

Two new regulatory properties arising from the transient phase kinetics of monocyclic enzyme cascades

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Taking as starting point a previous contribution about the kinetics of the transient phase and steady-state of monocyclic enzyme cascades, this paper suggest the definition and use of new regulatory modification properties involving the time elapses from the onset of the reaction to the attainment of the steady-state for a monocyclic enzyme cascade. A minimal set of simplifying assumptions allowing to derive analytical expressions for these properties has been used. From these general expressions we derive, as particular cases, other simpler expressions by using additional assumptions which have, therefore, a smaller range of validity. A discussion of the relationships between the kinetic parameters and concentrations needed to the additional assumption is observed is carried out. The goodness of our analysis has been tested by using numerical integration of the set of differential equation describing the kinetic behaviour of the cascade. The results obtained for a type of cascade are extrapolable to other different schemes of monocyclic enzyme cascades. Finally, a kinetic data analysis and an experimental design are suggested.

KEY WORDS: Enzyme kinetics, monocyclic cascades, transient phase, steady state, numerical integration

1. Introduction

Enzyme cascades are ubiquitous in biological systems. They play an important role in the regulation of many physiological processes, e.g. regulation of metabolism, repair of lesions, protection against infectants and evolution, regulation of neurotransmitter receptor function and the efficiency of synaptic transmission, or determination of the balance between cell activation and cell death. Enzyme cascades may be classified into non-cyclic and cyclic ones. The non-cyclic cascades are irreversible and unidirectional, involving activation of

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zymogens. Cyclic cascades are a common and important type of enzyme cascades which operate by allosterically regulated chemical modification/demodification of the active site of key metabolic enzymes. Cyclic cascades may be in turn classified as monocyclic, bicyclic and multicyclic cascades. Some examples of monocyclic cascades are the cascade involved in the modulation of the glycogen synthase and glycogen phosphorylase activity [1–9].

The steady state kinetics of monocyclic enzyme cascades under rapid equilibrium conditions have been extensively studied [2, 10–15]. The transient phase and steady state kinetic behaviour of these cascades both in strict conditions as well as under rapid equilibrium conditions was reported by Varón and Havsteen [16]. Nevertheless, these authors did not introduced parameters concerning the time elapsed to reach the steady state and did not test the goodness of their approached solutions obtained by analytical integration with those arising from the numerical integration of the corresponding set of differential equations describing the kinetic behaviour of the monocyclic cascade.

Therefore, the objectives of this communication are as follows:

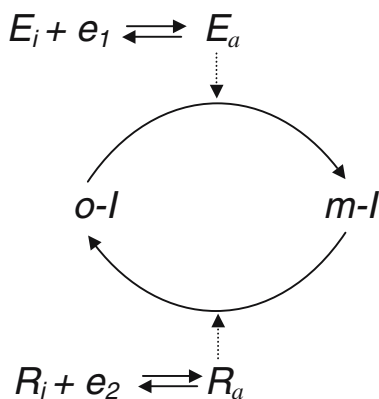
- (1) To define new time-parameters related with this type of cascades analogously as was made by Varón et al. [17] for bicyclic enzyme cascades.
- (2) To check the goodness of the kinetic analysis by comparison of the analytical results with those obtained by numerical integration using a specific software for enzyme reactions previously developed by Garcia-Sevilla et al. [18] which allows to simulate the kinetic behaviour of enzyme systems (e.g. any cyclic cascade) for any set of values of the rate constants and initial concentrations comparing them with the simulated progress curves obtained from the system of differential equations describing the kinetic behaviour of the cascade.
- (3) To suggest a kinetic data analysis and experimental design based on the expressions of new regulatory properties defined here.
- (4) To extend the results to different schemes of monocyclic enzyme cascades.

2. Materials and methods

The numerical integration was carried out using the Runge–Kutta–Fehlberg algorithm [19, 20] using a computer program implemented in Visual C++ 6.0 [18]. The above program was run on a PC-compatible computer based on a Pentium III/450 MHz processor with 128 Mbytes of RAM. Data thus obtained and the corresponding analytical solutions were plotted using the SigmaPlot Scientific Graphing System for Windows version 8.02.

3. The model of monocyclic cascade

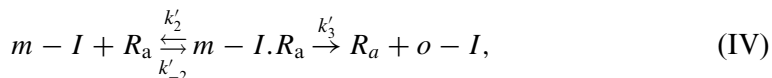
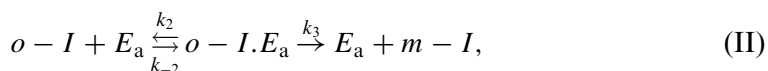
The model of monocyclic enzyme cascade object of this contribution is the well known one shown in the following scheme:



Scheme 1

where $E_a(R_a)$ and $E_i(R_i)$ are the active and inactive forms of the enzyme $E(R)$, e_1 and e_2 are the allosteric modifiers of the enzymes E and R and $o-I$ and $m-I$ are the original and modified forms, respectively, of the interconvertible enzyme I .

The set of reaction steps in Scheme 1 is:



Scheme 2

and the corresponding set of differential equations describing the kinetics of enzyme species involved in Scheme 2 is given in Appendix A.

3.1. Assumptions

The set of differential equations in Appendix A is not linear. Hence, it is very useful to make some reasonable assumptions yielding this system approximately linear and allowing analytical solutions. We assume, as other authors did to obtain steady-state equations [2, 16]:

Assumption 1. The reactions between the converter enzymes with their effectors (steps [I] and [III] in Scheme 2) are in rapid equilibrium.

Assumption 2.

$$[o - I.E_a] \ll [E_i], [E_a], \quad (1)$$

$$[m - I.R_a] \ll [R_i], [R_a], \quad (2)$$

From relations (1) and (2) as well as from Scheme 2, we deduce:

$$[E] \simeq [E_i] + [E_a], \quad (3)$$

$$[R] \simeq [R_i] + [R_a]. \quad (4)$$

Assumption 3. $[e_1]$ and $[e_2]$ are maintained at constant levels. This implies that the allosteric effectors e_1 and e_2 are present either in excess or continuously are produced and fed into the system at a rate commensurating with their conversion.

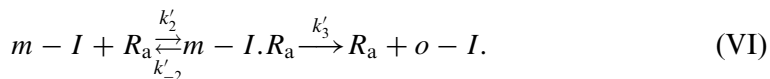
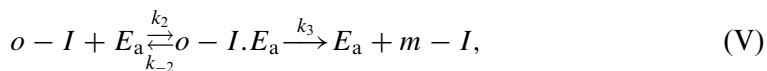
Assumptions 1–3 predict that the concentrations of E_a and R_a remain constant from the onset of the reaction. Their values are given by the following equations:

$$[E_a] = \frac{[E][e_1]}{K_1 + [e_1]}, \quad (5)$$

$$[R_a] = \frac{[R][e_2]}{K'_1 + [e_2]}, \quad (6)$$

where K_1 and K'_1 are the dissociation constants of E_a and R_a ($j = 1, 2, \dots, N$), respectively, i.e. $K_1 = k_{-1}/k_1$ and $K'_1 = k'_{-1}/k'_1$.

Hence, the kinetic study of Scheme 2 is equivalent to that of the following Scheme 3:



Scheme 3

The set of differential equations corresponding to Scheme 3 is:

$$\frac{d[o - I]}{dt} = -k_2[o - I][E_a] + k_{-2}[o - I.E_a] + k'_3[m - I.R_a], \tag{7}$$

$$\frac{d[o - I.E_a]}{dt} = -(k_{-2} + k_3)[o - I.E_a] + k_2[o - I][E_a], \tag{8}$$

$$\frac{d[m - I.R_a]}{dt} = -(k'_{-2} + k'_3)[m - I.R_a] + k'_2[m - I][R_a], \tag{9}$$

$$\frac{d[m - I]}{dt} = -k'_2[m - I][R_a] + k'_{-2}[m - I.R_a] + k_3[o - I.E_a], \tag{10}$$

where $[E_a]$ and $[R_a]$ are the constants given by equations (5) and (6) and, therefore, the set of differential equation (7)–(10) is linear.

Assumption 4. Bindings of the converter enzymes to the convertible ones are in rapid equilibrium from the onset of the reaction.

Rapid equilibrium assumptions in Scheme 3 requires that the first or pseudofirst-order rate constants involved in the reversible steps are much higher than the other ones and not very different [16, 21] i.e.:

$$k_2[E_a], k_{-2}, k'_2[R_a], k'_{-2} \gg k_3, k'_3, \tag{11}$$

$k_2[E_a], k_{-2}, k'_2[R_a], k'_{-2}$ mutually not very different.

4. Regulatory properties involving reaction time

To define new regulatory properties related with time elapsed to reach the steady state we start with the results for the time course of the fractional modification, FM, when rapid equilibrium in reversible steps in Scheme 3 prevails, previously reported by Varón and Havsteen [16]. The instantaneous fractional modification is defined as the quotient $[m - I]/[I]$ and is given by:

$$FM = (FM)_\infty(1 - e^{-\lambda t}), \tag{12}$$

where $(FM)_\infty$, the fractional modification at the steady state, i.e. when $t \rightarrow \infty$ and λ are:

$$(FM)_\infty = \frac{k_3 K'_2 [E_a]}{k_3 K'_2 [E_a] + k'_3 K_2 [R_a] + (k_3 + k'_3) [E_a] [R_a]}, \quad (13)$$

$$\lambda = -\frac{k_3 K'_2 [E_a] + k'_3 K_2 [R_a] + (k_3 + k'_3) [E_a] [R_a]}{K'_2 [E_a] + K_2 [R_a] + K_2 K'_2 + [E_a] [R_a]}, \quad (14)$$

where K_2 and K'_2 are the dissociation constants of $o-I.E_a$ and $m-I.R_a$, respectively, i.e. $K_2 = k_{-2}/k_2$ and $K'_2 = k'_{-2}/k'_2$.

Equation (12) allows an estimation of the time required by the system to reach the steady state. This is an important parameter because a cascade of high steady-state sensitivity but possessing a long transient time may, in real time, behave as a low sensitivity system. Conversely, a cascade of a moderate steady-state sensitivity could, in real time, display a considerable sensitivity, if it has a short transient phase. In the following we define the parameter *transient time*, T , of the interconvertible protein I of a monocyclic enzyme cascade system as the time reaction at which it is observed that:

$$(FM)_T = 0.9999(FM)_\infty. \quad (15)$$

If in equation (12) with $t = T$, equation (15) is taken into account we have, whenever that $(FM)_\infty \neq 0$ that:

$$T = -\frac{4 \ln 10}{\lambda}, \quad (16)$$

where λ is given by equation (14). If $(FM)_\infty = 0$ then T is not defined. If equation (14) is inserted into equation (16), we have:

$$T = \frac{4 (K'_2 [E_a] + K_2 [R_a] + K_2 K'_2 + [E_a] [R_a]) \ln 10}{k_3 K'_2 [E_a] + k'_3 K_2 [R_a] + (k_3 + k'_3) [E_a] [R_a]}. \quad (17)$$

Nevertheless, neither the knowledge of the $(FM)_\infty$ -value nor that of the transient time, T , is sufficient to define the efficiency of a monocyclic cascade. Therefore, we define the *mean regulation rate* of any of these cascades as:

$$M = \frac{(FM)_\infty}{T}. \quad (18)$$

If now equations (13) and (17) are inserted into equation (18), the result is:

$$M = \frac{k_3 K'_2 [E_a]}{4 (K'_2 [E_a] + K_2 [R_a] + K_2 K'_2 + [E_a] [R_a]) \ln 10}. \quad (19)$$

5. Results and discussion

The starting point of this contribution is equation (12), which gives the time course of the fractional modification of monocyclic enzyme cascades evolving according to Scheme 1. From this equation we have defined in this work two new parameters related with the efficiency of these cascades: the transient time, T (equation (17)) and the mean regulation rate, M (equation (19)).

From FM equal to $0.9999(FM)_\infty$ one can say that the system is practically at the steady state. The chose of 99.99 is arbitrary and any other percentage near 100 (except obviously 100 because in this case the transient time will be infinity) could be also chosen. The time that the system remains in the transient phase or, the same, the time required by the system to reach the steady state is the practical meaning of T . From this definition, M means the mean rate of increase of the fractional modification, FM . It is to be observed that M gives a more complete information than $(FM)_\infty$ alone or T alone.

In practice, T and M could easily be obtained from a fit of the experimental FM -values to equation (12), which allows to determine the parameters λ and $(FM)_\infty$. Hence, equation (16) gives the T -value and then, equation (18) furnishes the M -value.

5.1. Dependence of T and M on $[E_a]$ and $[R_a]$

Equations (17) and (19) show the dependence of T and M on $[E_a]$ and $[R_a]$ simultaneously. In the following we will use the notations $T_{[R_a]}$ and $M_{[R_a]}$ to indicate the function which gives the dependencies of T and M , respectively, on $[E_a]$ at a fixed $[R_a]$ -value. Likewise, we will use the notations $T_{[E_a]}$ and $M_{[E_a]}$ to indicate the function which gives the dependencies of T and M , respectively, on $[R_a]$ at a fixed $[E_a]$ -value.

According to equations (17) and (19), we have:

$$\lim_{[E_a] \rightarrow 0} T_{[R_a]} = \frac{4(K'_2 + [R_a]) \ln 10}{k'_3 [R_a]}, \tag{20}$$

$$\lim_{[E_a] \rightarrow \infty} T_{[R_a]} = \frac{4(K'_2 + [R_a]) \ln 10}{(k_3 + k'_3)[R_a] + k_3 K'_2}, \tag{21}$$

$$\lim_{[R_a] \rightarrow 0} T_{[E_a]} = \frac{4(K_2 + [E_a]) \ln 10}{k_3 [E_a]}, \tag{22}$$

$$\lim_{[R_a] \rightarrow \infty} T_{[R_a]} = \frac{4(K'_2 + [R_a]) \ln 10}{(k_3 + k'_3)[R_a] + k'_3 K_2}, \tag{23}$$

$$\lim_{[E_a] \rightarrow 0} M_{[R_a]} = 0, \tag{24}$$

$$\lim_{[E_a] \rightarrow \infty} M_{[R_a]} = \frac{k_3 K'_2}{4 (K'_2 + [R_a]) \ln 10}, \quad (25)$$

$$\lim_{[R_a] \rightarrow 0} M_{[E_a]} = \frac{k_3 [E_a]}{4 ([E_a] + K_2) \ln 10}, \quad (26)$$

$$\lim_{[R_a] \rightarrow \infty} M_{[E_a]} = 0 \quad (27)$$

For greater ease, in the following we will denote the limits $\lim_{[E_a] \rightarrow 0} T_{[R_a]}$, $\lim_{[E_a] \rightarrow \infty} T_{[R_a]}$, $\lim_{[R_a] \rightarrow 0} T_{[E_a]}$, $\lim_{[R_a] \rightarrow \infty} T_{[E_a]}$, $\lim_{[E_a] \rightarrow 0} M_{[R_a]}$, $\lim_{[E_a] \rightarrow \infty} M_{[R_a]}$, $\lim_{[R_a] \rightarrow 0} M_{[E_a]}$, and $\lim_{[R_a] \rightarrow \infty} M_{[E_a]}$ as $T_{[R_a]}^0$, $T_{[R_a]}^\infty$, $T_{[E_a]}^0$, $T_{[E_a]}^\infty$, $M_{[R_a]}^0$, $M_{[R_a]}^\infty$, $M_{[E_a]}^0$ and $M_{[E_a]}^\infty$, respectively.

In Figure 1 we have plotted the functions $T_{[R_a]}$ and $T_{[E_a]}$ for an arbitrary set of values of $[R_a]$ and $[E_a]$ and the equilibrium and rate constants involved. We have also plotted in figure 2 the functions $M_{[R_a]}$ and $M_{[E_a]}$ for the same arbitrary set of values of $[R_a]$ and $[E_a]$ and the equilibrium and rate constants used in figure 1. Note that T decreases when $[E_a]$ at a fixed $[R_a]$ -value or $[R_a]$ at a fixed $[E_a]$ -value increase.

5.2. Check of the goodness of our results

A test of these results by means of simulated progress curves is necessary to know the goodness of our approach and definitions. Equation (12) was obtained from the set of differential equations (7)–(10) under Assumptions 1–4. Thus, equation (12) is approximately valid under these assumptions. In figure 3 we indicate the time progress course of FM (i.e., the quotient $[m - I]/[I]$) obtained from equation (12) and from numerical integration of the set of differential Equations (7)–(10), assuming the constancy of $[E_a]$ and $[R_a]$ for the four arbitrary sets of values of $[I]$, $[E_a]$ and $[R_a]$ and the rate constants indicated in table 1 as cases 1–4. Likewise, equations (17) and (19) obtained from equation (12) have the same approached character as the latter. Table 2 gives the values of $(FM)_\infty$, T and M obtained from equations (13), (17) and (19), and from simulated progress curves in figure 3.

Discrepancies in figure 3 and table 2 between our results and those obtained from simulation (as in case 4) indicate that Assumption 4 is not observed for the chosen values as can be easily shown from the not fulfilment of relationship (11) for this case.

5.3. Kinetic data analysis

Experimental values of any of the parameters $T_{[R_a]}^0$, $T_{[R_a]}^\infty$ (obtained from a plot of $T_{[R_a]}$ versus $[E_a]$) $T_{[E_a]}^0$, $T_{[E_a]}^\infty$ (obtained from a plot of $T_{[E_a]}$ versus $[R_a]$), $M_{[R_a]}^\infty$ (obtained from a plot of $M_{[R_a]}$ versus $[E_a]$) and $M_{[E_a]}^0$ (obtained from a

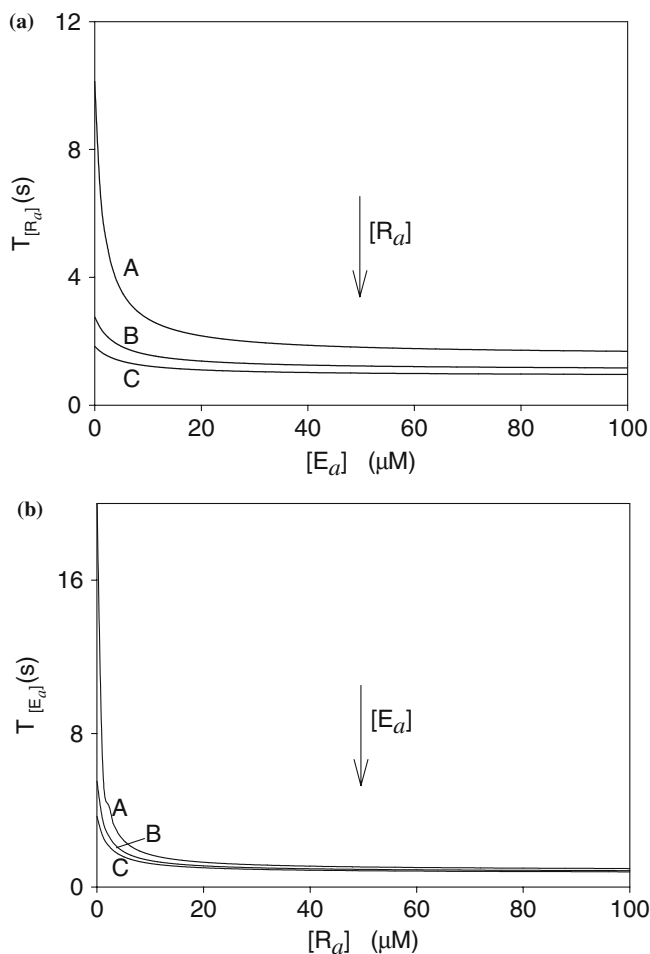


Figure 1. (a) Dependence of $T_{[R_a]}$ on $[E_a]$ at different fixed $[R_a]$ -values according to equation (17). Values of $[R_a]$ used in curves A–C where 1, 5 and $10 \mu\text{M}$, respectively. (b) Dependence of $T_{[E_a]}$ on $[R_a]$ at different $[E_a]$ values according to equation (17). Values of $[E_a]$ used in curves A–C, where 1, 5 and $10 \mu\text{M}$, respectively. Values of the equilibrium and rate constants where in all cases: $K_2 = K'_2 = 10^{-5} \text{ M}$, $k_3 = 5 \text{ s}^{-1}$, $k'_3 = 10 \text{ s}^{-1}$.

plot of $M_{[E_a]}$ versus $[R_a]$), and their corresponding expressions given by equations (20)–(23), (25) and (26) may be used to estimate the parameters involved. For example, from equation (26) we have that a plot of $1/(4M_{[E_a]}^0 \ln 10)$ versus $1/[E_a]$ is a straight line with the slope K_2/k_3 , and the intercept ordinate $1/k_3$ so that k_3 and K_2 are immediately obtained.

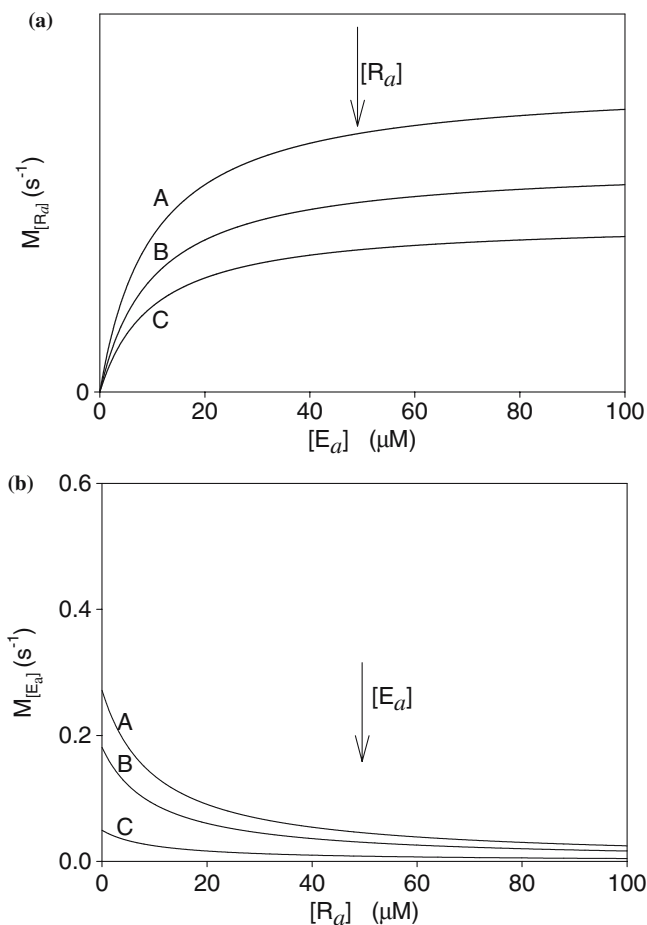


Figure 2. (a) Dependence of $M_{[R_a]}$ on $[E_a]$ at different fixed $[R_a]$ -values according to equation (19). Values of $[R_a]$ used in curves A–C, where 1, 5 and 10 μM, respectively. (b) Dependence of $M_{[E_a]}$ on $[R_a]$ at different $[E_a]$ values according to equation (19). Values of $[E_a]$ used in curves A–C, where 1, 5 and 10 μM, respectively. Values of the equilibrium and rate constants used where as figure 1.

Table 1

Values for the rate constants, $[E_a]$, $[R_a]$ and $[I]$ used for numerical integration of the set of differential equations giving the simulated time progress of FM. These values were also used for plots of equation (12) in the four different cases 1–4. On the two last columns, K_2 - and K'_2 - values for plotting equation (13) in each case are also given.

Case	$[I]$ (μM)	$[E_a]$ (μM)	$[R_a]$ (μM)	k_2 (μM ⁻¹ s ⁻¹)	k_{-2} (s ⁻¹)	k'_2 (μM ⁻¹ s ⁻¹)	k'_{-2} (s ⁻¹)	k_3 (s ⁻¹)	k'_3 (s ⁻¹)	K_2 (μM)	K'_2 (μM)
1	1	10	5	10	100	20	200	10	5	10	10
2	1	10	5	10	100	20	200	1	2	10	10
3	1	0.01	0.05	100	100	400	800	1	2	1	2
4	1	0.01	0.02	100	100	400	800	100	200	1	2

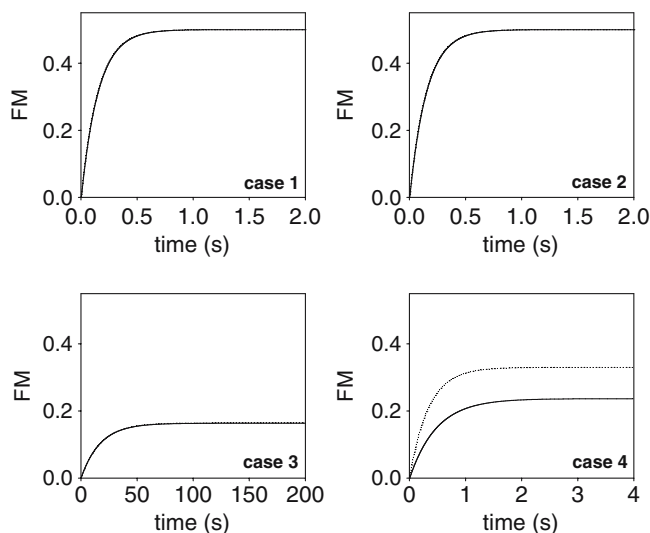


Figure 3. Time course equation of FM (i.e. the quotient $[m - I]/[I]$) obtained from simulation (—) and plotting equation (12) corresponding to each case (cases 1–4 in table 1) (...). Both curves practically overlap in cases 1–3 where Assumption 4 is observed, but not in case 4 where Assumption 4 is not observed (see table 4).

Table 2

Values obtained from the simulated progress curves and from equations (13), (17) and (19) for cases 1–4 in table 1.

Case	$(FM)_\infty$ (simulation)	$(FM)_\infty$ (equation (13))	T (s) (simulation)	T (s) (equation (17))	M (s^{-1}) (simulation)	M (s^{-1}) (equation (19))
1	0.5000	0.5000	1.3960	1.3816	0.3582	0.3619
2	0.2869	0.2857	7.2613	7.8946	0.0395	0.0362
3	0.1636	0.1646	157.20	156.20	0.0001	0.0001
4	0.2367	0.3300	3.7066	3.1008	0.0638	0.1064

5.4. Dependence of T and M on the concentrations of the converter enzymes E and R and of the allosteric effectors e_1 and e_2

Once shown the validity of our results we will give the dependence of T and M upon the converter enzymes E and R and upon the effectors e_1 and e_2 . If in equations (17) and (19) we insert equations (5) and (6), we have the dependencies required.

5.4.1. Monocyclic cascades particular cases Scheme 1

Figure 4 summarises four different schemes of monocyclic cascades which differ in the nature of the interactions between the allosteric effectors and the converter enzymes. For completeness we have also included Scheme 1 in figure 4.

Table 3

Expressions of $[E_a]$ and $[R_a]$ to be inserted into equations (13), (17) and (19) for Schemes 1 and 4–6. In all cases K_1 and K'_1 are dissociation equilibrium constants, i.e.: $K_1 = k_{-1}/k_1$ and $K'_1 = k'_{-1}/k'_1$.

Scheme	Steps in which the converter enzymes are involved	Expressions of $[E_a]$	Expressions of $[R_a]$
1	$E_i + e_1 \xrightleftharpoons[k_{-1}]{k_1} E_a$ and $R_i + e_2 \xrightleftharpoons[k'_{-1}]{k'_1} R_a$	$\frac{[E][e_1]}{K_1 + [e_1]}$	$\frac{[R][e_2]}{K'_1 + [e_2]}$
2	$E_i + e_1 \xrightleftharpoons[k_{-1}]{k_1} E_a$ and $R_a + e_2 \xrightleftharpoons[k'_{-1}]{k'_1} R_i$	$\frac{[E][e_1]}{K_1 + [e_1]}$	$\frac{K'_1[R]}{K'_1 + [e_2]}$
3	$E_a + e_1 \xrightleftharpoons[k_{-1}]{k_1} E_i$ and $R_i + e_2 \xrightleftharpoons[k'_{-1}]{k'_1} R_a$	$\frac{K_1[E]}{K_1 + [e_1]}$	$\frac{[R][e_2]}{K'_1 + [e_2]}$
4	$E_a + e_1 \xrightleftharpoons[k_{-1}]{k_1} E_i$ and $R_a + e_2 \xrightleftharpoons[k'_{-1}]{k'_1} R_i$	$\frac{K_1[E]}{K_1 + [e_1]}$	$\frac{K'_1[R]}{K'_1 + [e_2]}$

Note that in Schemes 1 and 4 the allosteric effector e_1 acts as an activator, whereas in Schemes 5 and 6 acts as an inhibitor. In Schemes 1 and 5 the allosteric effector e_2 acts as an activator, whereas in Schemes 4 and 6 acts as an inhibitor.

All results here obtained have been referred to Scheme 1. However equations (1)–(4) and (7)–(27) can also be directly applied to Schemes 4–6. Nevertheless, if

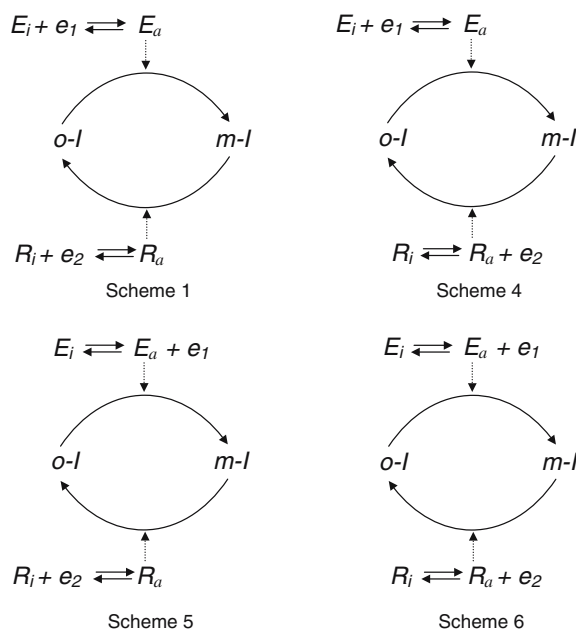


Figure 4. Simplified reactions patterns for different schemes to which equation (17) and (19) can be directly applied.

we want any of these equations, e.g. those for the transient time or the mean regulation rate as a function of the concentrations of the target converter enzymes and allosteric effectors, $[E_a]$ and $[R_a]$ in equations (5) and (6) must be replaced by the corresponding expressions (table 3). For the sake of completeness we also include Scheme 1 in table 3.

Appendix A

System of differential equations describing the evolution of the species involved in Scheme 2

$$\frac{d[E_i]}{dt} = -k_1 [E_i] [e_1] + k_{-1} [E_a], \quad (A1)$$

$$\frac{d[e_1]}{dt} = -k_1 [E_i] [e_1] + k_{-1} [E_a], \quad (A2)$$

$$\frac{d[E_a]}{dt} = - (k_{-1} + k_2 [o - I]) [E_a] + k_1 [E_i] [e_1] + (k_{-2} + k_3) [o - I.E_a], \quad (A3)$$

$$\frac{d[o - I]}{dt} = -k_2 [o - I] [E_a] + k_{-2} [o - I.E_a] + k'_3 [m - I.R_a], \quad (A4)$$

$$\frac{d[o - I.E_a]}{dt} = - (k_{-2} + k_3) [o - I.E_a] + k_2 [o - I] [E_a], \quad (A5)$$

$$\frac{d[m - I]}{dt} = -k'_2 [m - I] [R_a] + k'_{-2} [m - I.R_a] + k_3 [o - I.E_a], \quad (A6)$$

$$\frac{d[R_i]}{dt} = -k'_1 [R_i] [e_2] + k'_{-1} [R_a], \quad (A7)$$

$$\frac{d[e_2]}{dt} = -k'_1 [R_i] [e_2] + k'_{-1} [R_a], \quad (A8)$$

$$\frac{d[R_a]}{dt} = - (k'_{-1} + k'_2 [m - I]) [R_a] + k'_1 [R_i] [e_2] + (k'_{-2} + k'_3) [m - I.R_a], \quad (A9)$$

$$\frac{d[m - I.R_a]}{dt} = - (k'_{-2} + k'_3) [m - I.R_a] + k'_2 [m - I] [R_a]. \quad (A10)$$

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