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On the simple Michaelis–Menten mechanism for chemical reactions

Sara M.T. de la Selva [1], Eduardo Piña and L.S. García-Colín [2]

Departamento de Física, Universidad Autónoma Metropolitana-Iztapalapa,
C.P. 09340, P.O. Box 55-534, D.F., México

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Several mathematical properties associated with the simple Michaelis–Menten mechanism for enzymatic reactions are proven. In particular it is shown that the usual interpretation of the slope of the experimental Michaelis–Menten rate law in terms of the reaction constants of the mechanism can be obtained, in the approximation in which the total concentration of the enzyme is small compared with the Michaelis–Menten constant, independently of the ratio between the total initial concentrations of the enzyme and substrate. Furthermore, the ratio of the total concentration of the enzyme to the Michaelis–Menten constant allows for the elimination of a fast variable in a singular perturbation method, yielding the Michaelis–Menten rate law as a first order approximation.

1. Introduction

Since the work of Michaelis and Menten on enzymatic reactions, in 1913 [1], the idea exists that, for complex chemical reactions, there are basically two types of reacting species depending on their time behaviour: the long-lived reactives, also called slow variables, and the intermediates, short-lived ones or fast variables that for all practical purposes are undetectable in the time scale at which the long-lived ones are measured. This idea has been widely used [2] to relate a proposed mechanism to the experimentally established rate law that contains only the long-lived species and which, in general, does not conform itself to the kinetic mass action law of Guldberg and Waage.

The connection between the proposed mechanism involving intermediates and the experimental rate law has been made through the hypothesis [2–5] of "the pseudo steady state" which assumes that after a transient period the concentration of intermediates reaches an *almost* constant value. It also assumes that the concentration of the intermediates is constant through the remaining life of the reaction. In the case of Michaelis and Menten reactions, the transient period is considered to be a very short one, such that the whole measurable life of the reaction takes place in the steady state for the intermediate [5,6].

[1] Also at Universidad Iberoamericana-Santa Fe.

[2] Also at El Colegio Nacional.

The mathematical demonstrations of the theorems that a system of differential equations must satisfy in order that it justifies a pseudo steady state approximation have been worked out [7], including the Michaelis–Menten mechanism. Here we shall not dwell on this mathematical aspect of the problem; our main objective is to exhibit the appropriate parameter of smallness that leads from the mechanism to the rate law.

The pseudo steady state hypothesis has been justified as an approximation and a connection between the mechanism and the rate law has already been established for the Michaelis and Menten reactions without using the pseudo steady state hypothesis itself [8,9]. Indeed, it has been shown that under the assumption that the enzyme concentration e_0 is relatively small *with respect to the substrate concentration* s_0, the rate law is obtained as the degenerate case in a singular perturbation treatment of the kinetic equations of the mechanism. The same ratio was shown to be the one that makes the rate law compatible with the mechanism [7]. However, this result was found based upon a definition of the two time scales involved in terms of s_0. Perturbation techniques have also been used for other types of reactions [7,10,11] and for particular cases, namely, for particular values of the rate constants of the Michaelis–Menten mechanism [7,11–13].

An estimative evaluation of the two time scales associated with several Michaelis–Menten mechanisms, simple, reversible and with inhibitor, using the assumption of the pseudo steady state itself is available [5,14]. This estimate indicates that the ratio of the initial enzyme concentration to *the sum of the substrate initial concentration* s_0 *plus the Michaelis–Menten constant* K_s, is the parameter that, when small, leads, through the singular perturbation method, to the rate law. This was the first time that attention was drawn to the fact that the smallness parameter appropriate to recover the empirical rate law from the mechanism is not e_0/s_0. This estimate, however, is based on the pseudo steady state hypothesis itself. More complicated mechanisms of the Michaelis–Menten type [15,16] have been analyzed under the assumption $e_0/(s_0 + K_s) \ll 1$ when e_0/s_0 is not small.

In the present paper, we study the simple Michaelis–Menten mechanism, and demonstrate several of its properties in a systematic way. In particular we show that the exact expression for the asymptotic slope at equilibrium in coordinates, rate of production vs. substrate, is a function of the enzyme initial concentration and the Arrhenius constants of the mechanism only, with the substrate initial concentration s_0 playing no role at all. As a consequence, we are able to show, in an exact way, that when the concentration of enzyme e_0 is small compared to the Michaelis–Menten constant K_s, the slope at equilibrium calculated from the mechanism coincides with the slope from the rate law. A further consequence is that the rate law is recovered from the mechanism through the singular perturbation method when e_0/K_s is small. In this way we show that the appropriate criterion to obtain the Michaelis–Menten empirical law from the mechanism via perturbation analysis is the smallness in e_0/K_s, independently of the ratio e_0/s_0. Furthermore, the criterion $e_0/K_s \ll 1$ is stronger than $e_0/(s_0 + K_s) \ll 1$; in fact, whenever the condition $e_0/K_s \ll 1$ is satisfied, the condition $e_0/(s_0 + K_s) \ll 1$ is also satisfied; the converse, however, is not true. It can also be seen that when the method of perturbation analysis is employed using as a smallness parameter $e_0/s_0 \ll 1$, one is in fact using $e_0/K_s \ll 1$.

In 1985, Van Kampen [17] formalized and classified the equations pertaining to the physical processes that can be characterized by two different time scales. He established a general singular perturbation method for elimination of the fast variables (the short-lived ones) to derive the overall rate law of the process from the assumed mechanism. His scheme has been applied to several chemical reactions [18,19]. Here we apply it to the Michaelis–Menten system once we have found that the mechanism yields the near equilibrium experimental slope when the parameter $e_0/K_s \ll 1$ independently of the ratio e_0/s_0.

The geometric relation in the phase space of the rate laws obtained in the pseudo steady state and equilibrium state hypothesis for the Lindeman and enzymatic mechanisms and their numerical solutions has been studied in refs. [20–23]. In these references the whole basin of solutions in the phase space, the complex concentration versus the substrate concentration, for the simple Michaelis–Menten mechanism and more complicated enzymatic mechanisms were shown, and a functional iterative solution was presented that allows for the location of the attractor of the solutions. This allows for a different alternative method to solve the problem of numerical evaluation of the constants of the mechanism in terms of the empirical constants and initial concentrations. Several properties, inherent to the mechanism, are contained in refs. [20–23]. However we demonstrate and use some of them in a different context, to serve our above-stated purposes which are concerned with the smallness parameter that makes compatible the equations of the mechanism and the rate equation specifically via the elimination of a fast variable.

2. General properties

In this section we prove several features of the simple Michaelis–Menten mechanism. We particularly emphasize the values of the slope at different points in some of the different spaces in which the Michaelis and Menten mechanism can be related to experiments.

The conversion reaction of the substrate S into product P with the assistance of the enzyme E is represented by the following mechanism, the so-called simple Michaelis–Menten:

$$\mathrm{S} + \mathrm{E} \underset{k_2}{\overset{k_1}{\rightleftarrows}} \mathrm{C} \overset{k_3}{\rightarrow} \mathrm{P} + \mathrm{E}, \tag{2.1}$$

where C represents the intermediate (complex) and the k_i, $i = 1, 2, 3$, are the rate constants. According to the mass action law, the mechanism (2.1) implies the following equations for the time rate of change of the involved concentrations:

$$\frac{ds}{dt} = -k_1 se + k_2 c\,,$$

$$\frac{de}{dt} = -k_1 se + k_2 c + k_3 c\,,$$

$$\frac{dc}{dt} = k_1 se - k_2 c - k_3 c\,,$$

$$\frac{dp}{dt} = k_3 c\,. \tag{2.2}$$

Here we denote by lower-case letters the concentrations corresponding to the species represented by capital letters.

We notice that $d(e+c)/dt = 0$ and that $d(s+p+c)/dt = 0$. Therefore, there are two constants of motion and only two concentrations remain independent. If at an initial time, $t = 0$,

$$s(t=0) = s_0\,, \quad e(t=0) = e_0\,, \quad c(t=0) = c_0 \quad \text{and} \quad p(t=0) = 0\,, \tag{2.3}$$

the constants of motion are given by

$$e + c = e_0 + c_0 \quad \text{and} \quad s + c + p = s_0 + c_0\,. \tag{2.4}$$

We choose c and p as the independent concentrations, and the independent equations of motion are then given by

$$\dot{c} = k_1(s_0 + c_0 - p - c)(e_0 + c_0 - c) - (k_2 + k_3)c\,, \tag{2.5}$$

$$\dot{p} = k_3 c\,, \tag{2.6}$$

where the dot above the symbol denotes the time derivative.

Below, with the help of (2.4) we underline the mathematical properties of the involved variables, that will come useful later. From the physical fact that they are concentrations, the following inequalities must always be obeyed:

$$c \geqslant 0\,,$$

$$p \geqslant 0\,,$$

$$e_0 + c_0 - c \geqslant 0\,,$$

$$s_0 + c_0 - c - p \geqslant 0\,. \tag{2.7}$$

The set of eqs. (2.5) and (2.6) is subjected to the initial conditions (2.3), which imply that

$$\dot{c}(t=0) = k_1 s_0 e_0 \quad \text{and} \quad \dot{p}(t=0) = k_3 c_0\,. \tag{2.8}$$

On the other hand, calling Ω the time at which equilibrium has been achieved, we have

$$\dot{c}(t=\Omega)=0 \quad \text{and} \quad \dot{p}(t=\Omega)=0\,, \tag{2.9a}$$

which leads to the equilibrium values:

$$c(t=\Omega)=0 \quad \text{and} \quad p(t=\Omega)=s_0+c_0\,. \tag{2.9b}$$

From eq. (2.5) and the third inequality in (2.7) we find that $c(t)$ has a maximum $c(t_0)=c_m$ at time t_0; as a consequence, from eq. (2.6) we see that $\dot{p}(t)$ takes a maximum value,

$$\dot{p}(t=t_0)=k_3c_m\,, \tag{2.10}$$

and that therefore p has an inflexion point $p(t_0)=p_i$. From eq. (2.5) we obtain that at time t_0 the following relation holds:

$$k_1(s_0+c_0-p_i-c_m)(e_0+c_0-c_m)-(k_2+k_3)c_m=0\,. \tag{2.11}$$

Furthermore from the difference between eqs. (2.5) and (2.11) and due to the inequalities (2.7) it is easily shown that for $c<c_m$ and $p<p_i$,

$$\dot{c}>0 \tag{2.12}$$

must hold.

Up to now, from the conditions (2.3), (2.8), (2.9), the expression (2.10) and the property (2.12) we have some general information concerning the time behaviour of p and c. Also, because there is a maximum c_m and due to the first equilibrium condition in (2.9a), $\dot{c}$ must be negative for $t>t_0$. In fig. 1 we plot $c(t)$ and $p(t)$ where the above-mentioned features are exhibited. The figures shown in this paper are included here for the sake of illustration and clarity. Other authors [14] using different values for the parameters have obtained similar graphs.

We now focus on the problem of obtaining general information about $\dot{p}(s)$, since $(s,\dot{p})$ is the space of the Michaelis–Menten rate law. To achieve this goal, it is helpful to study first the behavior of $c(p)$. We start by noticing that since the right-hand sides of eqs. (2.5) and (2.6) are not explicit functions of time, the slope of $c(p)$, dc/dp, is given by

$$\frac{\dot{c}}{\dot{p}}=\frac{k_1}{k_3}\left\{\frac{(s_0+c_0-p-c)(e_0+c_0-c)}{c}-\frac{k_2+k_3}{k_1}\right\}. \tag{2.13}$$

Furthermore, with the help of the inequalities (2.7), after rearranging eq. (2.13), we find that the inequality

$$k_3\dot{c}/\dot{p}+k_2+k_3\geqslant 0 \tag{2.14}$$

must always hold. From expression (2.13) we now obtain the value of the slope at selected points of the curve $c(p)$. First, with the help of the initial conditions (2.3) and (2.8), we find that the slope at the origin is

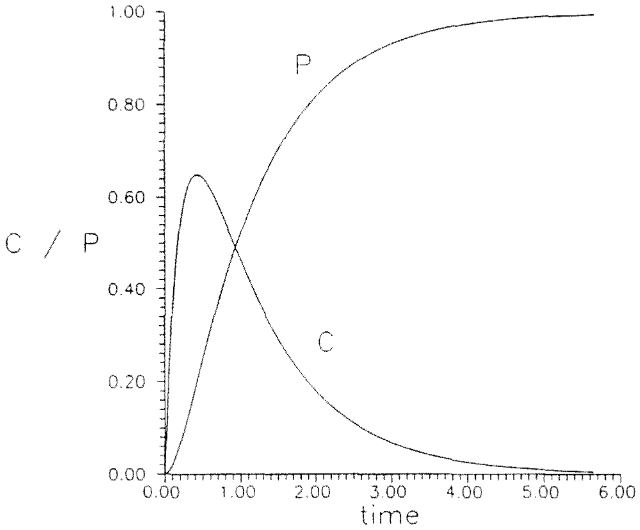

Fig. 1. Numerical solution of eqs. (2.5) and (2.6) with $k_1 = 1.0$, $k_2 = 0.02$, $k_3 = 0.98$, $e_0 = 5.0$, $c_0 = 0$, and $s_0 = 1.0$. C and P denote the complex and product concentrations, respectively.

$$\lim_{p\to 0} \dot{c}/\dot{p} = \lim_{t\to 0} \dot{c}/\dot{p} = \frac{k_1}{k_3}\left\{\frac{s_0}{c_0} - \frac{k_2+k_3}{k_1}\right\}. \tag{2.15}$$

Second, with the help of eq. (2.10), we verify that

$$(\dot{c}/\dot{p})_{c_m, p_i} = 0, \tag{2.16}$$

and third, that the value of the slope at the equilibrium point, ($p = s_0 + c_0$, $c = 0$) is given by the following limit:

$$\lim_{t\to\Omega} \dot{c}/\dot{p} = \lim_{t\to\Omega}\left(\frac{k_1}{k_3}\right)\left\{\frac{(s_0+c_0-p-c)(e_0+c_0-c)}{c} - \frac{k_2+k_3}{k_1}\right\}. \tag{2.17}$$

After applying the equilibrium conditions (2.9b), L'Hôpital's rule, and the definition

$$\mathbb{P} \equiv \lim_{t\to\Omega} \dot{c}/\dot{p} \tag{2.18}$$

to expression (2.17), we find the equation

$$k_3\mathbb{P}^2 + (k_1(e_0+c_0) + k_2 + k_3)\mathbb{P} + k_1(e_0+c_0) = 0, \tag{2.19}$$

whose solutions, both negative, are

$$\mathbb{P} = \frac{-(k_1(e_0+c_0)+k_2+k_3)}{2k_3} \pm \frac{[(k_1(e_0+c_0)+k_2+k_3)^2 - 4k_1k_3(e_0+c_0)]^{1/2}}{2k_3}. \tag{2.20}$$

In order to discern the physical meaning of these solutions, we subject them to condition (2.14), which implies, upon substitution of $2k_3\mathbb{P}$ from (2.20) into (2.14), that either one or both of the solutions, in order to be physically acceptable, must satisfy the inequality

$$k_2 + k_3 - k_1(e_0 + c_0) \pm [(k_1(e_0 + c_0) + k_2 + k_3)^2 - 4k_1k_3(e_0 + c_0)]^{1/2} \geqslant 0 .$$

This expression may be rewritten as

$$k_2 + k_3 - k_1(e_0 + c_0) \pm [(k_2 + k_3 - k_1(e_0 + c_0))^2 + 4k_1k_2(e_0 + c_0)]^{1/2} ,$$

from which we see that the inequality holds only for the plus sign. Thus, the physically acceptable expression for the slope at equilibrium in $c(p)$ is

$$\mathbb{P} = \frac{-(k_1(e_0 + c_0) + k_2 + k_3)}{2k_3} + \frac{[(k_1(e_0 + c_0) + k_2 + k_3)^2 - 4k_1k_3(e_0 + c_0)]^{1/2}}{2k_3} \tag{2.21}$$

and notice that

$$\mathbb{P} < 0 . \tag{2.22}$$

Moreover, as it is proved next,

$$\mathbb{P} \geqslant -1 . \tag{2.23}$$

Indeed, the solution $\mathbb{P}$ can be written as

$$\mathbb{P} = \frac{-(k_1(e_0 + c_0) + k_2 + k_3)}{2k_3} + \frac{(k_1(e_0 + c_0) + k_2 - k_3)}{2k_3} \times \left[1 + \frac{4k_2k_3}{(k_1(e_0 + c_0) + k_2 - k_3)^2}\right]^{1/2} , \tag{2.24}$$

where we see that in the case $k_2k_3 \to 0, \mathbb{P} = -1$, otherwise $\mathbb{P} > -1$.

The slope $\mathbb{P}$ can also be obtained from the eigenvalue of the slow manifold through linearized stability analysis [21] at equilibrium.

Also, from the difference between eqs. (2.13) and (2.16) we have that

$$\dot{c}/\dot{p} = \frac{k_1}{k_3}\left\{\frac{(s_0 + c_0 - p - c)(e_0 + c_0 - c)}{c} - \frac{(s_0 + c_0 - p_i - c_m)(e_0 + c_0 - c_m)}{c_m}\right\} , \tag{2.25}$$

from where it is immediately seen that for $c < c_m$ and $p < p_i$,

$$\dot{c}/\dot{p} > 0 . \tag{2.26}$$

Thus, from eqs. (2.15), (2.16), (2.21) and conditions (2.22) and (2.26) we have some general information about the behavior of $c(p)$ as predicted from the mechanism, independently of the particular physical cases one might want to consider with respect to relative concentrations and reaction constants values. Here again we have that because of conditions (2.16) and (2.22), $\dot{c}/\dot{p}$ must be negative for $t > t_0$. These features are illustrated in fig. 2.

We now proceed to investigate the consequences of the Michaelis–Menten mechanism (2.1) in the space $(s, \dot{p})$, where the mechanism, represented by eqs. (2.5) and (2.6), and the Michaelis–Menten rate law are to be compared. We already have some information concerning the behavior of $\dot{p}(s)$. In fact there is the initial point $(s = s_0, \dot{p} = k_3 c_0)$ because of conditions (2.3), a maximum point $(s_0 - p_i - c_m, k_3 c_m)$, and because of conditions (2.9), the equilibrium point $(s = 0, \dot{p} = 0)$. Therefore, the system evolves towards the origin of the coordinates and thus the equilibrium occurs at the origin. More information concerning the general shape of the curve $\dot{p}(s)$ is obtained from the values of the slope at the above-mentioned points. The equation for the slope $d\dot{p}/ds$ is obtained almost immediately from $\dot{p}/\dot{c}$. In fact, using the expression $d\dot{p}/ds$ and because of the properties of derivatives, we first have that

$$\frac{d\dot{p}}{ds} = \frac{\ddot{p}}{-\dot{p} - \dot{c}}, \tag{2.27}$$

and second, with the help of eq. (2.6), that

$$\frac{d\dot{p}}{ds} = -\frac{k_3 \dot{c}}{\dot{p} + \dot{c}}. \tag{2.28}$$

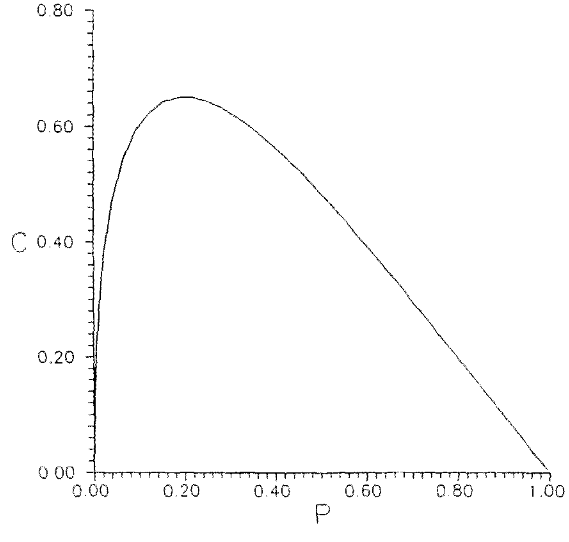

Fig. 2. Numerical solution of eqs. (2.5) and (2.6) with $k_1 = 1.0$, $k_2 = 0.02$, $k_3 = 0.98$, $e_0 = 5.0$, $c_0 = 0$ and $s_0 = 1.0$. Complex concentration vs. product concentration.

We now find, from eq. (2.28) and conditions (2.8), that the value of the initial slope, in the space $(s, \dot{p})$, the space of the rate law, is

$$\lim_{\substack{s\to s_0+c_0 \\ p\to 0}} \left[-\frac{k_3\dot{c}}{\dot{p}+\dot{c}}\right] = \lim_{t\to 0}\left[-\frac{k_3\dot{c}}{\dot{p}+\dot{c}}\right] = -\frac{k_3}{1+\frac{k_3 c_0}{k_1 s_0 e_0}} . \tag{2.29}$$

For the usual experimental conditions where $c_0 = 0$, the value of the initial slope is equal to the constant k_3.

We verify from eq. (2.28) that the slope at $(s_0 - p_i - c_m,\ k_3 c_m)$ is zero, and that at equilibrium eq. (2.28) becomes, using the definition (2.18),

$$\lim_{\substack{s\to 0 \\ c\to 0}} \frac{d\dot{p}}{ds} = \lim_{t\to\Omega}\frac{d\dot{p}}{ds} = -\frac{k_3\mathbb{P}}{1+\mathbb{P}} . \tag{2.30}$$

This last result is the exact value of the asymptotic slope of $\dot{p}(s)$ at equilibrium for all possible cases of different ratios of enzyme to substrate and for different k_i. Because of the inequalities (2.22) and (2.23) it will be always positive. Thus the slope of the Michaelis–Menten rate law must be recovered from eq. (2.30) in some approximation or as a particular case. This is shown in the next section along with two other near equilibrium possible cases.

3. Near equilibrium particular cases

In this section we show two of the possible approximations to the near equilibrium slope (2.30) that arise from the mechanism (2.2) and represent possible physical cases.

Let us first recall the empirical Michaelis–Menten rate law,

$$\dot{p}(s) = \frac{K_r e_0 s}{s + K_s} , \tag{3.1}$$

whose slope is given by

$$\left(\frac{d\dot{p}}{ds}\right)_r = \frac{K_r K_s e_0}{(s+K_s)^2} \tag{3.2a}$$

and which near equilibrium becomes

$$\lim_{t\to\Omega}\left(\frac{d\dot{p}}{ds}\right)_r = \lim_{s\to 0}\left(\frac{d\dot{p}}{ds}\right)_r = \frac{K_r e_0}{K_s} . \tag{3.2b}$$

Here K_r and K_s are empirical constants. We now want to show the approximation that yields eq. (3.2b) from eq. (2.30) and the ensuing identification of the k_i's in terms of the empirical constants. To do so, we need to take into account the information from the experiments that have led to the law (3.1). To our knowledge, in all the experiments reported in the literature, the initial complex concentration c_0 is

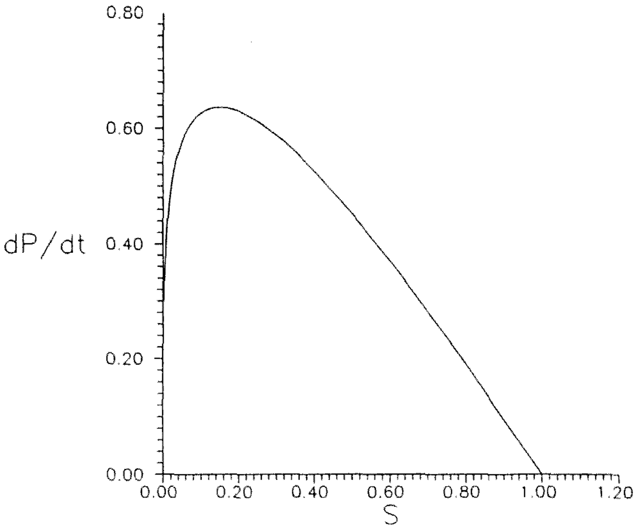

Fig. 3. Numerical solution of eqs. (2.5) and (2.6) with $k_1 = 1.0$, $k_2 = 0.02$, $k_3 = 0.98$, $e_0 = 5.0$, $c_0 = 0$, and $s_0 = 1.0$. Rate of production $\dot{p}$ vs. substrate concentration $s = s_0 - c - p$.

zero and the assertion is made that the enzyme concentration is low either with respect to the initial substrate concentration or with respect to the sum of initial substrate concentration plus the Michaelis–Menten. Since the initial substrate concentration does not play any role in eq. (2.30), we only have the possibility of trying approximations based on the relative values of e_0 and combinations of the k_i's. In what follows, we present two possible approximations of eq. (2.30), for the case $c_0 = 0$, involving e_0 and the k_i, that produce, for the slope at equilibrium, a ratio of constants multiplied by e_0. Both of them are independent of the value of the ratio e_0/s_0, and both can be compared with the empirical slope (3.2b). The justification to choose one of them as the correct one will be shown in the next section.

Let us consider the first case; assume that

$$e_0 \ll \frac{k_2 + k_3}{k_1} \, . \tag{3.3}$$

It is easily seen that this approximation implies that

$$\frac{k_1 k_3 e_0}{(k_1 e_0 + k_2 + k_3)^2} \ll 1$$

or, since the comparison is between orders of magnitude, that

$$\frac{4 k_1 k_3 e_0}{(k_1 e_0 + k_2 + k_3)^2} \ll 1 \, . \tag{3.4}$$

Therefore, in view of the inequality (3.4), the near equilibrium slope (2.21) becomes, to first approximation in $e_0 k_1/(k_2 + k_3)$,

$$\mathbb{P}_{MM} = -\frac{k_1 e_0}{k_1 e_0 + k_2 + k_3}, \tag{3.5}$$

where we have introduced the subscript MM to stress the fact that eq. (3.5) is a particular approximation to $\mathbb{P}$. Substituting this last result into eq. (2.30) we arrive at

$$\left(\lim_{t\to\Omega} \frac{d\dot{p}}{d(s)}\right)_{MM} = \frac{k_3 k_1 e_0}{k_2 + k_3}. \tag{3.6}$$

Comparison of both slopes, (3.2b) and (3.6), leads us to

$$\frac{k_3 k_1}{k_2 + k_3} = \frac{K_r}{K_s}. \tag{3.7}$$

If we identify K_s as it is usually done [4–6],

$$K_s \equiv \frac{k_2 + k_3}{k_1}, \tag{3.8}$$

then $K_r = k_3$ and the identification of the slope (3.6) with (3.2b) follows. The coincidence of slopes (3.2b) and (3.6) is exhibited in fig. 4, where we show the numerical solution of the mechanism's equations (2.5) and (2.6), in the $(s, \dot{p})$ space, superimposed to the graph of the Michaelis–Menten rate law, eq. (3.1). For fig. 4 we have used values for the k_i's and e_0 that satisfy condition (3.3) with $e_0/s_0 = 1$ to stress the fact that if condition (3.3) is satisfied, given the nature of the reacting species (that determines the k_i's), the slope (3.6) will be always obtained independently of the ratio e_0/s_0 and not only when e_0/s_0 is small.

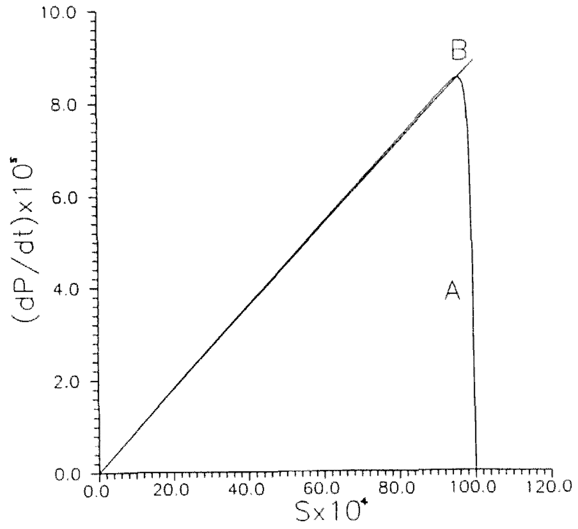

Fig. 4. A is the numerical solution of the eqs. (2.5) and (2.6) with $k_1 = 1.0$, $k_2 = 0.01$, $k_3 = 0.9$, $e_0 = 0.01$, $c_0 = 0$ and $s_0 = 0.01$. B is the solution of eq. (3.1) with $K_r = 0.9$ and $K_s = 1$.

The second case we consider is the high enzyme concentration approximation. Let us assume

$$k_1 e_0 \gg k_3 \,, \tag{3.9a}$$

which implies that

$$(k_1 e_0 + k_2) \gg k_3 \,, \tag{3.9b}$$

and rearrange the slope (2.21) to read

$$\mathbb{P} = \frac{-(k_1 e_0 + k_2 + k_3) + (k_1 e_0 + k_2)\left[1 + \frac{k_3(k_3 + 2k_2 - 2k_1 e_0)}{(k_1 e_0 + k_2)^2}\right]^{1/2}}{2k_3} \,. \tag{3.10}$$

We now use the inequality (3.9b) to approximate the root in eq. (3.10) to first order in $k_3/(k_1 e_0 + k_2)$. The result is

$$\mathbb{P} \cong -\frac{k_1 e_0}{k_1 e_0 + k_2} \,. \tag{3.11}$$

After substitution in (2.30) the equilibrium slope becomes

$$\left(\lim_{t \to \Omega} \frac{d\dot{p}}{ds}\right)_{HE} = \frac{k_1 k_3 e_0}{k_2} \,. \tag{3.12}$$

Further sub-cases are obtained from this result depending on the magnitude of k_2 as compared with k_3.

Since in both cases, low and high enzyme concentrations, the comparison is made with combinations of the k_i's and not with respect to the substrate concentration, as far as the equilibrium slope is concerned, the comparison could have been made also between expressions (3.2b) and (3.12) leading to the identification of K_r with k_3 and K_s with k_2/k_1 or to the identification K_r with k_1 and K_s with k_2/k_3 or any other identification. However, as it will be shown in the next section, following the idea expressed in refs. [8] and [18], the correct comparison is the one between expressions (3.2b) and (3.6) since it is the parameter $e_0 k_1/(k_2 + k_3)$ that allows for the recovery of the law (3.1) from the mechanism embodied by the eqs. (2.5) and (2.6) using a perturbative method.

It must be also pointed out that it has been shown [23] that the steady-state approximation, $\dot{c} = 0$, that yields eq. (3.1) is asymptotically a better description than the fast equilibrium hypothesis ($\dot{s} = 0$ in the space (s, c)), which leads to the same type of law as (3.1) but which we do not consider in the present communication. With regard to the evaluation of the separate k_i's, the reader must consult ref. [23].

4. Fast variable elimination

In this section we show that the Michaelis–Menten empirical rate law is obtained from the independent equations associated to the Michaelis–Menten mechanism near equilibrium, as the first order solution in the parameter $e_0k_1/(k_2+k_3)$. This is so because the equations satisfy the required form that exhibits the existence [8,17] of two different time scales when $e_0k_1/(k_2+k_3)$ is small. To see this feature, it is convenient to change from the representation (p,c) to (s,c). The two independent equations to be considered now are the first and the third one in (2.2), which with the help of (2.4), in the case $c_0 = 0$, become

$$\frac{ds}{dt} = -k_1 s(e_0 - c) + k_2 c\,, \tag{4.1}$$

$$\frac{dc}{dt} = k_1 s(e_0 - c) - (k_2 + k_3)c\,. \tag{4.2}$$

Recasting them into a dimensionless form with the help of the variables ν, σ, and τ, defined as follows:

$$t \equiv \frac{\tau}{k_1 e_0}\,, \quad c \equiv \nu e_0\,, \quad s \equiv \sigma\frac{k_2+k_3}{k_1}\,, \tag{4.3}$$

we get

$$\frac{d\sigma}{d\tau} = -\sigma(1-\nu) + \frac{k_2}{k_2+k_3}\nu \tag{4.4}$$

and

$$\frac{d\nu}{d\tau} = \frac{k_2+k_3}{k_1 e_0}\left[\sigma(1-\nu) - \nu\right]. \tag{4.5}$$

From eqs. (4.4) and (4.5) we see that as

$$\epsilon \equiv e_0\frac{k_1}{k_2+k_3} \tag{4.6a}$$

goes to zero, according to condition (3.3), $d\nu/d\tau \to \infty$ while $d\sigma/d\tau$ remains unchanged, thus identifying ν as the fast variable.

Following the standard procedure [17], we substitute

$$\nu(\tau) = \nu^{(0)}(\tau) + \epsilon\nu^{(1)}(\tau) + \epsilon^2\nu^{(2)}(\tau) + \dots \tag{4.6b}$$

in eq. (4.5) and require the different orders to be equal on both sides of the equation; the results $\nu^{(0)}$, $\nu^{(1)}$, ... thus obtained are substituted in eq. (4.4). To first order, namely ϵ^{-1}, from eq. (4.5) we obtain

$$\nu^{(0)} = \frac{\sigma^{(0)}}{1+\sigma^{(0)}} \tag{4.7a}$$

and upon substitution in eq. (4.4)

$$\frac{d\sigma}{d\tau} = -\frac{k_3}{k_2+k_3}\,\frac{\sigma^{(0)}}{1+\sigma^{(0)}}\,. \tag{4.7b}$$

Reverting to the original variables and remembering that $c_0 = 0$ for eq. (3.1), we arrive at the Michaelis–Menten rate law,

$$\frac{ds}{dt} = \frac{-k_3 e_0 s}{K_s + s}\,, \tag{4.8}$$

which in this approximation is the same as eq. (3.1). This can be seen upon substitution of eq. (4.7a) into eq. (2.6) after the transformation (4.3) is applied to it. To the next order in ϵ, we obtain

$$\frac{ds}{dt} = \frac{-k_3 e_0 s}{K_s + s}\left[1 - \frac{e_0 K_s s}{(K+s)^3} - \frac{e_0 k_2 K_s^2}{k_1 (K_s+s)^3}\right]. \tag{4.9}$$

The form of eqs. (4.4) and (4.5) and the result (4.8) show that indeed the concentration of complex c is a fast variable, namely a non-detectable one in the time scale of s. This is equivalent to saying that the sum of the rates of the complex decomposition, into the substrate and product, $k_2 + k_3$, is greater than its rate of formation $k_1 e_0$; not simply that $k_2 + k_3 \ll k_1$ [7]. The fact that the Michaelis–Menten rate law is recovered from the mechanism by a perturbative approximation in the parameter $e_0 k_1/(k_2+k_3)$ justifies the comparison made in (3.7).

With respect to the case when $k_3/k_1 e_0 \ll 1$, near equilibrium, we rewrite the equations for the rate of change of concentrations of the substrate, complex and product in (2.2) in terms of the parameter $\lambda \equiv k_3/k_1 e_0$. To do so we introduce the variables θ, ζ, π defined as

$$\theta = k_3 t\,, \quad s = \zeta e_0\,, \quad p = \pi e_0\,, \tag{4.10}$$

and find that it is the pair of equations, for the complex concentration and the product concentration, the one that exhibits a two time behavior with the former one as a fast variable and the latter as a slow one. The application of the standard procedure to these two equations leads to an altogether different rate law from the Michaelis–Menten one that has been reported in ref. [8] and discarded, as we also do, on the grounds that only if such a rate law is experimentally found, the comparison between (3.2b) and (3.12) is pertinent.

To examine the approximate behavior that the mechanism predicts near the beginning of the reaction (inner solution), following ref. [8] we define the time scale $\Sigma \equiv \tau/\epsilon$; but notice that here the smallness parameter is the one defined in (4.6a). In the Σ time scale, eqs. (4.4) and (4.5) become

$$\frac{d\sigma}{d\Sigma} = -\epsilon\sigma(1-\nu) + \epsilon\frac{k_2}{k_2+k_3}\nu\,, \tag{4.12}$$

$$\frac{d\nu}{d\Sigma} = [\sigma(1-\nu) - \nu] , \tag{4.13}$$

to which the initial conditions $\nu_0 = 0$ and $\sigma_0 = s_0/K_s$ apply. The solution to these equations is the same as the one reported in ref. [8]. This is enough to show that the method employed there applies with the perturbative parameter e_0/K_s, which is the true one yielding eq. (3.1) from the mechanism (2.1) independently of the ratio e_0/s_0. This fact further stresses the applicability of the rate law (3.1) to situations of high or low ratio e_0/s_0.

One might ask if the system of eqs. (4.1), (4.2) will exhibit a fast and a slow variable when $K_s/e_0 \ll 1$, thus allowing the elimination of the fast one. The answer is yes, but the resulting rate laws for this pseudo-steady state approximation are not Michaelis–Menten's. This can be verified by choosing the following dimensionless variables:

$$\theta \equiv (k_2 + k_3) , \quad s \equiv \zeta e_0 , \quad p \equiv \pi e_0 , \quad c \equiv \nu e_0 ,$$

and finding that the complex concentration equation and the product concentration equation constitute a pair of equations for fast and slow variables, respectively, when K_s/e_0 goes to zero.

For the sake of clarification, let us finally comment upon the conditions $e_0/s_0 \ll 1$ and $e_0/(s_0 + K_s) \ll 1$ that have been shown, by other authors [5,7–9,14], to make compatible the reaction mechanism and the Michaelis–Menten rate law. It happens that the selection of a smallness parameter is not unique. One must pay attention to additional information. What we have done here is to notice that the exact long time slope given by eq. (2.30) does not contain the initial condition s_0; there is no mathematical reason to introduce it.

But, one may resort to other sources of information. At the time when the condition $e_0/s_0 \ll 1$ was proposed [7,8], many experimental set-ups satisfied it, which resulted in Michaelis–Menten kinetics. Hence, the selection of dimensionless variables was made in such a way as to force the appearance of s_0. A moment of thought will convince the reader that the selection of dimensionless variables ν and τ as in (4.3) with $y \equiv s/s_0$ [8], which makes eqs. (4.1) and (4.2) look like a fast–slow pair with the small parameter e_0/s_0, does so because one is multiplying and dividing by s_0, but in fact it is $e_0k_1/(k_2 + k_3) \ll 1$ that is operating.

One can also consider the pseudo steady state hypothesis itself to find the explicit expressions for the two time scales, and from them exhibit $e_0/(s_0 + K_s)$ as the smallness parameter [5,14] even with the accompanying rules to choose smallness parameters, but the pseudo steady state is an approximation and, thus, has led to a less strong criterion that the one we find in a very simple but rigorous way from the mechanism without invoking the pseudo steady state hypothesis itself. Notice also that e_0/K_s is compatible with the exact long time slope from the reaction mechanism which does not contain s_0 whereas $e_0/(s_0 + K_s)$ is not. Let us stress the fact that K_s is a constant of the given reaction, whatever the initial conditions, whereas s_0 is a constant of the experiment, i.e. it may change with each experiment for the same reaction; the same can be said about e_0 but in contrast with s_0, e_0 is observed in the long-time slope.

5. Conclusions

In the present communication, we have demonstrated several properties and features of the simple Michaelis–Menten mechanism. The main ones are embodied by eqs. (2.12), (2.21), (2.29) and (2.30), which are exact results. We illustrate them with the help of figs. 1–3. Several approximations to eqs. (2.29) and (2.30) have been presented, and through them, it is possible to show that the condition under which the Michaelis–Menten mechanism yields the empirical rate law (3.1) from singular perturbation analysis is precisely the ratio e_0/K_s to be small compared to unity.

That this is a sufficient restriction in order to recover the Michaelis–Menten rate law from the mechanism, comes from the fact that the exact value of the near equilibrium slope in the space of the empirical rate law (s, $\dot{p}$), predicted by the mechanism, given by eq. (2.30) with eq. (2.21), depend *only* on the initial concentration of the enzyme and the Arrhenius constants of the reaction mechanism. Therefore the substrate concentration plays no role whatsoever in the approximations that follow from the exact slope. Also, within the context of the slow manifold method [21], it is shown that as a consequence of the fact that eqs. (2.2) are not explicitly dependent on the initial substrate concentration, the functional attractor equation also does not. Furthermore, the condition $e_0/(s_0 + K_s) \ll 1$ is a less stringent criterion, since whenever $e_0/K_s \ll 1$ holds, then $e_0/(s_0 + K_s) \ll 1$ also holds, but the converse is not true. Thus, it is because $e_0/K_s \ll 1$ is obeyed that $e_0/(s_0 + K_s) \ll 1$ is obeyed.

Finally, it has also been shown that the condition $K_s/e_0 \ll 1$ leads, through a fast variable elimination, to rate laws different from Michaelis–Menten's.

The conclusion is therefore, that the Michaelis–Menten mechanism is acceptable for a chemical reaction that yields the Michaelis–Menten experimental rate law, when the values of the Arrhenius constants, which depend on the chemical nature of the reacting species, are such that the ratio of initial concentration of the enzyme to the Michaelis–Menten constant is much smaller than unity, that is, when $e_0/K_s \ll 1$ holds regardless of the value of the ratio of initial enzyme concentration to initial substrate concentration.

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