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Enzyme kinetics of multiple alternative substrates

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An innovative theoretical approach that enables the complete characterisation of enzyme–substrate and enzyme–substrate–competitor reactions is generalised to systems with multiple alternative substrates. Based on the quasi-steady-state assumption, time-dependent closed form solutions are presented for cases with even, weak and mixed substrate competition. The analytic framework should facilitate the development of computational fitting procedures for progress curves, simplifying the measuring process and increasing the reliability of reaction constant estimates.

1. Introduction

The introduction of alternative substrates in enzyme processes is an important approach in the study of living materials and their transformations, as the induced perturbations allow a more detailed understanding of the normal initial states. In particular, alternative substrates are employed to bring out the features of enzyme active centres due to their selective and competitive reactions [38]. Driven by economic and environmental issues, much effort has been devoted to the industrial synthesis of enantiomerically pure compounds; in such enterprises, it is essential to estimate the kinetic parameters of the competing racemases in order to ensure efficient resolution [20].

Classical kinetic methods have been applied to enzyme systems involving no more than three substrates; e.g., the techniques of Daziel coefficients and Haldane relationships [17], which are essentially qualitative, and the more quantitative approaches of isotope exchange [4,28] and alternative substrates [7–10,27,24,41]. Further extensions to multiple alternative substrates are generally avoided since analysis gets rapidly complicated without adding much information to that obtained by studying the substrates individually. An approach to overcome this problem has been proposed [27] where alternative substrates are taken as competitors of a given substrate thus asserting that insight into the kinetics can be obtained by evaluating their competitive effects.

Previous work on multiple competition [1,6,15,16,35–38] has been mainly concerned with the development of graphical methods to estimate, within the Michaelis–

Menten (MM) framework, reaction constants and to elucidate the reactant interactions. Despite their virtues in data visualisation, diagnostics and education, such graphical methods can be tedious, of relatively poor accuracy and imply acquired expertise [6,14,18,25]. The general availability of powerful microcomputers, on the other hand, should now make approaches based on least-squares curve fitting procedures more attractive [11,12,39], although the hitherto lack of analytic expressions for progress curves hampers general acceptance as direct numerical integration can be computationally intensive [19,22,43].

Schnell and Mendoza [29] have revisited the MM formalism for the case of the basic enzyme–substrate system, and have found for the first time closed form solutions that characterise the complete evolution of both the reactant concentrations and their time derivatives. This analytic framework facilitates the development of least-squares fitting procedures to determine accurate reaction parameters from progress curves (for an independent verification, see [22]). Therefore, it proposes a more efficient and reliable experimental methodology based on the timing of a single decay curve instead of the usual lengthy estimates of initial velocity as a function of initial substrate concentration from several decay curves. This theoretical approach has been recently extended to describe the fully competitive enzyme–substrate–competitor system [30], obtaining closed form solutions for the three nominal cases of competition: even, weak and strong [26].

In the present report we generalise the work in [29,30] to the case of multiple alternative substrates. This is the first stage in a current systematic revision of more complicated enzyme reaction networks. Exclusive multiple competition is reduced to an n-substrate reaction system in order to make the mathematical formalism more compact, and is illustrated with solutions for the case of one and two substrates. The enzyme kinetics of such systems is briefly reviewed, followed by a derivation of the corresponding analytic solutions for the different cases of competition. Some conclusions of the work are also discussed.

2. The alternative n-substrate system

The multiple alternative substrates system can be reduced to the scheme

$$\mathrm{S}_i + \mathrm{E} \underset{k_{-i}}{\overset{k_i}{\rightleftharpoons}} \mathrm{ES}_i \overset{k_{2i}}{\rightarrow} \mathrm{E} + \mathrm{P}_i \tag{1}$$

with n substrates ($i = 1, \ldots, n$). E is the free enzyme, and S_i, ES_i and P_i respectively denote the ith substrate, enzyme–substrate complex and product. The parameters k_i, k_{-i} and k_{2i} are positive kinetic constants for the ith channel. Thus, if in an alternative substrate system $i = 1$ is taken to be the leading substrate, the system contains $(n-1)$ competitors ($i = 2, \ldots, n$).

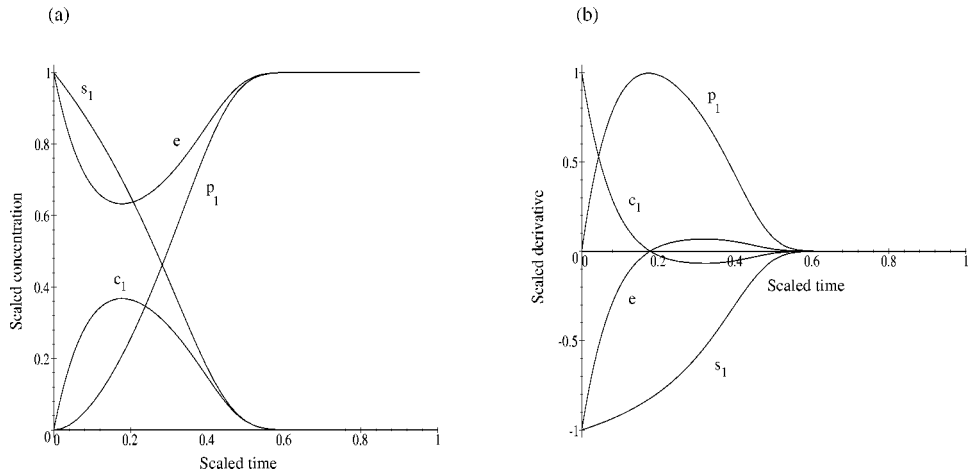

Figure 1. Characteristic curves for the enzyme–substrate reaction (1) with $n = 1$: (a) reactant concentrations and (b) time derivatives. The infinite time range has been reduced to the interval $(0, 1)$ by means of the exponential time scale $\tau = 1 - 1/\ln(t + \exp(1))$. The concentrations have been nondimensionally scaled as follows: free substrate $s_1 = [\mathrm{S}]_1/[\mathrm{S}_0]_1$; free enzyme $e = [\mathrm{E}]/[\mathrm{E}_0]$; complex $c_1 = [\mathrm{ES}]_1/[\mathrm{E}_0]$ and product $p_1 = [\mathrm{P}]_1/[\mathrm{S}_0]_1$. The time derivatives have been scaled to their absolute maximum value: $s_1 = \mathrm{d}[\mathrm{S}]_1/\mathrm{d}t$; $e = \mathrm{d}[\mathrm{E}]/\mathrm{d}t$; $c_1 = \mathrm{d}[\mathrm{ES}]_1/\mathrm{d}t$ and $p_1 = \mathrm{d}[\mathrm{P}]_1/\mathrm{d}t$.

For the basic enzyme–substrate reaction ($n = 1$), a time-dependent closed form solution for the substrate concentration has been derived [29]:

$$\left[\mathrm{S}'\right]_1(t) = W\left(\left[\mathrm{S}'_0\right]_1 \exp\left(-\kappa_1 t + \left[\mathrm{S}'_0\right]_1\right)\right), \tag{2}$$

where W is the *omega function* W (see the appendix), $[\mathrm{S}']_1 \equiv [\mathrm{S}]_1/K_1$ is the substrate *reduced concentration* (with initial value $[S'_0]_1$) and $\kappa_1 \equiv v_1^{\max}/K_1$ is the *first-order rate constant*. The familiar *MM constant* and *maximum velocity* are defined, respectively, as

$$K_1 \equiv \frac{k_{-1} + k_2}{k_1} \quad \text{and} \quad v_1^{\max} \equiv k_2[\mathrm{E}_0]. \tag{3}$$

Expression (2) describes the substrate decay during its complete duration ($0 \leqslant t < \infty$), and its effectiveness in experimental data fitting is ensured by the availability of highly efficient algorithms for W [2,3,21]. Moreover, as shown in figure 1, characteristic progress curves for all reactants and their time derivatives can be henceforth generated.

Similar solutions for the fully competitive enzyme–substrate–competitor system ($i = 1, 2$) have also been formulated [30]. Making use of the *competition matrix* $\delta_{ij} \equiv \kappa_j/\kappa_i$ to classify different levels of competition [26,30], the solution for evenly competitive substrates ($\delta_{12} \approx 1$) is given by

$$\left[\mathrm{S}'\right]_i = \frac{[\mathrm{S}'_0]_i}{[\mathrm{S}'_0]_1 + [\mathrm{S}'_0]_2} W\left(\left(\left[\mathrm{S}'_0\right]_1 + \left[\mathrm{S}'_0\right]_2\right) \exp\left(-\kappa_i t + \left[\mathrm{S}'_0\right]_1 + \left[\mathrm{S}'_0\right]_2\right)\right). \tag{4}$$

It may be appreciated that the reduced substrate concentrations keep the constant ratio $[S'_0]_1/[S'_0]_2$ throughout the reaction. For weak competition ($\delta_{12} \ll 1$) the solutions now take the form

$$\left[S''\right]_1(t) = W\left(\left[S''_0\right]_1 \exp\left(-\widetilde{\kappa}_1 t + \left[S''_0\right]_1\right)\right), \tag{5}$$

$$\left[S''\right]_2(t) = \left[S''_0\right]_2 \left(\frac{[S'']_1(t)}{[S''_0]_1}\right)^{\delta_{12}}, \tag{6}$$

where $[S'']_i \equiv [S]_i/\widetilde{K_i}$ and $\widetilde{\kappa_i} \equiv v_i^{\max}/\widetilde{K_i}$ are now referred to as the *apparent reduced concentration* and *apparent first-order rate constant*, respectively, normalised to the *apparent MM constant*

$$\widetilde{K_i} \equiv K_i \left(1 + \frac{[S_0]_j}{K_j}\right). \tag{7}$$

Therefore, the fast substrate concentration decays in a very similar fashion to the isolated case, i.e. as "apparently" alone, but the competition causes an increase of the MM constant [32]; on the other hand, the competitor decay corresponds to that of the fast substrate but severely attenuated by a power of δ_{12}. Characteristic curves for this system are depicted in figures 2 and 3. The last case of strong competition ($\delta_{12} \gg 1$) is trivial as its solutions are essentially equations (5) and (6) with permuted indices noting that $\delta_{21} = 1/\delta_{12}$. An interesting result that emerges from the formalism is the role played by the apparent MM constants which is only of noteworthy importance in systems with uneven competition.

3. Enzyme kinetics

By applying the law of mass action, the time evolution of the n-substrate system (1) is described by the nonlinear coupled differential equations

$$\frac{d[S]_i}{dt} = -k_i[E][S]_i + k_{-i}[ES]_i, \tag{8}$$

$$\frac{d[E]}{dt} = \sum_{i=1}^{n} \left(-k_i[E][S]_i + (k_{-i} + k_{2i})[ES]_i\right), \tag{9}$$

$$\frac{d[ES]_i}{dt} = k_i[E][S]_i - (k_{-i} + k_{2i})[ES]_i, \tag{10}$$

$$\frac{d[P]_i}{dt} = k_{2i}[ES]_i \tag{11}$$

with $i = 1, \ldots, n$ and initial conditions at $t = 0$

$$\left([S]_i, [E], [ES]_i, [P]_i\right) = \left([S_0]_i, [E_0], 0, 0\right). \tag{12}$$

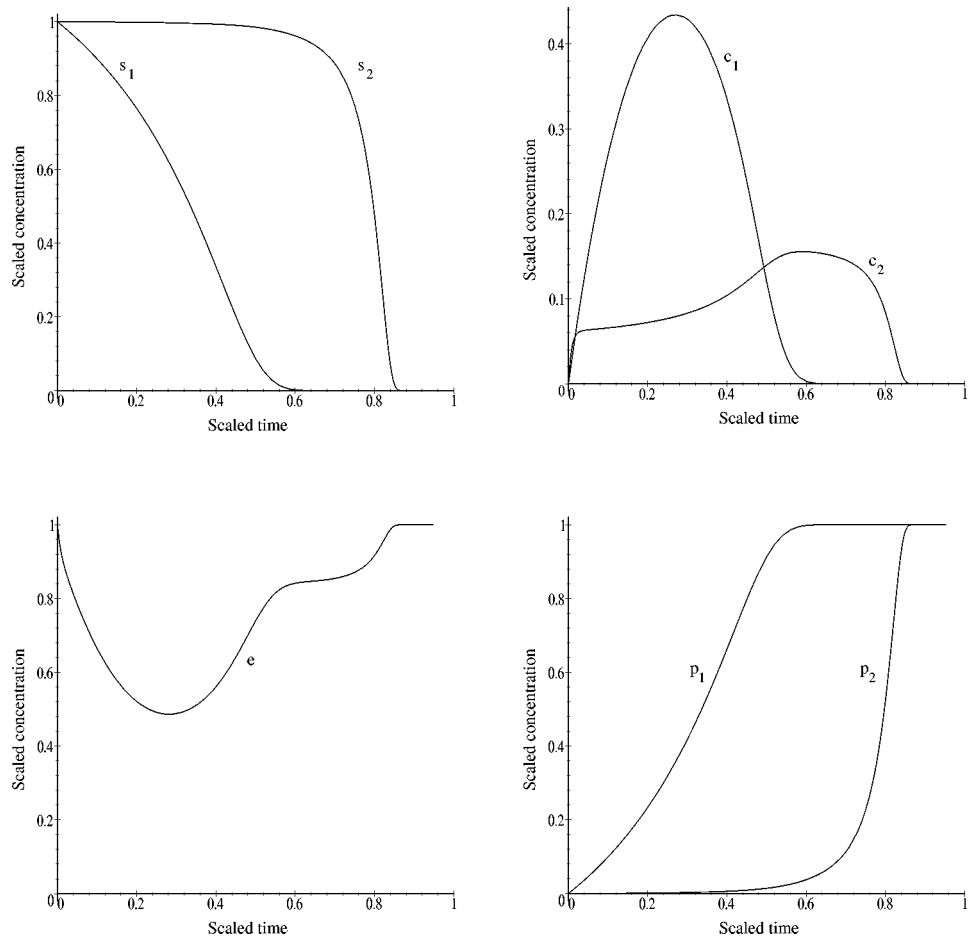

Figure 2. Progress curves for an enzyme–substrate–competitor reaction of the form (1) with $n = 2$. In this reaction a weak competitor ($\delta_{12} \ll 1$) is assumed. The infinite time range has been reduced to the interval $(0, 1)$ by means of the exponential time scale $\tau = 1 - 1/\ln(t + \exp(1))$. The reactant concentrations have been nondimensionally scaled as follows: free substrate $s_i = [\mathrm{S}]_i/[\mathrm{S}_0]_i$; free enzyme $e = [\mathrm{E}]/[\mathrm{E}_0]$; complex $c_i = [\mathrm{ES}]_i/[\mathrm{E}_0]$ and product $p_i = [\mathrm{P}]_i/[\mathrm{S}_0]_i$. The subscripts 1 and 2 denote, respectively, the substrate and weak competitor.

Since the enzyme E is a catalyst, its total concentration (free plus combined) must be a constant. This conservation law is readily expressed by adding equations (9) and (10):

$$\frac{\mathrm{d}[\mathrm{E}]}{\mathrm{d}t} + \sum_{i=1}^{n} \frac{\mathrm{d}[\mathrm{ES}]_i}{\mathrm{d}t} = 0 \quad \Rightarrow \quad [\mathrm{E}](t) + \sum_{i=1}^{n} [\mathrm{ES}]_i(t) = [\mathrm{E}_0]. \tag{13}$$

Also, at any time the sum of the concentrations of the ith free substrate $[\mathrm{S}]_i$, its complex $[\mathrm{ES}]_i$ and product $[\mathrm{P}]_i$ must be equal to the initial substrate concentration $[\mathrm{S}_0]_i$;

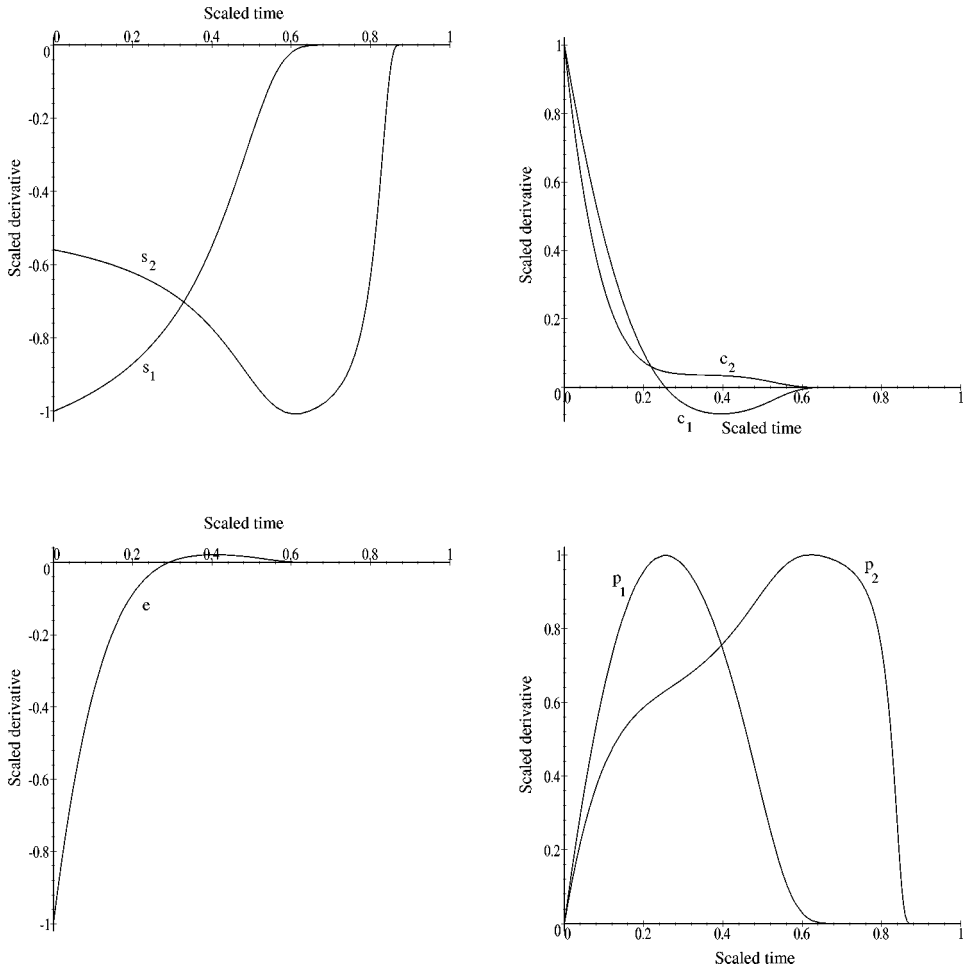

Figure 3. Time derivatives for an enzyme–substrate–competitor reaction of the form (1) with $n = 2$. In this reaction a weak competitor ($\delta_1 \ll 1$) is assumed. The infinite time range has been reduced to the interval $(0, 1)$ by means of the exponential time scale $\tau = 1 - 1/\ln(t + \exp(1))$. The time derivatives have been scaled to their absolute maximum value: $s_i = \mathrm{d[S]}_i/\mathrm{d}t$; $e = \mathrm{d[E]}/\mathrm{d}t$; $c_i = \mathrm{d[ES]}_i/\mathrm{d}t$ and $p_i = \mathrm{d[P]}_i/\mathrm{d}t$. The subscripts 1 and 2 denote, respectively, the substrate and weak competitor.

that is, by adding equations (8), (10) and (11) it may be shown that

$$\frac{\mathrm{d[S]}_i}{\mathrm{d}t} + \frac{\mathrm{d[ES]}_i}{\mathrm{d}t} + \frac{\mathrm{d[P]}_i}{\mathrm{d}t} = 0 \quad \Rightarrow \quad [\mathrm{S}]_i(t) + [\mathrm{ES}]_i(t) + [\mathrm{P}]_i(t) = [\mathrm{S}_0]_i. \tag{14}$$

Thus the conservation laws reduce the system of differential equations (8)–(11) to two equations for S_i and ES_i,

$$\frac{\mathrm{d[S]}_i}{\mathrm{d}t} = -k_i \left([\mathrm{E}_0] - \sum_{j=1}^{n} [\mathrm{ES}]_j \right) [\mathrm{S}]_i + k_{-i} [\mathrm{ES}]_i, \tag{15}$$

$$\frac{\mathrm{d}[\mathrm{ES}]_i}{\mathrm{d}t} = k_i\left([\mathrm{E}_0] - \sum_{j=1}^{n}[\mathrm{ES}]_j\right)[\mathrm{S}]_i - (k_{-i} + k_{2i})[\mathrm{ES}]_i. \tag{16}$$

This nonlinear system is complicated to solve, but further simplifications can be introduced if the quasi-steady-state approximation (QSSA) is obeyed.

As previously discussed [5,23,29–34,40], the QSSA implies that after initial transients, $t > t_{C_i}$ say, the complex concentrations remain approximately constant. That is, in the slow-time regime it can be assumed that

$$\frac{\mathrm{d}[\mathrm{ES}]_i}{\mathrm{d}t} \approx 0, \tag{17}$$

resulting in the expression

$$[\mathrm{ES}]_i = \frac{[\mathrm{E}_0][\mathrm{S}']_i}{1 + \sum_j [\mathrm{S}']_j}, \tag{18}$$

where $[\mathrm{S}']_i \equiv [\mathrm{S}]_i/K_i$ is the reduced concentration with the MM constant being defined as

$$K_i \equiv \frac{k_{-i} + k_{2i}}{k_i}. \tag{19}$$

Substituting (18) in (11) and simplifying the conservation law (14) with the assumption (17) yields the following differential equation:

$$\frac{\mathrm{d}[\mathrm{S}']_i}{\mathrm{d}t} = \frac{-\kappa_i[\mathrm{S}']_i}{1 + \sum_j [\mathrm{S}']_j}, \tag{20}$$

where

$$\kappa_i \equiv \frac{v_i^{\max}}{K_i} = \frac{k_{2i}[\mathrm{E}_0]}{K_i} \tag{21}$$

is the first-order rate constant.

The QSSA also implies that during the transient time, $t < t_{C_i}$, there is not a significant fraction of the substrate bound to the enzyme, that is,

$$[\mathrm{S}]_i \approx [\mathrm{S}_0]_i, \tag{22}$$

leading to the important conclusion that (20) is valid in both the fast- and slow-time regimes. Therefore, solutions valid during the total span of the reaction ($0 < t < \infty$) can be obtained from the two equations

$$\frac{\mathrm{d}[\mathrm{S}']_i}{\mathrm{d}t} = \frac{-\kappa_i[\mathrm{S}']_i}{1 + \sum_j [\mathrm{S}']_j}, \tag{23}$$

$$[\mathrm{ES}]_i = \frac{[\mathrm{E}_0][\mathrm{S}']_i}{1 + \sum_j [\mathrm{S}']_j}\big(1 - \exp(-\lambda_i t)\big), \tag{24}$$

where λ_i is the constant

$$\lambda_i = k_i K_i \left(1 + \sum_{j=1}^{n} [\mathrm{S}'_0]_j \right). \tag{25}$$

Equation (23) can be decoupled by introducing the competition matrix [26,30]

$$\delta_{ij} \equiv \frac{\kappa_j}{\kappa_i} \tag{26}$$

which, for each pair of substrates, provides a measure of their relative rates. We now obtain the following differential equation:

$$\frac{\mathrm{d}[\mathrm{S}']_j}{\mathrm{d}[\mathrm{S}']_i} = \delta_{ij} \frac{[\mathrm{S}']_j}{[\mathrm{S}']_i}, \tag{27}$$

which is easily integrable to result in the useful working relation

$$\frac{[\mathrm{S}']_j(t)}{[\mathrm{S}'_0]_j} = \left(\frac{[\mathrm{S}']_i(t)}{[\mathrm{S}'_0]_i} \right)^{\delta_{ij}}. \tag{28}$$

Moreover, noting that the competition matrix obeys the following constraints:

$$\delta_{ii} = 1, \tag{29}$$

$$\delta_{ji} = \frac{1}{\delta_{ij}}, \tag{30}$$

$$\delta_{ji'} = \frac{\delta_{ii'}}{\delta_{ij}}, \tag{31}$$

the reaction system is then completely specified by the row vector

$$\delta_i = (\delta_{i1}, \delta_{i2}, \ldots, \delta_{in}). \tag{32}$$

Equation (28) allows us to conclude that if the competition vector (32) is known for the ith substrate, then the complete reaction system ($i = 1, \ldots, n$) is characterised by solely measuring the concentration decay of the ith substrate. The time behaviour of the latter can be obtained from the solution of the uncoupled equation

$$\frac{\mathrm{d}[\mathrm{S}']_i}{\mathrm{d}t} = \frac{-\kappa_i [\mathrm{S}']_i}{1 + \sum_j [\mathrm{S}'_0]_j ([\mathrm{S}']_i / [\mathrm{S}'_0]_i)^{\delta_{ij}}} \tag{33}$$

with the initial condition $([\mathrm{S}']_i) = ([\mathrm{S}'_0]_i)$ at $t = 0$. Conversely, if the time decays of all the substrates are measured, then the competition matrix can be in principle reconstructed.

Following [33,34] and generalising the results in [29,30], two sets of time scales are considered for the reaction system: a set related to the duration of the initial transients, t_{C_i}, and a second containing the times taken for significant changes in

the S_i concentrations, t_{S_i}, that provides a measure of the quasi-steady-state period. Using (24), the individual fast-time scales are given by $t_{C_i} = \lambda_i^{-1}$, namely,

$$t_{C_i} = \frac{1}{k_i K_i (1 + \sum_j [S_0']_j)}. \tag{34}$$

The slow-time scales are determined by dividing the total change in substrate concentration $[S_0]_i$ by the maximum rate of substrate change $|d[S]_i/dt|_{max}$,

$$t_{S_i} = \frac{1 + \sum_j [S_0']_j}{\kappa_i}; \tag{35}$$

hence, the competition matrix can also be written in terms of the ratio of the slow-time scales,

$$\delta_{ij} = \frac{t_{S_i}}{t_{S_j}}. \tag{36}$$

We are now in a position to establish the general conditions of the QSSA for the reaction scheme under consideration. Extending the prescription in [33,34] for the basic enzyme reaction ($n = 1$) to the multiple substrate case ($i = 1, \ldots, n$), we require that

$$\left| \frac{\Delta[S']_i}{[S_0']_i} \right| \approx \frac{t_{C_i}}{[S_0']_i} \left| \frac{d[S']_i}{dt} \right|_{max} \ll 1, \tag{37}$$

which implies that the QSSA is valid if the following condition is obeyed:

$$\max_{i=1,\ldots,n} \left(\frac{[E_0]}{K_i (1 + \sum_j [S_0']_j)} \right) \ll 1. \tag{38}$$

4. Solutions

In order to derive closed form solutions for the alternative substrates, we start by integrating (33) to obtain the relation

$$\kappa_i t = \sum_{j=1}^{n} \frac{[S_0']_j}{\delta_{ij}} \left(1 - \left(\frac{[S']_i}{[S_0']_i} \right)^{\delta_{ij}} \right) - \ln \left(\frac{[S']_i}{[S_0']_i} \right). \tag{39}$$

In the case of the single substrate reaction, the usefulness of this progress-curve equation to estimate reaction constants from experimental data has been recently discussed [22,29].

4.1. Even competition ($\delta_i \approx 1$)

In a similar fashion to [30], (39) is rearranged after substituting $\delta_{ij} = 1$ to obtain the solution for the time evolution of the reduced concentration of the ith substrate in terms of the omega function W,

$$\left[\mathrm{S}'\right]_i(t) = \frac{[\mathrm{S}'_0]_i}{\sum_j [\mathrm{S}'_0]_j} W\left(\left(\sum_j \left[\mathrm{S}'_0\right]_j\right) \exp\left(-\kappa_i t + \sum_j \left[\mathrm{S}'_0\right]_j\right)\right). \tag{40}$$

This relation shows that in even competition the substrate reduced concentrations at any time are essentially determined by their initial fractional reduced concentrations and first-order rate constants, thus keeping the proportion

$$\left[\mathrm{S}'\right]_1 : \left[\mathrm{S}'\right]_2 : \left[\mathrm{S}'\right]_3 : \cdots : \left[\mathrm{S}'\right]_n = \left[\mathrm{S}'_0\right]_1 : \left[\mathrm{S}'_0\right]_2 : \left[\mathrm{S}'_0\right]_3 : \cdots : \left[\mathrm{S}'_0\right]_n \tag{41}$$

throughout the reaction.

4.2. Weak competition ($\delta_i \ll 1$)

For the case where the competition vector $\delta_i \ll 1$, a Taylor expansion shows that, to first order in δ_{ij},

$$\left(\frac{[\mathrm{S}']_i}{[\mathrm{S}'_0]_i}\right)^{\delta_{ij}} = 1 + \delta_{ij} \ln\left(\frac{[\mathrm{S}']_i}{[\mathrm{S}'_0]_i}\right) + \mathrm{O}(\delta_{ij})^2. \tag{42}$$

Equation (39) then reduces to

$$\kappa_i t = \left[\mathrm{S}'_0\right]_i - \left[\mathrm{S}'\right]_i - \left(1 + \sum_{j \neq i} \left[\mathrm{S}'_0\right]_j\right) \ln\left(\frac{[\mathrm{S}']_i}{[\mathrm{S}'_0]_i}\right), \tag{43}$$

which leads to the following expression for the time evolution of the concentration of the ith substrate:

$$\left[\mathrm{S}'\right]_i(t) = \left(1 + \sum_{j \neq i} \left[\mathrm{S}'_0\right]_j\right) W\left(\frac{[\mathrm{S}'_0]_i}{1 + \sum_{j \neq i} [\mathrm{S}'_0]_j} \exp\left(\frac{-\kappa_i t + [\mathrm{S}'_0]_i}{1 + \sum_{j \neq i} [\mathrm{S}'_0]_j}\right)\right). \tag{44}$$

Making use of relation (28), the solution for the jth substrate ($j \neq i$) is given by

$$\left[\mathrm{S}'\right]_j(t) = \left[\mathrm{S}'_0\right]_j \left(\frac{[\mathrm{S}']_i(t)}{[\mathrm{S}'_0]_i}\right)^{\delta_{ij}}. \tag{45}$$

Furthermore, (44) can be rewritten as

$$\left[\mathrm{S}''\right]_i(t) = W\left(\left[\mathrm{S}''_0\right]_i \exp\left(-\widetilde{\kappa}_i t + \left[\mathrm{S}''_0\right]_i\right)\right), \tag{46}$$

where $[S'']_i \equiv [S]_i/\widetilde{K_i}$ is the apparent reduced concentration and $\widetilde{\kappa_i} \equiv v_i^{\max}/\widetilde{K_i}$ the apparent first-order rate constant normalised to the apparent MM constant

$$\widetilde{K_i} = K_i\left(1 + \sum_{j\neq i}\left[S'_0\right]_j\right). \tag{47}$$

The ith MM constant displays an increase due to the statistical factor that accounts for the distribution of enzyme between the E and ES_j constituents [32]. Since expression (46) has a similar form to that for the single substrate reaction (see equation (2)), the fast competitor substrate behaves as "apparently" alone, and the competition is adequately taken into account by means of the apparent MM constant.

4.3. Mixed competition

Rather than considering the specific case of strong competition, which as previously discussed is trivial, we consider the more general case of mixed competition with l evenly competitive alternative substrates substrates, $\delta_{ij} \approx 1$ for $j = 1, \ldots, l$, in the presence of $(n - l)$ weak competitor substrates, $\delta_{ij} \ll 1$ for $j = (l + 1), \ldots, n$. The concentration decay of the $i = 1, \ldots, l$ competitor substrates is now expressed as

$$\begin{aligned}\left[S'\right]_i(t) = {} & \frac{[S'_0]_i}{\sum_{j=1}^{l}[S'_0]_j}\left(1 + \sum_{j=l+1}^{n}\left[S'_0\right]_j\right) \\ & \times W\left(\frac{\sum_{j=1}^{l}[S'_0]_j}{1+\sum_{j=l+1}^{n}[S'_0]_j}\exp\left(\frac{-\kappa_i t + \sum_{j=1}^{l}[S'_0]_j}{1+\sum_{j=l+1}^{n}[S'_0]_j}\right)\right)\end{aligned} \tag{48}$$

while the concentrations of the weak competitor substrates, $j = (l + 1), \ldots, n$, can again be derived from relation (28):

$$\left[S'\right]_j(t) = \left[S'_0\right]_j\left(\frac{[S']_i(t)}{[S'_0]_i}\right)^{\delta_{ij}}. \tag{49}$$

The effect of the $(n - l)$ weak competitor substrates can again be incorporated in the apparent MM constant

$$\widetilde{K_i} = K_i\left(1 + \sum_{j=l+1}^{n}\left[S'_0\right]_j\right) \tag{50}$$

to provide simplified expressions for the apparent reduced concentrations of the n substrates:

$$\left[S''\right]_i(t) = \frac{[S''_0]_i}{\sum_{j=1}^{l}[S''_0]_j}W\left(\left(\sum_{j=1}^{l}\left[S''_0\right]_j\right)\exp\left(-\widetilde{\kappa_i}t + \sum_{j=1}^{l}\left[S''_0\right]_j\right)\right) \quad (i \leqslant l), \tag{51}$$

$$\left[S''\right]_j(t) = \left[S''_0\right]_j\left(\frac{[S'']_i(t)}{[S''_0]_i}\right)^{\delta_{ij}} \qquad (i > l). \tag{52}$$

5. Discussion

We have developed a general and compact formalism that fully describes the reaction of multiple alternative substrates. The analytic solutions that have been derived for each case of competition are certainly a considerable advance from previous work that mainly relied on involved graphical methods or numerically integrated equations. From a qualitative point of view, such solutions bring out in a clear fashion the collective kinetic behaviour of a set of multiple substrates competing for an enzyme. Furthermore, as recently illustrated [22], they can also be exploited to implement non-linear least-squares fitting procedures that can result in estimates of reaction constants of quantitative reliability. It is worth emphasising that since the proposed curve fitting is aimed at concentration decays rather than velocities, experimental methodologies should be greatly simplified. Since the present work is part of a systematic revision of enzyme reaction networks, we are confident that the formalism can be extended to tackle multimode inhibition enzyme kinetics that will be reported elsewhere.

Appendix

The *omega function*, $W(x)$, is defined as the function satisfying the following transcendental equation [13,42]:

$$W(x)\exp\big(W(x)\big) = x \quad \text{for } x \geqslant -\exp(-1). \tag{53}$$

A plot of $W(x)$ (see figure 4) shows that for $-\exp(-1) \leqslant x < 0$ the function has two possible real values. From these dual values, two real branches of $W(x)$ can be defined: the upper branch $W(x) \geqslant -1$ and the lower branch $W(x) \leqslant -1$. For convenience, we only consider here the upper branch.

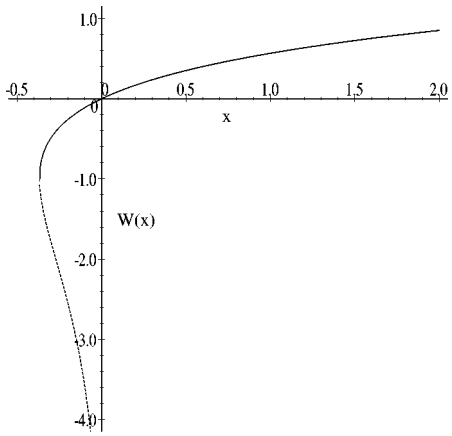

Figure 4. Representation of the $W(x)$ function, showing the upper branch (solid curve) and the lower branch (dotted curve).

By rewriting expression (53) as $W(x) = x/\exp(W(x))$, the omega function W can be expressed as a continued fraction for $|W(x)| < 1$,

$$W(x) = \frac{x}{\exp(W(x))} = \frac{x}{\exp\dfrac{x}{\exp(W(x))}} = \cdots = \frac{x}{\exp\dfrac{x}{\exp\dfrac{x}{\exp\dfrac{x}{\ddots}}}} \tag{54}$$

or, it can be rewritten as $W(x) = \ln(x/W(x))$ leading to the alternative continued fraction for $|W(x)| > 1$,

$$W(x) = \ln\frac{x}{\ln(W(x))} = \ln\frac{x}{\ln\dfrac{x}{\ln(W(x))}} = \cdots = \ln\frac{x}{\ln\dfrac{x}{\ln\dfrac{x}{\ln\dfrac{x}{\ddots}}}}. \tag{55}$$

The exponential continued fraction (54) can be used to approximate $W(x)$ around $x = 0$; however, a polynomial continued fraction approximation is more effective [2,3]. A series expansion of equation (53) at $x = -\exp(-1)$ yields

$$W(x) = -1 + \sqrt{v} - \left(\frac{1}{3} - \frac{11}{72}\sqrt{v}\right)v - \left(\frac{43}{540} - \frac{769}{17280}\sqrt{v}\right)v^2 + \mathrm{O}\left(v^3\right), \tag{56}$$

where $v = 2 + 2\exp(1)x$. Converting this series into a continued fraction yields

$$W(x) = -1 + \frac{\sqrt{v}}{1 + \dfrac{\sqrt{v}}{3 + m}}, \tag{57}$$

where

$$m = \frac{a\sqrt{v}}{b + \sqrt{v}}. \tag{58}$$

Adjusting a and b to minimise the error in the interval $-\exp(-1) \leqslant x \leqslant 2$ gives

$$a = \frac{4 - 3\sqrt{2} + b(2\sqrt{2} - 3)}{\sqrt{2} - 2}, \tag{59}$$

$$b = \frac{28769}{6237}\left(v + \frac{17035}{15549}\right)^{1/4}. \tag{60}$$

For $x > 2$, the following truncated form of the logarithmic continued fraction (55) also provides a satisfactory approximation:

$$W(x) = \ln\frac{x}{\dfrac{x}{\ln^n(x)}}, \tag{61}$$

where

$$n = \exp\left(-\frac{c}{d + \ln(x)}\right) \tag{62}$$

with

$$c = \frac{42887}{38139}, \qquad d = \frac{9737}{23046}. \tag{63}$$

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