NEW ENZYMATIC KINETIC
RELATING MICHAELIS-MENTEN MECHANISMS

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Received: 27 June 2005 Modified: 27 July 2005 Accepted: 25 September 2005

SUMMARY

In the context of Michaelis-Menten mechanisms a new related enzymatic kinetic is proposed. It provides an exponential form of the instantaneous velocity for a reaction counting for the competitive and uncompetitive inhibitions of the enzyme and enzyme-substrate complex, respectively, as well for the reversible overall process. Such an approach is suited for progress curves analysis of the substrate and product concentrations.

Keywords: enzymatic kinetic, Michaelis-Menten mechanisms, inhibitions, binomial and exponential series expansions.

INTRODUCTION

From over one century the enzyme kinetics and the reliability of their kinetic parameters' estimates appear as a fascinating filed of research due to its immediate applications to the experimental data analysis.

Briefly, we are reminded the pioneering work of Adrian Brown from 1902 [1], who demonstrated that as the sucrose concentration is much higher than that of the β-fructofuranosidase the reaction rate of hydrolysis of sucrose becomes independent of the sucrose concentration.
Facing similar behavior of enzymatic kinetics, contemporary, Victor Henri had proposed a reversible reaction scheme between an enzyme $E$ and a substrate $S$, giving the enzyme-substrate complex $ES$, and releasing irreversibly the product $P$ [2]:

$$
E + S \xrightleftharpoons[k_{-1}]{k_1} \overset{k_2}{\rightarrow} P + E
$$

(1)

The mathematical conditions under which the flow of the enzymatic reaction occurs was basically solved by Leónor Michaelis and Maude Menten in 1913 [3], that despite little variants over time [4], reduce under the steady state condition:

$$
\frac{d}{dt}[ES] \equiv 0,
$$

(2)

leaving with an expression for the velocity of product formation:

$$
v_0 = \left( \frac{d}{dt}[P] \right)_{t=0} = \frac{V_{\text{max}}[S]}{K_M + [S]},
$$

(3)

known as the Michaelis-Menten equation [5].

However, the kinetic parameters that appears in eq. (3), namely the maximum velocity, relating with the initial enzyme concentration $[E]_0$ through

$$
V_{\text{max}} = k_2[E]_0,
$$

(4)

and the so called Michaelis-Menten constant,

$$
K_M = \frac{k_{-1} + k_2}{k_1},
$$

(5)

have become the most used biochemical parameters to determine the enzymatic kinetics.

Nevertheless, one difficulty in their determination regards their indirect relationship prescribing that at substrate concentration $[S] = K_M$ the reaction velocity is half-maximal:

$$
[S] = K_M \Rightarrow v_0 = \frac{V_{\text{max}}}{2}.
$$

(6)

The main problem with the Michaelis-Menten equation is that it accounts only for the velocity of the initial time of the reaction. The information that is outside the first moments of the progress curve is virtually lost or neglected. More, such way, when velocities are measured it is usually to determine one velocity from each experimental assay. In special in the case of sensitive enzymes, this procedure is difficult to be performed.
Another complication of the Michaelis-Menten equation (3) is that, even describing a kinetic, differs than the ordinary chemical ones by its rectangular hyperbola shape, rather by an expected exponential form, as is plotted in the Figure 1.

Figure 1. The plot of the initial velocity $v_0$ of a simple Michaelis-Menten reaction of Eq. (1) versus the substrate concentration $[S]$.

Finding the correspondent exponential equation formulation for the Michaelis-Menten equation (3) as well the exponential expression for a more general enzymatic scheme including the inhibition and the reversible reaction of the product formation in Eq. (1), at any time, not only restricted to the initial one, stand as the main purposes of the present work.

**METHOD**

Finding a suitable exponential form of the Michaelis-Menten equation (3) requires a procedure that preserves its kinetic parameters, i.e. $V_{max}$ and $K_M$.

When we focus on $V_{max}$, the saturating regime applies,

$$\frac{K_M}{[S]} \equiv x << 1,$$  \hspace{1cm} (7)

which allows to the replacing of the binomial with an exponential function, based upon their equivalent asymptotical forms:
Within the prescription (8) the MM equation (3) can be firstly arranged as:

\[
(1 + x)^{-1} \approx 1 - x + x^2 - x^3 + ... \\
\approx 1 - x + \frac{1}{2!}x^2 - \frac{1}{3!}x^3 + ... \\
\approx e^{-x} 
\]

(8)

To be then transformed to the new equation:

\[
v_0 = \frac{V_{\text{max}}[S]}{K_M + [S]} = V_{\text{max}} \frac{1}{1 + \frac{K_M}{[S]}} = V_{\text{max}} \left(1 + \frac{K_M}{[S]}\right)^{-1} 
\]

(9)

to be then transformed to the new equation:

\[
v_0^* = V_{\text{max}} \exp \left(-\frac{K_M^*}{[S]}\right).
\]

(10)

Nevertheless, even preserving \(V_{\text{max}}\), we have to take into account the modification of the Michaelis-Menten constant, that now displays the feature to correspond with the substrate concentration at which the initial velocity acquires the \(1/e\) of \(V_{\text{max}}\) instead of half-maximum:

\[
[S] = K_M^* \Rightarrow v_0^* = \frac{V_{\text{max}}}{e} < \frac{V_{\text{max}}}{2}.
\]

(11)

Such discrepancy can be further avoided if the relation between the new and old Michaelis-Menten constants is searched imposing on (10) the conditions:

\[
[S] = K_M & v_0^* = \frac{V_{\text{max}}}{2}
\]

(12)

to give the result:

\[
K_M^* = K_M \ln 2
\]

(13)

Now, with (13) in (10) the new Michaelis-Menten-type kinetic law is obtained under the form:

\[
v_0^* = V_{\text{max}} \exp \left(-\frac{K_M}{[S]} \ln 2\right)
\]

(14)

which preserves all the kinetic parameter features of the initial Michaelis-Menten equation (3), as can be clearly visualized also from the Figure 2.
As it can be seen from the Figure 2, the present approach displays a lower kinetics, until the substrate concentration equal the value of $K_M$. As the substrate concentration exceeds the value of $K_M$ the curve move toward $V_{max}$ value quicker than the classical Michaelis-Menten curve. This stands as an experimentally advantage since the interest to find more rapid $V_{max}$ value and not to reach very quickly $K_M$ value.

**RESULTS**

According with many enzymatic reactions the Michaelis-Menten mechanism has to be relaxed to the back reaction of the product jointly with the alteration of the enzyme activity by the so called inhibitors $I$.

Consequently, worth to find out the overall kinetic of the combined enzymatic phenomena through the general Michaelis-Menten type mechanism:

$$
\[ \begin{array}{c}
E + S \xleftrightarrow[k_1]{k_2} ES \xrightarrow[k_{-1}]{k_{-2}} P + E \\
\updownarrow K_I \quad \updownarrow K_{IS'} \\
\updownarrow E + I \xrightarrow{k_{IS'}} ESI \xrightarrow[k_{IS'}]{} P + EI
\end{array} \]
$$

(15)
in which, apart from the new appearing rate constant $k_{-2}$ of the product that back reacts to form the substrate, there are involved also the inhibition equilibrium constants,

$$K_I = \frac{[EI]}{[E]} , \quad K_{IS} = \frac{[ES][I]}{[ESI]}$$

(16)
corresponding to the competitive and uncompetitive inhibitions, respectively.

In order to solve the kinetic of the mechanism (15), the enzyme mass conservation condition, between the initial $[E]_0$ and the instantaneous total $[E]_T$ enzyme concentrations,

$$[E]_0 = [E]_T = [E] + [ES] + [EI] + [ESI]$$

(17)

has to be combined with the steady state condition (2) of the present reaction scheme:

$$\frac{d}{dt}[ES] \equiv 0 = k_1[E][S] + k_{-2}[P][E] - (k_{-1} + k_2)[ES].$$

(18)

Firstly, alternative relationships between the enzyme and the enzyme-substrate complex concentrations results from (18) under the forms:

$$[E] = \frac{k_{-1} + k_2}{k_1[S] + k_{-2}[P]} [ES] \quad \quad [ES] = \frac{k_1[S] + k_{-2}[P]}{k_{-1} + k_2} [E]$$

(19)

Additionally, the inhibitor-binding dissociation constants (16) release the expressions for the concentrations:

$$[EI] = \frac{[I]}{K_I} [E], \quad [ESI] = \frac{[I]}{K_{IS}} [ES]$$

(20)
of the enzyme-inhibitor and the enzyme-substrate-inhibitor complexes, respectively.

Going now to replace the relations (19) and (20) into the total enzyme conservation law (17) we arrive at the simple expression:

$$[E]_T = \left( \alpha \frac{k_{-1} + k_2}{k_1[S] + k_{-2}[P]} + \alpha' \right) [ES]$$

(21)

where the inhibition parameters $\alpha$ and $\alpha'$ were introduced by the definitions:

$$\alpha = 1 + \frac{[I]}{K_I} , \quad \alpha' = 1 + \frac{[I]}{K_{IS}}$$

(22)

The expression (21) is further rearranged to its most convenient form, as:

$$[ES] = \frac{k_1[S] + k_{-2}[P]}{\alpha (k_{-1} + k_2) + \alpha' (k_1[S] + k_{-2}[P])} [E]_T$$

(23)
in order to be properly inserted into the instantaneous velocity of the overall reaction:

\[ v = -\left(\frac{d}{dt}[S]\right) = k_1[S][E] - k_{-1}[ES] \]  

(24)

to give

\[ v = \frac{k_1k_2[S] - k_{-1}k_2[P]}{\alpha(k_{-1} + k_2) + \alpha(k_1[S] + k_{-2}[P])}[E]_T \]  

(25)

when also the first relation of (19) was employed.

Since dividing both the numerator and denominator of equation (25) by \((k_{-1} + k_2)\)

further arrangement of this equation is provided:

\[ v = \frac{k_2}{k_{-1} + k_2} \left(\frac{k_1}{k_{-1} + k_2} [S] - \frac{k_{-2}}{k_{-1} + k_2} [P]\right) \]  

\[ + \alpha \left(\frac{k_1}{k_{-1} + k_2} [S] + \frac{k_{-2}}{k_{-1} + k_2} [P]\right) \]  

\[ = \frac{k_1k_2[S] - k_{-1}k_2[P]}{\alpha(k_{-1} + k_2) + \alpha(k_1[S] + k_{-2}[P])}[E]_T \]  

(26)

The velocity equation (26) is suited to be rewritten, by following parameters

analogously with the constants (4) and (5) of the Michaelis-Menten equation (3),

\[ K_M^S \equiv \frac{k_{-1} + k_2}{k_1}, \quad K_M^P \equiv \frac{k_{-1} + k_2}{k_{-2}}, \quad V'_f = k_2[E]_T, \quad V'_r = k_{-1}[E]_T \]  

(27)

under the compact form:

\[ v = \frac{V'_f}{K_M^S} \frac{[S]}{K_M^S} - \frac{V'_r}{K_M^P} \frac{[P]}{K_M^P} \]  

\[ + \alpha \left(\frac{[S]}{K_M^S} + \frac{[P]}{K_M^P}\right) \]  

(28)

From this point we apply the previous described method of transforming the hyperbolic type equation (28) into an exponential one.

For doing this, it is firstly split into the forward and backward overall velocity components by appropriate factorizations,

\[ v = \frac{V'_f}{\alpha' \left(1 + \frac{K_M^S}{\alpha' [S]} \frac{[S]}{[S]}\right) + \frac{K_M^S}{\alpha' [S]} \frac{[S]}{[S]}} - \frac{V'_r}{\alpha' \left(1 + \frac{K_M^P}{\alpha' [P]} \frac{[P]}{[P]}\right) + \frac{K_M^P}{\alpha' [P]} \frac{[P]}{[P]}} \]  

(29)
from where, there is easy to perform the envisaged transformation to give:

\[
\frac{V^*}{V_{\text{max}}} = \frac{1}{\alpha'} \left( \frac{K_M^{+S}}{[S]} \left( \frac{\alpha}{\alpha'} \frac{[P]}{K_P^{+S}} \right) - \frac{K_M^{+P}}{[P]} \left( \frac{\alpha}{\alpha'} \frac{[S]}{K_M^{+S}} \right) \right),
\]

(30)

the correspondent “star” expression of (29), where the appearing “star” Michaelis-Menten constants are derived from their counterparts from (27) simply by multiplication with the factor \( \ln 2 \), as the recipe (13) requires.

\[\text{DISCUSSION}\]

The overall velocity expression (30) stands as the present generalization of the Michaelis-Menten equation for the enzymatic reaction (15).

Aiming to interpret it let’s first observe that no temporal assumption or limitation was applied, being therefore an instantaneous result, valid at any time in the course of the reaction, being therefore suited for further temporal integration to find the progress curves, numerically or analytically.

Nevertheless, some limiting cases worth to be here analyzed.

By inspection of the expression (30), under the initial conditions:

\[\{[P] = 0, [S] = [S]_0\}\]

(31)

one immediately gets the simple result:

\[\frac{V_0^{*f}}{V_{\text{max}}} = \frac{1}{\alpha'} \left( \frac{K_M^{+S}}{[S]} \left( \frac{\alpha}{\alpha'} \right) \right),\]

(32)

from where, the previous Michaelis-Menten “star” formulation (14) is recognized when neglecting the inhibition processes (\( \alpha = \alpha' = 1 \)).

On the contrary, when the forward reaction is completely consumed, so to speak when the entirely substrate is becoming a product,

\[\{[S] = 0, [P] \neq 0\}\]

(33)
in principle at the infinite time from the initialization of the enzyme reaction, the backward reaction dominates the process with the velocity

$$v_{0*}^r = -\frac{1}{\alpha'} V_{\text{max}} \exp\left( -\frac{\alpha' K_M^{*P}}{\alpha' [P]} \right).$$  (34)

Cumulating the extreme cases of velocities, (32) and (34), into the general equation (30), it can be accordingly written as:

$$v^* = v_0^* e^{-\frac{K_M^{*S}}{[P]} K_M^{*P} \frac{[S]}{[P]} K_M^{*P} \frac{[S]}{[P]} + v_0^* e^{-\frac{K_M^{*S}}{[P]} K_M^{*P} \frac{[S]}{[P]}}.$$

The form (35) of the overall velocity of the reaction (15) is particularly meaningful. That because, from it, there is clear that the Michaelis-Menten case $v_0^*$ appears to be modulated by the exponential factor of the product formation $[P]$, to which further adds the reversible kinetics.

This is an interesting analytical form of the generalized Michaelis-Menten equation that cannot be derived from the hyperbolic form (28).

More, due to the product formation presence, in fact, the velocity equation (35), even not explicitly, accounts for the time evolution of the concerned enzymatic reaction. Therefore, the equation (35) allows of representing on a single graph the overall velocity instants as the substrate concentration $[S]$ is consumed from its initial value $[S]_0$ to the product $[P]$.

To better emphasis on the latter aspect, let’s roughly represent the shape of the velocity (35) for a particular pilot case.

For doing that in the expression (35) all considered parameters and constants are replaced by their definitions until the working form:

$$v^* = \frac{1}{\alpha'} [E]_0 \left\{ -\frac{\alpha' k_2 e^{\alpha' k_1 \ln 2 [S]_0 - [S]}}{[S]} - \frac{\alpha' k_1 e^{\alpha' k_2 \ln 2 [S]_0 - [S]}}{[S]} \right\},$$

where also the product instantaneous concentration was replaced with that of the initial and instantaneous substrate by the relation:

$$[P] = [S]_0 - [S].$$  (37)

If the parameters that appear in (36) are chosen to be:
\[ k_1 = k_2 = 10^8 [M^{-1}s^{-1}], \]
\[ k_{-1} = k_2 = 10^2 [s^{-1}], \]
\[ [E]_0 = 1 \mu M, [S]_0 = 100 \mu M, \] \hspace{1cm} (38)

The resulting velocities are functions only of the substrate concentration \([S]\) with the same shapes, represented in the Figure 3, but having different amplitudes depending on the values of the inhibition constants (22).

Nevertheless, the Figure 3 differs significantly from the Michaelis-Menten velocity-versus-substrate concentration plots, see Figure 1 for instance, from many points of view.

Basically, Figure 3 accounts for the temporal evolution curves, due to the consumption of the substrate concentration from its initial value until the complete formation of the product when it achieves zero value.

Figure 3. The plot of the instantaneous overall velocity (36), under the conditions (38), versus the substrate concentration variation between its maximal initial value (on the right extreme on the abscise) and its complete consumption (the origin point on the abscise) to form the product in an enzymatic reaction of type (15), for the mixed \((\alpha = 1.5, \alpha' = 1.25, \text{the dashed line})\) and without \((\alpha = \alpha' = 1, \text{the thick line})\) inhibition, respectively.
Another feature displayed in Figure 3 reveals the oscillatory behavior of the enzymatic reaction based on the scheme (15), between the substrate consumption and product formation. The particular conditions in which the overall reaction is merely orientated to the products rather backwards to the substrate, as well as the influence of the enzymatic activity on the factors appearing in (36) equation, together with analytically or numerically determination of the parameters that governs the progress curves of substrate concentration, are the perspectives to be next explored, and will be reported in the subsequent communications.

**Conclusion**

One of the main challenges regarding the enzyme-substrate reactions stands as to determine the kinetic parameters, such as the maximum velocity of reaction or the affinity constants, with a high degree of reliability. This may, the progress curves or the time dependent forms of the substrate and product concentrations have become a valuable alternative to the measuring the initial velocity of reaction in a well-known Michaelis-Menten enzymatic mechanism. However, such enterprises have been proved to be dependent on the analytical frame in which the enzymatic kinetic is described.

In this work we have identified the basic hyperbolic shape of the Michaelis-Menten type kinetics as the main source of analytical limitations techniques in the experimental analysis of data, and we replace it with a more reliable kinetic form as the exponential functions reveal.

Following this line, a new enzymatic kinetic was founded relating with the basic Michaelis-Menten description as the limiting case.

The present approach opens the perspective of treating the enzymatic reactions and activity within a consistent unitarily biochemical kinetic.

**Acknowledgements**

Authors gratefully acknowledge Prof. Adrian Chiriac from West University of Timisoara for many useful discussions.
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