} \leq s + p + q \leq S_0, E_{01} - S_0 \leq e_1\}.
\]

$\delta_{m,n}$ is the Kronecker delta given in expression (8) and $c_1$, $c_2$ and $e_2$ are given by the random variables from equations (20) as:

\[
c_1 = E_{01} - e_1, \quad c_2 = S_0 - E_{01} - s - p - q + e_1, \quad e_2 = E_{01} + E_{02} - S_0 + s + p + q - e_1.
\]

The solution to this system of equations is:

\[
P(s, p, q, e_1) = \frac{1}{\alpha} \frac{(k_3/k_2)^{s+e_1}(k_6/k_3)^{s+e_1}(k_6/k_5)^{s+e_1}+p(k_7/k_8)^q}{s!p!q!e_1!e_2!c_1!c_2!}.
\]

where $\Omega_3$ is given in expression (22), $c_1$, $c_2$ and $e_2$ are given in equations (23) and $\alpha$ is a normalizing constant.
It is convenient at this point to rewrite the equations in another form. Let:
\[ x' = (s, e_1, p, q, e_1, e_2, c_2) = (x_1, \ldots, x_7). \] (25)
the reactants,
\[ x'_1 = (s, e_1, p, q). \] (26)
the random variables,
\[
\begin{bmatrix}
1 & 0 & 0 & 0 \\
1 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 \\
0 & 0 & 1 & 0 \\
0 & 0 & 0 & 1 \\
\end{bmatrix}
\]
A
\[
\begin{bmatrix}
1 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 \\
0 & 1 & 0 & 0 \\
0 & 0 & 1 & 0 \\
0 & 0 & 0 & 1 \\
\end{bmatrix}
\]
B
\[
\begin{bmatrix}
A_1 \\
A_2 \\
\end{bmatrix}
\]
and
\[
\begin{bmatrix}
0 & 0 & 0 & 1 \\
0 & 0 & 0 & 0 \\
\end{bmatrix}
\]
B,
\[
\begin{bmatrix}
B_1 \\
B_2 \\
\end{bmatrix}
\]
A and B are formed by placing a 1 in row i and column j if reactant \( x_i \) is converted to another reactant in step j; A specifies conversions in the forward direction and B in the reverse direction. Using this notation, equation (21) can now be rewritten as:
\[
\sum_{i=1}^{4} \left( k_{2j-1} \prod_{i=1}^{7} (x_{i}^{a_{ij}}) + k_{2j} \prod_{i=1}^{7} (x_{i}^{b_{ij}}) \right) P(x_1) = \sum_{j=1}^{4} \left( 1 - \delta_{x_1 + A_{1j} - B_{1j}, \Omega_3} \right) k_{2j-1} \prod_{i=1}^{7} \left[ (x_i + 1)^{a_{ij}} \right] P(x_1 + A_{1j} - B_{1j}) \]
\[
+ \sum_{j=1}^{4} \left( 1 - \delta_{x_1 - A_{1j} + B_{1j}, \Omega_3} \right) k_{2j} \prod_{i=1}^{7} \left[ (x_i + 1)^{b_{ij}} \right] P(x_1 - A_{1j} + B_{1j}), \]
\]
where \( \Omega_3 \) is given in expression (22). \( A_{1j} \) and \( B_{1j} \) are columns of \( A_1 \) and \( B_1 \), \( a_{ij} \) and \( b_{ij} \) are elements of A and B and \( \delta_{m,n} \) is now:
\[
\delta_{m,n} = \begin{cases} 
0 & m \in n, \\
1 & m \notin n. 
\end{cases}
\] (29)

Also using the new notation, the probability function (24) is:
\[
P(x_1) = \frac{1}{\alpha} \prod_{j=1}^{4} \left( \frac{k_{2j-1}}{k_{2j}} \right) g(x_1) \prod_{i=1}^{7} x_i!, \quad x_1 \in \Omega_3.
\] (30)
where:
\[
g(x_1) = (B_1 - A_1)^{-1} x_1.
\] (31)
and \( \alpha \) is a normalizing constant.
5. A General Biochemical System

The probability function for a general biochemical system results from following the same procedures used for the example systems. Instead of a model of mechanism(s), an \( m \times 1 \) vector, \( \mathbf{x} \), of reactants and corresponding \( m \times n \) matrices, \( \mathbf{A} \) and \( \mathbf{B} \), of conversions specify this system of \( n \) steps with \( m \) reactants. Each of the \( n \) steps proceeds at forward and reverse rates, \( k_{+j} \) and \( k_{-j} \), \( j = 1, 2, \ldots, n \). A set of \( m - n \) conservation equations restrict the concentration ranges of the reactants.

The elements of \( \mathbf{x} \) represent the reactants in the system; the first \( n \) elements are the random variables. \( \mathbf{A} \) and \( \mathbf{B} \) are formed by placing a 1 in row \( i \) and column \( j \) if \( x_i \) undergoes a conversion to another reactant in step \( j \) and a 0 otherwise; \( \mathbf{A} \) specifies conversions in the forward direction and \( \mathbf{B} \) in the reverse direction. The \( m - n \) conservation equations are linear combinations of reactants for which the same linear combinations of rows of \( \mathbf{B} - \mathbf{A} \) produce rows of zeros. Each of \( \mathbf{x} \), \( \mathbf{A} \) and \( \mathbf{B} \) may be partitioned into separate matrices:

\[
\mathbf{x} = \begin{bmatrix} x_1 \\ \vdots \\ x_2 \end{bmatrix}, \quad \mathbf{A} = \begin{bmatrix} A_1 \\ \vdots \\ A_2 \end{bmatrix}, \quad \mathbf{B} = \begin{bmatrix} B_1 \\ \vdots \\ B_2 \end{bmatrix}
\]

where \( x_1 \) is \( n \times 1 \) and \( A_1 \) and \( B_1 \) are each \( n \times n \). For the theory to hold, the system must be such that \( m \geq n \) (at least as many reactants as steps), \( \mathbf{B} - \mathbf{A} \) is non-singular and \( k_{+j} \) and \( k_{-j} \) are non-zero for \( j = 1, 2, \ldots, n \).

Making the same assumptions as before, the conversions between states and the probabilities of each occurring for a general system are:

\[
\begin{align*}
\mathbf{x}'_{i} & \to \mathbf{x}'_{j} \\
(k_{+j} \prod_{i=1}^{m} (x_i + 1)^{a_{ij}} \Delta t + o(\Delta t)) & \quad j = 1, 2, \ldots, n \\
(k_{-j} \prod_{i=1}^{m} (x_i + 1)^{b_{ij}} \Delta t + o(\Delta t)) & \quad j = 1, 2, \ldots, n \\
1 - \sum_{j=1}^{n} \left( k_{+j} \prod_{i=1}^{m} x_i^{a_{ij}} + k_{-j} \prod_{i=1}^{m} x_i^{b_{ij}} \right) \Delta t + o(\Delta t)
\end{align*}
\]

where \( A_{1j} \) and \( B_{1j} \) are columns of \( A_1 \) and \( B_1 \) and \( a_{ij} \) and \( b_{ij} \) are elements of \( A \) and \( B \).

Using the forward Kolmogorov equations and setting the time derivative to zero (as for the example systems), the system of linear equations reduces...
to:
\[
\sum_{j=1}^{n} \left( k_{+j} \prod_{i=1}^{m} x_{i}^{a_{ij}} + k_{-j} \prod_{i=1}^{m} x_{i}^{b_{ij}} \right) P(x_1)
\]
\[
= \sum_{j=1}^{n} \left( 1 - \delta_{x_{i}+A_{1j}-B_{1j},\Omega} \right) k_{+j} \prod_{i=1}^{m} (x_{i}+1)^{a_{ij}} P(x_{1} + A_{1j} - B_{1j})
\]
\[
+ \sum_{j=1}^{n} \left( 1 - \delta_{x_{i}+A_{1j}+B_{1j},\Omega} \right) k_{-j} \prod_{i=1}^{m} (x_{i}+1)^{b_{ij}} P(x_{1} - A_{1j} + B_{1j}), \quad x_1 \in \Omega.
\]

where \( \Omega \) is determined by the conservation equations and initial conditions, \( A_{1j}, B_{1j}, a_{ij} \) and \( b_{ij} \) are defined above and \( \delta_{m,n} \) is given in expression (29). The first example system derivation considers the uniqueness of the function.

Equation (33) is satisfied if each of the \( 2n \) terms on the left side equals a different term on the right side; i.e. if:

\[
\sum_{j=1}^{n} \left( 1 - \delta_{x_{i}+A_{1j}-B_{1j},\Omega} \right) k_{+j} \prod_{i=1}^{m} (x_{i}+1)^{a_{ij}} P(x_{1} + A_{1j} - B_{1j}) = k_{-j} \prod_{i=1}^{m} (x_{i}+1)^{b_{ij}} P(x_{1} - A_{1j} + B_{1j}).
\]

(34) \( j = 1, 2, \ldots, n, \quad x_1, x_1 - A_{1j} + B_{1j} \in \Omega. \)

(The other \( n \) terms yield identical equations.) Assuming \( P(x_1) \neq 0 \) and \( k_{-j} \neq 0. \)

\[
\frac{P(x_{1} - A_{1j} + B_{1j})}{P(x_1)} = \prod_{i=1}^{m} \frac{x_{i}^{a_{ij}}}{(x_{i}+1)^{b_{ij}}} k_{+j}, \quad j = 1, 2, \ldots, n.
\]

For some \( j \), when \( b_{ij} = 1, x_{i}+1 \) occurs in the denominator on the right in this ratio and in the argument of the numerator probability function on the left; when \( a_{ij} = 1, x_{i} \) occurs in the numerator of the ratio on the right and \( x_{i} - 1 \) in the argument of the numerator probability function on the left. This occurs if the probability function contains

\[
\left[ \prod_{i=1}^{m} x_{i}! \right]^{-1},
\]

so that:

\[
P(x_1) = \frac{\prod_{j=1}^{n} \left( \frac{k_{+j}}{k_{-j}} \right)^{g_j(x_1)}}{\prod_{i=1}^{m} x_{i}!}, \quad x_1 \in \Omega.
\]

where:

\[
g_j(x_1 - A_{1j} + B_{1j}) - g_j(x_1) = 1
\]
\[
g_j(x_1 - A_{1j} + B_{1j}) - g_j(x_1) = 0, \quad j' = 1, 2, \ldots, n, \quad j' \neq j.
\]

The vector \( \mathbf{g} \) has this property if:

\[
\mathbf{g}(x_1) = (B_1 - A_1)^{-1} x_1.
\]

The probability function given in equation (36) is the product of independent
Poisson functions conditioned on the conservation equations. The moments remain to be determined.

Considering the reversible Michaelis–Menten mechanism, as did Staff (1970),

\[ S + E \xrightarrow{k_1} C \xrightarrow{k_2} P + E, \]

as did Staff (1970),

\[ \begin{bmatrix} s \\ p \\ c \\ e \end{bmatrix} = \begin{bmatrix} x_1 \\ \vdots \\ x_2 \end{bmatrix}, \quad A = \begin{bmatrix} 1 & 0 \\ 0 & 0 \\ 0 & 1 \\ 1 & 0 \end{bmatrix}, \quad B = \begin{bmatrix} 0 & 1 \\ 1 & 0 \end{bmatrix}, \quad \begin{bmatrix} A_1 \\ A_2 \end{bmatrix}, \quad \begin{bmatrix} B_1 \\ B_2 \end{bmatrix} \]

The conservation equations,

\[ e + c = E_0 + C_0, \quad s + p + c = S_0 + P_0 + C_0, \]

result from consideration of \( B - A \),

\[ B - A = \begin{bmatrix} -1 & 0 \\ 0 & 1 \\ \ldots \\ 1 & -1 \\ -1 & 1 \end{bmatrix} \]

since the sums of rows three and four and of rows one, two and three are rows of zeros. \( S_0, P_0, C_0 \) and \( E_0 \) are initial concentrations of \( s, p, c \) and \( e \), respectively. Taking the inverse,

\[ (B_1 - A_1)^{-1} = \begin{bmatrix} -1 & 0 \\ 0 & 1 \end{bmatrix} \]

Therefore,

\[ g(x_1) = \begin{bmatrix} -s \\ p \end{bmatrix}. \]

Letting \( P_0 = C_0 = 0 \) as did Staff, from equation (36),

\[ P(s, p) = \frac{1}{\zeta} \frac{(k_1/k_2)^s (k_3/k_4)^p}{s!p!c!e!}, \quad (s, p) \in \Omega, \]

where:

\[ c = S_0 - s - p, \quad e = E_0 - S_0 + s + p, \]

and

\[ \Omega = \{(s, p) : s = 0, 1, \ldots, S_0, p = 0, 1, \ldots, S_0, S_0 - E_0 \leq s + p \leq S_0\} \]
in agreement with Staff. Since the means of $s$ and $p$, derived from the probability function, are:

$$
\mu_s = \frac{k_2}{k_1} \beta, \quad \mu_p = \frac{k_3}{k_4} \beta,
$$

(48)

where $\beta$ is a constant, the ratio:

$$
\frac{\mu_p}{\mu_s} = \frac{k_1 k_3}{k_2 k_4} = K_m.
$$

(49)

where $K$ is the equilibrium constant for the reaction.

6. Discussion

The state of the art of analyzing biochemical systems would be greatly improved by the development of a comprehensive probability theory for both open and closed systems. Functions of rate constants could be estimated from in vivo measurements of concentrations of intermediate reactants, eliminating the need for the assumption that enzymes operate identically in vitro as in vivo (Walter, 1966, preface). A larger proportion of the total number of mutations that occur could be detected by statistical testing procedures applied to intermediate data than are detectable by electrophoresis. Nei (1975) estimates that 70% of all mutations result in amino acid changes while only 30% are detectable by electrophoresis. The source of altered enzyme activity resulting from a mutation could possibly be determined by testing for a change in the amount of enzyme, thereby simplifying an otherwise difficult problem (Harris, 1975). Regulatory enzymes could be located with statistical rigor, not obtainable using crossover plots (Rolleston, 1972). Finally, models of enzyme mechanisms could be tested against alternatives using in vivo data.

Analyses, which employ the probability function derived here, are limited to those that can use data from a closed (in vitro) system at equilibrium. Functions of rate constants, or equilibrium constants in this case, can be estimated. Both detecting mutant enzymes and testing models for enzyme mechanisms require that enzyme properties be reflected in the data. One would not expect this to be true at equilibrium since the equilibrium constant does not depend on the enzyme. The location of regulatory enzymes and the determination of enzyme level necessitate in vivo data. The probability function finds its main importance as the first step in the development of a more complete theory, one which includes open biochemical systems.

On the other hand, while the applications of this theory are limited, the systems to which it applies are numerous and varied. Three restrictions are required to develop the theory. Two of them qualify easily since most
reactions can be considered reversible and closed systems generally have as many reactants as steps. The third restriction, that $B_i - A_i$ be non-singular, eliminates certain allosteric mechanisms, yet, in the cases tried, a solution proves possible if particular rate constants are equated. There is no limit to the length of the pathway, to the number of reactants or to the mechanisms assumed for the reactions as long as these three restrictions are met.

Consideration of the plausible uses and shortcomings of this theory leads to proposals for its extension. Measurement error will have to be incorporated for the function to be practical since the variation included in the present model may not nearly represent all that occurs in the data (Heyde & Heyde, 1971). The applications will increase with extension to open systems. Logically, the reactants in the model should have a continuous density rather than discrete probabilities, for that is the nature of their measurements. Including compartmentation (as in mitochondria or in different cells), variable enzyme levels (as with degradation), or more than one set of rate constants (as with heterozygotes), may add useful, though unessential, features.

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