Estimating Enzyme Kinetic Parameters from Apparent $K_M$s and $V_{max}$s

Simon Brown, Noorzaid Muhamad and David C Simcock

Abstract—The kinetic properties of enzymes are often reported using the apparent $K_M$ and $V_{max}$ appropriate to the standard Michaelis-Menten enzyme. However, this model is inappropriate to enzymes that have more than one substrate or where the rate expression does not apply for other reasons. Consequently, it is desirable to have a means of estimating the appropriate kinetic parameters from the apparent values of $K_M$ and $V_{max}$ reported for each substrate. We provide a means of estimating the range within which the parameters should lie and apply the method to data for glutamate dehydrogenase from the nematode parasite of sheep Teladorsagia circumcincta.

Keywords—enzyme kinetics, glutamate dehydrogenase, interval analysis, parameter estimation.

I. INTRODUCTION

It is standard practice in the analysis of enzyme kinetics to employ the Michaelis-Menten mechanism [1], which may or may not be appropriate [3]. This model involves the irreversible conversion of a single substrate into a single product. While this may be appropriate to isomerases (E.C. 5), for example, the model need not apply perfectly, because many isomerases catalyse reversible reactions (of course this deficiency can be minimised if the product concentration is sufficiently low that the rate of the reverse reaction is negligible). However, most enzyme reactions involve two reactants, for example many dehydrogenases require a substrate and NAD(P)+ or NAD(P)H; some enzymes catalyse reactions involving three reactants [4]; and a theoretical analysis of reactions involving four reactants has been reported [5].

Despite these limitations, it is common to see [6] analyses of kinetics in which the standard Michaelis-Menten expression for the rate ($v$) of an enzyme-catalysed reaction is related to the substrate concentration ($s$)

$$v = \frac{V_{max}s}{K_M + s},$$

and two kinetic parameters, the Michaelis constant ($K_M$) and the maximum rate of the reaction ($V_{max}$). The $K_M$ and $V_{max}$ usually have the definitions derived from the analysis of Briggs and Haldane [7]. These analyses are applied to enzymes with more than one substrate, such as dehydrogenases, despite the fact that the mechanism of such an enzyme can not be that of a purely Michaelis-Menten enzyme [8].

We show that the apparent $K_M$s and $V_{max}$s obtained from analyses based on (1) are distinct from the parameters that appear in the rate equations. The apparent $K_M$s and $V_{max}$s reported in the literature depend on the conditions in which they were measured (as we show in section II). In particular, they depend on the substrate concentrations employed. Since the conditions vary between reports, apparent $K_M$s and $V_{max}$s can rarely be compared directly. The kinetic parameters describing the catalytic mechanism of the enzyme are independent of substrate concentration, at least, and so values from different analyses can be compared more reliably. It is, therefore, desirable to be able to estimate the latter from the apparent $K_M$s and $V_{max}$s that are often reported in the literature [2, 9] and are collected in at least one valuable database [6]. Here, we use interval analysis [10] to provide a means of estimating the bounds of the parameters using the apparent $K_M$s and $V_{max}$s, and apply the methods to the oxidative and reductive reactions of glutamate dehydrogenase (E.C. 1.4.1.3).

II. THEORY

In general, the unidirectional, steady-state reaction rate ($v$) involving $n$ substrates $s_i$, $i = 1, 2, … n$, binding to the enzyme in any order might be written

$$v = \frac{V_{max}}{1 + \sum_{i=1}^{n} \alpha_i s_i + \sum_{i=1}^{n-1} \beta_{ij} s_i s_j + \sum_{i=1}^{n-2} \sum_{j<k} \gamma_{ijk} s_i s_j s_k + \ldots}, \tag{2}$$

where $V_{max}$ is the maximum rate of the reaction and the $\alpha_i$s, $\beta_{ij}$s and $\gamma_{ijk}$s are functions of the rate constants of the mechanism [4, 5]. In the denominator of (2), the number of terms corresponding to the $s_i$s taken $k = 1, 2, … n$ at a time is given by $n!(n-k)!k!$. Including $V_{max}$ this amounts to a total of $2^n$ unknowns. Often one or more of the terms in the denominator is omitted as determined by the mechanism, as is the case for the reductive amination catalysed by glutamate dehydrogenase (section IIIIB). Clearly, (2) can exhibit a range of behaviour [11]. In order that the most appropriate of these is identified, reliable estimates of the unknowns are required. However, $2^n$ is large
even for small \( n \) and, as has been demonstrated, just a few parameters have the potential to yield a huge variety of behaviours [12].

The apparent \( K_M \) and \( V_{\text{max}} \) can be estimated for each substrate from (2). For \( s_i \), for example, they are

\[
K_M^{(i)} = \alpha_i + \sum_{j=1}^{n} \frac{\beta_{ij}}{S_j} + \sum_{j=1}^{n} \sum_{k=1}^{n} \gamma_{jik} S_j S_k + \ldots
\]

\[
1 + \sum_{i=1}^{n} \alpha_i + \sum_{i=2}^{n} \sum_{j=1}^{n} \frac{\beta_{ij}}{S_i} + \sum_{i=2}^{n} \sum_{j=1}^{n} \sum_{k=1}^{n} \gamma_{ijk} S_i S_j S_k + \ldots
\]

and

\[
V_{\text{max}}^{(i)} = \frac{V_{\text{max}}}{1 + \sum_{i=1}^{n} \alpha_i + \sum_{i=2}^{n} \sum_{j=1}^{n} \frac{\beta_{ij}}{S_i} + \sum_{i=2}^{n} \sum_{j=1}^{n} \sum_{k=1}^{n} \gamma_{ijk} S_i S_j S_k + \ldots}
\]

respectively. For each of the \( n \) substrates, specific apparent \( K_M \)s and \( V_{\text{max}} \)s can be determined, that can be equated to the appropriate form of (3) and (4). From an experimental perspective, for (3) and (4), only \( s_i \)s are varied and all the other \( s_j \)s are held constant. However, unless all the \( s_i \)s are present at saturating concentrations, the apparent \( K_M \) for substrate \( m \) are not equivalent to any of the parameters and the apparent \( V_{\text{max}} \) for substrate \( m \) is not equal to \( V_{\text{max}} \) respectively. On the other hand, if the \( s_i \)s, for \( i > 1 \), are very large, it is easy to see from (3) and (4) that the apparent \( K_M \) and \( V_{\text{max}} \) for \( s_i \) are \( \alpha_i \) and \( V_{\text{max}} \) respectively.

Of course, in principle, the apparent \( K_M \) and \( V_{\text{max}} \) can be estimated for each \( s_i \), yielding 2\( n \) values. For \( n = 2 \), it is possible to determine the 2\( n \) unknowns in (2) using the \( n \) estimates of \( K_M \) and \( V_{\text{max}} \). For \( n = 3 \), the 2\( n \) unknowns outnumber the \( n \) estimates of \( K_M \) and \( V_{\text{max}} \), but it is often the case that the reaction mechanism renders one or more of the parameters irrelevant (section III). So, if \( m \) parameters are unnecessary, then if \( 2^n - 2n - m = 0 \) it is possible to estimate the parameters in the rate expression. In fact, since the last term in the denominator of (2) is just \( \alpha_1 \), all the other parameters can be expressed in terms of \( \alpha_1 \), which can be estimated by nonlinear regression from the estimates of the \( K_M \)s and \( V_{\text{max}} \)s. This means that the system can be solved if \( m \) – 1 parameters are redundant and the condition becomes \( 2^n - 2n - m = 1 \).

III. APPLICATION TO GLUTAMATE DEHYDROGENASE

Glutamate dehydrogenase (E.C. 1.4.1.3) catalyses the reversible oxidative deamination of glutamate

\[
\text{glutamate} + \text{NAD(P)}^+ + \text{H}_2\text{O} = \alpha\text{-ketoglutarate} + \text{NAD(P)H} + \text{NH}_4^+ \quad (5)
\]

where the oxidant can be \( \text{NAD}^+ \) or \( \text{NADP}^+ \) with differing reaction efficiency, although in some species separate \( \text{NAD}^+ \)- and \( \text{NADP}^+ \)-dependent enzymes are synthesised [13] and more complex combinations have also been reported [14].

Careful analysis of the kinetics of the oxidative reaction indicate that glutamate binds before \( \text{NAD(P)}^+ \) [15-17], leading to the model shown in Fig. 1A. The kinetics of the reductive reaction indicate that \( \text{NAD(P)}H \) binds before \( \alpha\text{-ketoglutarate} \) and followed by \( \text{NH}_4^+ \) [15-17], as shown in Fig 1B.

![Fig. 1 Mechanisms of the oxidative deamination (A) and reductive amination (B) reactions of glutamate dehydrogenase (5).](image-url)

A. The oxidative deamination reaction

Based on the mechanism in Fig 1A, the rate of the oxidative deamination reaction, written in the same form as (2), but following the notation conventionally used in biochemistry [18], is

\[
v = \frac{V_{\text{max}}}{1 + \frac{K_a}{a} + \frac{K_s}{s} + \frac{K_{sa}}{sa}}, \quad (6)
\]

where \( s \) and \( a \) are the concentrations of glutamate and \( \text{NAD(P)}^+ \), respectively, the \( K_s \)s (corresponding to the \( \alpha_s \)s and \( \beta_i \)s in (2)) are functions of the rate constants derived from the mechanism and \( V_{\text{max}} \) is the maximum rate of the reaction. Since \( n = 2 \), there are 4 parameters \( (V_{\text{max}}, K_a, K_s, K_{sa}) \) and two pairs of apparent \( K_M \) and \( V_{\text{max}} \) it is possible to estimate the parameters.

The numerical values for the apparent \( K_M \)s and \( V_{\text{max}} \)s are not equivalent to the \( K_s \)s and \( V_{\text{max}} \)s in (6), as is clear from (3) and (4). However, apparent \( K_M \)s and \( V_{\text{max}} \)s obtained by fitting standard Michaelis-Menten expressions to the \( v \)-[substrate] data can be used to estimate the parameters in (6).

From (6), the apparent \( K_M \)s for \( \text{NAD(P)}^+ \) and glutamate obtained at a constant value of \( s \) or \( a \), respectively, are
respectively. Of course, it is possible to express (7-10) as a system of linear equations such as

\[
\begin{align*}
K_a^* &= \frac{K_a s + K_{sa}}{s + K_a}, \\
K_a^* &= \frac{K_a^* a + K_{sa}^*}{a + K_a},
\end{align*}
\]  

respectively, and the apparent \( V_{\text{max}} \)s are

\[
V_{\text{max}}^a = \frac{V_{\text{max}}^s}{s + K_a^*},
\]

and

\[
V_{\text{max}}^s = \frac{V_{\text{max}}^a a}{a + K_a^*},
\]

(10)

but it is clear from (11) that any physically plausible value of \( K_a^* \) yielding parameters \( s \in 9^\circ \) is consistent with (7-10).

However, (11) does prompt a strategy to determine physically plausible bounds on the parameters. This involves eliminating one parameter from appropriate combinations of (7-10) and then solving the resultant expression for a second parameter. Since this parameter must be positive, a bound on the remaining parameter is determined by the expression. Since all the bounds must be correct, those defining the innermost region, consistent with all parameters being greater than zero, must represent the region within which the best estimate is located. Note that we have not employed the experimental error estimates in estimating the parameters, but it would not be difficult to do so.

To illustrate this method, we describe the case for the oxidative deamination reaction in some detail. Lower bounds on \( K_a \) can be obtained by (i) eliminating \( K_a^* \) from (7) and (8) and solving for \( K_a \), to obtain

\[
K_a > \frac{K_a^* s - K_a^* a}{s + K_a^*},
\]

(12)

(ii) eliminating \( K_a \) from (7) and (8), and solving for \( K_a^* \), to yield

\[
K_a > \frac{K_a^* (s + K_a^* a)}{s - K_a^* s K_a^*},
\]

(13)

and (iii) eliminating \( V_{\text{max}}^* \) from (9) and (10) we obtain the expression

\[
K_a = \left( a + K_a \left( \frac{V_{\text{max}}^*}{V_{\text{max}}^* s - 1} \right) \right) s,
\]

(14)

and since \( K_a > 0 \) and \( s \geq 0 \), the term in brackets must be positive, so

\[
K_a > \left( \frac{V_{\text{max}}^*}{V_{\text{max}}^* s - 1} \right) a. \]

(15)

An upper limit for \( K_a \) can be obtained by substituting (14) into the ratio of (7) and (8) to yield an expression for \( K_a^* \) and requiring that it is positive

\[
K_a < \frac{\left( \frac{V_{\text{max}}^*}{V_{\text{max}}^* s - 1} \right) a}{\frac{V_{\text{max}}^* + \frac{V_{\text{max}}^*}{s}}{V_{\text{max}}^* s}}.
\]

(16)

Combining (12), (13), (15) and (16) yields

\[
K_a = \left( \frac{\left( \frac{K_a^* a - K_{a^*} s}{s + K_a^* a} \right) \left( \frac{K_a^* s + K_{a^*} a}{s + K_a^* s K_a^* a} \right) \left( \frac{V_{\text{max}}^*}{V_{\text{max}}^* s - 1} \right) a}{\frac{V_{\text{max}}^*}{s} K_{a^*} a - V_{\text{max}}^* s K_a^* a} \right) s.
\]

(17)

where the square brackets contain the bounds as [minimum, maximum], as is the usual convention in interval analysis [10].

Following similar procedures with (7), (8) and (14) yields expressions for \( K_a^* \),

\[
K_a^* = \left( \frac{\left( \frac{K_a^* a - K_{a^*} s}{a + K_{a^*}} \right) \left( \frac{K_a^* s + K_{a^*} a}{s - K_{a^*} s K_a^* a} \right) \left( \frac{V_{\text{max}}^*}{V_{\text{max}}^* s - 1} \right) a}{\frac{V_{\text{max}}^*}{s} K_{a^*} a - V_{\text{max}}^* s K_a^* a} \right) s.
\]

(18)
From the analysis in section II, one would expect that \(2^3\) maximum rate of the reaction.

Substituting the appropriate bounds from (17) into (9-10) yields an expression for \(V_{\text{max}}\)

\[
V_{\text{max}} = \left[ \frac{V_{\text{max}},a^s K_{\text{M}},a^s - V_{\text{max}},a^s K_{\text{M}},a^s}{V_{\text{max}},a^s K_{\text{M}},a^s - V_{\text{max}},a^s K_{\text{M}},a^s} \right].
\]

(Equations 17-20) are used. Substituting the appropriate bounds from (17) into (9-10) yields an expression for \(V_{\text{max}}\).

But only six of the seven parameters because the reaction mechanism involves ordered substrate binding (Fig 1B). However, only six of the seven parameters can be estimated from the \(V\)-[substrate] data. The apparent \(K_{\text{S}}\) and \(V_{\text{max}}\) have the forms

\[
K_{\text{M}}^k = \frac{K_k + K_a + \delta \frac{K_{\text{a}}}{j} + \kappa}{1 + \frac{K_k}{j}},
\]

(22)

where \(\delta \in \{0, 1\}\), and

\[
V_{\text{max}} = \left[ \frac{V_{\text{max}}}{1 + \frac{K_k}{j}} (1 - \delta) \frac{V_{\text{max}}}{j} \right],
\]

(23)

respectively, for \((i, j, k, \delta, K_k) \in \{(n, p, r, 0, K_n), (n, r, p, 0, K_r), (r, p, n, 1, K_r)\}\) and if \(K_{\text{S}} = K_{\text{b}}\) then \(K_{\text{M}}^k = K_{\text{b}}\) or vice versa.

We note in passing that (23) implies that

\[
V_{\text{max}} > \max \left( V_{\text{max}}^n, V_{\text{max}}^p, V_{\text{max}}^r \right)
\]

(24)

and that (22) implies that it is unlikely, but not impossible, that \(K_{\text{M}}^k = K_k\), which is the case if

\[
K_k = \frac{K_k + \frac{K_{\text{a}}}{j} + \kappa}{K_{\text{a}} + K_k (1 - \delta) K_{\text{a}}^{-1}}.
\]

(25)

For the oxidative reaction, the constant values of the concentrations of glutamate and NAD(P)\(^+\) were \(s = 5 \text{ mM}\) and \(a = 1 \text{ mM}\), respectively. For the reductive reactions, the constant values of the concentrations of \(k\)-ketoglutarate, \(\text{NH}_4^+\) and NAD(P)\(^+\) were \(p = 5 \text{ mM}\), \(n = 40 \text{ mM}\) and \(r = 1 \text{ mM}\), respectively.

Both of these implications are to be expected. For example, \(V_{\text{max}}\) is an asymptote rather than something that can be observed directly, as is clear from the standard Michaelis-Menten mechanism and the associated definitions: \(V_{\text{max}}\) can be observed only when all of the enzyme is in form of the enzyme-substrate complex, which is logically improbable [19].

An iterative approach was adopted to obtain estimates of the \(K_{\text{S}}\) and \(V_{\text{max}}\). Eliminating the denominators of (22) and (23) yields

\[
\begin{pmatrix}
K_{\text{M}}^k \\
V_{\text{max}}^n \\
V_{\text{max}}^p \\
V_{\text{max}}^r
\end{pmatrix} = \frac{1}{V_{\text{max}}} \begin{pmatrix}
1 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & n & 0 & n p & K_k \\
0 & 1 & 0 & 0 & 1 & 0 & n r & 0 & n p & K_p \\
0 & 0 & 1 & r & 0 & p & r p & 0 & 1 & 0 & 1 & K_r \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0
\end{pmatrix}
\]

(26)

which is ill-posed. However, parameter estimates can be obtained using a Lagrange multiplier [20] combined with Karush-Kuhn-Tucker optimality conditions and assuming that (24) can be replaced with

\[
V_{\text{max}} = \max \left( V_{\text{max}}^n, V_{\text{max}}^p, V_{\text{max}}^r \right)
\]

in the initial iteration. Having obtained a set of parameter estimates, a new estimate of \(V_{\text{max}}\) obtained from

\[
V_{\text{max}} = \frac{1}{np + K_k p + K_p n + K_r \delta} V_{\text{max}}^r
\]

(27)

was used in the Lagrange multiplier to obtain an updated set of parameter estimates. After nine iterations the \(V_{\text{max}}\) changed.
by less than $2 \times 10^{-5}$ and the other parameters changed by less than $10^{-3}$.

Following the strategy employed for the oxidative reaction, we seek to establish bounds on the parameters. The expressions are easily obtained, but are quite extensive and are reproduced, for reference, in the Appendix. From (23) three sets of bounds for $V_{\text{max}}$ (corresponding to $(i, j, k, \delta, K_o) \in \{(n, p, r, 0, K_o), (n, r, p, 0, K_o), (r, p, n, 1, K_o)\}$) can be obtained in the form

$$V_{\text{max}} = \left[ V_{\text{max}} - \min \left( \frac{K_i K_j}{j} (1 - \delta) \frac{K_o'}{ij} \right) \right]^{k_M},$$

where the third argument of min() is ignored if $\delta = 1$.

Equation (23) can be used in a similar way to estimate some of the bounds on $K_o$ (Appendix), but others can be derived from (22), for example

$$K _ k = \left\{ 1 + \frac{K_i}{j} + \frac{K_i}{i} (1 - \delta) \frac{K_o'}{ij} \right\} K_M - K_{o,i} + \delta K'_o + j + \kappa$$

so

$$K _ k = \left[ K_k - \min \left( \frac{K_i}{j} + \frac{K_i}{i} (1 - \delta) \frac{K_o'}{ij} \right) \right] \frac{K_M}{k}$$

where the third argument of min() is ignored if $\delta = 1$. Once again we seek the innermost sets of bounds.

While the families of bounds represented by (28), (30) and (36-40) depend on the values of other unknown parameters, the iterative approach described here yields a reasonable set of estimates that are shown in Table II.

IV. CONCLUSION

The apparent $K_{o,i}$ and $V_{\text{max}}$ obtained from fitting the Michaelis-Menten function (1) to $v$-[substrate] data are distinct from the $K_{o,i}$ and $V_{\text{max}}$ for more complex models. Because the apparent $K_{o,i}$ and $V_{\text{max}}$ obtained for most enzymes depend on the concentrations of all the substrates (3-4), as well as the assay conditions, they can be compared directly only rarely. We provide an approach that enables the use of the apparent values to estimate the bounds of the values of kinetic parameters of more complex models without requiring access to the original raw data.

APPENDIX

In addition to (28) and (30) the following expressions can be obtained from (22) and (23):

$$K _ o = \left\{ 1 + \frac{K_j}{j} + \frac{K_i}{i} (1 - \delta) \frac{K_o'}{ij} \right\} \frac{K_M}{i} - K_{j,i} + \delta K'_o + \kappa$$

Recall that (28), (30) and (36-40) have to be applied for $(i, j, k, \delta, K_o) \in \{(n, p, r, 0, K_o), (n, r, p, 0, K_o), (r, p, n, 1, K_o)\}$ and if $K_o = K_{o,i}$ then $K'_o = K_o$ or vice versa.
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