

The total quasi-steady-state approximation is valid for reversible enzyme kinetics

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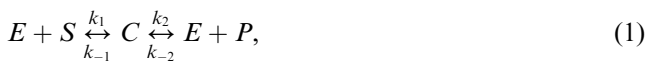
Abstract

The Briggs–Haldane approximation of the irreversible Michaelis–Menten scheme of enzyme kinetics is cited in virtually every biochemistry textbook and is widely considered the classic example of a quasi-steady-state approximation. Though of similar importance, the reversible Michaelis–Menten scheme is not as well characterized. This is a serious limitation since even enzymatic reactions that go to completion may be reversible. The current work derives a total quasi-steady-state approximation (tQSSA) for the reversible Michaelis–Menten and delineates its validity domain. The tQSSA allows the derivation of uniformly valid approximations for the limit of low enzyme concentrations, $E_T \ll S_T + K_M$, and under certain more restrictive conditions also for high enzyme concentrations such that $S_T \ll E_T + K_M$. Using these simple analytical approximations, a sequential experimental–theoretical method is suggested for unambiguously estimating all the kinetic parameters of the reversible Michaelis–Menten scheme. © 2003 Elsevier Ltd. All rights reserved.

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1. Introduction

The Michaelis–Menten scheme (Michaelis and Menten, 1913) is extensively used in biochemistry to describe enzymatic processes in solution. This scheme reads



where E and C denote the free and substrate-bound enzyme, respectively, S denotes the free substrate and P denotes the product, k_1 is the rate constant of formation of the enzyme substrate complex, k_{-1} the rate constant of dissociation of the enzyme–substrate complex, k_2 is the catalysis rate constant, and k_{-2} is the rate constant of enzyme–product complex formation. Kinetic scheme (1) represents the simplest reversible enzymatic reaction in which the enzyme–substrate complex is the same as the enzyme–product complex. Early studies on enzyme kinetics used the limiting case of scheme (1) where the enzyme–product binding on rate approaches zero ($k_{-2} = 0$) to study irreversible, consumptive, enzymatic

reactions. Classic works by investigators like Michaelis and Menten (1913) and van Slyke and Cullen (1914) were concerned with for example the action of urease or invertase–catalysis of sucrose. However, many biochemical pathways of interest, for example in the regulation of carbohydrate metabolism, are regenerative, and therefore often cyclical and necessarily reversible. Examples of reversible enzyme catalytic reactions that are well described by kinetic scheme (1) can be found in the literature (Alberty, 1959; Sellin and Mannervik, 1983; Duggleby, 1994). However, due to the added mathematical complexity in analysing reversible reactions, even this restricted case has received much less attention than the irreversible case.

For enzymatic reactions in closed systems, kinetic scheme (1) implies the following conservation relationships:

$$E_T = E + C, \quad (2)$$

$$S_T = S + C + P, \quad (3)$$

where E_T and S_T are, respectively, the total concentrations of the enzyme and the substrate in the system. Applying the law of mass action to kinetic scheme (1),

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and using Eqs. (2) and (3) to eliminate E and P , yields the following set of differential equations:

$$\frac{dS}{dt} = -k_1(E_T - C)S + k_{-1}C, \quad (4)$$

$$\frac{dC}{dt} = k_1((E_T - C)S - K_M C) + k_{-2}(E_T - C)(S_T - S - C), \quad (5)$$

where we employed the standard notation

$$K_M = \frac{k_{-1} + k_2}{k_1}. \quad (6)$$

Typically, enzymatic reactions are studied subject to the following uniform initial conditions:

$$(E, S, C) = (E_T, S_T, 0), \quad t = 0. \quad (7)$$

Namely, at the beginning of the experiment the enzyme is free and there are no products.

Starting with Briggs and Haldane (1925), the quasi-steady-state approximation (QSSA), also referred to as the pseudo-steady-state hypothesis, has proven fruitful in the analysis of Eqs. (4)–(7). Approximate analytical solutions and simple parameter estimation schemes for the case of *irreversible* enzyme kinetics (IREK) can be obtained. Segel (1988) used scaling analysis to argue that the Briggs–Haldane approximation is valid whenever $E_T \ll K_M + S_T$ (a rigorous proof is given in Segel and Slemrod, 1989), and Schnell and Mendoza (1997) derived uniformly valid approximations for this case. More recently, Borghans et al. (1996) introduced the total QSSA (tQSSA) and showed that its domain of validity overlaps that of the Briggs–Haldane approximation and extends to include the case of high enzyme concentrations, $E_T + S_T \gg k_2/k_1$. In this context, the term *total* refers to the fact that the tQSSA yields an equation for the total substrate concentration. Schnell and Maini (2000) showed that the reverse QSSA (rQSSA) of IREK is valid at high enzyme concentrations such that $E_T \gg \max(k_2/k_1, S_T)$ and derived uniformly valid approximations for this case. In this context, the term *reverse* refers to the fact that the rQSSA is derived by assuming that $dS/dt \approx 0$ during the QSS phase (Segel and Slemrod, 1989). Recently, Tzafiriri (2003) rederived and corrected the tQSSA of IREK. Approximate solutions of the irreversible limit of Eqs. (4)–(7) were derived and proved to be uniformly valid whenever the Briggs–Haldane approximation or the rQSSA are valid, and when both of these approximations break down, for example, whenever $K_M > E_T > K \gg S_T$.

The problem of reversible enzyme kinetics (REK) is substantially less well characterized. Haldane (1930) derived the QSSA of REK for the case where the bound enzyme concentration is negligible, but did not consider its solution. Alberty and Koerber (1957) used the implicit solution of the Haldane equation to derive

short time approximations of the product concentration. Miller and Alberty (1958) analytically solved the case where substrate and product binding of enzyme were balanced ($k_{-2} = k_1$) and used standard perturbation theory to extend this solution for cases such that $k_1/k_{-2} \ll 1$ and $S_T \gg E_T$. Moreover, these authors demonstrated that in the limit of low enzyme concentrations such that $E_T \ll S_T \approx K_M$, the exact solution of the case $k_{-2} = k_1$ is well approximated by the Briggs–Haldane approximation of IREK. For example, these authors showed that the Briggs–Haldane approximation was within 1% error of the exact analytical solution when the concentration of enzyme exceeded that of the substrate by at least four log orders ($E_T = 10^{-4} S_T$). These results were later corroborated by Fraser and Roussel (1994) using dynamic systems analysis. Moreover, these authors analysed the divergence of the Briggs–Haldane approximation from the slow manifold at lower relative concentration ratios. Similarly, Stayton and Fromm (1979) conducted a numerical study of Eqs. (4)–(7) for cases such that $k_{-2} \leq k_1$ and concluded that enzyme excess ($E_T \leq 100 S_T$) was also the key requirement for ensuring the validity of the Haldane approximation. Palsson and Lightfoot (1984) non-dimensionalized Eqs. (4)–(7) and used linear stability theory to analyse them. They concluded that the Haldane approximation is valid whenever $E_T \ll K_M \ll S_T$. Schauer and Heinrich (1979) derived approximations for the fast pre-steady-state transient and used them to derive criteria for the validity of the Haldane approximation. However, numerical evaluation of these criteria was only carried out for IREK and yielded the validity domain of the Briggs–Haldane approximation in the (S_T, E_T) plane. Using criteria for the validity of the QSSA similar to those suggested by Schauer and Heinrich (1979), Segel (1988) found that the Haldane approximation is valid provided that $\varepsilon k_{-2}/k_1 \ll K_M + S_T$, where $\varepsilon \equiv E_T/(K_M + S_T)$. Moreover, Segel noted that $\varepsilon \ll 1$ guarantees the validity of the Briggs–Haldane approximation and concluded that the validity of the Briggs–Haldane approximation implies the validity of the Haldane approximation.

Building upon recent analysis of the tQSSA of the IREK (Tzafiriri, 2003), the current work seeks to obtain the tQSSA of the REK, to define its domain of validity and to solve it analytically for the extreme cases of high and low enzyme concentrations (as defined below). The motivation for this analysis is that as the initial transient of the IREK is obtained by neglecting the concentration of products, it can also be used to approximate the REK. This observation is not new (Segel, 1988). However, its significance can only be fully appreciated in light of the recent finding that the validity of the approximation for the initial transient guarantees the validity of the tQSSA of IREK.

2. The total quasi-steady-state approximation

Following Borghans et al. (1996) we introduce the transient total substrate concentration

$$\bar{S} = S + C \tag{8}$$

and add Eqs. (4) and (5) to obtain

$$\frac{d\bar{S}}{dt} = -k_2C + k_{-2}(E_T - C)(S_T - \bar{S}). \tag{9}$$

Substituting Eq. (8) into Eq. (5) and introducing the definition

$$\sigma \equiv \bar{S} + (k_{-2}/k_1)(S_T - \bar{S}) \tag{10}$$

we obtain an equation for the concentration of bound enzyme

$$\frac{dC}{dt} = k_1(C - C_+(\sigma))(C - C_-(\sigma)), \tag{11}$$

where

$$C_{\pm}(\sigma) = \frac{(E_T + K_M + \sigma) \pm \sqrt{(E_T + K_M + \sigma)^2 - 4E_T\sigma}}{2} \tag{12}$$

are the roots of the quadratic equation

$$C^2 - (E_T + K_M + \sigma)C + E_T\sigma = 0. \tag{13}$$

The variable σ allows us to emphasize the analogy between Eqs. (11)–(13) and their IREK counterparts (Tzafiriri, 2003) and thereby suggests that the former can be similarly analysed. Moreover, the fact that $\sigma = \bar{S}$ for $k_{-2} = 0$ makes it straightforward to recover the IREK limit from the analysis presented below.

Initial conditions (7) imply that during the initial transient we can substitute $\sigma = S_T$ into Eq. (11) to obtain

$$\frac{dC}{dt} = k_1(C - C_+(S_T))(C - C_-(S_T)). \tag{14}$$

The solution of this Riccati equation is

$$C_i = C_-(S_T) \left(\frac{1 - e^{-t/t_C}}{1 - (C_-(S_T)C_+(S_T))e^{-t/t_C}} \right), \tag{15}$$

where we defined

$$t_C^{-1} \equiv k_1(C_+(S_T) - C_-(S_T)) \\ = k_1\sqrt{(E_T + K_M + S_T)^2 - 4E_T S_T}. \tag{16}$$

As a criterion for the validity of the initial transient approximation (ITA), we shall require that this approximation is self-consistent. Namely, we shall require that the substitution of $C_i(t)$ into Eq. (9) yields $\sigma(t) \approx \bar{S} \approx S_T$ or (Segel, 1988)

$$1 \gg \frac{|S_T - \sigma(t)|}{S_T}, \quad t \leq t_C. \tag{17}$$

As emphasized by Segel (1972), inconsistent approximations are generally invalid, while consistent approximations are

generally valid unless the problem is ill conditioned. By ill conditioned we mean that a small error in the problem can lead to large errors in the solution. Thus, Eq. (17) is a necessary condition for the validity of the ITA, and very likely a sufficient condition as well, since this problem does not seem to be ill conditioned.

The self-consistency criterion expressed by Eq. (17) can be made explicit by invoking the *mean value theorem* of differential calculus (Courant and John, 1965), which guarantees the existence of $\theta \in (0, t)$ such that $\sigma(t) = S_T + (d\sigma/dt)_{\theta}t$. Thus, using the mean value theorem and Eqs. (9) and (10) we can rewrite criterion (17) in the form

$$1 \gg \left| 1 - \frac{k_{-2}}{k_1} \right| \left(\frac{k_2 C_i(\theta) - k_{-2}(E_T - C_i(\theta))(S_T - \bar{S}(\theta))}{S_T} \right) t, \tag{18}$$

$\theta \in (0, t)$.

One immediate implication of Eq. (18) is that the ITA is guaranteed to be valid whenever $k_{-2} = k_1$. Indeed, for the specific case $k_{-2} = k_1$, Eq. (15) corresponds to the *exact* solution of Eq. (11) obtained by Miller and Alberty (1958). Whenever the prefactor $|1 - k_{-2}/k_1|$ in Eq. (18) is not small, this equation has to be simplified further, since at this point \bar{S} is still undetermined. One way of achieving the necessary simplification is by noting that

$$k_2 C_i(\theta) \geq k_2 C_i(\theta) - k_{-2}(E_T - C_i(\theta))(S_T - \bar{S}(\theta)) \geq 0 \tag{19}$$

and

$$C_i(t) \leq C_-(S_T), \quad 0 < t \leq \infty. \tag{20}$$

Finally, noting that the initial transient corresponds to $t \leq t_C$ we obtain the following condition for the validity of the ITA:

$$\varepsilon \equiv \left| 1 - \frac{k_{-2}}{k_1} \right| \left(\frac{k_2 C_-(S_T)}{S_T} \right) t_C = \left| 1 - \frac{k_{-2}}{k_1} \right| \varepsilon_{IR} \ll 1, \tag{21}$$

where ε_{IR} is the value of ε in the case of IREK. Thus, up to the prefactor $|1 - k_{-2}/k_1|$, Eq. (21) is exactly the same as the criterion for the validity of ITA in the case of IREK (Tzafiriri, 2003). We therefore conclude that whenever $|1 - k_{-2}/k_1| \leq 1$ (e.g. $0 \leq k_{-2}/k_1 \leq 2$), the validity of the ITA of IREK guarantees the validity of the initial transient for REK. Moreover, as shown in Appendix B of Tzafiriri (2003), $\varepsilon_{IR}(S_T, E_T)$ has a global maximum at $S_T = 0$, $E_T = K_M$, such that

$$\varepsilon_{IR}(S_T, E_T) \leq \varepsilon_{IR}(0, K_M) = \frac{K|1 - k_{-2}/k_1|}{4K_M} \\ \leq \frac{|1 - k_{-2}/k_1|}{4}, \tag{22}$$

where

$$K \equiv k_2/k_1 \tag{23}$$

is the so-called *Van Slyke–Cullen* constant (van Slyke and Cullen, 1914). This entails that the ITA is always at least roughly valid when $0 \leq k_{-2}/k_1 \leq 2$, and that the smaller the ratio K/K_M , the better the approximation. Moreover, Eq. (22) implies that the validity of the ITA can be guaranteed by a criterion depending only on the kinetic parameters that appear in kinetic scheme (1), namely

$$\frac{K|1 - k_{-2}/k_1|}{4K_M} \ll 1. \tag{24}$$

This is noteworthy, since the ratio K/K_M is very small for many metabolic enzymes (see Chapter 5 of Atkinson, 1977).

Assuming that condition (21) is satisfied, we note that Eq. (15) implies that $C_i(t)$ grows and in a time of order t_C approaches the maximal asymptotic value implied by the initial conditions, $C_-(S_T)$, which in turn implies that the enzyme–substrate complex eventually enters a QSS such that

$$\frac{dC}{dt} \approx 0, \quad t > t_C \tag{25a}$$

and

$$C \approx C_-(\sigma), \quad t > t_C. \tag{25b}$$

Moreover, since the validity of Eq. (21) guarantees that the fractional decrease of \bar{S} is negligible during the initial transient, the total QSSA (tQSSA) reduces the problem to a single nonlinear differential equation

$$\frac{d\bar{S}}{dt} \approx -k_2 C_-(\sigma) + k_{-2}(E_T - C_-(\sigma))(S_T - \bar{S}), \tag{26}$$

$t > t_C,$

subject to the initial condition

$$\bar{S} = S_T, \quad t = t_C. \tag{27}$$

For the tQSSA to be uniformly valid for $t > 0$, the induction period prior to the QSS, t_C , must be much shorter than, $t_{\bar{S}}$, the time-scale for the depletion of \bar{S} during the *beginning* of the QSS phase

$$\delta \equiv \frac{t_C}{t_{\bar{S}}} \ll 1. \tag{28}$$

Namely, Eq. (28) guarantees that we can substitute $t_C = 0$ into Eqs. (26) and (27) and moreover, that

$$C \approx C_-(\sigma) \left(\frac{1 - e^{-t/t_C}}{1 - (C_-(S_T)/C_+(S_T))e^{-t/t_C}} \right), \quad 0 < t < \infty. \tag{29}$$

For IREK, $k_{-2} = 0$, so that Eqs. (26) and (27) imply $t_{\bar{S}} \approx S_T/(k_2 C_-(S_T))$ and $\delta \approx \epsilon_{IR}$ (Tzafiriri, 2003). However, when $k_{-2} > 0$ the term $k_{-2}(E_T - C_-(\sigma))(S_T - \bar{S})$ may be significant and it is difficult to obtain a priori estimates of $t_{\bar{S}}$ and δ . Henceforth, we shall therefore restrict our analysis of REK to three specific cases for which the tQSSA of IREK has been shown to be valid

and moreover for which $C_-(\sigma)$ can be approximated by a more manageable form which allows the closed form integration of Eqs. (26) and (27). Hence, $\epsilon \ll |1 - k_{-2}/k_1|$ for each of these cases which guarantees the validity of the ITA provided that $k_{-2}/k_1 = O(1)$. Thus, to determine whether the tQSSA is also valid for these cases we only need to estimate δ .

2.1. Uniformly valid approximations

2.1.1. Low enzyme concentrations

As shown in (Tzafiriri, in press), the criterion

$$E_T \ll S_T + K_M. \tag{30}$$

guarantees the validity of the tQSSA of the IREK and moreover that Eq. (12) reduces to

$$C_-(\sigma) \approx \frac{E_T \sigma}{K_M + \sigma}, \quad C_+(\sigma) \approx \sigma + K_M. \tag{31}$$

Correspondingly, Eq. (26) reduces to

$$\frac{d\bar{S}}{dt} \approx \frac{E_T(k_{-2}K_D S_T - (k_{-2}K_D + k_2)\bar{S})}{K_M + (k_{-2}/k_1)S_T + (1 - (k_{-2}/k_1))\bar{S}} \tag{32}$$

where we introduced the standard notation

$$K_D \equiv k_{-1}/k_1. \tag{33}$$

Substituting the identity $\bar{S} = S_T - P$ into both sides of Eq. (32) yields

$$\frac{dP}{dt} \approx \frac{E_T(k_2\bar{S} - k_{-2}K_D P)}{K_M + \bar{S} + (k_{-2}/k_1)P}. \tag{34}$$

Substituting S instead of \bar{S} in Eq. (34) reduces it to the celebrated Haldane equation (Haldane, 1930). Moreover, whenever Eq. (30) is strictly valid, $C \ll \bar{S}$ and Eqs. (32)–(34) reduce to the Haldane equation. Henceforth, we shall therefore refer to Eqs. (31) and (32) as the *total Haldane* approximation. Integrating Eq. (32) we find

$$\bar{S} = \bar{S}_{ss} + (c - a) W\left(\left(\frac{a}{c - a}\right)e^{(a-b)t/(c-a)}\right), \tag{35}$$

where W is the Lambert W function, defined as the real valued solution of $W(x)e^{W(x)} = x$ (Corless et al., 1996) and we employed the simplifying notations

$$\begin{aligned} \bar{S}_{ss} &\equiv \frac{k_{-2}K_D S_T}{k_2 + k_{-2}K_D}, \quad a \equiv S_T - \bar{S}_{ss}, \\ b &\equiv \frac{E_T(k_2 + k_{-2}K_D)}{1 - (k_{-2}/k_1)}, \quad c \equiv \frac{S_T + K_M}{1 - (k_{-2}/k_1)}. \end{aligned} \tag{36a–d}$$

Moreover, Eq. (31) implies that $C_-(S_T)/C_+(S_T) \ll 1$ and $t_C^{-1} \approx k_1(S_T + K_M)$, so that Eqs. (15) and (29) reduce to

$$C_i \approx \frac{E_T S_T}{K_M + S_T} (1 - e^{-k_1(S_T + K_M)t}) \tag{37}$$

and

$$C \approx \frac{E_T \sigma}{K_M + \sigma} (1 - e^{-k_1(S_T + K_M)t}), \tag{38}$$

where $\sigma \equiv \bar{S} + (k_{-2}/k_1)(S_T - \bar{S})$ and \bar{S} is given by Eq. (35). It is noteworthy that results (31)–(32), (35) and (38) reduce to the correct IREK approximations (Tzafiriri, 2003).

The validity of the *classical* Haldane approximation (e.g. Eq. (34)) has been studied by others. Miller and Alberty (1958) and Stayton and Fromm (1979) showed by direct means that the criterion $E_T \ll S_T$ guarantees the validity of the classical Haldane approximation and Segel (1988) used scaling analysis to argue that Eq. (30) implies its validity, but did not demonstrate this numerically.

Here we note that Eq. (31) reduces Eq. (21) to

$$\varepsilon \approx \frac{|1 - k_{-2}/k_1|KE_T}{(K_M + S_T)^2} < \frac{|1 - k_{-2}/k_1|E_T}{K_M + S_T} \quad (39)$$

and that Eqs. (35) to (36) imply

$$\begin{aligned} \delta &= \frac{bt_C}{c - a} = \left(\frac{E_T}{K_M + S_T} \right) \frac{(k_2 + k_{-2}K_D)^2}{k_1K_M(k_2 + k_{-2}(K_D + S_T))} \\ &\leq \left(\frac{E_T}{K_M + S_T} \right) \frac{K + (k_{-2}/k_1)K_D}{K_M}. \end{aligned} \quad (40)$$

Hence, the validity of Eq. (31) implies that $\varepsilon \ll |1 - k_{-2}/k_1|$ and $\delta \ll (K/K_M) + (k_{-2}/k_1)(K_D/K_M)$, which suggests that the validity of the ITA and the total Haldane approximation can be guaranteed by ensuring that $E_T/(K_M + S_T)$ is sufficiently small. In lieu of an estimate of k_{-2} , the requirement $E_T/(K_M + S_T) \leq 0.01$ should suffice.

Fig. 1 considers a case where Eq. (35) is valid ($E_T/(S_T + K_M) = 0.03$) and compares the *total* Haldane approximation to the numerical solution of Eqs. (4)–(7) for k_{-2}/k_1 values ranging from 0 to 5. As can be seen, the total Haldane approximation reproduces the qualitative behavior of the exact solutions, which is manifested by correlation coefficients that are all very near unity. In order to get a better measure of the correspondence of the approximations to the exact solutions we calculated the corresponding R^2 values. For the total Haldane approximations illustrated in Figs. 1A and B we find, respectively, $R^2 = (0.9998, 0.9970, 0.9865)$ and $R^2 = (0.9995, 0.9978, 0.9959)$ for the range $k_{-2}/k_1 = (0, 1.0, 5.0)$. Using $\sqrt{1 - R^2}$ as a measure of a characteristic relative error, these R^2 values imply $(\Delta\bar{S}/\bar{S}) = (0.016, 0.055, 0.116)$ and $(\Delta C/C) = (0.022, 0.047, 0.064)$. It is noteworthy that these errors are on the order of the validity parameter $E_T/(S_T + K_M)$ and that, in accord with Eq. (40), $\Delta\bar{S}/\bar{S}$ and $\Delta C/C$ both increase with k_{-2}/k_1 . Moreover, it is noteworthy that for the cases shown in Fig. 1A, $(\Delta\bar{S}/\bar{S}) = (0.057, 0.250, 0.660)$, which implies that \bar{S} is better approximated by Eq. (35) than by S . Thus, the Briggs–Haldane approximation and the classical Haldane approximation are both invalid for the cases shown in Fig. 1.

Although solutions in terms of the Lambert W function can be used for parameter fitting purposes

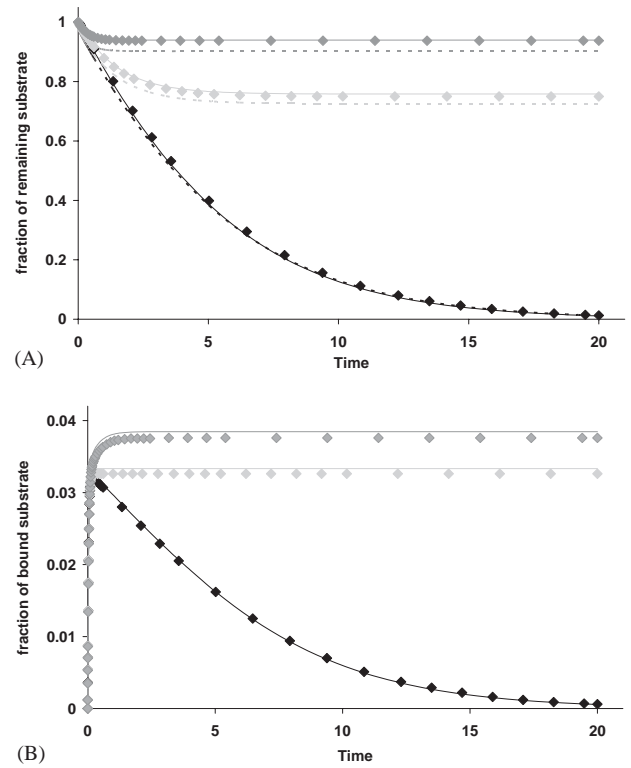


Fig. 1. Fraction of free (S/S_T , panel A), enzyme-bound (C/S_T , panel B) and total (\bar{S}/S_T , panel A) substrate as a function of k_{-2}/k_1 . The numerical solution of Eqs. (4)–(7) (diamonds and dashes) are contrasted with the tQSSA, Eqs. (35) and (38) (solid lines) in both panels. For the cases depicted in panel A, Eq. (35) is a better approximation of \bar{S} than the numerical solution of Eq. (4) (dashes), and consequently of the Briggs–Haldane approximation and the classical Haldane approximation. The parameter values used in these simulations guarantee the validity of Eq. (30) [$k_1 = 1, k_{-1} = 15, k_2 = 5, S_T = 10$ and $E_T = 1$ ($K_M = 20, K = 5$) and $k_{-2}/k_1 = 0$ (black), $k_{-2}/k_1 = 1.0$ (light gray) and $k_{-2}/k_1 = 5.0$ (dark gray)].

(Goudar et al., 1999; Goudar and Ellis, 2001), this is a rather cumbersome process. Moreover, since Eq. (35) is not very revealing we shall now consider its simplification. We begin by noting that

$$\begin{aligned} c - a &\equiv K_M + (k_{-2}/k_1)S_T + (1 - k_{-2}/k_1)\bar{S}_{ss} \\ &\equiv K_M + \sigma_{ss} \geq K_M \end{aligned} \quad (41a)$$

and

$$\begin{aligned} \frac{a}{c - a} &\equiv \left(\frac{S_T}{K_M + \sigma_{ss}} \right) \left(\frac{k_2}{k_2 + k_{-2}K_D} \right) \\ &\leq \frac{S_T}{K_M + \sigma_{ss}} \leq \frac{S_T}{K_M}. \end{aligned} \quad (41b)$$

Thus, the condition

$$K_M \gg S_T, \quad (42)$$

guarantees that $a/(c - a) \ll 1$ so that (Corless et al., 1996)

$$W(x) \approx x, \quad x \equiv \left(\frac{a}{c - a} \right) e^{(a - bt/c - a)} \ll 1. \quad (43)$$

Consequently Eq. (35) reduces to

$$\bar{S} \approx \bar{S}_{ss} + ae^{(-bt/c-a)t} = \bar{S}_{ss} + (S_T - \bar{S}_{ss})e^{-t/t_{\bar{S}}} \quad (44a)$$

with

$$t_{\bar{S}} \equiv \frac{b}{c} \approx \frac{K_M}{E_T(k_2 + k_{-2}K_D)}. \quad (44b)$$

Moreover, Eq. (42) implies that Eq. (38) reduces to

$$C \approx (E_T/K_M)(1 - e^{-(k_{-1}+k_2)t}) \times ((k_{-2}/k_1)S_T + (1 - k_{-2}/k_1)\bar{S}). \quad (45)$$

Fig. 2 compares the numerical solution of Eqs. (4)–(7) to the evaluation of Eqs. (44)–(45) for a case such that $E_T/(S_T + K_M) = 0.05$ and $S_T/K_M = 0.05$. Using $\sqrt{1 - R^2}$ as a measure of the relative errors for the examples in Fig. 2 yields $(\Delta\bar{S}/\bar{S}) = (0.048, 0.081, 0.146)$ and $(\Delta C/C) = (0.091, 0.171, 0.234)$ for the range $k_{-2}/k_1 = (0, 0.5, 2.0)$. Although these errors are low for $k_{-2} = 0$, they increase with k_{-2}/k_1 and eventually become larger than the validity parameters, $E_T/(S_T +$

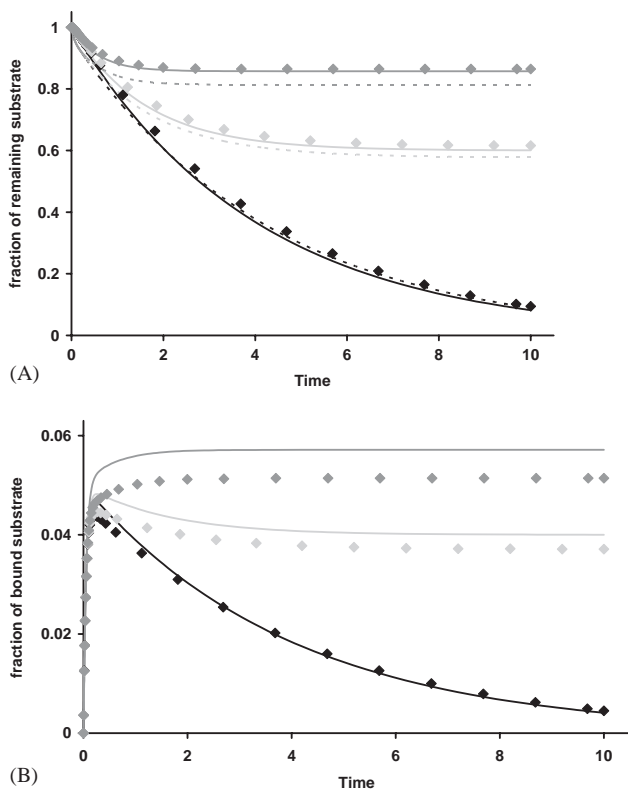


Fig. 2. Fraction of free (S/S_T , panel A), enzyme-bound (C/S_T , panel B) and total (\bar{S}/S_T , panel A) substrate as a function of k_{-2}/k_1 . The numerical solution of Eqs. (4)–(7) (diamonds and dashes) are contrasted with the tQSSA, Eqs. (44) and (45) (solid lines) in both panels. For the cases depicted in panel A, Eqs. (44) is a better approximation of \bar{S} than the numerical solution of Eq. (4) (dashes), and consequently of the Briggs–Haldane approximation and the classical Haldane approximation. The parameter values used in these simulations guarantee the validity of Eqs. (30) and (42) [parameter values as in Fig. 1 except $S_T = 1$ and $k_{-2}/k_1 = 0$ (black), $k_{-2}/k_1 = 0.5$ (light gray) and $k_{-2}/k_1 = 2.0$ (dark gray)].

$K_M)$ and S_T/K_M . For comparison, the corresponding estimates for Eqs. (35) and (38) are, respectively, $(\Delta\bar{S}/\bar{S}) = (0.033, 0.069, 0.125)$ and $(\Delta C/C) = (0.033, 0.080, 0.122)$, which demonstrates once more that Eq. (30) guarantees the validity of the total Haldane approximation for a wide range of k_{-2}/k_1 values. Finally, inspection of Fig. 2A demonstrates for this case as well that \bar{S} is more closely approximated by the tQSSA than by the exact value of S .

2.1.2. High enzyme concentrations

As shown in (Tzafiriri, 2003), each of the criteria

$$E_T + K_M \gg S_T \quad \text{and} \quad K \ll K_M, \quad (46)$$

$$E_T \gg S_T \quad \text{and} \quad E_T \gg K_M \approx K \quad (47)$$

guarantee the validity of the tQSSA of IREK and moreover that Eq. (12) reduces to

$$C_-(\sigma) \approx \left(\frac{E_T}{E_T + K_M} \right) \sigma, \quad C_+(\sigma) \approx E_T + K_M. \quad (48)$$

Correspondingly, Eq. (26) reduces to

$$\frac{d\bar{S}}{dt} \approx - \left(\frac{k_2 E_T}{E_T + K_M} \right) \sigma + \frac{k_{-2} E_T (E_T + K_M - \sigma)}{E_T + K_M} (S_T - \bar{S}). \quad (49)$$

The fact that σ is linear in \bar{S} (see Eq. (11)) implies that the right-hand side of Eq. (49) is quadratic in \bar{S} and hence that Eq. (49) is a solvable Riccati equation. Instead of deriving the closed form solution of Eq. (49), we note that the validity of either of Eqs. (46)–(47) implies that we can neglect σ compared to $E_T + K_M$ in the second bracketed term on the right-hand side of Eq. (49). Namely,

$$\begin{aligned} \frac{d\bar{S}}{dt} \approx & k_{-2}(E_T + K_D) \left(\frac{E_T}{E_T + K_M} \right) S_T - (k_2 + k_{-2}) \\ & \times (E_T + K_D) \left(\frac{E_T}{E_T + K_M} \right) \bar{S}. \end{aligned} \quad (50)$$

This equation implies the following steady-state value:

$$\bar{S}_{ss} \approx \frac{(E_T + K_D)S_T}{E_T + K_D + k_2/k_{-2}}. \quad (51)$$

Integrating Eq. (50) we obtain

$$\begin{aligned} \bar{S} \approx & \bar{S}_{ss} + (S_T - \bar{S}_{ss})e^{-t/t_{\bar{S}}}, \\ t_{\bar{S}} = & \frac{E_T + K_M}{(k_2 + k_{-2}(E_T + K_D))E_T}. \end{aligned} \quad (52)$$

Moreover, Eq. (48) implies that $C_-(S_T)/C_+(S_T) \ll 1$ and $t_C^{-1} \approx k_1(E_T + K_M)$, so that

$$C \approx \frac{E_T((k_{-2}/k_1)S_T + (1 - k_{-2}/k_1)\bar{S})}{E_T + K_M} \times (1 - e^{-k_1(E_T + K_M)t}). \quad (53)$$

It is noteworthy that Eqs. (52) and (53) reduce to the correct IREK approximations (Tzafiriri, 2003). Regarding

the validity of these approximations for $k_{-2} > 0$ we note that Eq. (48) reduces Eq. (21) to

$$\varepsilon \approx \frac{|1 - k_{-2}/k_1|KE_T}{(K_M + E_T)^2} < \frac{|1 - k_{-2}/k_1|K}{K_M + E_T} \quad (54)$$

and that Eqs. (45) and (49) imply that

$$\begin{aligned} \delta &\approx \left(\frac{E_T}{K_M + E_T}\right) \frac{K + (k_{-2}/k_1)(K_D + E_T)}{K_M + E_T} \\ &< \left(\frac{E_T}{K_M + E_T}\right) \left(\frac{K}{K_M + E_T} + \frac{k_{-2}}{k_1}\right). \end{aligned} \quad (55)$$

Thus, Eqs. (46) and (47) both imply that $\varepsilon \ll |1 - k_{-2}/k_1|$. Consequently, the strict validity of either of Eqs. (46) and (47) guarantees the validity of the ITA (e.g. that $\varepsilon \ll 1$) for any given k_{-2} . Moreover, the strict validity of either of Eqs. (46) and (47) reduces Eq. (55) to the form

$$\delta \approx \left(\frac{E_T}{K_M + E_T}\right) \left(\frac{k_{-2}}{k_1}\right). \quad (56)$$

Thus, Eq. (47) implies that $\delta \approx k_{-2}/k_1$. Consequently, Eq. (47) guarantees the validity of the tQSSA *only* for $k_{-2}/k_1 \ll 1$. For example, this implies that Eqs. (52) and (53) are valid for studying the interconversion of hemimercaptal of glutathione and methylglyoxal and S-D-lactoylglutathione at high glyoxalase I concentrations ($K/K_M = 0.997$ and $k_{-2}/k_1 = 0.03$, Sellin and Manner-*vik*, 1983). In contrast, Eq. (46) implies nothing about the prefactor $E_T/(K_M + E_T)$ and consequently can also guarantee the validity of the tQSSA for $k_{-2}/k_1 = O(1)$. However, rewriting Eq. (51) in the form

$$\begin{aligned} \bar{S}_{ss} &\approx S_T \left(1 - \frac{K}{K + (k_{-2}/k_1)(E_T + K_D)}\right) \\ &= S_T \left(1 - \frac{KE_T}{(E_T + K_M)^2 \delta}\right) \end{aligned} \quad (57)$$

demonstrates that $\delta = O(1)$ implies that $\bar{S}_{ss} \approx S_T$ and vice versa. Note, that although Eq. (57) was obtained by assuming the validity of the tQSSA, this is actually a much more general result, since $dC/dt = 0$ at the true steady state. Thus, although $\delta = O(1)$ implies that the tQSSA is invalid, the errors incurred by Eq. (52) are guaranteed to be small and therefore do not affect the validity of Eq. (53). Namely, $\delta = O(1)$ implies that Eqs. (52) and (53) reduce to the corresponding ITA. We therefore conclude that Eqs. (52) and (53) may be safely used whenever either of Eqs. (46) and (47) is valid, but that they only possess real information content for $\delta \ll 1$.

Fig. 3 compares the numerical solution of Eqs. (4)–(7) to Eqs. (52)–(53) for a case such that $S_T/(E_T + K_M) = 0.03$ and $K/K_M = 0.25$, so that Eq. (43) is only *roughly* valid. Moreover, $E_T/(S_T + K_M) = 0.3$ for this example and, indeed, the total Haldane approximation (dashes) is rather poor. The $\sqrt{1 - R^2}$ relative errors associated with Eqs. (52) and (53) in Fig. 3 are $(\Delta\bar{S}/\bar{S}) = (0.107, 0.189, 0.325)$ and $(\Delta C/C) = (0.137, 0.025, 0.069)$ for the range $k_{-2}/k_1 = (0, 0.5, 2.0)$. We see that whereas

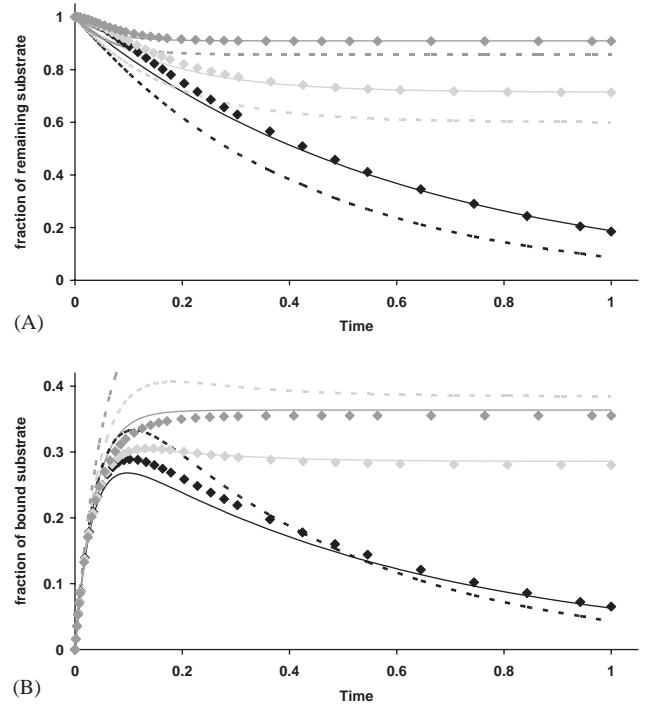


Fig. 3. Fraction of total (\bar{S}/S_T , panel A) and enzyme-bound (C/S_T , panel B) substrate as a function of k_{-2}/k_1 . The numerical solution of Eqs. (4)–(7) (diamonds) are contrasted with the tQSSA for high enzyme concentrations, Eqs. (52) and (53) (solid lines) and the tQSSA of low enzyme concentrations, Eqs. (35) and (38) (dashes) in both panels. The parameter values used in these simulations guarantee the validity of Eq. (46) [$k_1 = 1$, $k_{-1} = 15$, $k_2 = 5$, $S_T = 1$ and $E_T = 10$ ($K_M = 20$, $K = 5$) and $k_{-2}/k_1 = 0$ (black), $k_{-2}/k_1 = 0.5$ (light gray) and $k_{-2}/k_1 = 2.0$ (dark gray)].

Eq. (53) yields a good approximation of C for the whole range of k_{-2}/k_1 values, Eq. (52) loses its accuracy with increasing k_{-2}/k_1 values, which is consistent with Eq. (55). Moreover, this example demonstrates the importance of the requirement $K \ll K_M$ in Eq. (46) (Eq. (55)). Indeed, for a case similar to the one considered in Fig. 2 ($k_1 = 1$, $k_{-1} = 18$, $k_2 = 2$, $S_T = 1$ and $E_T = 10$) such that $K/K_M = 0.11$ we find $(\Delta\bar{S}/\bar{S}) = (0.099, 0.141, 0.164)$ and $(\Delta C/C) = (0.042, 0.037, 0.046)$ for the range $k_{-2}/k_1 = (0, 0.5, 2.0)$ (results not shown).

Fig. 4 compares the numerical solution of Eqs. (4)–(7) to Eqs. (52)–(53) for a case such that $S_T/E_T = 0.02$ and $K_M/E_T = 0.04$, so that Eq. (46) is valid. Moreover, $E_T/(S_T + K_M) = 16.7$ for this example and, indeed, the total Haldane approximation (dashes) is exceedingly poor. The $\sqrt{1 - R^2}$ relative errors associated with Eqs. (52) and (53) in Fig. 4 are $(\Delta\bar{S}/\bar{S}) = (0.068, 0.134, 0.268)$ and $(\Delta C/C) = (0.069, 0.034, 0.005)$ for $k_{-2}/k_1 = (0, 0.1, 0.5)$. In agreement with the general analysis, $(\Delta C/C)$ is always small and decreases with k_{-2}/k_1 , whereas $(\Delta\bar{S}/\bar{S})$ increases with k_{-2}/k_1 and can reach non-negligible values. A close inspection of Fig. 3A reveals that as predicted, the error associated with Eq. (52) is concentrated in the early phase, before C

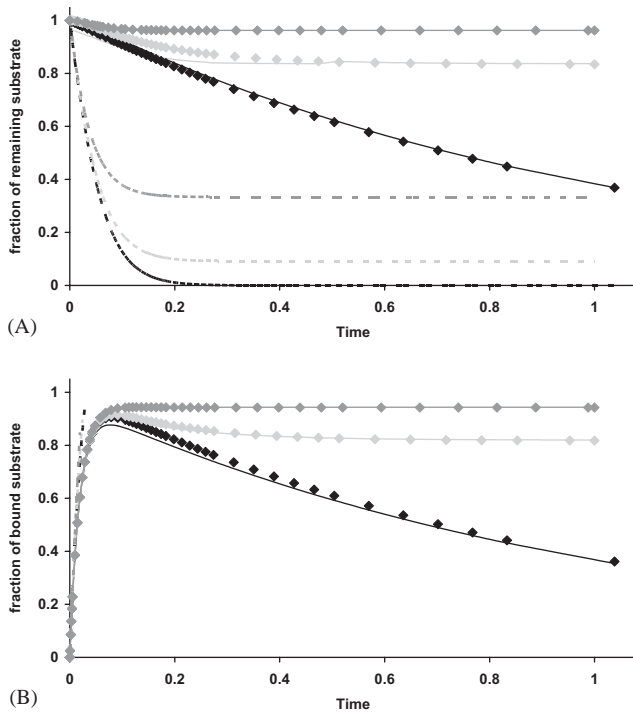


Fig. 4. Fraction of total (\bar{S}/S_T , panel A) and enzyme-bound (C/S_T , panel B) substrate as a function of k_{-2}/k_1 . The numerical solution of Eqs. (4)–(7) (diamonds) are contrasted with the tQSSA for high enzyme concentrations, Eqs. (52) and (53) (solid lines) and the tQSSA of low enzyme concentrations, Eqs. (35) and (38) (dashes) in both panels. The parameter values used in these simulations guarantee the validity of Eq. (47) [$k_1 = 1$, $k_{-1} = 1$, $k_2 = 1$, $S_T = 1$ and $E_T = 50$ ($K_M = 2$, $K = 1$) and $k_{-2}/k_1 = 0$ (black), $k_{-2}/k_1 = 0.1$ (light gray) and $k_{-2}/k_1 = 0.5$ (dark gray)].

attains its maximum and that the subsequent plateau is well approximated by Eq. (52). This behavior correlates well with the fact that $\delta = (0.019, 0.113, 0.490)$ for $k_{-2}/k_1 = (0, 0.1, 0.5)$. Since \bar{S} only varies by 4% for the $k_{-2}/k_1 = 0.5$ case in Fig. 3A, the errors associated with Eq. (52) during early times translate to insignificant errors associated with Eq. (53). Finally, it is interesting to note that the $k_{-2}/k_1 = 0.5$ case in Fig. 4B does not display an observable maximum, whereas a long accepted hypothesis has been that C displays a maximum whenever $k_{-2}/k_1 < 1$ (Miller and Alberty, 1958). However, previous analysis was restricted to low enzyme concentrations (where it is consistent with our current analysis), whereas our analysis of the high enzyme concentration case indicates that regardless of the value of the ratio k_{-2}/k_1 , the concentration of enzyme–substrate complex does not have a maximum whenever $\delta = O(1)$.

3. Sequential parameter estimation

Although Eqs. (35) and (39) have sufficient degrees of freedom for fitting all the parameters that appear in

kinetic scheme (1), the fact that these approximations are based on the Lambert W functions makes this procedure somewhat cumbersome (Goudar et al., 1999; Goudar and Ellis, 2001). We shall therefore derive here a simple alternative to this approach.

We begin by noting the finding that whenever $E_T < S_T + K_M$ the ITA of C is independent of k_{-2} . This suggests that it is possible to obtain a direct estimate of k_1 and K_M by fitting the time dependent rise of the enzyme–substrate complex to Eq. (38), just as is in the case of IREK (Schnell and Mendoza, 1997). Once K_M has been determined in such a manner, one can conduct an experiment under conditions of low substrate and enzyme concentrations, such that Eqs. (30) and (42) are both valid. In this case Eq. (44) is valid and an estimate of $t_{\bar{S}} = (K_M/E_T(K_D k_{-2} + k_2))$ yields an indirect estimate of the combination $\kappa \equiv K_D k_{-2} + k_2$. Since $K_M - \kappa = k_{-1}(1 - k_{-2}/k_1)$, accurate estimates of K_M and κ can be used to determine if $k_{-2} > k_1$ or $k_{-2} < k_1$. Moreover, Eq. (36a) can be rearranged as $k_{-2}K_D = \kappa \bar{S}_{ss}/S_T$. Hence, once an estimate of κ exists a simple measurement of P_{ss} can provide an estimate of $k_{-2}K_D$ and consequently of $k_2 = \kappa - k_{-2}K_D$, $k_{-1} = k_1 K_M - k_2$, $K_D \equiv k_{-1}/k_1$ and $k_{-2} = (\kappa - k_2)/K_D$. We therefore see that using such a sequential approach allows the estimation of all the parameters appearing in kinetic scheme (1) from experiments at low enzyme concentrations such that $E_T \ll S_T + K_M$, for which the total Haldane approximation is guaranteed to be valid. Subsequently, the actual validity of the kinetic scheme can be tested by using these parameter estimates to numerically simulate different experimental conditions.

4. Discussion and conclusions

Since every chemical reaction is reversible under specific conditions, kinetic scheme (1) has been suggested as a possible generalization of the irreversible Michaelis–Menten scheme (Haldane, 1930; Alberty, 1959). As has been pointed out (Miller and Alberty, 1958), the condition for a reaction following kinetic scheme (1) to go to completion is that the product of the rate constants for forward reactions substantially exceed the product of the rate constants for the reverse reactions ($k_1 k_2 \gg k_{-1} k_{-2}$). This criterion puts no restriction on the value of k_{-2} . For example, if $k_{-2} = k_1$, the reaction would essentially go to completion provided that $k_2 \gg k_1$. Thus, the observation that a reaction goes to completion (e.g. $\lim_{t \rightarrow \infty} P = S_T$) does *not* imply that $k_{-2} = 0$. Consequently, in many cases, product inhibition could potentially be explained by kinetic scheme (1) rather than letting $k_{-2} = 0$ and adding a separate reaction to account for inhibition (Miller and Alberty, 1958; Alberty, 1959; Duggleby, 1994). This has been realized in the past; however, in the absence of simple

analytical approximations for reactions that satisfy kinetic scheme (1), the analysis of experiments was achieved either by graphical methods based on the implicit solution of the Haldane approximation (Alberty and Koerber, 1957) or by coupling the numerical solution of Eqs. (4)–(7) to an optimization code.

While graphical methods are simple to use, they are known to change the error structure of the data resulting in distortion of the plot. Garfinkel et al. (1977) have discussed this problem and its possible solutions. An additional drawback of using QSS based graphical methods to interpret data is that QSS rate laws are *not* unique. For example, Haldane's classical rate law actually applies to Michaelis–Menten schemes with *any* number of intermediates (Alberty and Koerber, 1957). Thus, fitting QSS rate laws using graphical methods or otherwise can only yield limited information regarding the underlying kinetic scheme. In principle, direct numerical fitting of transient data to kinetic equations can be used to determine the underlying kinetic scheme and to estimate the kinetic parameters. Although this direct approach is somewhat cumbersome, it is increasingly employed due to its generality and the availability of the necessary software (Frieden, 1993; Dang and Frieden, 1997; Kuzmic, 1996; Mendes and Kell, 1998). However, one limitation of direct numerical fitting of kinetic schemes to experimental data is that in most cases it proves impossible to fit all the kinetic parameters from a small number of experiments. This is not surprising, since the existence of a QSS implies that only a subset of the kinetic parameters have observable effects on the kinetics (Stayton and Fromm (1979) demonstrated this explicitly for REK). Thus, in the absence of a good characterization of the kinetic equations to be fitted, it is impossible to plan a logical experimental strategy for parameter fitting and a brute force numerical approach is both cumbersome and inefficient.

Consequently, there has recently been a renewed effort to derive uniformly valid approximations for IREK (Schnell and Mendoza, 1997, 2000; Schnell and Maini, 2000; Tzafiriri et al., 2002; Tzafiriri, 2003) as a means of unambiguously estimating kinetic parameters from carefully designed experiments wherein the kinetics of P are followed for long times, well into the QSS phase, whereas C is measured during the fast initial transient. However, none of the existing literature has dealt with a detailed analysis of REK. The current work set out to bridge this gap between our understanding of IREK and REK using the tQSSA formalism, since for IREK the domain of validity of the tQSSA has been shown to overlap and extend both the Briggs–Haldane approximation and the rQSSA (Borghans et al., 1996; Tzafiriri, 2003). A central result of the current work is that Eq. (30) guarantees the validity of the tQSSA of REK, whereas Eqs. (46) and (47) guarantee the validity

of the ITA, but only guarantee the validity of the tQSSA for a subset of the cases $k_{-2} < k_1$. This implies that in contrast to the case of IREK, there is no a priori justification for estimating k_2 from experiments at high enzyme concentrations. This finding has prompted the derivation in the current work of a simple sequential parameter estimation scheme which is solely based on experiments at low enzyme concentrations such that $E_T \ll S_T + K_M$. Thus, our analysis suggested a rational theoretical–experimental approach for identification and estimation of all the kinetic parameters, which is simpler and more effective than direct numerical fitting.

Although our analysis of the high enzyme concentration case was not crucial for parameter estimation purposes, it is noteworthy. First, the finding that even at high enzyme concentrations the initial rise in C , almost up to its maximal value, is practically independent of k_{-2} is somewhat surprising. Second, the long accepted assertion that C always displays a maximum if and only if $k_{-2} < k_1$ (Miller and Alberty, 1958) is verified for low enzyme concentrations such that Eq. (30) holds, but refuted at high enzyme concentrations. Namely, our analysis indicates that whenever either of Eqs. (46) and (47) is valid C will not display an experimentally observable maximum for $k_{-2} < k_1$ such that $\delta = O(1)$. An example of a case where $k_{-2}/k_1 = 0.5$, but C does not display a maximum is illustrated in Fig. 4B.

As noted in Section 2.1.1, the total Haldane approximation reduces to the classical Haldane approximation whenever $E_T \ll S_T + K_M$, since the latter condition guarantees that $C \ll S$ (and consequently $S_T - P = \tilde{S} \approx S$). However, as illustrated in Figs. 1A and 2A, the approximation $S_T - P \approx S$, which reduces the total Haldane approximation to the classical Haldane approximation can be rather poor even when the ratio $E_T/(S_T + K_M)$ is small. Thus, even at low enzyme concentrations the tQSSA yields approximations that are of wider validity than the classical Haldane approximation and has the additional advantage of yielding a tight criterion for the validity of the ITA. The latter point is especially important in light of the finding that the ITA of C is in fact of wider validity than the tQSSA. The wider validity of the total Haldane approximation makes it a better choice than the classical Haldane approximation whenever there is ambiguity regarding the concentration enzyme. For example, such an ambiguity is inherent to in situ enzyme assays with permeabilized cells. This invasive technique, developed by Sols and co-workers, is based on cell membrane permeabilization to low-molecular-weight compounds (Reeves and Sols, 1973; Serrano et al., 1973; Cordeiro and Ponces Fereire, 1995). Free diffusion of low-molecular-weight molecules from and into the cells allows the elimination of endogenous substrates, providing controlled enzyme assay conditions at physiological

enzyme concentrations. The development of this in situ technique was driven by the appreciation that intracellular enzyme concentrations may be markedly higher than enzyme concentrations in standard in vitro assays, so that the validity of the classical Briggs–Haldane and Haldane approximations is questionable (Sols and Marco, 1970). Nevertheless, in lieu of a simple alternative, the classical Haldane equation is still used for analysing such assays (Martins et al., 2001). In light of our finding that the total Haldane approximation (or the tQSSA for that matter) is valid in a significantly larger domain of parameter space than the classical Haldane approximation, we suggest that in such cases the tQSSA results, Eqs. (35)–(38) or Eqs. (51)–(53), be used instead of the classical Haldane equation.

The advantages of the tQSSA stem from the fact that it makes use of both conservation equations of the problem and a more accurate approximation of the initial kinetics. Namely, the tQSSA satisfies the Principle of Minimal Simplification (Kruskal, 1963; Segel and Slemrod, 1989), which states that in the process of simplifying a system of equations no term should be neglected without good reason. These ideas have been used to analyse similar problems (Borghans et al., 1996; Tzafiriri et al., 2002) and should carry through to the analysis of other serial kinetic schemes.

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