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Steady-state kinetic behaviour of two- or *n*-enzyme systems made of free sequential enzymes involved in a metabolic pathway

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Abstract

The overall rate of functioning of a set of free sequential enzymes of the Michaelis–Menten type involved in a metabolic pathway has been computed as a function of the concentration of the initial substrate under steady-state conditions. Curves monotonically increasing up to a saturation plateau have been obtained in all cases. The shape of these curves is sometimes, but not usually, close to that of a hyperbola. Cases exist in which the overall rate of reaction becomes quasi proportional to the concentration of initial substrate almost up to the saturation plateau, which never occurs with individual enzymes. Increasing the number of enzymes sequentially involved in a metabolic pathway does not seem to generate any particularly original behaviour compared with that of two-enzyme systems. ***To cite this article: G. Legent et al., C. R. Biologies 329 (2006).***

Résumé

Comportement cinétique en conditions stationnaires de systèmes bi- ou multienzymatiques constitués d'enzymes libres intervenant séquentiellement dans une voie métabolique. La vitesse globale de fonctionnement d'un ensemble d'enzymes michaéliennes impliquées séquentiellement dans une voie métabolique a été calculée en fonction de la concentration du substrat initial en conditions stationnaires. Des courbes monotones croissantes jusqu'à un plateau de saturation ont toujours été obtenues. Ces courbes ont parfois, mais rarement, une forme proche de celle d'une hyperbole. Il arrive que la vitesse globale de réaction devienne quasi proportionnelle à la concentration du substrat initial pratiquement jusqu'au plateau de saturation, ce qui ne se produit pas lorsqu'une seule enzyme intervient. Il ne semble pas qu'augmenter le nombre d'enzymes différentes impliquées dans la transformation du substrat initial en produit final génère des comportements originaux par rapport aux systèmes à deux enzymes. ***Pour citer cet article : G. Legent et al., C. R. Biologies 329 (2006).***

Keywords: Enzyme kinetics; Metabolic pathways; Modelling

Mots-clés : Cinétique enzymatique ; Voies métaboliques ; Modélisation

Abbreviations: FDS, functioning-dependent structure.

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1. Introduction

Proteins involved in metabolic pathways are often distributed non-randomly as multimolecular assemblies that may range from quasi-static, multi-enzyme complexes to transient, dynamic protein associations [1–6]. A *functioning-dependent structure* (FDS) is such an enzyme complex that forms and maintains itself as a result of its action in accomplishing a task [7]. Moreover, in the case of an extremely simplified model system, we have inferred that such FDSs may display unexpected kinetic properties under steady-state conditions [7]. Before endeavouring to build a complete theory of FDS functioning under realistic conditions, it was necessary to study the behaviour of free (i.e. non-engaged in a FDS) enzymes involved sequentially in a metabolic pathway. This is the aim of the present contribution, in which the calculations have been made according to the classical approach of enzyme kinetics [8].

2. The model of free sequential enzymes

A model of free sequential enzymes of the Michaelis–Menten type is represented in Fig. 1, in the case of a two-enzyme system catalysing the transformation of an initial substrate S_1 into a final product S_3. This model system is made of two reaction circuits, where the first and second circuits correspond to the activity of the first and second enzyme, E_1 and E_2, respectively. Clearly, it would be easy to model three-, four-, ..., n-enzyme systems by adding a third, fourth, ..., nth reaction circuit corresponding to enzymes E_3, E_4, ..., E_n, respectively (not shown).

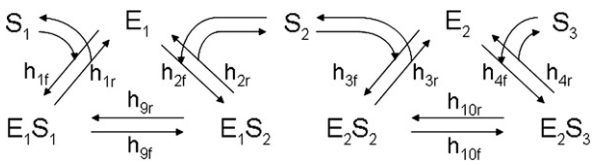

Fig. 1. The model of free sequential enzymes in the case of a two-enzyme system. The first enzyme, E_1, binds to the initial substrate, S_1, to form the enzyme–substrate complex, E_1S_1. Within this complex, E_1 transforms S_1 into its product, S_2, resulting in the transformation of E_1S_1 into E_1S_2, and then E_1S_2 liberates S_2, thus regenerating E_1. In similar manner, the second enzyme E_2 binds S_2; transforms S_2 into S_3 and finally liberates the final product, S_3. h_{if} and h_{ir} are the forward and reverse rate-constants; their numbering, i, has been chosen in such a way as to be consistent with that for the FDSs (in preparation). Note that h_{1f}, h_{2f}, h_{3f} and h_{4f} are expressed in $\mathrm{mol}^{-1}\ \mathrm{s}^{-1}\ \mathrm{m}^3$, whereas all the other rate constants are expressed in s^{-1}.

3. Numerical simulations in the case of a two-enzyme system

The numerical simulations have consisted of studying the dependence of the rate, v, of the overall reaction of transformation of S_1 into S_3, on the concentration of S_1 for various values of the forward and reverse rate constants, h_{if} and h_{ir} (see Fig. 1). In practise, we have used dimensionless expressions of all the variables and parameters. For the definition of these dimensionless quantities, see Appendix A. Independent steady-state equations have been obtained by writing down the mass balance of the species involved, i.e.

$$\mathrm{d}e_1/\mathrm{d}\tau = k_{1r}\cdot e_1 s_1 + k_{2r}\cdot e_1 s_2 - (k_{1f}\cdot s_1 + k_{2f}\cdot s_2)\cdot e_1 = 0 \quad (1)$$

$$\mathrm{d}e_2/\mathrm{d}\tau = k_{3r}\cdot e_2 s_2 + k_{4r}\cdot e_2 s_3 - (k_{3f}\cdot s_2 + k_{4f}\cdot s_3)\cdot e_2 = 0 \quad (2)$$

$$\mathrm{d}s_2/\mathrm{d}\tau = k_{2r}\cdot e_1 s_2 + k_{3r}\cdot e_2 s_2 - (k_{2f}\cdot e_1 + k_{3f}\cdot e_2)\cdot s_2 = 0 \quad (3)$$

$$e_1 + e_1 s_1 = e_{1t} \quad (4)$$

$$e_2 + e_2 s_2 = e_{2t} \quad (5)$$

In these equations, k_{if}, k_{ir}, τ, e_1, e_2, s_1, s_2, s_3, e_1s_1, e_1s_2, e_2s_2 and e_2s_3 are the dimensionless expressions of the forward and reverse rate constants, h_{if} and h_{ir}, of the time, t, and of the concentrations of E_1, E_2, S_1, S_2, S_3, E_1S_1, E_1S_2, E_2S_2, and E_2S_3.

To write down the steady-state conditions of functioning of the system, we have assumed that external mechanisms supply S_1 and remove S_3 as and when they are consumed and produced, respectively, such that S_1 is maintained at a constant concentration ($s_1 = \text{constant}$) and S_3 at a zero concentration ($s_3 = 0$). Under such steady-state conditions, v is measured indifferently by the rate of consumption of S_1 or the rate of production of S_3, i.e. for the dimensionless expression of v:

$$v = k_{1f}\cdot e_1 s_1 - k_{1r}\cdot e_1 s_1 = k_{4r}\cdot e_2 s_3 - k_{4f}\cdot e_2 s_3 \quad (6)$$

Moreover, two relationships have to be taken into account between the rate constants. One is imposed by how the dimensionless quantities have been defined (see Appendix A, Eq. (A.9)),

$$k_{1r} \equiv 1 \quad (7)$$

and the other is a consequence of the principle of detailed balance, i.e.:

$$k_{1f}\cdot k_{2r}\cdot k_{3f}\cdot k_{4r}\cdot k_{9f}\cdot k_{10f}/k_{1r}\cdot k_{2f}\cdot k_{3r}\cdot k_{4f}\cdot k_{9r}\cdot k_{10r} = K \quad (8)$$

in which K is the equilibrium constant of the overall reaction of S_1 into S_3.

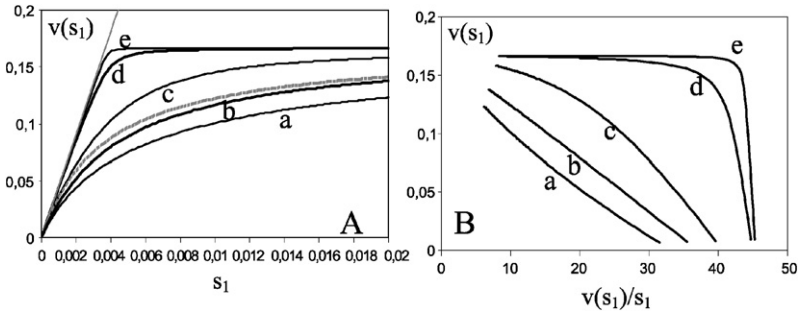

Fig. 2. Examples of computed curves in the case of a two-enzyme system with $s_3 = 0$. (A) Curves represented in the system of coordinates $\{s_1, v\}$. The parameter values are $e_{1t} = e_{2t} = 0.5$, $K = 100$, $k_{1f} = 10$, $k_{2r} = k_{3f} = 100$, $k_{9f} = k_{10f} = k_{3r} = k_{4r} = k_{9r} = k_{10r} = 1$, $k_{1r} \equiv 1$ (Eq. (7)), k_{4f} calculated according to Eq. (8), and $k_{2f} = 10$ (curve a), 5 (curve b), 1 (curve c), 0.1 (curve d) and 0.01 (curve e). The straight, dashed line is the tangent at origin of curve (e). The curve in dotted line is the hyperbola with the same tangent at origin and the same plateau as curve (e). (B) Same data as in (A), expressed in the system of coordinates $\{v/s_1, v\}$.

The numerical simulations of the dependence of v on s_1 (with $s_3 = 0$) for a variety of values of the rate constants have been carried out using the MAPLE software to solve the algebraic system (Eqs. (1) to (5)). We have always found, as expected from the calculation of the first derivative of v (not shown), that the $\{s_1, v\}$ curves were increasing monotonically up to a saturation plateau (see examples in Fig. 2A). Moreover, in these numerical simulations, we have never found any curve exhibiting one or several inflexion points. Sometimes, the shape of the curves was close to that of a hyperbola as shown by the fact that the corresponding curves in the system of coordinates $\{v/s_1, v\}$ (Fig. 2B) were close to straight lines (see curve b); however, most often this was not the case (see especially curves d and e). When adjusting straight lines to the computed curves in Fig. 2A, in the range of s_1 values from 0 to the abscissa, s_{int}, of the point of intersection of the tangent at origin with the saturation plateau of each curve (see Eq. (B.5) in Appendix B for the determination of s_{int}), the values of the regression coefficient, r^2, of these linear adjustments were 0.9608, 0.9682, 0.9843, 0.9991, and 0.9999 for curves (a) to (e), i.e. for k_{2f}-values varying from 10 (curve a) to 0.01 (curve e). This means that decreasing the value of k_{2f} tends to render the reaction rate, v, proportional to the concentration of initial substrate, s_1; moreover, with a k_{2f} value as low as 0.01, it appears that proportionality remains valid (curve e practically undistinguishable from its tangent at origin) almost up to the saturation plateau. It is also visible in the figure that the linear approximation thus observed at a low k_{2f} value extends far beyond the quasi-linear zone that is known to exist at the beginning (s_1 close to zero) of a hyperbolic $\{s_1, v\}$ curve (compare curve (e) with the hyperbolic curve drawn in dotted line in Fig. 2A).

Increasing k_{1f}/k_{1r} or k_{4f}/k_{4r} and/or decreasing k_{3f}/k_{3r} have the same effect as decreasing k_{2f}/k_{2r}, i.e. this tends to favour the proportional response of v as a function of s_1. Increasing k_{10f}/k_{10r} tends to increase the value of the saturation plateau. In our present approach, the equilibrium constant, K, of the overall reaction of transformation of S_1 into S_3 is involved only in the calculation of k_{4f} from the other rate constants (Eq. (8)); since we have imposed $s_3 = 0$, the value of k_{4f}, and consequently that of K, has no effect on the result of the numerical simulations.

4. Numerical simulations in the case of an *n*-enzyme system

As an example of an n-enzyme system, we have studied the case of a system made of four free enzymes. We have always found $\{s_1, v\}$ curves (*i*) monotonically increasing up to a saturation plateau and (*ii*) sometimes exhibiting an extended region where v was proportional to s_1. On total, the results were never qualitatively very different from those observed with two-enzyme systems.

5. Discussion and conclusion

With a system of two free sequential enzymes of the Michaelis–Menten type in a metabolic pathway, we have always observed in our numerical simulations that the $\{s_1, v\}$ curves were monotonically increasing up to a saturation plateau. Depending on the values of the rate constants, the curve shape varied from quasi-hyperbolic to extremely non-hyperbolic, including cases in which v became quasi proportional to s_1 almost up to the saturation plateau (which never occurs with individual enzymes). Choices of values of the rate constants either increasing the efficiency of enzyme E_1 in the transformation of S_1 to S_2 (high values of k_{1f}/k_{1r} and low values of k_{2f}/k_{2r}) or decreasing the efficiency of enzyme E_2 in the transformation of S_2 to S_3 (low values of

k_{3f}/k_{3r} and high values of k_{4f}/k_{4r}) favoured the appearance of this quasi-proportional response. The behaviour of four-enzyme systems was qualitatively not very different from that of two-enzyme systems.

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Appendix A. Definition of dimensionless quantities in the case of a two-enzyme system

Dimensionless quantities have been defined by normalising all concentrations (with [X] = concentration of X) to the sum of the total concentrations of E_1 and E_2, $[E_{1t}]+[E_{2t}]$, and all time values to $1/h_{1r}$. As a consequence, the molar fractions of enzymes E_1 and E_2 are:

$$e_{1t} = [E_{1t}]/\big([E_{1t}]+[E_{2t}]\big)$$
$$e_{2t} = [E_{2t}]/\big([E_{1t}]+[E_{2t}]\big), \quad \text{with } e_{1t}+e_{2t}=1 \tag{A.1}$$

the dimensionless concentrations of all the substances involved are

$$s_1 = [S_1]/\big([E_{1t}]+[E_{2t}]\big)$$
$$s_2 = [S_2]/\big([E_{1t}]+[E_{2t}]\big)$$
$$s_3 = [S_3]/\big([E_{1t}]+[E_{2t}]\big) \tag{A.2}$$
$$e_1 = [E_1]/\big([E_{1t}]+[E_{2t}]\big)$$
$$e_2 = [E_2]/\big([E_{1t}]+[E_{2t}]\big) \tag{A.3}$$
$$e_1 s_1 = [E_1S_1]/\big([E_{1t}]+[E_{2t}]\big)$$
$$e_1 s_2 = [E_1S_2]/\big([E_{1t}]+[E_{2t}]\big) \tag{A.4}$$
$$e_2 s_2 = [E_2S_2]/\big([E_{1t}]+[E_{2t}]\big)$$
$$e_2 s_3 = [E_2S_3]/\big([E_{1t}]+[E_{2t}]\big) \tag{A.5}$$

the dimensionless expression, τ, of time, t, is

$$\tau = t\cdot h_{1r} \tag{A.6}$$

The dimensionless expression of constants h_{1f} to h_{4f} is:

$$k_{1f} = (h_{1f}/h_{1r})\cdot\big([E_{1t}]+[E_{2t}]\big)$$
$$k_{2f} = (h_{2f}/h_{1r})\cdot\big([E_{1t}]+[E_{2t}]\big)$$
$$k_{3f} = (h_{3f}/h_{1r})\cdot\big([E_{1t}]+[E_{2t}]\big)$$

and

$$k_{4f} = (h_{4f}/h_{1r})\cdot\big([E_{1t}]+[E_{2t}]\big) \tag{A.7}$$

while the dimensionless expression of h_{9f}, h_{10f} and all the reverse constants h_{ir} are

$$k_{9f} = h_{9f}/h_{1r}, \quad k_{10f} = h_{10f}/h_{1r} \quad \text{and}$$
$$k_{ir} = h_{ir}/h_{1r} \tag{A.8}$$

with, obviously,

$$k_{1r} = h_{1r}/h_{1r} \equiv 1 \tag{A.9}$$

Appendix B

The steady-state rate of transformation of S_1 into S_3 at saturation, v_{max} ($s_1=\infty$), and the slope at origin, $v'(0)$ ($s_1=0$), can be derived from Eqs. (1) to (5). Define Q by:

$$Q = k_{4r}\cdot k_{10f}\cdot(k_{2r}+k_{9f}+k_{9r})\cdot e_{2t} - k_{2r}\cdot k_{9f}\cdot(k_{4r}+k_{10f}+k_{10r})\cdot e_{1t} \tag{B.1}$$

If $Q<0$, then

$$v_{max} = k_{4r}\cdot k_{10f}\cdot e_{2t}/(k_{4r}+k_{10f}+k_{10r}) \tag{B.2}$$

If $Q>0$, then

$$v_{max} = k_{2r}\cdot k_{9f}\cdot e_{1t}/(k_{2r}+k_{9f}+k_{9r}) \tag{B.3}$$

and it is easily checked that the two v_{max} values are identical to each other when $Q=0$. Moreover, the slope at origin is written:

$$v'(0) = k_{1f}\cdot k_{2r}\cdot k_{3f}\cdot k_{4r}\cdot k_{9f}\cdot k_{10f}\cdot e_{1t}\cdot e_{2t} \Big/\big(k_{1r}\cdot k_{2f}\cdot k_{9r}\cdot(k_{3r}\cdot k_{4r}+k_{3r}\cdot k_{10r}+k_{4r}\cdot k_{10f})\cdot e_{1t} + k_{3f}\cdot k_{4r}\cdot k_{10f}\cdot(k_{1r}\cdot k_{2r}+k_{1r}\cdot k_{9r}+k_{2r}\cdot k_{9f})\cdot e_{2t}\big) \tag{B.4}$$

Eventually, the concentration of initial substrate, s_{int}, which corresponds to the intercept of the tangent at origin with the saturation plateau, is written as:

$$s_{int} = v_{max}/v'(0) \tag{B.5}$$

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