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INFLUENCE OF PREMIXING ON TRANSIENT-PHASE KINETICS FOR TWO-SUBSTRATE ENZYME SYSTEMS

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SUMMARY

Transient-phase kinetic equations are worked out, using the Laplace transform method, for two-substrate enzyme reactions occurring by the Theorell–Chance, ping pong bi bi and ordered ternary-complex mechanisms (the equations for the random ternary-complex mechanism are too complex to be useful). Two sets of premixing conditions were considered: (i) E not premixed with the first substrate A, *i.e.*, $E \mid A \mid B$, $E \mid A + B$ or $E + B \mid A$; and (ii) E premixed with the first substrate A, *i.e.*, $E + A \mid B$. For each mechanism and premixing conditions two cases were treated: (a) enzyme concentration limiting, *i.e.*, $e_0 \ll a_0, b_0$, and (b) concentration of first substrate A limiting, $a_0 \ll e_0, b_0$. In all cases (a) gives rise to a transient phase followed by a steady state, the transient phase being represented by two or more exponential terms. In all cases (b) gives no steady state; the concentrations of products X and Y rise to a final value of a_0 in a manner represented by two or more exponential terms. Premixing of type (ii) leads to a more rapid initial rise than that of type (i), in Cases a and b. Some results on horse liver alcohol dehydrogenase are shown to be consistent with the equations derived for a Theorell–Chance mechanism; there is no evidence for the participation of two types of active sites on the enzyme.

INTRODUCTION

In a recent paper[1] we have presented equations for the pre-steady-state and steady-state kinetics of two-substrate systems occurring by the following mechanisms: (1) Theorell–Chance, (2) ping pong bi bi, (3) ordered ternary complex, and (4) random ternary complex.

It was assumed that the concentrations of the substrates A and B were in great excess of that of the enzyme E, and that the three ingredients E, A and B were mixed together at zero time.

In the present paper we derive the equations that apply to certain pre-mixing procedures. In the case of the first three mechanisms, one substrate, which we denote as A, interacts first with the enzyme, the other, B, being involved later in the reaction.

The pre-steady-state kinetics will therefore be modified if we premix E and A, and then add B at zero time. On the other hand, for these three mechanisms, premixing E and B will lead to the same pre-steady-state kinetics as bringing E, A and B together at zero time.

Equations for the various cases are derived in the present paper and attention is also given to the situation in which the concentration of A is much less than that of E and B. It will be seen that the resulting equations lead to criteria for distinguishing between the various mechanisms on the basis of transient-phase studies under different conditions of premixing. The equations for the random ternary-complex mechanisms have been omitted from the present paper, since they are too complicated to be useful in interpreting experimental data; however, certain qualitative conclusions are drawn about the influence of different premixing conditions.

THEORETICAL

Figs 1, 2 and 3 show the Theorell–Chance, ping pong bi bi and ordered ternary-complex mechanisms, and give the initial concentrations of the various species. Two special cases are considered:

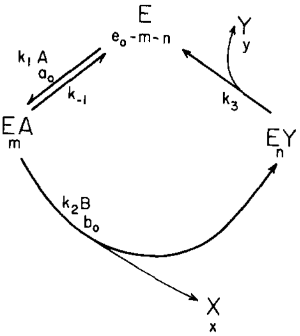

Fig. 1. The Theorell–Chance mechanism, showing the concentrations of the various species for the case a_0, $b_0 \gg e_0$.

(i) both substrates in excess of enzyme;

a_0, $b_0 \gg e_0$;

(ii) enzyme and substrate B in excess of substrate A;

e_0, $b_0 \gg a_0$

The concentrations of the various species for these special cases are shown, for the three mechanisms, in Table I.

Two different premixing conditions are considered:

(i) $E \mid A \mid B$, $E \mid A + B$ or $E + B \mid A$. The vertical stroke indicates mixing at $t = 0$, the positive sign premixing; thus $E \mid A + B$ means that A and B are premixed, their mixed solutions being added to E at $t = 0$. By definition B is the second substrate involved in the reaction; premixing of B with A, or of B with E, therefore, does not affect the kinetics. The equations for this case were given in the previous paper[1].

TABLE I

CONCENTRATIONS AT TIME t

	E	A	B	EA	EA'	EB	EAB	$EA'B$	EY	X	Y
Theorell–Chance											
1. $a_0, b_0 \gg e_0$	e_0-m-n	a_0	b_0	m	—	—	—	—	n	x	y
2. $b_0, e_0 \gg a_0$	e_0	$a_0-m-n-y$	b_0	m	—	—	—	—	n	x	y
Ping pong bi bi											
3. $a_0, b_0 \gg e_0$	$e_0-m-n-z$	a_0	b_0	m	n	—	z	—	—	x	y
4. $e_0, b_0 \gg a_0$	e	$a_0-m-n-y-z$	b_0	m	$e_0-z-m-e$	—	—	z	—	x	y
Ordered ternary complex											
5. $a_0, b_0 \gg e_0$	$e_0-m-n-z$	a_0	b_0	m	—	—	n	—	z	x	y
6. $e_0, b_0 \gg a_0$	e_0	$a_0-m-n-y-z$	b_0	m	—	—	n	—	z	x	y

(ii) $E + A \mid B$. This type of premixing will affect the kinetics, in that there will be more rapid reaction in the transient phase.

Theorell–Chance mechanism

The Theorell–Chance mechanism is shown in Fig. 1, and the equations for the rates of formation of X (the first product produced) and Y (the product later produced) are given in Table II, for a_0, $b_0 \gg e_0$ and the two premixing conditions stated above. The equations were obtained by the use of the Laplace transform method[1–4]. The constants λ_1 and λ_2 are always positive quantities, and we arbitrarily define λ_1 as the one having the larger value. It is easily shown that λ_1 must be greater than k_3; λ_2 can be either greater or less than k_3.

TABLE II

RATE EQUATIONS FOR THE THEORELL–CHANCE MECHANISM, WITH a_0, $b_0 \gg e_0$

λ_1 and λ_2 are the negatives of the roots of the quadratic equation

$P^2 + (k_1a_0 + k_{-1} + k_2b_0 + k_3)P + k_3(k_1a_0 + k_{-1} + k_2b_0) + k_1k_2a_0b_0 = 0.$

Premixing	*Formation of X*	*Formation of Y*
$E\|A\|B$ $E\|A + B$ or $E + B\|A$	$x = \dfrac{k_1k_2k_3e_0a_0b_0}{k_3(k_1a_0+k_{-1}+k_2b_0)+k_1k_2a_0b_0}t$ $- \dfrac{k_1k_2e_0a_0b_0(\lambda_1-k_3)}{\lambda_1^2(\lambda_1-\lambda_2)}(1-e^{-\lambda_1 t})$ $+ \dfrac{k_1k_2e_0a_0b_0(\lambda_2-k_3)}{\lambda_1^2(\lambda_1-\lambda_2)}(1-e^{-\lambda_2 t})$	$y = \dfrac{k_1k_2k_3e_0a_0b_0}{k_3(k_1a_0+k_{-1}+k_2b_0)+k_1k_2a_0b_0}t$ $+ \dfrac{k_1k_2k_3e_0a_0b_0}{\lambda_1^2(\lambda_1-\lambda_2)}(1-e^{-\lambda_1 t})$ $- \dfrac{k_1k_2k_3e_0a_0b_0}{\lambda_2^2(\lambda_1-\lambda_2)}(1-e^{-\lambda_2 t})$
$E + A\|B$	$x = \dfrac{k_1k_2k_3e_0a_0b_0}{k_3(k_1a_0+k_{-1}+k_2b_0)+k_1k_2a_0b_0}t$ $- \dfrac{k_1k_2e_0a_0b_0(1-\lambda_1/k_{-1})(\lambda_1-k_3)}{\lambda_1^2(\lambda_1-\lambda_2)}(1-e^{-\lambda_1 t})$ $+ \dfrac{k_1k_2e_0a_0b_0(1-\lambda_2/k_{-1})(\lambda_2-k_3)}{\lambda_2^2(\lambda_1-\lambda_2)}(1-e^{-\lambda_2 t})$	$y = \dfrac{k_1k_2k_3e_0a_0b_0}{k_3(k_1a_0+k_{-1}+k_2b_0)+k_1k_2a_0b_0}t$ $+ \dfrac{k_1k_2k_3e_0a_0b_0(1-\lambda_1/k_{-1})}{\lambda_1^2(\lambda_1-\lambda_2)}(1-e^{-\lambda_1 t})$ $- \dfrac{k_1k_2k_3e_0a_0b_0(1-\lambda_2/k_{-1})}{\lambda_2^2(\lambda_1-\lambda_2)}(1-e^{-\lambda_2 t})$

Analysis of the experimental data with reference to the equations for x and y was discussed in our earlier paper.

If, on the other hand, E and A are premixed, the equilibrium $E + A \rightleftharpoons EA$ is established; if m_0 is the value of m at $t = 0$ we have the equilibrium equation

$$k_1 a_0 e_0 = k_{-1} m_0 \tag{1}$$

whence

$$m_0 = \frac{k_1 a_0 e_0}{k_{-1}} \tag{2}$$

The procedure for solving the differential equations for this case is similar to that for the previous case[1], the only modification being that in replacing the differentials by operators P, the transformation is $P_m - P_{m0}$. The solutions for this case are shown in the lower part of Table II.

It is to be noted that for both sets of premixing conditions there is a biphasic exponential rise of x or y to the steady state, the steady state being identical in the two cases. However, in the second case the exponential rise is more rapid; this is because of the equilibrium concentration of EA that is established during the premixing. A study of the pre-steady state thus permits one to distinguish A (the first substrate) from B; premixing with A will enhance the pre-steady-state kinetics, while premixing with B will either leave it unaffected or (if an abortive complex is formed with B) will actually reduce the pre-steady-state rate.

The equations for the situation in which the concentration of A is limiting (e_0, $b_0 \gg a_0$) are shown in Table III, for the two sets of premixing conditions. λ_1 and λ_2 are positive, with $\lambda_1 > \lambda_2$. It is to be noted that there is now no steady state, x and y rising to the value a_0. The exponential rise is biphasic for x, triphasic for y.

TABLE III

RATE EQUATIONS FOR THE THEORELL-CHANCE MECHANISM, WITH e_0, $b_0 \gg a_0$

λ_1 and λ_2 are the negatives of the roots of the quadratic equation
$P^2 + (k_1 e_0 + k_{-1} + k_2 b_0)P + k_1 k_2 e_0 b_0 = 0$.

Premixing	*Formation of X*	*Formation of Y*
$E\vert A\vert B$	$x = a_0 + \frac{k_1 k_2 e_0 a_0 b_0}{\lambda_1(\lambda_1 - \lambda_2)} e^{-\lambda_1 t}$	$y = a_0 - \frac{k_1 k_2 k_3 e_0 a_0 b_0}{\lambda_1(\lambda_1 - \lambda_2)(\lambda_1 - k_3)} e^{-\lambda_1 t}$
$E\vert A + B$	$- \frac{k_1 k_2 e_0 a_0 b_0}{\lambda_2(\lambda_1 - \lambda_2)} e^{-\lambda_2 t}$	$+ \frac{k_1 k_2 k_3 e_0 a_0 b_0}{\lambda_2(\lambda_1 - \lambda_2)(\lambda_2 - k_3)} e^{-\lambda_2 t}$
or $E + B\vert A$		$- \frac{k_1 k_2 k_3 e_0 a_0 b_0}{k_3(\lambda_1 - k_3)(\lambda_2 - k_3)} e^{-k_3 t}$
$E + A\vert B$	$x = a_0 + \frac{k_1 k_2 e_0 a_0 b_0 (1 - \lambda_1/k_{-1})}{\lambda_1(\lambda_1 - \lambda_2)} e^{-\lambda_1 t}$	$y = a_0 - \frac{k_1 k_2 k_3 e_0 a_0 b_0 (1 - \lambda_1/k_{-1})}{\lambda_1(\lambda_1 - \lambda_2)(\lambda_1 - k_3)} e^{-\lambda_1 t}$
	$- \frac{k_1 k_2 e_0 a_0 b_0 (1 - \lambda_2/k_{-1})}{\lambda_2(\lambda_1 - \lambda_2)} e^{-\lambda_2 t}$	$+ \frac{k_1 k_2 k_3 e_0 a_0 b_0 (1 - \lambda_2/k_{-1})}{\lambda_2(\lambda_1 - \lambda_2)(\lambda_1 - k_3)} e^{-\lambda_2 t}$
		$- \frac{k_1 k_2 k_3 i e_0 a_0 b_0 (1 - k_3/k_{-1})}{k_3(\lambda_1 - k_3)(\lambda_2 - k_3)} e^{-k_3 t}$

Analysis of experimental results for this situation could be carried out as follows. A plot of ln x against t is biphasic, the slopes of the two lines being $-\lambda_1$ and $-\lambda_2$. From the equation at the top of Table III

$$\lambda_1 + \lambda_2 = k_1e_0 + k_{-1} + k_2b_0 \tag{3}$$

and

$$\lambda_1\lambda_2 = k_1k_2e_0b_0 \tag{4}$$

so that a plot of $\lambda_1 + \lambda_2$ against e_0 gives k_1, and against b_0 gives k_2; k_{-1} can then be obtained. Alternatively, $\lambda_1\lambda_2$ could be plotted against e_0b_0. A similar procedure could be applied to y.

It is to be seen that the different premixing procedures give similar behavior (x biphasic, y triphasic, no steady state). However, with a_0 limiting, premixing E and A gives a faster exponential rise than $E \mid A \mid B$, $E \mid A + B$, *etc.*

Ping pong bi bi mechanism

This mechanism is shown in Fig. 2, which gives the concentrations corresponding to the condition a_0, $b_0 \gg e_0$ (*cf.* also Table I). The resulting expressions for this condition are given in the upper part of Table IV; they were worked out in detail in the previous paper[1]. The λ values are the negatives of the roots of the equation

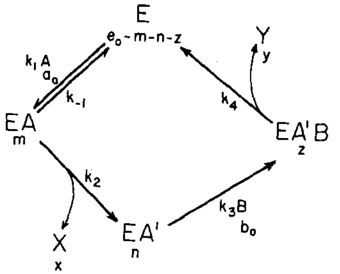

Fig. 2. The ping pong bi bi mechanism, showing the concentrations of the various species for the case a_0, $b_0 \gg e_0$.

$$P^3 + MP^2 + LP + Q = 0 \tag{5}$$

with

$$M = k_1a_0 + k_{-1} + k_2 + k_3b_0 + k_4 \tag{6}$$

$$L = k_3k_4b_0 + (k_3b_0 + k_4)(k_1a_0 + k_{-1} + k_2) + k_1k_2a_0 \tag{7}$$

$$Q = k_3k_4b_0(k_1a_0 + k_{-1} + k_2) + k_1k_2k_4a_0 + k_1k_2k_3a_0b_0 \tag{8}$$

The premixing of E and A gives rise to the product X; a study of its formation in the absence of B is a good way of arriving at the constants k_1, k_{-1} and k_2. The kinetic equation during this premixing process is

$$x = e_0 + \frac{k_1k_2e_0a_0}{\lambda_1(\lambda_1-\lambda_2)} e^{-\lambda_1 t} - \frac{k_1k_2e_0a_0}{\lambda_2(\lambda_1-\lambda_2)} e^{-\lambda_2 t} \tag{9}$$

TABLE IV

RATE CONSTANTS FOR THE PING PONG BI BI MECHANISM, WITH a_0, $b_0 \gg e_0$

Premixing	*Formation of X*	*Formation of Y*
$E \mid A \mid B$	$x = \dfrac{k_1k_2k_3k_4e_0a_0b_0}{k_1k_2k_4a_0+k_3k_4(k_{-1}+k_2)b_0+k_1k_3(k_2+k_4)a_0b_0}\, t$	$y = \dfrac{k_1k_2k_3k_4e_0a_0b_0}{k_1k_2k_4a_0+k_3k_4(k_{-1}+k_3)b_0+k_1k_3(k_2+k_4)a_0b_0}\, t$
$E \mid A + B$ or $E + B \mid A$	$-\sum\limits_{i=1}^{3} \dfrac{k_1k_2e_0a_0(\lambda_i-k_3b_0)(\lambda_i-k_4)}{\lambda_i^2(P-\lambda_i)}\,(1-e^{-\lambda_i t})$	$-\sum\limits_{i=1}^{3} \dfrac{k_1k_2k_3k_4e_0a_0b_0}{\lambda_i^2(P-\lambda_i)}\,(1-e^{-\lambda_i t})$
	$x = x_0 + \dfrac{k_1k_2k_3k_4e_0a_0b_0}{k_3k_4b_0+k_1k_2k_4a_0+k_1k_2k_3a_0b_0}\, t$	$y = \dfrac{k_1k_2k_3k_4e_0a_0b_0}{k_3k_4b_0+k_1k_2k_4a_0+k_1k_2k_3a_0b_0}\, t$
$E + A \mid B$	$-\sum\limits_{i=1} \dfrac{k_1k_2k_3k_4e_0a_0b_0}{\lambda_i^2(P-\lambda_i)}\,(1-e^{-\lambda_i t})$	$-\sum\limits_{i=1}^{3} \dfrac{k_3k_4e_0b_0[\lambda_i^2-\lambda_i(k_1a_0+k_{-1}+k_2)+k_1k_2a_0]}{\lambda_i^2(P-\lambda_i)}\,(1-e^{-\lambda_i t})$

where $-\lambda_1$ and $-\lambda_2$ are the roots of

$$P^2 + (k_1a_0 + k_{-1} + k_2)P + k_1k_2a_0 = 0 \tag{10}$$

The constants k_1, k_{-1} and k_2 can readily be obtained, by analogous methods to those described above and in our previous paper[1].

At the end of the $E + A$ premixing, E has all been converted into EA'; the initial concentration of this is therefore e_0. The kinetic equations for the rates of formation of X and Y for this case are given in the lower part of Table IV; the λ values are the negatives of the roots of the equation

$$P^3 + MP^2 + NP + Q = 0 \tag{11}$$

with

$$M = k_1a_0 + k_{-1} + k_2 + k_3b_0 + k_4 \tag{12}$$

$$N = k_1k_2a_0 + (k_3b_0 + k_4)\,(k_1a_0 + k_{-1} + k_2) \tag{13}$$

$$Q = k_3k_4b_0 + k_1k_2k_3a_0b_0 + k_1k_2k_4a_0 \tag{14}$$

The determination of rate constants can be carried out as previously. It will be noted that for this case of $E + A \mid B$ the steady-state rates are the same for X as for Y, but that they are different from those for $E \mid A \mid B$.

The equations for the case in which the concentration of A is limiting (e_0, $b_0 \gg a_0$) are given in Table V. The λ_1 and λ_2 values for the first premixing case are the negatives of the roots of

$$P^2 + P(k_1e_0 + k_{-1} + k_2) + k_1k_2e_0 = 0 \tag{15}$$

and λ_3 is k_3b_0. In the case of $E + A$ premixing, A is all converted into EA' during the premixing, and there is no further production of X after B is added; the equation for the formation of Y is given in Table V.

Again, we may wish to measure X formation during premixing of E with A, in the absence of B. The equation for this, with $e_0 \gg a_0$, is

$$x = a_0 + \frac{k_1k_2e_0a_0}{\lambda_1(\lambda_1-\lambda_2)}\,e^{-\lambda_1 t} - \frac{k_1k_2e_0a_0}{\lambda_2(\lambda_1-\lambda_2)}\,e^{-\lambda_2 t} \tag{16}$$

where λ_1 and λ_2 are the negatives of the roots of the equation

$$P^2 + (k_1e_0 + k_{-1} + k_2)P + k_1k_2e_0 = 0 \tag{17}$$

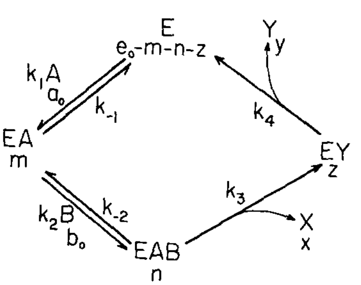

Fig. 3. The ordered ternary-complex mechanism, showing the concentrations of the various species for the case a_0, $b_0 \gg e_0$.

TABLE V

RATE CONSTANTS FOR THE PING PONG BI BI MECHANISM ,WITH e_0, $b_0 \gg a_0$

Premixing	*Formation of X*	*Formation of Y*
$E\|A\|B$ $E\|A+B$ or $E+B\|A$	$x = a_0 + \frac{k_1k_2e_0a_0}{\lambda_1(\lambda_1-\lambda_2)} e^{-\lambda_1 t} - \frac{k_1k_2e_0a_0}{\lambda_2(\lambda_1-\lambda_2)} e^{-\lambda_2 t}$	$y = a_0 + \frac{k_1k_2k_3k_4e_0a_0b_0}{\lambda_1(\lambda_1-\lambda_2)(\lambda_1-\lambda_3)(\lambda_1-k_4)} e^{-\lambda_1 t} - \frac{k_1k_2k_3k_4e_0a_0b_0}{\lambda_2(\lambda_1-\lambda_2)(\lambda_2-\lambda_3)(\lambda_2-k_4)} e^{-\lambda_2 t} + \frac{k_1k_2k_3k_4e_0a_0b_0}{\lambda_3(\lambda_1-\lambda_3)(\lambda_2-\lambda_3)(\lambda_3-k_4)} e^{-\lambda_3 t} - \frac{k_1k_2k_3k_4e_0a_0b_0}{k_4(\lambda_1-k_4)(\lambda_2-k_4)(\lambda_3-k_4)} e^{-k_4 t}$
$E+A\|B$	No further formation of X	$y = a_0 - \frac{k_4a_0}{k_4-k_3b_0} e^{-k_3 b_0 t} + \frac{k_3b_0a_0}{k_4-k_0b_0} e^{-k_4 t}$

TABLE VI

RATE CONSTANTS FOR THE ORDERED TERNARY COMPLEX MECHANISM, WITH a_0, $b_0 \gg e_0$

The λ values are the negatives of the roots of the polynominal

$P^3+MP^2+LP+Q = 0$

with

$M = k_1a_0+k_4+k_{-1}+k_{-2}+k_3+k_2b_0$

$L = k_4(k_1a_0+k_{-1}+k_2b_0+k_{-2}+k_3)+k_{-2}(k_1a_0+k_{-1})+k_3(k_1a_0+k_{-1}+k_2b_0)+k_1k_2a_0b_0$

$Q = k_{-2}k_4(k_1a_0+k_{-1})+k_3k_4(k_1a_0+k_{-1}+k_2b_0)+k_1k_2k_4a_0b_0+k_1k_2k_3a_0b_0$

Premixing	*Formation of X*	*Formation of Y*
$E\|A\|B$	$x = \frac{k_1k_2k_3k_4e_0a_0b_0}{k_{-2}k_4(k_1a_0+k_{-1})+k_3k_4(k_1a_0+k_{-1}+k_2b_0) + k_1k_2(k_3+k_4)a_0b_0} t$	$y = \frac{k_1k_2k_3k_4e_0a_0b_0}{k_{-2}k_4(k_1a_0+k_{-1})+k_3k_4(k_1a_0+k_{-1}+k_2b_0) + k_1k_2(k_3+k_4)a_0b_0}$
$E\|A+B$ or $E+B\|A$	$-\sum_{i=1}^{3} \frac{k_1k_2k_3k_4e_0a_0b_0(k_4-\lambda_i)}{\lambda_j^2(P-\lambda_j)} (1-e^{-\lambda_i t})$	$-\sum_{i=1}^{3} \frac{k_1k_2k_3k_4e_0a_0b_0}{\lambda_i^2(P-\lambda_i)} (1-e^{-\lambda_i t})$
	$x = \frac{k_1k_2k_3k_4e_0a_0b_0}{k_{-2}k_4(k_1a_0+k_{-1})+k_3k_4(k_1a_0+k_{-1}+k_2b_0) + k_1k_2(k_3+k_4)a_0b_0} t$	$y = \frac{k_1k_2k_3k_4e_0a_0b_0}{k_{-2}k_4(k_1a_0+k_{-1})+k_3k_4(k_1a_0+k_{-1}+k_2b_0) + k_1k_2(k_3+k_4)a_0b_0}$
$E+A\|B$	$-\sum_{i=1}^{3} \frac{k_1k_2k_3e_0a_0b_0(1-\lambda_i/k_{-1})(k_4-\lambda_i)}{\lambda_i^2(P-\lambda_i)} (1-e^{-\lambda_i t})$	$-\sum_{i=1}^{3} \frac{k_1k_2k_3k_4e_0a_0b_0(1-\lambda_i/k_{-1})}{\lambda_i^2(P-\lambda_i)} (1-e^{-\lambda_i t})$

TABLE VII

RATE CONSTANTS FOR THE ORDERED TERNARY COMPLEX MECHANISM, WITH e_0, $b_0 \gg a_0$

The values are the negatives of the roots of the polynominal

$P^3 + MP^2 + LP + Q = 0$

with

$M = k_1e_0 + k_{-1} + k_2b_0 + k_{-2} + k_3$

$L = (k_{-2} + k_3)(k_1e_0 + k_{-2}) + k_2k_3b_0 + k_1k_2e_0b_0$

$Q = k_1k_2k_3b_0e_0.$

Premixing	*Formation of X*	*Formation of Y*
$E\|A\|B$, $E\|A+B$ or $E+B\|A$	$x = a_0 - \sum_{i=1}^{3} \frac{k_1k_2k_3e_0a_0b_0}{\lambda_i(P-\lambda_i)} e^{-\lambda_i t}$	$y = a_0 - \sum_{i=1}^{4} \frac{k_1k_2k_3k_4e_0a_0b_0}{\lambda_i(P-\lambda_i)} e^{-\lambda_i t}$
$E+A\|B$	$x = a_0 - \sum_{i=1}^{3} \frac{k_1k_2k_3e_0a_0b_0(1-\lambda_i/k_{-1})}{\lambda_i(P-\lambda_i)} e^{-\lambda_i t}$	$y = a_0 - \sum_{i=1}^{4} \frac{k_1k_2k_3k_4e_0a_0b_0(1-\lambda_i/k_{-1})}{\lambda_i(P-\lambda_i)} e^{-\lambda_i t}$

The results for this case are easily analyzed to give k_1, k_{-1} and k_2.

This ping pong bi bi mechanism is easily distinguished from the others on the basis of transient-phase studies, since it is the only one in which product X is produced in the absence of one reactant B. The reactants A and B are readily distinguished in this way, and rate constants are easily determined.

Ordered ternary-complex mechanism

The various solutions for the ordered ternary-complex mechanism (Fig. 3) are given in Tables VI and VII. Analysis of results can be carried out by methods similar to those considered previously. Again, premixing of E with A gives a more rapid pre-steady state period; premixing of E with B has no effect.

Random ternary-complex mechanism

Even the steady-state equation[5] for the random ternary-complex mechanism (Fig. 4) is extremely complicated, too much so to be applied to experimental results. The pre-steady-state equations are even more complicated; they can be worked out

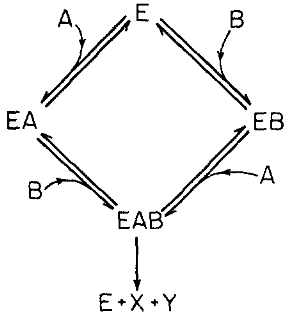

Fig. 4. The random ternary-complex mechanism.

by the Laplace transform method, but would be useless for interpreting experimental results.

On the qualitative side it can be noted that for this mechanism premixing of E with either A or B will lead to no change in steady-state kinetics, but to an acceleration of the pre-steady-state kinetics relative to $E \mid A \mid B$, $E \mid A + B$, *etc.* This allows a clear distinction of this mechanism from the other three, for which $E + B$ premixing cannot increase rates (it may decrease them, if an abortive EB complex is formed).

DISCUSSION

The main conclusions of the present work are summarized in Table VIII. They can be further summarized as follows:

TABLE VIII

SUMMARY OF KINETIC EQUATIONS

Initial mixing conditions	*Formation of X*	*Formation of Y*	*Remarks*
$E\mid A\mid B;\ E\mid A + B;\ E + B\mid A;\ a_0, b_0 \gg e_0$	Biphasic	Biphasic	Steady state established
$E + A\mid B;\ a_0, b_0 \gg e_0$	Biphasic	Biphasic	Faster rise to steady state
$E\mid A\mid B;\ E\mid A + B;\ E + B\mid A;\ e_0, b_0 \gg a_0$	Biphasic	Triphasic	No steady state
$E + A\mid B;\ b_0, e_0 \gg a_0$	Biphasic	Triphasic	No steady state; faster rise
Ping pong bi bi			
$E\mid A\mid B;\ E\mid A + B;\ E + B\mid A;\ a_0, b_0 \gg e_0$	Triphasic	Triphasic	Steady state
$E + A\mid B;\ a_0, b_0 \gg e_0$	Triphasic	Triphasic	Faster rise to steady state
$E\mid A\mid B;\ E\mid A + B;\ E + B\mid A;\ e_0, b_0 \gg a_0$	Biphasic	Quadriphasic	No steady state
$E + A\mid B;\ e_0, b_0 \gg a_0$	No further formation of X	Biphasic	No steady state; faster production of Y
Ordered ternary complex			
$E\mid A\mid B;\ E\mid A + B;\ E + B\mid A;\ a_0, b_0 \gg e_0$	Triphasic	Triphasic	Steady state
$E + A\mid B;\ a_0, b_0 \gg e_0$	Triphasic	Triphasic	Faster rise to steady state
$E\mid A\mid B;\ E\mid A + B;\ A + B\mid A;\ e_0, b_0 \gg a_0$	Triphasic	Quadriphasic	No steady state
$E + A\mid B;\ e_0, b_0 \gg a_0$	Triphasic	Quadriphasic	No steady state, faster rise

(1) When the concentration of E is limiting, there is always a steady state preceded by a phase consisting of the sum of exponentials.

(2) When the concentration of the first substrate A is limiting there is no steady state; the concentrations of X and Y rise to a limiting value equal to the initial concentration of A, the rise being represented by the sum of exponentials. (The case of B being limiting was also investigated, but no analytical solution appears to be possible: presumably, there would again be no steady-state, the concentrations of X and Y rising to the initial concentration of B.)

(3) Premixing of E and B affects the kinetics only in the case of the random ternary-complex mechanism; it leads to more rapid initial reaction as compared with $E \mid A \mid B$, *etc.*

(4) Premixing E and A leads to product (X) formation only in the case of the ping pong mechanism.

(5) Premixing E and A followed by addition of B leads to more rapid initial

reaction, but does not affect the number of phases, except for the ping pong bi bi mechanism.

(6) Premixing does not affect the steady-state kinetics, except for the ping pong bi bi mechanism; this is a special case, because the starting material is now altered.

Results for horse liver alcohol dehydrogenase

Recently some transient-phase studies[6–9] have been carried out with horse liver alcohol dehydrogenase, and in particular, Luisi and Favilla[9] have studied the effect of premixing the enzyme and co-enzyme. The results have been interpreted as implying that there are two catalytic sites on the enzyme. However, the theoretical equations derived in the present paper show that no such conclusion is necessary; the experimental results are consistent with a Theorell–Chance mechanism occurring at a single catalytic site.

The main experimental results of the studies, with premixing of E and A (co-enzyme), can be summarized as follows, with reference to the theoretical treatment of the present paper:

(1) With $e_0 \gg a_0$ or b_0 there is a biphasic exponential formation of product X, completion of the reaction corresponding to depletion of the limiting reagent. This is consistent with the equation given in Table III for the Theorell–Chance mechanism. Two catalytic sites are not required to explain the biphasic character.

(2) During the biphasic formation of product X there is an initial exponential formation of approximately half of the product, followed by a slower exponential formation of the other half. This is explained by the equation in Table III if λ_1 and λ_2 are fairly close to one another. The exponential terms (amplitude factors) are then approximately equal, leading to the result that $a_0/2$ will be produced initially followed by the remaining $a_0/2$.

(3) A similar result was found with a_0, $b_0 \gg e_0$ and $E + A \mid B$; in this case a steady state is attained. There was an initial rapid production of X, followed by slower exponential processes; this is predicted by the equations in Table II.

(4) The amplitudes of the pre-exponential terms under the above conditions were proportional to e_0, a_0 and b_0. This is predicted by the equations in Table II.

We conclude that the results are fully explained by the equations in Tables II and III of the present paper, and that the transient-phase studies provide no evidence for two catalytic sites.

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