The kinetic effect of product instability in a Michaelis–Menten mechanism with competitive inhibition

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Abstract

In most kinetic studies it is assumed that both the reactant and the products are stable. However, under certain conditions spontaneous decomposition or deterioration caused by one of the participating species occurs. Studies, in which a species (the free enzyme, the enzyme–substrate complex, an inhibitor or the product of the reaction) is unstable, have appeared in the literature. However, to our knowledge, the enzymatic systems, in which a competitive inhibition and a decomposition or transformation of the products take place simultaneously, have not been studied so far. In this paper, we present a kinetic analysis of an enzyme reaction that follows a Michaelis–Menten mechanism, in which the free enzyme suffers a competitive inhibition simultaneously with the decomposition of the immediate product. In this study, we have linearised the differential equations that describe the kinetics of the process. Under the assumption of limiting concentration of enzyme, we have obtained and tested the explicit equation describing the time dependence of the product concentration using numerical calculus. With this equation and the experimental progress curve of the product, we constructed an easy procedure for the evaluation of the principal kinetic parameters of the process. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Enzyme inhibition; Enzyme reactions; Kinetic parameter; Progress curves; Unstable product

1. Introduction

Studies, in which a single species (the free enzyme, the substrate, the enzyme–substrate complex, an inhibitor or the product of the reaction) is unstable, have already been carried out (Duggleby, 1986, 1994; Topham, 1990; Garrido-del Solo et al., 1993, 1994, 1996; Varón et al., 1993, 1998). Only a few examples of enzymatic reactions, in which the products are unstable and undergo decomposition by reactions of first or second order have so far been described in the
literature (Childs and Bardsley, 1975; Claiborne and Fridovich, 1979; Boekelheide et al., 1980; Frère et al., 1982; Garcia-Carmona et al., 1982; Escribano et al., 1985).

However, these analyses were performed under specific assumptions regarding the ratio of the concentrations of enzyme and substrate and omitted the case of the effect of a combination of species decomposition and competitive inhibition. Since the structures of substrates, intermediates and products are related for mechanistic reasons, such a case is bound to play a role in many enzyme systems. Hence, the analysis of experimental data must take that situation into account. We recommended a simple procedure for the evaluation of the kinetic parameters associated with a common case.

A recent example of the slow inhibition of an enzyme due to product instability is the foetal ethanol intoxication. Apparently, ethanol activates a gangliosidase, the progressively decomposing products of which inhibit the enzyme. The result is failing neuritogenesis that leads to severe retardation of the motoric and sensoric development of infants suffering from prenatal intoxication by alcohol consumed in even moderate doses by the mother (three to five glasses of beer or equivalent beverages corresponding to 5–25 mM ethanol in the blood) during the second trimester of the pregnancy (Rosenberg and Noble, 1994).

A more general example of the inhibition of an enzyme by a decomposing inhibitor is the classical allosteric feedback inhibition of a metabolic key enzyme by a metabolite, e.g. the inhibition of phosphofructokinase by citrate (and ATP) (Hers and van Schaftingen, 1981).

2. Theory

2.1. Notation and definitions

\[ [X] \] concentration of the species X (X = E, free enzyme; S, substrate; I, inhibitor; ES, enzyme–substrate complex; EI, enzyme–inhibitor complex; P, immediate product of the enzymatic reaction; R, inactive species, in which the product was transformed, respectively)

\[ [X]_0 \] initial concentration of the species X

\[ [X]_\infty \] concentration of the species X at infinite time

\[ [P]_{\text{max}} \] maximal value of [P]

\[ t_{\text{max}} \] time corresponding to \([P]_{\text{max}}\)

\[ t_{\text{inflex}} \] time at which the concentration of P passes an inflexion point

\[ k_i \] rate constants \((i = 1–5)\)

\[ K_m \] Michaelis constant, \((k_2 + k_5)/k_1\)

\[ j \] Rate constant to the decomposition of the product

Scheme 1 shows the reaction scheme of the process that we shall analyse.

We assumed the prevalence of the conditions described by the following relationship:

\[
[S]_0, [I]_0 \gg [E]_0 \quad [P] + [R] \ll [S]_0
\] (1)

Hence, the differential equations Eqs. (A1)–(A7) of Appendix A, describing the kinetics of the enzymatic system of Scheme 1, can yield an approximate analytical solution. Under these conditions, we can assume that:

\[
[S] \approx [S]_0 \quad [I] \approx [I]_0
\] (2)

Then, the system of differential equations Eqs. (A1)–(A7) can be transformed into Eq. (A.8). The analytical solution of the latter equation for the product P and the species R are:
\[ P = [P]_\infty + \sum_{h=1}^{3} \gamma_h e^{\delta_h t} \quad (3) \]
\[ [R] = \beta + at + \sum_{h=1}^{3} r_h e^{\delta_h t} \quad (4) \]

where \( \delta_h \) (\( h = 1–3 \)) are the eigenvalues of the matrix of coefficients of the Eq. (A.8), i.e. the roots of the equation:

\[ (\lambda + j) (\lambda^2 + F_1 \lambda + F_2) = 0 \quad (5) \]

in which:

\[ F_1 = k_4[S]_0 + k_5[I]_0 + k_2 + k_4 + k_5 \quad (6) \]
\[ F_2 = k_1k_4[S]_0 + (k_3[I]_0 + k_9)(k_2 + k_5) \quad (7) \]
\[ a = \frac{k_1k_4[S]_0[E]_0}{\lambda_1 \lambda_2} \quad (8) \]
\[ [P]_\infty = \frac{a}{j} \quad (9) \]
\[ \beta = \frac{k_1k_4[S]_0[E]_0}{j[\lambda_4 (\lambda_1 + \lambda_2) + \lambda_1 \lambda_2] - k_4 \lambda_1 \lambda_2} \quad (10) \]

The parameters \( \gamma_i \) (\( i = 1–3 \)) and \( r_i \) (\( i = 1–3 \)) are given in Appendix A.

One of the roots of Eq. (5) is equal to \(-j\). The other two, which we shall name \( \lambda_1 \) and \( \lambda_2 \), are the roots of the equation \( \lambda^2 + F_1 \lambda + F_2 = 0 \).

If we admit that \( k_4[S]_0 \) and \( k_5 \) are much smaller than \( k_1[I]_0 \) and \( k_2 \), which is true in the case of a slow-binding inhibition (Szedlaczek and Duggleby, 1995; Sculley et al., 1996), and when we assume that a pseudo steady state exists in the catalytic route from the onset of the reaction (Waley, 1980; Tatsunami et al., 1981; Topham, 1990; Wang, 1990), i.e. when \( k_1[S]_0, \ k_2, \ k_5 > k_4[I]_0, \ k_9, \ j \), then, using the (Eq. (A.12)) of Appendix A, it is easy to show that:

\[ k_4 < |\lambda_1| < (k_4[I]_0 + k_4) \quad (11) \]
\[ \lambda_2 \approx -(k_1[S]_0 + k_2 + k_5) \quad (12) \]
\[ |\lambda_2| > |\lambda_1| \quad (13) \]

Now, Eq. (3) can be simplified to:

\[ [P] = [P]_\infty + \gamma e^{-\beta t} - ([P]_\infty + \gamma) e^{\beta t} \quad (14) \]

where:

\[ [P]_\infty = \frac{k_4k_4[S]_0[E]_0}{j(K_m k_3[I]_0 + k_4(K_m + [S]_0))} \quad (15) \]
\[ \lambda = -\left( k_4 + \frac{K_m k_5[I]_0}{K_m + [S]_0} \right) \quad (16) \]
\[ \gamma = \frac{k_4(j - k_4)[S]_0[E]_0}{j(K_m k_5[I]_0 - (j - k_4)(K_m + [S]_0))} \quad (17) \]

If we denote \( \omega_0 \) as \((d[P]/dt)\) at \( t = 0 \), then we obtain from the Eq. (14):

\[ \omega_0 = \frac{k_4[S]_0[E]_0}{K_m + [S]_0} \quad (18) \]

From Eqs. (15), (16) and (18) we then obtain:

\[ \frac{k_4}{j} = \frac{[P]_\infty \lambda}{\omega_0} \quad (19) \]

### 3. Materials and methods

The simulated curves were obtained by the numerical solution of the differential equations Eqs. (A1–A7) using a set of arbitrary but realistic rate-constants and initial concentrations according to the conditions defined by Eq. (1). The numerical solution was obtained by the Adams–Moulton method in combination with the Runge–Kutta fourth-order formulas (Gerald and Wheatley, 1989; Garrido-del Solo et al., 1992). The analytical solutions were obtained using Laplace transformation (Darvey, 1977; Jacquez, 1985).

### 4. Results and discussion

In this paper, we have, under the conditions described by Eq. (1), derived the kinetic Eqs. (3) and (4), which describe the evolution of the species \( P \) and \( R \) in the enzyme reaction that is shown in Scheme 1.

Fig. 1a shows the progress curve of the product according to the Eq. (3) and is obtained by numerical solution. It can be noted that the two curves overlap. The Fig. 1a shows that the progress curve of the product reaches a maximal value, \([P]_{\text{max}}\), when \( t = t_{\text{inf}} \) and that it goes through an inflexion point, \([P]_{\text{inflex}}\), at \( t = t_{\text{inflex}} \). If
Fig. 1. (a) Plot of Eq. (3) and the simulated progress curve of the product obtained by numerical solution of the system of differential equations Eqs. (A1)–(A7). The two curves overlap. (b) Plot of \( \frac{d[P]}{dt} \) against time. In both figures, the singular points \( [P]_{max} \) and \( [P]_{inflex} \) are shown. The values used for the initial concentrations and for the rate constants were, \( [E]_0 = 1 \) nM, \( [I]_0 = 10 \) mM, \( [S]_0 = 1 \) mM, \( k_1 = 1 \times 10^6 \) M\(^{-1}\) s\(^{-1}\), \( k_2 = 500 \) s\(^{-1}\), \( k_3 = 5000 \) s\(^{-1}\), \( k_4 = 0.01 \) s\(^{-1}\), \( k_5 = 100 \) s\(^{-1}\) and \( j = 0.1 \) s\(^{-1}\).

we denote with \( t_\infty \) the \( t \) value preceding \( t_{max} \), at which \( [P] = [P]_\infty \), then the following equations can be obtained easily:

\[
[P]_{max} - [P]_\infty = \gamma \left( 1 + \frac{j}{\lambda} \right) e^{-\beta_{max}}
\]  
\[ (20) \]

\[
t_{max} = \frac{1}{j + \lambda} \ln \left( -\frac{j\gamma}{\lambda(\gamma + [P]_\infty)} \right)
\]  
\[ (21) \]

\[
\lambda = \frac{\ln \left( \frac{\gamma(\gamma + [P]_\infty)}{t_\infty} \right)}{t_\infty} - j
\]  
\[ (22) \]

The \( t_\infty \) value can be useful, since it allows the evaluation of \( [P]_\infty \) without reaching the end of the reaction, i.e. the \( [P]_\infty \) value can be obtained from \( t_{max} \) and \( t_{inflex} \). The latter is equal to the concentration of the product, when \( t = t_\infty = 2t_{max} - t_{inflex} \) (see Fig. 1a).

The evaluation of the co-ordinates of the singular points of the progress curve of \( P \) can be carried out, for example, from a plot of the \( d[P]/dt \) against time, since the intersection point of the curve obtained with the abscises axis was located at \( t = t_{max} \). The value of \( t \) corresponding to the minimum value of the curve \( d[P]/dt \) was equal to \( t_{inflex} \) (see Fig. 1b).

4.1. Particular cases

The particular cases of Scheme 1 can be obtained by insertion of the corresponding values for the rate constants, i.e. the Schemes 2–4 of Table 1, and their associated equations, will emerge with \( j = 0 \) and \( k_4 = 0 \), respectively (see Table 1).

In the case in which the disappearance of the product is due to its irreversible reaction with a reactant \( Y \) ([\( Y]_0 \gg [P] + [R]) \), in which \( [Y]_0 \approx [Y] \) along the entire course of the reaction, then the constant \( j \) may be substituted for \( k_j[Y]_0 \) in the equations, where \( k_j \) is the rate constant corresponding to the process of transformation of the product.

5. Kinetic data analysis

In this contribution, we suggest a procedure that allows the evaluation of the more important kinetic parameters of the enzymatic reaction presented for Scheme 1, if the conditions defined by Eqs. (1) and (2) are fulfilled. At first, several series of progress curves of \( P \) must be obtained for different initial concentrations of substrate, free enzyme and inhibitor. If the \([S]_0\) value is kept constant, then a series of progress curves of \( P \), for different values of \([I]_0\), at \([E]_0\) constant, may be obtained (see Fig. 2a). Next, \([S]_0\) was varied, while \([I]_0\) was kept constant.
From these progress curves, the linear and non-linear parameters of Eq. (14) can be obtained. For each curve \( \ln([P]_\infty - [P]) \) against \( t \) was plotted. The points were fitted to a straight line by linear regression. From the slope of the latter, we obtained one of the non-linear parameters of Eq. (14). The linear parameter \( \gamma \) was obtained from the ordinate intercept. The other non-linear parameter can be obtained from Eq. (22). Once the non-linear parameters have been obtained, they can be used as initial estimates for a fitting by non-linear regression of the experimental data to the Eq. (14). The \( j \) value can also be obtained from an independent assay. According to Eq. (15), the plot of \( [E]_0/[P]_\infty \) against \( 1/[S]_0 \), at constant \( [I]_0 \), allows the evaluation of \( k_5 \). The constants \( k_5 \) and \( K_m \) can also be evaluated directly from a plot of \( [E]_0/[S]_0 \) versus \( 1/[S]_0 \). From the plot of \( -\lambda \) against \( [I]_0 \) (keeping \( [S]_0 \) constant) we obtained \( k_3 \) and \( k_4 \) (see Fig. 3). The evaluation of \( \alpha_0 \) generally presents difficulties that can be avoided, if this parameter is obtained indirectly from a plot of \( [I]_0 \) against \( 1/[P]_\infty \) (keeping \( [S]_0 \) constant). The intersection of the straight line obtained with the abscissa permitted the evalua-

Table 1
Particular cases corresponding to Scheme 1

Scheme 2:

\[
\begin{align*}
E + S &\overset{k_1}{\rightleftharpoons} ES &\longrightarrow & E + P \\
&\overset{k_2}{\rightleftharpoons} &\text{ES} &\longrightarrow & E + P \\
+ &\overset{1}{\longrightarrow} &\text{I} &\longrightarrow & R \\
k_3[I]_0 &\downarrow &\text{ES} &\longrightarrow & E + P
\end{align*}
\]

Scheme 3:

\[
\begin{align*}
E + S &\overset{k_1}{\rightleftharpoons} ES &\longrightarrow & E + P \\
&\overset{k_2}{\rightleftharpoons} &\text{ES} &\longrightarrow & E + P \\
+ &\overset{1}{\longrightarrow} &\text{I} &\longrightarrow & R \\
k_3[I]_0 &\downarrow &\text{ES} &\longrightarrow & E + P
\end{align*}
\]

Scheme 4:

\[
\begin{align*}
E + S &\overset{k_1}{\rightleftharpoons} ES &\longrightarrow & E + P \\
&\overset{k_2}{\rightleftharpoons} &\text{ES} &\longrightarrow & E + P \\
+ &\overset{1}{\longrightarrow} &\text{I} &\longrightarrow & R \\
k_3[I]_0 &\downarrow &\text{ES} &\longrightarrow & E + P
\end{align*}
\]

Fig. 2. (a) Progress curves of the product at different initial concentrations of inhibitor. Note that the \( [P]_\infty \) value decreases with the increase of the initial concentration of inhibitor. The values used for the initial concentrations of \( [E]_0 \) and \( [S]_0 \) as well as for the rate constants were the same as those used in Fig. 1; (b) schematic plot of \( [I]_0 \) against \( 1/[P]_\infty \) according to Eq. (A.16). The intersection of the straight line obtained with the abscissa permits the evaluation of \( \alpha_0 \) since, according to Eqs. (A.16) and (18), \( a = j/\alpha_0 \).
tion of $x_0$ since, according to Eqs. (15) and (18), $a = j/x_0$ (see Fig. 2b).

The plot of $-\lambda$ against $[I]_0$ (keeping $[S]_0$ constant) permitted a discrimination between the various particular cases. Eq. (16) shows that, regardless of whether the straight line obtained, passes through the origin of the reference system or not, and regardless of whether $\lambda$ varies with $[I]_0$ or not, the discrimination mentioned above will be possible. Sometimes, Eq. (A.16) is interesting, since the $[P]_\infty$ value can be fixed by modifying the initial concentration of the inhibitor.

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Appendix A

Differential equations describing the kinetics of Scheme 1:

\[
\frac{d[E]}{dt} = -k_1[E][S] + (k_2 + k_3)[ES] - k_4[E][I] + k_4[EI]
\]

(A.1)

\[
\frac{d[ES]}{dt} = k_1[E][S] - (k_2 + k_3)[ES]
\]

(A.2)

\[
\frac{d[EI]}{dt} = k_3[ES] - k_4[EI]
\]

(A.3)

\[
\frac{d[P]}{dt} = k_5[ES] - j[P]
\]

(A.4)

\[
\frac{d[S]}{dt} = k_2[ES] - k_1[E][S]
\]

(A.5)

\[
\frac{d[I]}{dt} = k_4[EI] - k_3[E][I]
\]

(A.6)

\[
\frac{d[R]}{dt} = j[P]
\]

(A.7)
\[
\begin{bmatrix}
\frac{d[E]}{dt} \\
\frac{d[ES]}{dt} \\
\frac{d[EF]}{dt} \\
\frac{d[P]}{dt} \\
\frac{d[R]}{dt}
\end{bmatrix}
= \begin{bmatrix}
-(k_1[S]_0 + k_2[I]_0) & k_2 & k_4 & 0 & 0 \\
0 & k_1[S]_0 & -(k_2 + k_3) & 0 & 0 \\
0 & 0 & -k_4 & 0 & 0 \\
0 & 0 & 0 & -j & 0 \\
0 & 0 & 0 & j & 0
\end{bmatrix}
\begin{bmatrix}
[E] \\
[ES] \\
[EF] \\
[P] \\
[R]
\end{bmatrix}
\quad (A.8)
\]

\[
\gamma_h = \frac{k_2k_5}{\lambda_0} \frac{1}{3} \sum_{q=1}^{\lambda_h} (\lambda_q - \lambda_0) \quad (h = 1-3)
\quad (A.9)
\]

\[
\gamma_h = \frac{k_2k_5}{\lambda_0} \frac{1}{3} \sum_{q=1}^{\lambda_h} (\lambda_q - \lambda_0) \quad (h = 1-3)
\quad (A.10)
\]

\[
\gamma_1 + \gamma_2 = -F_1
\]

\[
\gamma_1\gamma_2 = F_2
\quad (A.11)
\]

\[
\lambda_1 = -F_1 + \sqrt{F_1^2 - 4F_2}
\]

\[
\lambda_2 = -F_1 - \sqrt{F_1^2 - 4F_2}
\quad (A.12)
\]

\[
t_{\infty} = \frac{1}{j + \lambda} \ln \left( \frac{\gamma}{\gamma + [P]_{\infty}} \right)
\quad (A.13)
\]

\[
t_{\text{inflex}} = \frac{1}{j + \lambda} \ln \left( \frac{\gamma}{\gamma + [P]_{\infty}} \frac{j}{\lambda} \right)
\quad (A.14)
\]

\[
t_{\text{max}} - t_{\infty} = t_{\text{inflex}} - t_{\text{max}} = \frac{1}{j + \lambda} \ln \left( \frac{-j}{\lambda} \right)
\quad (A.15)
\]

Eq. (15) also can be written as:

\[
[I]_0 = \frac{k_2k_5}{jK_mk_3} \frac{1}{[P]_{\infty}} - \frac{k_4(K_m + [S]_0)}{K_mk_3}
\quad (A.16)
\]

References


