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ORIGINAL PAPER

Is there anything left to say on enzyme kinetic constants and quasi-steady state approximation?

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Abstract In this paper we re-examine the commonly accepted meaning of the two kinetic constants characterizing any enzymatic reaction, according to Michaelis-Menten kinetics. Expanding in terms of exponentials the solutions of the ODEs governing the reaction, we determine a new constant, which corrects some misinterpretations of current biochemical literature.

Keywords Michaelis-Menten kinetics · Quasi-steady state approximations · Asymptotic expansions

1 Introduction

The question addressed in the title of this paper is not merely a rethoric one. Our answer, of course, is definitely *yes*: we do think that there is still a lot of room in this field. Formulated more than one century ago, the Michaelis-Menten-Briggs-Haldane approximation, or standard quasi-steady state approximation (sQSSA) [7,24,33], still represents a milestone in the mathematical modeling of enzymatic reactions. Nevertheless, the hypothesis of quasi-steady state is crucial for the interpretation of the reaction and must be handled with much care. It is based on the assumption that

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the complex can be considered "substantially" constant, but this statement has led to many misinterpretations of the model. In fact, as Heineken et al. showed in [15], the correct mathematical interpretation of the quasi-steady state assumption is that when we expand asymptotically the solutions of the ODEs governing the process with respect to an appropriate parameter, the sQSSA is the zero order approximation of the solution. As already observed by Briggs and Haldane by a chemical point of view, when the parameter of the expansion is sufficiently small this approximation is valid. Heineken et al. used the parameter given by the ratio of the initial concentrations of enzyme E and substrate S, obtaining the well-known chemical requirement.

In 1987 Fraser [13] pointed out that, geometrically speaking, the steady state assumption for chemical reactions is an approximation in the phase space to the slow manifold, i.e., the singular trajectory which strongly attracts all fast transient flow. He also described an iterative scheme to approximate this singular trajectory without any restrictions on the rate constants of the system. The same arguments were applied to the Michaelis-Menten mechanism by Calder and Siegel [8]. In 1988 Segel [32] and in 1989 Segel and Slemrod [33] obtained the Michaelis-Menten approximation expanding the solutions in terms of a new parameter, including the Michaelis constant and showing that the sQSSA is valid in a wider range of parameters than the one supposed before. However it is well known that while *in vitro* the condition on the concentrations can be easily fulfilled, *in vivo* it is not always respected [1,34–36], in particular when the reaction is not isolated but is part of complex reaction networks. This means that, though very useful, this approximation cannot always be applied.

Michaelis-Menten kinetics has recently become one of the most important tools in the field of Systems Biology and in particular of mathematical modeling of intracellular enzyme reactions, but in most literature any *apriori* analysis of the applicability of sQSSA is absent, even in very complex reaction networks. This fact has led to several problems concerning the study of particular phenomena, like oscillations [12,28], bistability [10], ultrasensitivity [26] or Reverse Engineering [29]. Following [20], recent papers [2,6,10,11,22,26–28,38–40] have introduced and explored a new approximation, called total quasi-steady state approximation (tQSSA), which has been shown to be always roughly valid in the case of an isolated reaction. Nevertheless, since it is in any case an approximation, also the tQSSA can dramatically fail, as shown in [28], in more complex mechanisms, involving more than one reaction, but it is doubtless that it is valid in a much wider range of parameter than the sQSSA [10,27–29,31].

One of the main problems of the mathematical treatment of the sQSSA is the misinterpretation of the hypothesis that the complex time concentration has zero derivative. Many papers and even monographies tend to indicate, probably for the sake of simplicity, the "substantial" equilibrium as a real equilibrium [14,21,30,42], which is obviously not true; in this case any simplification can be definitely misleading. As observed in [15], p. 97, this use of the equations seems scandalous to any mathematicians and can bring to results which are absolutely inconsistent and false. In this work we want to re-examine some mathematical aspects of Michaelis-Menten reaction and of the sQSSA, trying to clarify some aspects of the enzyme reactions; in particular we discuss the biochemical and mathematical meaning of the tQSSA, comparing it with the sQSSA, then we analyse the consequences of the misuse of the sQSSA, recon-

sidering the meaning of the two kinetic constants V_{max} and K_M; finally we introduce an expansion in terms of exponentials, which is valid for every choice of the parameters and enzyme initial concentrations; this expansion is the most appropriate to approximate the asymptotic behavior of the solution for large values of t, in absence of product degradation; moreover we use it to solve a serious incoherence present in literature, related to the biochemical interpretation of the constant K_M.

2 Notations, definitions and main known results

The model of biochemical reactions was set forth by Henri [16–18] and Michaelis and Menten [24] and further developed by Briggs and Haldane [7]. This formulation considers a reaction where a substrate S binds an enzyme E reversibly to form a complex C. The complex can then decay irreversibly to a product P and the enzyme, which is then free to bind another molecule of the substrate. This process is summarized in the scheme

$$E + S \underset{d}{\overset{a}{\rightleftarrows}} C \xrightarrow{k} E + P, \tag{1}$$

where a, d and k are kinetic parameters (supposed constant) associated with the reaction rates: a is the second order rate constant of enzyme-substrate association; d is the rate constant of dissociation of the complex; k is the catalysis rate constant. Following the mass action principle, which states that the concentration rates are proportional to the reactant concentrations, the formulation leads to an ODE for each complex and substrate involved. We refer to this as the *full system*. From now on we will indicate with the same symbols the names of the enzymes and their concentrations. The ODEs describing (1) are

$$\begin{aligned} \frac{dS}{dt} &= -a(E_T - C)S + d\,C, \\ \frac{dC}{dt} &= a(E_T - C)S - (d + k)C, \end{aligned} \tag{2}$$

with initial conditions

$$S(0) = S_T, \quad C(0) = 0, \tag{3}$$

and conservation laws

$$E + C = E_T, \quad S + C + P = S_T. \tag{4}$$

Here E_T is the total enzyme concentration assumed to be free at time $t = 0$. Also the total substrate concentration, S_T, is free at $t = 0$. This is called the Michaelis-Menten (MM) kinetics [3,24]. Let us observe that (2)–(4) asimptotically admits only the trivial solution given by $C = S = 0$, $P = S_T$ and $E = E_T$. This means that all the substrate eventually becomes product due to the irreversibility, while the enzyme eventually is free and the complex concentration tends to zero. Assuming that the complex concentration is approximately constant after a short transient phase leads

to the usual Michaelis-Menten (MM) approximation, or *standard quasi-steady state approximation* (sQSSA): we have an ODE for the substrate while the complex is assumed to be in a quasi-steady state (i. e., $\frac{dC}{dt} \approx 0$):

$$C \cong \frac{E_T \cdot S}{K_M + S}, \qquad \frac{dS}{dt} \cong -kC \cong -\frac{V_{max} S}{K_M + S}, \quad S(0) = S_T, \tag{5}$$

where

$$V_{max} = k\, E_T, \quad K_M = \frac{d + k}{a}. \tag{6}$$

and K_M is the *Michaelis constant*. Applying a quasi-steady approximation reduces not only the dimensionality of the system, passing from two equations (full system) to one (MM approximation or sQSSA). It reduces also its stiffness and thus speeds up numerical simulations greatly, especially for large networks as found *in vivo*. It allows also a theoretical investigation of the system which cannot be obtained with the numerical integration of the full system. Moreover, the kinetic constants in (1) are usually not known, whereas finding the kinetic parameters for the MM approximation is a standard in vitro procedure in biochemistry. See e.g., [3] for a general introduction to this approach. We stress here that this is an approximation to the full system, and that it is valid only under suitable hypotheses, e.g., when the enzyme concentration is much lower than either the substrate concentration or the Michaelis constant K_M, i.e. (see, e.g., [33])

$$\varepsilon_{MM} := \frac{E_T}{S_T + K_M} \ll 1 \tag{7}$$

This condition is usually fulfilled for in vitro experiments, but often breaks down *in vivo* [1,34–36]. We refer to [31] for a nice, general review of the kinetics and approximations of (1). It is useful to quote also the recent papers [10,12,25,28,41] which discuss the applicability of the sQSSA. In order to solve this problem, Laidler [20], discussing the mathematical theory of the transient phase, found expressions for the behavior of P in the quasi-steady state and found several sufficient conditions for the applicability of the approximations. These conditions were much more general than $\frac{E_T}{S_T} \ll 1$. The importance of Laidler's results can be understood comparing his approach to a recent one, based on the *total quasi-steady state approximation* (tQSSA). It was introduced by Borghans et al. [6] and refined by Tzafriri [38] for isolated reactions. It arises introducing the *total substrate*

$$\overline{S} = S + C, \tag{8}$$

and assuming that the complex is in a quasi-steady state as for the sQSSA. Reaction (1) then gives the tQSSA [6,20]:

$$\frac{d\overline{S}}{dt} \cong -k\, C_-(\overline{S}), \quad \overline{S}(0) = S_T, \tag{9}$$

where

$$C_-(\overline{S}) = \frac{(E_T + K_M + \overline{S}) - \sqrt{(E_T + K_M + \overline{S})^2 - 4E_T\overline{S}}}{2}. \tag{10}$$

Numerical integration of (9) gives the time behavior of $\overline{S}$ and then (8) and (10) give the corresponding C and S. Tzafriri [38] showed that the tQSSA (9) is valid whenever

$$\varepsilon_{tQSSA} := \frac{K}{2S_T}\left(\frac{E_T + K_M + S_T}{\sqrt{(E_T + K_M + S_T)^2 - 4E_T S_T}} - 1\right) \ll 1, \tag{11}$$

(where $K = \frac{k}{a}$), and that this is at least roughly valid for any sets of parameters, in the sense that $\varepsilon_{tQSSA} \leq \frac{K}{4K_M} \leq \frac{1}{4}$. This means that, for *any* combination of parameters and initial conditions, (9) gives a decent approximation to the full system (2). The parameter K is known as the Van Slyke-Cullen constant. The *dissociation constant* $K_D = \frac{d}{a}$ [3] is related to the previous kinetic constants by the simple formula $K_D = K_M - K$. Let us remark that, in recent literature, the sQSSA is applied to complex enzyme reaction networks, like, e.g., the MAPK cascade, without any *a priori* analysis on its applicability, setting to zero not only the derivatives of the complex concentrations, but also, surprisingly, the complex concentrations themselves (see, e.g., [9,19,23]). This produces serious inconsistencies with experimental observations and has resulted in the discovery of the so-called "substrate sequestration" hypothesis [4,5], which states that the enzyme can sequester a significant amount of substrate by binding to it, making this sequestered fraction of the substrate no longer accessible to other kinases. The importance of the choice of $\bar{S}$ as one of the system variables lies in the fact that substrate sequestration is naturally included in the total substrate. Indeed, the latter takes into account both the free and the "sequestered" substrate.

3 Use and misuse of the standard Quasi-Steady State Approximation (sQSSA)

The roles of V_{max}, the maximal reaction velocity, and K_M, the Michaelis constant, become essential when characterizing biochemical reactions *in vitro* as well as *in vivo*. Moreover, the description of cooperative reactions, inhibition and many other biochemical processes have up to now exploited the fundamental ideas of the MM scheme, i.e., the sQSSA and the parameters V_{max} and K_M (see, e.g., [3]). However, these approximations cannot be expected to be valid *in vivo*.

The dependence of the product velocity

$$v := \frac{dP}{dt} = kC \tag{12}$$

on the concentration of S is based on the *a priori* (and not always true) assumption that the sQSSA is valid. In this case

$$v = kC \cong \frac{V_{max} \cdot S}{K_M + S}. \tag{13}$$

Consequently V_{max} is usually intended as the limit of the "initial velocity" for the S concentration tending to infinity and K_M as the value of S such that

$$v(S = K_M) = \frac{V_{max}}{2}. \tag{14}$$

Since the tQSSA is much more appropriate than the sQSSA, we can use formula (10) and very simple algebra to define in a more appropriate way K_M (if $S_T > K_M$): (i) when the value of the total substrate is equal to $\overline{S} = K_M + \frac{E_T}{2}$, then the rate of P is equal to $\frac{V_{max}}{2}$:

$$v\left(\overline{S} = K_M + \frac{E_T}{2}\right) = \frac{V_{max}}{2} \tag{15}$$

This result can also be found in [37]. Let us remark, by the way, that if we used the Tzafriri approximating formula, we would obtain the following definition:
(ii) when the value of the total substrate is equal to $\overline{S} = K_M + E_T$, then the velocity of P is equal to $\frac{V_{max}}{2}$:

$$v\left(\overline{S} = K_M + E_T\right) = \frac{V_{max}}{2} \tag{16}$$

Then the estimate given by (16) becomes largely incorrect for high values of E_T.

4 The equilibrium constant revisited

Though the sQSSA is based on the approximation $\frac{dC}{dt} \cong 0$, several biochemistry textbooks (see e.g., [14,21,30,42]), in order to simplify the mathematics, consider the approximation as a true equality, leading to a misinterpretation of the QSSA. As a consequence, the Michaelis constant is determined by equating to zero the right hand side of the second equation of (2) [14,30,42], obtaining

$$K_M = \frac{E \cdot S}{C} = \frac{(E_T - C) \cdot S}{C}. \tag{17}$$

Actually, the derivative of C is equal to zero only at time $t = t_{max}$, when C reaches its maximum value. Consequently we cannot declare that the right hand side in (17) remains constant. On the other hand, we could interpret K_M as the equilibrium value for $\frac{E \cdot S}{C}$, reached for large t (supposing that no degradation, product inhibition or back reaction phenomena are involved), in the same way as the dissociation constant K_D is interpreted in the original Michaelis-Menten reaction, where $k = 0$ [30]. Actually, while this last reaction, which is completely reversible, reaches a steady-state where both S and C are different from zero, in reaction (1), as remarked above, S and C tend to zero and consequently we cannot use (17), which gives an undefined ratio, for $t \to \infty$. Thus the equality $K_M = \frac{E \cdot S}{C}$ is valid for every reaction only at $t = t_{max}$. We can however try to solve the indetermination of the ratio for $t \to \infty$ in the following way. After the transient phase, all the reactants seem to follow asymptotically an exponential behavior, with negative exponent. If we suppose that the asymptotic

decay of C is proportional to $e^{-\alpha t}$, for some α, formula (12) implies that also $S_T - P$ will be asymptotically proportional to $e^{-\alpha t}$. By means of the conservation laws (4) we can conclude that also S and $E_T - E$ will follow the same asymptotic behavior as C. Thus let us expand S and C in powers of $e^{-\alpha t}$: we have

$$S(t) = S_0 + S_1\, e^{-\alpha t} + S_2\, e^{-2\alpha t} + o(e^{-2\alpha t}) \tag{18}$$

$$C(t) = C_0 + C_1\, e^{-\alpha t} + C_2\, e^{-2\alpha t} + o(e^{-2\alpha t}) \tag{19}$$

After some computations, we get then

$$S_{as}(t) \cong S_1\, e^{-\alpha t} \tag{20}$$

$$C_{as}(t) \cong \frac{\alpha}{k-\alpha}\, S_1\, e^{-\alpha t} \tag{21}$$

where

$$\alpha = \frac{a}{2}(K_M + E_T)\left[1 - \sqrt{1 - \frac{4kE_T}{a(K_M + E_T)^2}}\right] \tag{22}$$

There is still an unknown parameter, S_1, which could be estimated from experimental data via a least-squares procedure.

We are now in position to state the main results of this section.

Theorem 4.1 *For* $t \to \infty$

$$\frac{E\,S}{C}(t) \cong \frac{E_{as}\,S_{as}}{C_{as}}(t) \to \left(\frac{k-\alpha}{\alpha}\right) E_T =: K_W \tag{23}$$

The constant K_W, here introduced for the first time, gives the exact asymptotic value of the ratio $\frac{E\,S}{C}$ and, in contrast with biochemical literature [14,30,21,42], in general is different from K_M. This result is clearly illustrated in Figs. 1, 2, and 3, where we

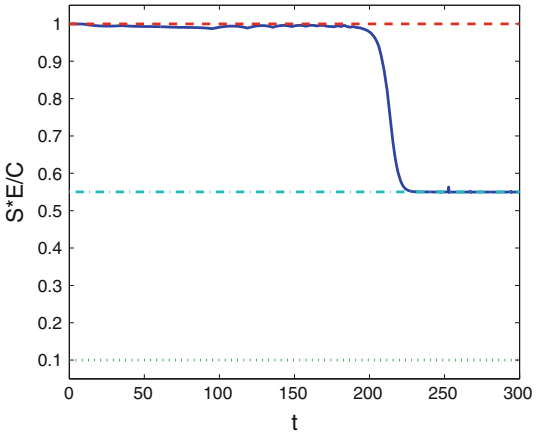

Fig. 1 Plot of $\frac{E\,S}{C}$ for $a = 1$, $k = 0.9$, $d = 0.1$, $S_T = 100$, $E_T = 0.55$. *Solid line* numerical solution of the full system, *dashed* K_M, *dashed-dotted* K_W, *dotted* K_D. Parameters and initial conditions were chosen to give $K_W = \frac{K_M + K_D}{2}$

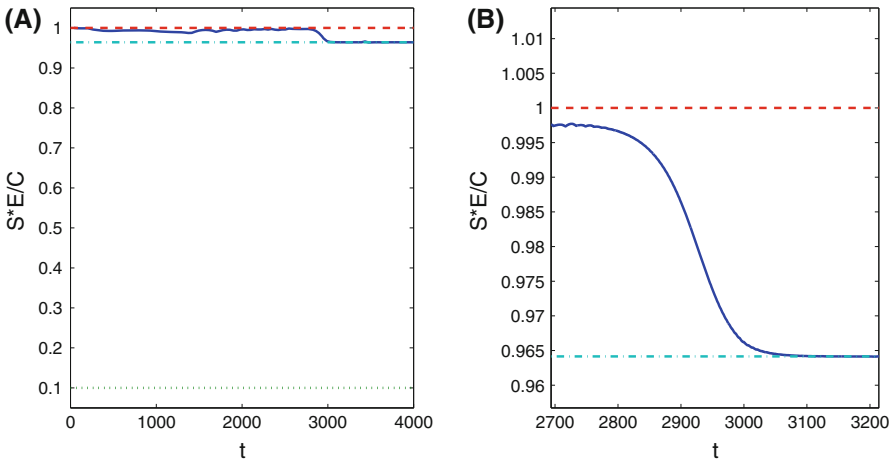

Fig. 2 **a** Plot and **b** zoom of $\frac{E\,S}{C}$ for $a = 1, k = 0.9, d = 0.1, S_T = 100,\ E_T = 0.04$. *Solid line* numerical solution of the full system, *dashed* K_M, *dashed-dotted* K_W, *dotted* K_D

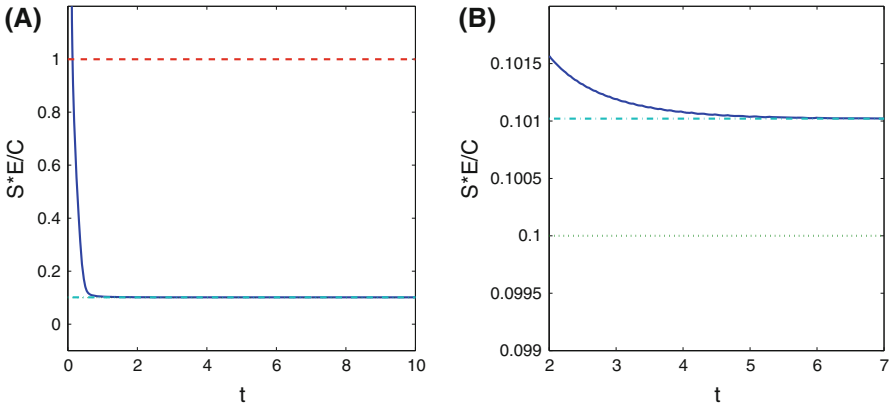

Fig. 3 **a** Plot and **b** zoom of $\frac{E\,S}{C}$ for $a = 1, k = 0.9, d = 0.1, S_T = 100,\ E_T = 89$. *Solid line* numerical solution of the full system, *dashed* K_M, *dashed-dotted* K_W, *dotted* K_D

have plotted the time course of the ratio $\frac{E\,S}{C}$, where the values E, S, C are obtained by the numerical integration of system (2)–(4). Finally, let us state some important properties of K_W.

Theorem 4.2 *For any admissible choice of the kinetic parameters and the initial conditions, the following inequalities hold:*

$$K_D \leq K_W \leq K_M. \tag{24}$$

Varying appropriately the parameter values, we can obtain for K_W every value between K_D and K_M. In particular,

Theorem 4.3 *For any admissible choice of the kinetic parameters and for any* $\bar{K} \in (K_D\,, K_M)$*, there exists* $\bar{E}_T$ *such that* $\frac{ES}{C} \to \bar{K}$ *when* $t \to \infty$.

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