Biochimica et Biophysica Acta, 364 (1974) 181–192

BBA 67315

THE BEHAVIOUR OF COUPLED ENZYME SYSTEMS IN THE TRANSIENT AND STEADY-STATE REGIONS OF THE REACTION

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(Received May 27th, 1974)

SUMMARY

The analytical solutions for the pre-steady-state phase of a coupled enzymic reaction, where two types of conditions are fulfilled, are developed. To illustrate the types of solutions obtainable, only single- and double-intermediate enzyme mechanisms are considered; the method can clearly be extended to more complex cases.

The differential equations describing the coupled reactions are linearized by utilizing two methods similar to those previously used in the case of single enzymes (Gutfreund, H. (1955) Disc. Far. Soc. 20, 167; Laidler, K. J. (1955) Can. J. Chem. 33, 1614; Swoboda, P. A. T. (1957) Biochim. Biophys. Acta 23, 70; Hijazi, N. H. and Laidler, K. H. (1972) Can. J. Chem. 50, 1440).

The differential equations are solved by the Laplace–Carson operator procedure to give solutions that express concentrations of reactants as a function of time (Rodiguin, N. M. and Rodiguina, E. N. (1964) Consecutive Chemical Reactions: Mathematical Analysis and Development (Sneider, R. F., ed.), Engl. edn, D. van Nostrand, Princeton, N.J.). Methods, using a digital computer and the analytical solutions developed herein, are described for the fitting of experimental data from coupled reactions in order to evaluate individual rate constants for the first enzyme; given that the second enzyme can be studied independently and the eigen values controlling the exponential decay of the pre-steady state are known.

Mention is made of the use of the equations developed with regard to detecting possible heterologous enzyme interactions, in coupled enzymic assays, studied in the pre-steady state.

INTRODUCTION

Until now there have been no attempts to derive kinetic equations that adequately describe the pre-steady state behaviour of coupled enzyme systems, although there have been a number of procedures published that deal with the steady-state region of the reaction (McClure [6], Hess and Wurster [7], Easterby [8], Kuchel et al. [9]).

Steady-state analysis of data from coupled systems utilizing the methods cited in the references above, can only yield the steady-state parameters, V and K_m and

steady-state isomerization constants. Only by an analysis of the transient phases of each reaction can the individual unitary rate constants be obtained.

Analytical solutions of the differential equations that describe the simple Michaelis–Menten enzyme mechanism can only be obtained under two conditions: (a) when the initial substrate concentration is much greater than the initial enzyme concentration, and (b) where the enzyme concentration is much greater than the initial substrate concentration.

These conditions enable the concentration of the species in highest concentration to be considered a constant. The conditions therefore convert the otherwise second-order non-linear (second degree in the first derivative) differential equation into a linear form, which is readily solvable.

In the first case, (a), the solution describes a transient phase at early times in the reaction followed later by a steady state of *ES* complex. In the second case, (b), there is no steady state of any reacting species and the reaction can only be followed by more specialised fast reaction techniques.

Both conditions and forms of solution are used in this paper with reference to coupled enzyme systems. The solutions are formulae that describe the concentration of the various chemical species as explicit functions of time, and are thus in the form required for analysing experimental data.

CASE 1. LOW ENZYME CONCENTRATIONS; SINGLE-INTERMEDIATE ENZYMES

Consider the case of two single-intermediate enzymes acting consecutively. This can be described by the following reaction scheme

$$\begin{aligned} E + S &\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \\ F + P &\underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} FP \xrightarrow{k_4} F + R \end{aligned} \tag{1}$$

In the transient phase of an enzymic reaction the amount of product formed is of the same order of magnitude as the amount of enzyme present. If the concentration of the coupling enzyme $[F]$, is sufficiently high, then during this transient phase (and perhaps longer) the amount of F bound as FP will be much smaller than the concentration of free enzyme, $[F]$. Under these conditions, $[F]$ can be regarded as a constant and equal to the initial concentration $[F_0]$, i.e. $[F] \gg [FP]$, $[F] \approx [F_0]$. The additional assumption made in this case is $[S_0] \gg [E_0]$ and so $[S] \approx [S_0]$ for the time domain of interest. The differential equations that describe the above system are given below in which the differential $(\mathrm{d}/\mathrm{d}t)$ has been replaced by the Laplace–Carson operator ψ [5] and the transformed time-dependent species by lower-case letters.

$$\psi[e] - \psi[E_0] = -k_1[e]\,[S_0] + (k_{-1} + k_2)\,[es] \tag{2a}$$
$$\psi[es] = k_1[e]\,[S_0] - (k_{-1} + k_2)\,[es] \tag{2b}$$
$$\psi[p] = k_2[es] - k_3[F_0]\,[p] + k_{-3}[fp] \tag{2c}$$
$$\psi[fp] = k_3[F_0]\,[p] - (k_{-3} + k_4)\,[fp] \tag{2d}$$
$$\psi[r] = k_4[fp] \tag{2e}$$

It is possible to derive expressions for the chemical species in terms of the various rate constants, the operator ψ, and the initial enzyme and substrate concentrations. For instance, from Eqns 2a and 2b, the transform for $[e]$ is

$$[e] = \frac{(\psi + k_E)\,[E_0]}{(\psi + \lambda_1)} \qquad (k_E = k_{-1} + k_2) \tag{3a}$$

where $\lambda_1 = k_1[S_0] + k_{-1} + k_2$. The other transforms are

$$[es] = \frac{k_1\,[E_0]\,[S_0]}{(\psi + \lambda_1)} \tag{3b}$$

$$[p] = \frac{k_1 k_2\,[E_0]\,[S_0]\,(\psi + k_F)}{(\psi + \lambda_1)\,(\psi + \lambda_2)\,(\psi + \lambda_3)} \tag{3c}$$

where $k_F = k_{-3} + k_4$

$$[fp] = \frac{k_1 k_2 k_3\,[E_0]\,[S_0]\,[F_0]}{(\psi + \lambda_1)\,(\psi + \lambda_2)\,(\psi + \lambda_3)} \tag{3d}$$

$$[r] = \frac{k_1 k_2 k_3 k_4\,[E_0]\,[S_0]\,[F_0]}{\psi(\psi + \lambda_1)\,(\psi + \lambda_2)\,(\psi + \lambda_3)} \tag{3e}$$

where

$$\lambda_2 = \frac{A + \sqrt{A^2 - 4B}}{2}$$

$$\lambda_3 = \frac{A - \sqrt{A^2 - 4B}}{2}$$

$$A = k_3[F_0] + k_{-3} + k_4$$

$$B = k_3 k_4 [F_0]$$

The behaviour of the final product $[R]$ with time is obtained from its respective transform. The original for $[r]$ is obtained from tables of the Laplace–Carson transforms and their inverses [5] and is given by

$$[R] = \frac{\prod_{i=1}^{4} k_i\,[E_0]\,[F_0]\,[S_0]}{\lambda_1\lambda_2\lambda_3} - P_1\,(1 - e^{-\lambda_1 t}) - P_2\,(1 - e^{-\lambda_2 t}) - P_3\,(1 - e^{-\lambda_3 t}) \tag{4a}$$

where

$$P_i = \frac{\prod_{i=1}^{4} k_i\,[E_0]\,[F_0]\,[S_0]}{\lambda_i^2 \prod_{\substack{j=1 \\ j \neq i}}^{3} (\lambda_j - \lambda_i)}$$

Substitution for the λ_i in the first term of Eqn 4a gives

$$[R] = v_0\,t - \sum_{i=1}^{3} P_i\,(1 - e^{-\lambda_i t}) \tag{4b}$$

where

$$v_0 = \frac{k_2 [E_0] [S_0]}{[S_0] + K_E}$$

and

$$K_E = \frac{(k_{-1} + k_2)}{k_1}.$$

Each constant P_i can be expressed in terms of only v_0 and the λ_i

$$P_i = \frac{v_0 \prod_{i=1}^{3} \lambda_i}{\lambda_i^2 \prod_{\substack{j=1 \\ j \neq i}}^{3} (\lambda_j - \lambda_i)}$$

Hence

$$[R] = v_0 \left(t - \sum_{i=1}^{3} \frac{(1 - e^{-\lambda_i t})}{\lambda_i^2} \frac{\prod_{j=1}^{3} \lambda_i}{\prod_{\substack{j=1 \\ j \neq i}}^{3} (\lambda_j - \lambda_i)} \right) \tag{4c}$$

and hence the equation for $[R]$ is fully described by the four constants v_0, λ_1, λ_2 and λ_3.

If the concentration of the second enzyme is high enough then it is possible that the concentration of the first product, $[P]$, will remain much lower than the K_m of the second enzyme for appreciable times even into the steady-state region of the reaction. Under these conditions the amount of enzyme F bound as FP will remain much less than the concentration of free enzyme $[F]$ and hence Eqn 4b will remain valid. As t increases the exponential terms become vanishingly small (λ_1, λ_2 and λ_3 are all positive) and the equation reduces to

$$[R] = v_0 t - \sum_{i=1}^{3} P_i$$

Therefore, as t increases a plot of $[R]$ versus t becomes linear with a slope, v_0, equal to the velocity of the first reaction. The linear region when extrapolated back cuts the t axis at

$$t = \tau = \sum_{i=1}^{3} P_i / v_0$$

and intersects the $[R]$ axis at

$$[R] = -\sum_{i=1}^{3} P_i = -v_0 \tau = \beta$$

Since, in general $\lambda_2 \gg \lambda_3$, it follows that

$$\lambda_3 \approx \frac{\lambda_2 \lambda_3}{\lambda_2 + \lambda_3} = \frac{k_3 k_4 [F_0]}{k_3 [F_0] + k_{-3} + k_4} = \frac{V_F}{[F_0] + K_F} \approx \frac{V_F}{K_F} \tag{5}$$

where V_F and K_F are the steady-state parameters V and K_m for the second reaction. If $\lambda_1 \gg \lambda_3$, then $P_3 \gg P_1$, P_2 and the intercept on the $-[R]$ axis approximates to P_3, but

$$P_3 = \frac{v_0\lambda_1\lambda_2\lambda_3}{\lambda_3^2(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)} \approx \frac{v_0}{\lambda_3} = \frac{v_0 K_F}{V_F}$$

Under these particular conditions the intercept on the $-[R]$ axis $v_0 K_F/V_F$ agrees with the steady-state treatment [7, 8].

Fig. 1 shows the behaviour of the analytical solution (Eqn 4b) for various concentrations of the second enzyme compared with the solution of the original differential equations using a numerical integration procedure. The numerical integration was carried out using a computer simulation program (Elmore and Roberts, unpublished work) incorporating a subroutine specifically designed for the solution of

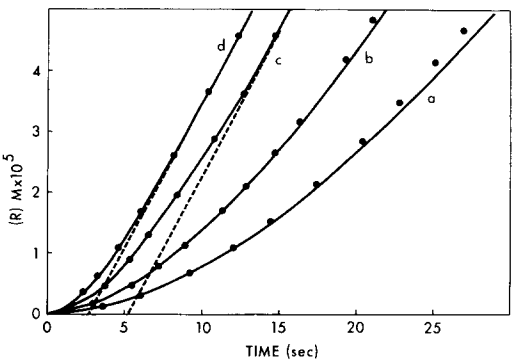

Fig. 1. A comparison of the analytical solution (●) given by Eqn 4c with the solution (------) obtained by the numerical integration of the basic differential equations given in Eqn 2. For all curves, a, b, c and d, $k_1 = 10^7$, $k_{-1} = 10^4$, $k_2 = 10^3$, $k_3 = 2 \cdot 10^7$, $k_{-3} = 2 \cdot 10^4$, $k_4 = 2 \cdot 10^3$, $[S_0] = 10^{-3}$ M and $[E_0] = 10^{-8}$ M. The various concentrations of the second enzyme, $[F_0]$, are: a, $2 \cdot 10^{-8}$ M; b, $4 \cdot 10^{-8}$ M; c, $1 \cdot 10^{-7}$ M; and d, $2 \cdot 10^{-7}$ M. The slopes of the dotted lines in Curves c and d give the velocity (v_0) of the respective reactions after attainment of the steady state of $[P]$ and the intercept on the t axis yields τ; expressions for v_0 and τ are given in the text under Case 1.

stiff equations (Gear [10]). The data is input in the form of chemical equations as in Eqn 1 with the relevant kinetic constants and initial concentrations. Obviously, for Curve a and to a lesser extent b in Fig. 1, the concentration of the coupling enzyme $[F_0]$ is too low for the assumptions made in the derivation of the analytical solution to remain valid into the steady-state region.

From Eqn 4b

$$[R] - v_0 t - \beta = \sum_{i=1}^{3} P_i e^{-\lambda_i t} \tag{6}$$

and so a plot of log $([R] - v_0 t - \beta)$ against t will have three linear phases corresponding to the regions where λ_1, λ_2 or λ_3 dominates. Over long times Eqn 6 reduces to a single exponential and hence the λ_i and P_i corresponding to the slowest exponential decay, can be obtained from the slope and ordinal intercept of the log plot. Subtraction of these values ($P_j e^{-\lambda_j t}$) from the left-hand side of Eqn 6 and plotting

$\log([R] - v_0 t - \beta - P_j e^{-\lambda_j t})$ against t allows the next exponential to be determined. In some cases the curves will only show one or two linear sections. This can arise because one or more of the λ_i are too fast to be followed by the experimental apparatus or because two of the λ_i are similar in value. In either case complete analysis of the system by the above method would be impossible.

Since the second enzyme is chosen because its product can be conveniently monitored, λ_2 and λ_3 can be determined from an independent study of the reaction of F with P, (pre-steady state and steady state) under the same conditions as the coupled system, e.g. pH, ionic strength, buffer type. The pre-steady-state study of the "auxiliary" or second enzyme can be carried out with $[P_0] \gg [F_0]$ or $[F_0] \gg [P_0]$. In the latter case the transform for $[R]$ is

$$[r] = \frac{k_3 k_4 [F_0] [P_0]}{(\psi + \lambda_2)(\psi + \lambda_3)} \tag{7a}$$

and the original is therefore

$$[R] = \frac{k_3 k_4 [F_0] [P_0]}{\lambda_2 \lambda_3} - P_1 e^{-\lambda_2 t} - P_2 e^{-\lambda_3 t} \tag{7b}$$

where

$$P_1 = \frac{k_3 k_4 [F_0] [P_0]}{\lambda_2 (\lambda_3 - \lambda_2)}$$

and

$$P_2 = \frac{k_3 k_4 [F_0] [P_0]}{\lambda_3 (\lambda_2 - \lambda_3)}.$$

The definitions of λ_2 and λ_3 under the conditions of $[F_0] \gg [P_0]$ are the same as Eqn 3. From Eqn 7b the first term is simply $[P_0]$ hence

$$[P] = [P_0] - \frac{\lambda_3 e^{-\lambda_2 t}}{(\lambda_3 - \lambda_2)} - \frac{\lambda_2 e^{-\lambda_3 t}}{(\lambda_2 - \lambda_3)}$$

The data can be analysed by the logarithmic plots outlined previously. The individual rate constants for the second reaction can be obtained from plots of $(\lambda_2 + \lambda_3)$ and $\lambda_2 \lambda_3$ against $[F_0]$, and making use of the steady-state data, $V_F = k_4[F_0]$ and $K_F = (k_{-3} + k_4)/k_3$. If $\lambda_2 \gg \lambda_3$ then from Eqn 5 a plot of $1/\lambda_3$ against $1/[F_0]$ will have a slope of K_F/k_4 and an ordinal intercept of $1/k_4$. The determination of λ_2 and λ_3 from an independent study of the second reaction leaves only λ_1 unknown in Eqn 4b.

The method suggested here is to use a computer to generate results using the analytical solution of the differential equations, Eqn 4b. These are very readily programmed on a small computer and are very convenient for demand terminal use in conjunction with a plotter. In the case of the coupled reaction discussed above, with v_0 known from the linear region of the reaction, it only remains to adjust the value of λ_1 until an optimum fit is obtained to the experimental data. The assumptions made in the derivation of the analytical solution can be checked by determining v_0 for various concentrations of $[F_0]$. The steady-state velocity will increase to a maximum limiting velocity (v_0) with increasing $[F_0]$ and there will be a corresponding decrease

in the intercept β. If there is no change in v_0 with increasing $[F_0]$ then the analytical solution is valid for the experimental system under examination.

The second and more general, but tedious approach is to numerically integrate the original differential equations for differing values of k_1, k_{-1}, and k_2 and the result compared with the experimental curve. The values of the rate constants can be refined manually or automatically (Curtis and Chance [11])and the process repeated. Since values of K_E and V_E can be obtained from the variation of the steady-state velocity v_0 with initial substrate concentration $[S_0]$, the values of the rate constants are not independent but are related by the definitions of K_E and V_E. Even so, the process of simulation and optimization for enzyme systems in which the differential equations are usually "stiff", can be quite slow and tedious with respect to computing time.

However, by judicious control of experimental conditions, in particular with respect to relative enzyme concentrations, what normally is a major computing exercise, reduces to a very simple one, using the analytical solutions described herein.

Finally, analog computers can be used for fitting data from coupled and other reactions. They are more susceptible to operator interaction, but the general scarcity of large computers of this type necessitates an alternative approach.

CASE 2. LOW ENZYME CONCENTRATIONS; DOUBLE-INTERMEDIATE ENZYMES

The analytical method can easily be extended to the case of two coupled, three-step enzyme reactions, as indicated in the following scheme.

$$\begin{aligned} E + S &\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow[P_1]{k_2} EP \xrightarrow{k_3} E + P \\ F + P &\underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} FP \xrightarrow[R_1]{k_5} FR \xrightarrow{k_6} F + R \end{aligned} \tag{8}$$

The double-intermediate mechanism allows the possibility of the second enzyme being coupled with either the first product P_1, or with the second product P. The second reaction can also be monitored by observing the formation of R_1 or R, for the scheme shown above. The transform for $[r]$ is found to be

$$[r] = \frac{k_1 k_2 k_3 k_4 k_5 k_6 [E_0] [F_0] [S_0]}{\psi(\psi + \lambda_1)(\psi + \lambda_2)(\psi + \lambda_3)(\psi + \lambda_4)(\psi + \lambda_5)} \tag{9}$$

where

$$\lambda_1 = (\mathrm{A} + \sqrt{\mathrm{A}^2 - 4\mathrm{B}})/2$$
$$\lambda_2 = (\mathrm{A} - \sqrt{\mathrm{A}^2 - 4\mathrm{B}})/2$$
$$\mathrm{A} = k_1[S_0] + k_{-1} + k_2 + k_3$$
$$\mathrm{B} = k_1(k_2 + k_3)([S_0] + k_m^E)$$
$$k_m^E = k_3(k_{-1} + k_2)/(k_1(k_2 + k_3))$$
$$\lambda_3 = (\mathrm{C} + \sqrt{\mathrm{C}^2 - 4\mathrm{D}})/2$$
$$\lambda_4 = (\mathrm{C} - \sqrt{\mathrm{C}^2 - 4\mathrm{D}})/2$$
$$\mathrm{C} = k_4[F_0] + k_{-4} + k_5$$
$$\mathrm{D} = k_4 k_5 [F_0]$$
$$\lambda_5 = k_6$$

Since $\lambda_1 \lambda_2 = k_1 (k_2 + k_3) ([S_0] + K_m^E)$, and $\lambda_3 \lambda_4 = k_4 k_5 [F_0]$, the original for $[R]$ is

$$[R] = v_0 t - \sum_{i=1}^{5} \frac{(1 - e^{-\lambda_i t})}{\lambda_i^2} \frac{\prod_{i=1}^{5} \lambda_i}{\prod_{\substack{j=1 \\ j \neq i}}^{5} (\lambda_j - \lambda_i)}$$

The behaviour of an $[R]$ versus t curve is similar to that for the simpler scheme. With the additional exponential terms due to the new complexes EP and FR, it is even more essential to be able to study the second reaction alone in order to eliminate some of the otherwise unknown λ_i values.

CASE 3. HIGH ENZYME CONCENTRATIONS; SINGLE-INTERMEDIATE ENZYMES

The reaction scheme outlined in Eqn 1 can also be studied under conditions where the concentrations of both enzymes are sufficiently high that they can be regarded as constant, i.e. $[E_0] \gg [S_0]$ and $[F_0] \gg [P]$ (at any time of interest during the reaction). The operator equation for $[e]$ Eqn 2a is replaced by one for $[s]$

$$\psi[s] - \psi[S_0] = -k_1 [E_0] [s] + k_{-1} [es] \tag{10}$$

and the equation for $[es]$ in Eqn 2b is changed to

$$\psi[es] = k_1 [E_0] [s] - (k_{-1} + k_2) [es]$$

the remaining equations are the same as Eqns 2c, 2d and 2e.

The transform for $[s]$ is found by rearranging Eqn 10, to be

$$[s] = \frac{[S_0] \psi(\psi + k_E)}{(\psi + \lambda_1)(\psi + \lambda_2)}$$

where

$$\lambda_1 = (A + \sqrt{A^2 - 4B})/2$$
$$\lambda_2 = (A - \sqrt{A^2 - 4B})/2$$
$$A = k_1 [E_0] + k_{-1} + k_2$$
$$B = k_1 k_2 [E_0]$$

The behaviour of the final product $[R]$, with respect to time, is given by the inverse of the transform

$$[r] = \frac{k_1 k_2 k_3 k_4 [E_0] [F_0] [S_0]}{(\psi + \lambda_1)(\psi + \lambda_2)(\psi + \lambda_3)(\psi + \lambda_4)} \tag{11}$$

where λ_3 and λ_4 are defined as in Eqn 3.

The solution for $[R]$ from Eqn 11 is therefore

$$[R] = \frac{k_1 k_2 k_3 k_4 [E_0] [F_0] [S_0]}{\lambda_1 \lambda_2 \lambda_3 \lambda_4} - \sum_{i=1}^{4} P_i e^{-\lambda_i t}$$

where

$$P_i = \frac{\prod_{i=1}^{4} k_i [E_0] [S_0] [F_0]}{\lambda_i \prod_{\substack{j=1 \\ j \neq i}}^{4} (\lambda_j - \lambda_i)}$$

Substituting for $\lambda_1 \lambda_2 = k_1 k_2 [E_0]$ and $\lambda_3 \lambda_4 = k_3 k_4 [F_0]$ the equation for $[R]$ becomes

$$[R] = [S_0] \left(1 - \sum_{i=1}^{4} e^{-\lambda_i t} \prod_{\substack{j=1 \\ j \neq i}}^{4} \frac{\lambda_j}{(\lambda_j - \lambda_i)} \right) \tag{12}$$

Under these conditions, there is no steady state, the product $[R]$ rises from zero in a complicated exponential fashion to a final concentration $[S_0]$. The behaviour of $[R]$ for a particular arbitrary set of rate constants and initial concentrations is shown in Fig. 2.

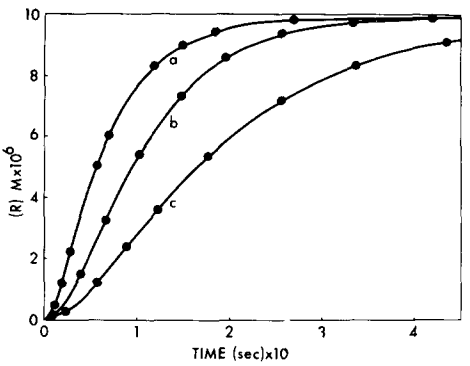

Fig. 2. A comparison of the analytical solution (●) given by Eqn 12 with the solution (———) obtained by numerical integration of the basic differential equations of the scheme described in Case 3. For all curves, $k_1 = 10^7$, $k_{-1} = 10^4$, $k_2 = 10^3$, $k_3 = 2 \cdot 10^7$, $k_{-3} = 2 \cdot 10^4$, $k_4 = 2 \cdot 10^3$, $[S_0] = 10^{-5}$ M, $[F_0] = 10^{-5}$ M. The various concentrations of the first enzyme, $[E_0]$, are: a, $7.5 \cdot 10^{-6}$ M; b, $2 \cdot 10^{-5}$ M; and c, $8 \cdot 10^{-5}$ M.

In general $\lambda_1 \gg \lambda_2$ and $\lambda_3 \gg \lambda_4$, and under these conditions

$$\lambda_2 \approx \frac{\lambda_1 \lambda_2}{\lambda_1 + \lambda_2} = \frac{k_1 k_2 [E_0]}{k_1 [E_0] + k_{-1} + k_2} = \frac{V_E}{[E_0] + K_E}$$

$$\lambda_4 \approx \frac{\lambda_3 \lambda_4}{\lambda_3 + \lambda_4} = \frac{k_3 k_4 [F_0]}{k_3 [F_0] + k_{-3} + k_4} = \frac{V_F}{[F_0] + K_F}$$

and hence plots of $1/\lambda_2$ against $1/[E_0]$ and $1/\lambda_4$ against $1/[F_0]$ will give the rate constants k_2, k_4 and the K_m values, K_E and K_F, for the two enzymes. Independent experimental study of the second reaction will greatly assist in the analysis of the coupled reaction data by computer fitting as outlined previously.

CASE 4. HIGH ENZYME CONCENTRATIONS; DOUBLE INTERMEDIATE ENZYMES

For the three-step mechanisms, in which the enzyme concentrations are sufficiently high ($[E_0] \gg [S_0]$, and $[F_0] \gg [P]$), the transform for $[R]$ is

$$[r] = \frac{k_1 k_2 k_3 k_4 k_5 k_6 [E_0] [F_0] [S_0]}{(\psi + \lambda_1)(\psi + \lambda_2)(\psi + \lambda_3)(\psi + \lambda_4)(\psi + \lambda_5)(\psi + \lambda_6)}$$

The original for $[R]$ is therefore

$$[R] = [S_0] - \sum_{i=1}^{6} P_i e^{-\lambda_i t}$$

where

$$P_i = \frac{\prod_{1=i}^{6} k_i [E_0] [F_0] [S_0]}{\lambda_i^2 \prod_{\substack{i=1 \\ j \neq i}}^{6} (\lambda_j - \lambda_i)}$$

$$\lambda_1 = (A + \sqrt{A^2 - 4B})/2$$
$$\lambda_2 = (A - \sqrt{A^2 - 4B})/2$$
$$A = k_1 [E_0] + k_{-1} + k_2$$
$$B = k_1 k_2 [E_0]$$
$$\lambda_3 = k_3$$
$$\lambda_4 = (C + \sqrt{C^2 - 4D})/2$$
$$\lambda_5 = (C - \sqrt{C^2 - 4D})/2$$
$$C = k_4 [F_0] + k_{-4} + k_5$$
$$D = k_4 k_5 [F_0]$$
$$\lambda_6 = k_6$$

hence

$$[R] = [S_0] \left(1 - \sum_{i=1}^{6} e^{-\lambda_i t} \prod_{\substack{j=1 \\ j \neq i}}^{6} \frac{\lambda_j}{(\lambda_j - \lambda_i)}\right)$$

There are now three exponentials associated with each enzyme, two of the λ_i (λ_3 and λ_6) are independent of enzyme concentration. Since λ_1 is usually much greater than λ_2, and $\lambda_4 \gg \lambda_5$ plots of $1/\lambda_2$ against $1/[E_0]$ and $1/\lambda_5$ against $1/[F_0]$ will yield k_2, k_4, K_E ($= (k_{-1} + k_2)/k_1$) and K_F ($= (k_{-4} + k_5)/k_4$). Finally, additional information is available from the steady-state relationships, e.g.

$$K_m^E = \frac{k_3}{(k_2 + k_3)} \cdot \frac{(k_{-1} + k_2)}{k_1} = \frac{k_3}{(k_2 + k_3)} \cdot K_E$$

and

$$k_{cat}^E = \frac{k_2 k_3}{(k_2 + k_3)}$$

These can be utilized to approach more closely a unique fit to the experimental data.

DISCUSSION

We have presented the solution of the differential equations that describe coupled enzymic reactions using their unitary rate constants. The enzyme models chosen are of the simplest form and conditions imposed on the theoretical schemes, in order to facilitate solutions of the corresponding differential equations, are very reasonable and experimentally easily attained. The mathematical requirement, in Cases 3 and 4, that $[E_0] \gg [S_0]$ and $[F_0] \gg [P]$, is a sufficient but not necessary condition for the analytical solution to fit the numerically simulated curve. Thus in Fig. 2, Curve a, where $[E_0] = 0.75 \cdot [S_0]$, the analytical solution still corresponds closely to the real solution. This arises because, with the parameters chosen, the amount of enzyme bound as ES is small and the enzyme is continuously recycled, so the approximation that $[E] \approx [E_0]$ is still valid.

From the development of Eqn 5 and subsequent discussion, it is apparent that the solution for the system where the second enzyme is in great excess over the first, reduces to that given by Barwell and Hess [12] and Easterby [8] for steady-state systems of the same kind; provided certain highly reasonable relationships exist between the unitary rate constants.

The important solution of the equations, giving final product as a function of time, consists of a linear term in t and a sum of exponentials with negative exponents in t; the number of exponential terms is one less than the number of steps in the reaction pathway.

Methods of computing individual rate constants from experimental data of coupled reactions are discussed; this requires a decision on an enzyme model and the use of the appropriate solution to the differential equations describing the scheme. By using the computer approach presented, data can be rapidly fitted.

The applicability of the analytical results presented is not exclusive for systems only assayable as couples, but can also be used for systems where there is suspicion that the enzymes catalysing the consecutive reactions may heterogeneously interact. In the latter circumstance, if the enzymes had been studied independently, it would be possible to predict their behaviour as a couple using the equations presented. Any deviations, with due consideration taken of the various necessary controls, from predicted behaviour, would be attributable to an alteration in the reaction mechanism. Such interactions are more likely to be observable using the high enzyme, experimental method (Cases 3 and 4). A favourable experimental outcome may enable, by the use of procedures discussed herein, the elucidation of the particular step or steps in the coupled reaction, that is affected by the enzyme–enzyme interaction.

ACKNOWLEDGEMENT

We wish to thank Professor L. W. Nichol for his helpful comments and suggestions during the preparation of this manuscript.

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