

Confidence bands for rational rate equations

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Abstract. The standard Michaelis-Menten expression for the rate of an enzyme-catalysed reaction (v) can be extended using the ratio of two polynomials in the substrate concentration (s). Such rational functions are nonlinear and their behaviour can vary considerably. Comparisons of two such functions requires more than a simple consideration of a particular coefficient, especially given the coefficients do not necessarily have any mechanistic significance. A second issue is the identification of particular coefficients that contribute significantly to the overall error of the estimate of v at a given s . As the number of coefficients rises this becomes increasingly difficult. To address the first of these issues we provide a general expression for the estimated confidence band of an arbitrary rational function of this type. We employ sensitivity analysis to address the second issue and provide general expressions for the coefficient elasticities. We apply these in the analysis of the kinetics of chymotrypsin and muscle lactate dehydrogenase.

Keywords: confidence band, enzyme kinetics, rational function, sensitivity analysis.

1 INTRODUCTION

More than 5700 enzymes have been catalogued [1], of these, the mechanism has been characterised for only a fraction. Nevertheless, a large number of enzyme mechanisms have been analysed [2] and, as we have pointed out previously [3], the reaction rate (v) expression of some of these can be summarised as

$$v = \frac{V_{\max}}{1 + \sum_{i=1}^n \frac{\alpha_i}{s_i} + \sum_{i=1}^{n-1} \sum_{i < j \leq n} \frac{\beta_{ij}}{s_i s_j} + \sum_{i=1}^{n-2} \sum_{i < j < k} \sum_{j < k \leq n} \frac{\gamma_{ijk}}{s_i s_j s_k} + \dots} \quad (1)$$

for a unidirectional reaction involving n substrates s_i , $i = 1, 2, \dots, n$, binding to the enzyme in any order. In (1) V_{\max} is the maximum rate of the reaction and the α_i s, β_{ij} s and γ_{ijk} s are functions of the rate constants of the enzyme mechanism. Clearly, such expressions are not really general, do not include every case and are not especially

simple. Bardsley *et al.* [4] took a different approach by considering the behaviour of rational functions

$$v_{pq} = \frac{\sum_{i=1}^p \alpha_i s^i}{\sum_{j=0}^q \beta_j s^j} \quad (2)$$

where the coefficients ($\alpha_i s$ and $\beta_j s$) are non-negative constants and $p \leq q$. Equations (1) and (2) differ in several ways. First, (2) involves only a single substrate rather than n substrates (1), although more substrates could be incorporated [5]. Second, each version of (1) corresponds to an enzyme mechanism, whereas this need not be the case for (2). Of course specific cases of (2) do correspond to a mechanism. For example if $p = q = 1$, (2) is just the Michaelis-Menten rate equation

$$v = \frac{V_{\max} s}{K_m + s} \quad (3)$$

[6, 7], in which case $\alpha_1 = V_{\max}$, $\beta_0 = K_m$ and $\beta_1 = 1$, if $p = 1$ and $q = 2$ (2) corresponds to a substrate inhibition model [8] and if $p = q = 2$ (2) can be interpreted in terms of an allosteric mechanism [4, 9]. Third, (2) has an extraordinary range of behaviour [10] even for small (≤ 4) p and q , as Bardsley *et al.* [4] illustrate.

In their analysis of the behaviour of (2), Bardsley *et al.* [4] used the Eadie-Hofstee transformation (in which v is plotted against $v s^{-1}$)

$$v = -K_m \frac{v}{s} + V_{\max} \quad (4)$$

[11-13] which was originally devised to transform (3) into a linear form. This is a well-established approach that continues to be used despite the problems inherent in the transformations [14-16]. While (4) may be the most reliable of the transformations [14, 16], the untransformed function (3) gives a better indication of the biologically relevant behaviour and more reliable parameter estimates can be obtained from it [17]. Data corresponding to (2) with $p > 1$ and $q > 1$ can be highly varied (Figure 1A), even when transformed in this way (4) (Figure 1B).

In comparing enzyme kinetics analysed using (2) it is not enough to compare coefficients (α_k and β_k) using the specific coefficient error estimates ($\varepsilon_{\alpha k}$ and $\varepsilon_{\beta k}$), instead a confidence band should be used [8, 18]. While this is generally the case, it is especially important for nonlinear functions such as (2). There are two reasons for this. The first is that the behaviour of (2) is difficult to predict without carrying out some mathematical analysis, which means that it is not necessarily obvious which coefficient is significant. The second is that the coefficients do not necessarily have any mechanistic significance, as we have already discussed, so there is no particular reason for selecting any one of the coefficients.

A second issue associated with the use of (2) is the identification of those coefficients that contribute to the error of the estimated v_{pq} at a given s . This is important because the error is partitioned among the coefficients in a complicated manner that depends on s and on the individual $\varepsilon_{\alpha k}$ or $\varepsilon_{\beta k}$.

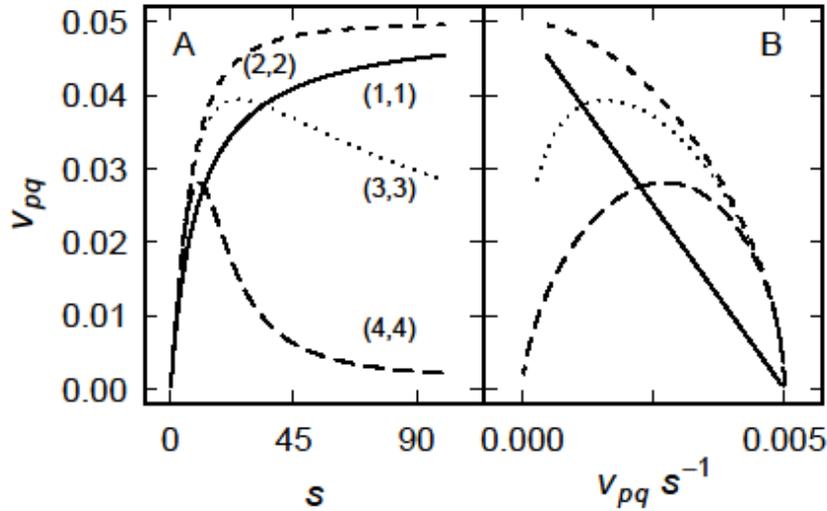


Figure 1. Some examples of the behaviour of (2) for $p = q = 1, 2, 3, 4$ in the untransformed (A) and Eadie-Hofstee (B) coordinates. The values of (p, q) are specified in (A). Coefficient values are $\alpha = (0.1, 0.01, 1 \times 10^{-5}, 1 \times 10^{-6})$ and $\beta = (20, 2, 0.2, 0.002, 0.001)$.

Here, we summarise some of the characteristics of (2) that are useful in the subsequent analysis. We then provide a general expression for the confidence band of (2) and employ sensitivity analysis to provide a means of identifying the most influential coefficient errors. Finally, we apply this analysis to the kinetics of muscle lactate dehydrogenase and chymotrypsin.

2 SOME CHARACTERISTICS OF EQUATION (2)

As is clear from Figure 1A, the range of behaviour of (2) can be considerable. For convenience, the characteristics of (2) include the following.

- a. As the coefficients of (2) are positive, $v_{pq} \geq 0$, where the equality holds when $s = 0$.
- b. At very high substrate concentrations v_{pq} is

$$\lim_{s \rightarrow \infty} v_{pq} = \begin{cases} \frac{\alpha_p}{\beta_q} & p = q \\ 0 & p < q \end{cases} \quad (5)$$

- c. If $p < q$, v_{pq} passes through a maximum (v_{opt}), but this can also occur if $p = q$ (Figure 1A). Writing (2) as $v_{pq} = f(s)/g(s)$ for simplicity, v_{opt} occurs when $s = s_{max}$, which is given by a root of the polynomial in s

$$g(s)f'(s) - g'(s)f(s) = 0, \quad (6)$$

and

$$v_{\text{opt}} = \frac{f(s_{\text{max}})}{g(s_{\text{max}})} = \frac{f'(s_{\text{max}})}{g'(s_{\text{max}})}. \quad (7)$$

For v_{22} , for example, s_{max} is a positive root of the quadratic

$$(\alpha_2\beta_1 - \alpha_1\beta_2)s_{\text{max}}^2 + 2\alpha_2\beta_0s_{\text{max}} + \alpha_1\beta_0 = 0$$

which is easily calculated. However, we observe that the existence of a real positive root ($s_{\text{max}} > 0$) requires that $\alpha_1\beta_2 > \alpha_2\beta_1$ since all the coefficients are non-negative. If there is a maximum, then (7) yields

$$v_{\text{opt}} = \frac{\alpha_1 + 2\alpha_2s_{\text{max}}}{\beta_1 + 2\beta_2s_{\text{max}}} < \frac{\alpha_1}{\beta_1},$$

where the inequality arises from the requirement that $\alpha_1\beta_2 > \alpha_2\beta_1$, from which $\alpha_2/\alpha_1 < \beta_2/\beta_1$. From the combination of this with (5) we infer that there will be a maximum if $\alpha_1/\beta_1 > \alpha_2/\beta_2$. In the case of v_{21} , for which $\alpha_2 = 0$, the positive root of the quadratic is $s_{\text{max}} = \sqrt{\beta_0/\beta_2}$ and so

$$v_{\text{opt}} = \frac{\alpha_1}{\beta_1 + 2\sqrt{\beta_0\beta_2}}.$$

For larger p and q the expressions become more complicated, if necessary the existence of positive real roots of (6) may be inferred from Descartes' rule of signs.

- d. Consistent with one measure of the efficiency of a Michaelis-Menten enzyme

$$\lim_{s \rightarrow 0} \frac{dv_{pq}}{ds} = \frac{\alpha_1}{\beta_0}, \quad (8)$$

which is called the 'kinetic power' by Keleti and Welch [19] for (3).

3 CONFIDENCE BAND

Following the approach we have previously employed [18], the confidence band of (2) can be estimated using

$$\varepsilon_v = \sqrt{\sum_{k=1}^p \left(\frac{\partial v}{\partial \alpha_k} \varepsilon_{\alpha k} \right)^2 + \sum_{k=1}^q \left(\frac{\partial v}{\partial \beta_k} \varepsilon_{\beta k} \right)^2}, \quad (9)$$

where the $\varepsilon_{\alpha k}$ and $\varepsilon_{\beta k}$ are the error estimates of the corresponding α_k and β_k , respectively. The derivatives of (2) are

$$\frac{\partial v_{pq}}{\partial \alpha_k} = \frac{s^k}{\sum_{j=0}^q \beta_j s^j} = \frac{s^k}{\sum_{i=1}^p \alpha_i s^i} v \quad (10)$$

and

$$\frac{\partial v_{pq}}{\partial \beta_k} = -\frac{s^k \sum_{i=1}^p \alpha_i s^i}{\left(\sum_{j=0}^q \beta_j s^j\right)^2} = -\frac{s^k}{\sum_{j=0}^q \beta_j s^j} v. \quad (11)$$

After substituting (10) and (11) into (9) and using (2) to simplify the expression we obtain

$$\varepsilon_v = \frac{v}{\sum_{i=1}^p \alpha_i s^i} \sqrt{\sum_{k=1}^p \varepsilon_{\alpha k}^2 s^{2k} + v^2 \sum_{k=0}^q \varepsilon_{\beta k}^2 s^{2k}}. \quad (12)$$

For the many models for which $p = q$ [4], (12) becomes

$$\varepsilon_v = \frac{v}{\sum_{j=1}^p \alpha_j s^j} \sqrt{\sum_{k=0}^p (\varepsilon_{\alpha k}^2 + v^2 \varepsilon_{\beta k}^2) s^{2k}}, \quad (13)$$

where $\varepsilon_{\alpha 0} = 0$.

4 SENSITIVITY ANALYSIS

While (12) and (13) are useful in constructing the approximate confidence band for v_{pq} , they are less useful in estimating the contribution of specific $\varepsilon_{\alpha k}$ s and $\varepsilon_{\beta k}$ s to ε_v . An intuitively attractive approach to estimating these contributions would be to calculate the proportional change in v_{pq} in response to a proportional change in a coefficient. This is the basis of sensitivity analysis which provides a way of characterising the potential impact of errors in the estimates of the coefficients of (2) [20-24].

A proportional change in v_{pq} in response to a proportional change in a coefficient is measured by the coefficient elasticity (λ). The elasticities of (2) are

$$\lambda_{\alpha_k} = \frac{\alpha_k}{v_{pq}} \frac{\partial v_{pq}}{\partial \alpha_k} = \frac{\partial \ln v_{pq}}{\partial \ln \alpha_k} = \frac{\alpha_k s^k}{\sum_{i=1}^p \alpha_i s^i}, \quad (14)$$

for $k = 1, 2, \dots, p$, and

$$\lambda_{\beta_k} = \frac{\beta_k}{v_{pq}} \frac{\partial v_{pq}}{\partial \beta_k} = \frac{\partial \ln v_{pq}}{\partial \ln \beta_k} = -\frac{\beta_k s^k}{\sum_{j=0}^q \beta_j s^j}, \quad (15)$$

for $k = 1, 2, \dots, q$, from which it is clear that $\lambda_{\alpha_k} \geq 0$, $\lambda_{\beta_j} \leq 0$, $\sum_k \lambda_{\alpha_k} = 1$, $\sum_j \lambda_{\beta_j} = -1$ and $\sum_k \lambda_{\alpha_k} + \sum_j \lambda_{\beta_j} = 0$. In effect, λ measures the proportional change in v_{pq} ($\partial v_{pq}/v_{pq}$) in response to a proportional change in a coefficient (say, $\partial \alpha/\alpha$ for α) which depends on s consistent with (13-14). Irrespective of p and q , the elasticity of α_1 and β_0 declines with increasing s and, consistent with (5), the elasticities of α_p and β_q either approach ± 1 or are increasing at $s = 100$, the highest value shown (Figure 2). At intermediate s , the elasticities of the remaining coefficients rise and fall in turn (Figure 2), naturally the s at which a particular λ

reaches an extremum is determined by the relative magnitudes of the coefficients (13-14).

An alternative graphical approach [21] is to plot v_{pq} for each coefficient as the coefficient is varied systematically from $\Delta\alpha_k = (\alpha_k - \varepsilon_{\alpha k})/\alpha_k$ to $\Delta\alpha_k = (\alpha_k + \varepsilon_{\alpha k})/\alpha_k$ (and similarly for β_k) while holding each of the other coefficients at its average value. Inevitably, when $\Delta\alpha_k = 0$ or $\Delta\beta_k = 0$ for any k , v_{pq} is the value given by (2) when the coefficients are maintained at their estimated value. Of course, v_{pq} will increase with increasing α_k (10) and decrease with increasing β_k (11) since all coefficients are non-negative. For example, Figure 3 shows the results of this analysis for four different s , assuming $p = q = 3$ and that the coefficients are the same as those in Figure 2C. At each s the point at which all seven curves intersect corresponds to $\Delta\alpha_k = 0$ or $\Delta\beta_k = 0$. At $s = 5$, where $\lambda_{\beta_0} < \lambda_{\beta_1} < \lambda_{\beta_2}$ (Figure 2C), the curve for $\Delta\beta_1$ is steeper than that for $\Delta\beta_2$ (Figure 3A), but this is reversed at $s = 45$ (Figure 3C) or $s = 90$ (Figure 3D) where $\lambda_{\beta_0} > \lambda_{\beta_1} > \lambda_{\beta_2}$ (Figure 2C). At $s = 10$, where $\lambda_{\alpha_1} = \lambda_{\alpha_2}$ and $\lambda_{\beta_0} = \lambda_{\beta_1} = \lambda_{\beta_2}$ (Figure 2C), the curves for $\Delta\alpha_1$ and $\Delta\alpha_2$ are superimposed, as are those for $\Delta\beta_0$, $\Delta\beta_1$ and $\Delta\beta_2$ (Figure 3B). So, the elasticities plotted in Figure 2 provide an indication of the relative sensitivities of v_{pq} to variation in the coefficients. However, the elasticities take no account of the error of the coefficient estimates, but the graphical approach illustrated in Figure 3 remedies this.

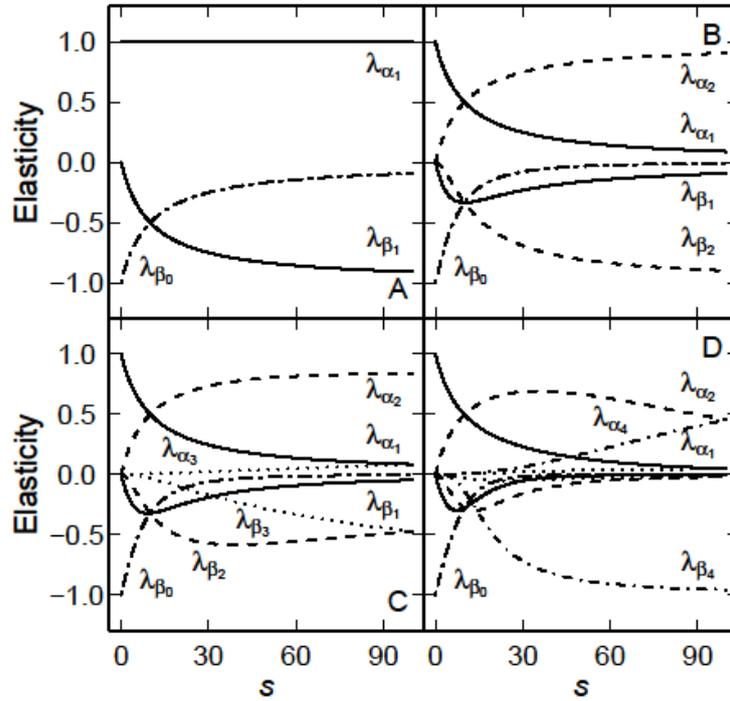


Figure 2. Elasticities of v_{pq} as a function of substrate concentration (s) for $(p, q) = (1, 1)$ (A), $(2, 2)$, (B), $(3, 3)$ (C) and $(4, 4)$ (D). The λ_{α_i} and λ_{β_i} were calculated using (14) and (15), respectively, assuming the coefficient values were $\alpha = (0.1, 0.01, 1 \times 10^{-5}, 1 \times 10^{-6})$ and $\beta = (20, 2, 0.2, 0.002, 0.001)$. The corresponding $v_{pq}S$ are shown in Figure 1A. Not all of the curves are labelled in (D), but in all panels (A-D), $\lambda_{\alpha_i} \geq 0$ and $\lambda_{\beta_i} \leq 0$: λ_{β_0} — — —; $\lambda_{\alpha_1}, \lambda_{\beta_1}$ — — —; $\lambda_{\alpha_2}, \lambda_{\beta_2}$ - - -; $\lambda_{\alpha_3}, \lambda_{\beta_3}$ ····; $\lambda_{\alpha_4}, \lambda_{\beta_4}$ - · - · - ·.

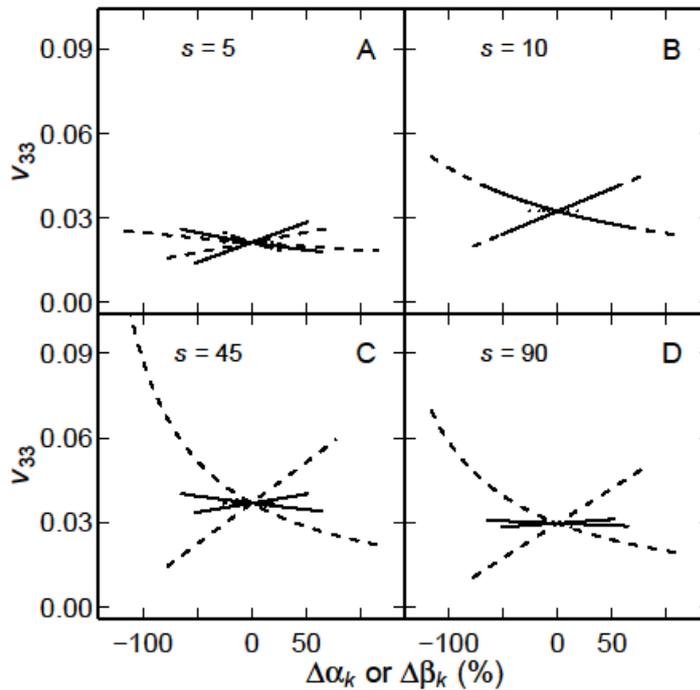


Figure 3. The effect on v_{33} of varying each coefficient over the range indicated ($\Delta\alpha_k$ or $\Delta\beta_k$) while holding all other coefficients constant and $s = 5, 10, 45$ or 90 (A, B, C and D, respectively). The coefficient values were $\alpha = (0.1, 0.01, 1 \times 10^{-5})$ and $\beta = (20, 2, 0.2, 0.002)$ and the standard deviations were $\varepsilon_\alpha = (0.02, 0.003, 1 \times 10^{-6})$ and $\varepsilon_\beta = (2, 0.5, 0.09, 0.0001)$. The v_{pq} is shown in Figure 1A and the elasticities are shown in Figure 2C. In all panels (A-D), curves corresponding to $\Delta\alpha_k$ and $\Delta\beta_k$ have positive and negative gradients, respectively: $\Delta\beta_0$ - - -; $\Delta\alpha_1, \Delta\beta_1$ - - -; $\Delta\alpha_2, \Delta\beta_2$ - - -; $\Delta\alpha_3, \Delta\beta_3$ ····.

5 APPLICATION

The kinetics of chymotrypsin (E.C. 3.4.4.5) are relatively complex [4, 25, 26], but the measurements of Bardsley *et al.* [4] were adequately described by (2) using $p = q = 2$ (Figure 4A). The error of the coefficient estimates is significant (standard deviations were up to 200% of the coefficient estimate) and ε_i is proportional to s (Figure 4A), which is also apparent in the Eadie-Hofstee coordinates (Figure 4B).

Since $\alpha_1\beta_2 < \alpha_2\beta_1$, v_{22} does not pass through a maximum, consistent with the asymptotic rate given by (5) ($\alpha_2/\beta_2 = 0.068$). This is also reflected in the coefficient elasticities, which are dominated by α_2 and β_2 even at $s = 2$ (Figure 4C). At $s = 1$, the contributions of α_1 and β_0 are small and α_2 and β_2 are dominant (Figure 4D), and this is reversed at very low s (Figure 4C).

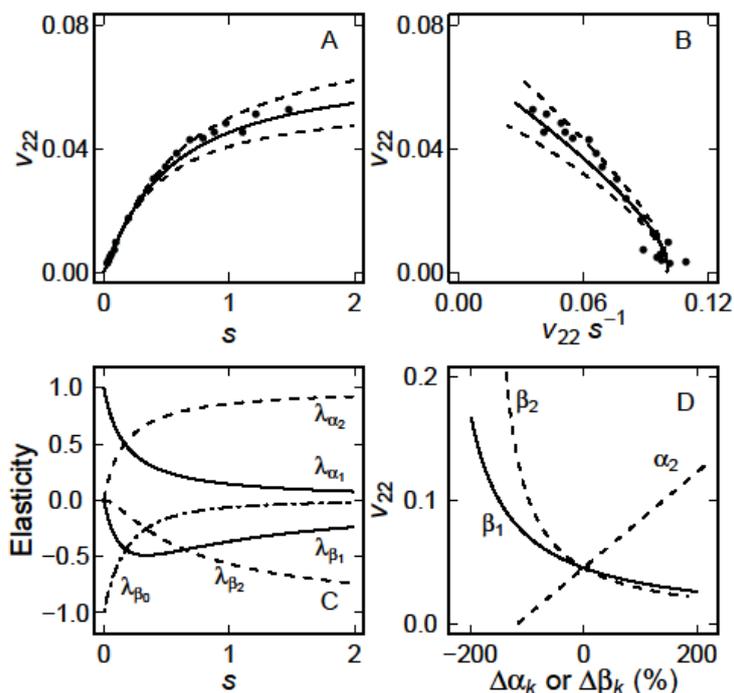


Figure 4. Properties of v_{22} for chymotrypsin based on the coefficient estimates of Bardsley *et al.* [4]. Panels (A) and (B) show the untransformed and Eadie-Hofstee transformed reaction rate estimates (solid curves) with approximate 85% confidence bands (dashed curves) and the data (\bullet) [4]. Panel (C) shows the elasticities of all the coefficients ($\lambda_{\alpha_i} \geq 0$ and $\lambda_{\beta_i} \leq 0$: λ_{β_0} - - - -; λ_{α_1} , λ_{β_1} - - - -; λ_{α_2} , λ_{β_2} - - - -; λ_{α_3} , λ_{β_3} \cdots). Panel (D) shows the effect on v_{22} of varying each coefficient over the range indicated ($\Delta\alpha_k$ or $\Delta\beta_k$) while holding all other coefficients constant and $s = 1$. The λ_{α_i} and λ_{β_i} were calculated using (14) and (15), respectively, assuming the coefficient values were $\alpha = (0.1, 0.6)$ and $\beta = (1, 5.7, 8.8)$ and the corresponding standard deviations were $\varepsilon_{\alpha} = (0.008, 1.3)$ and $\varepsilon_{\beta} = (0, 11.3, 16.2)$ [4]. The dimensions of α_k and β_k are $\text{mM}^{-k} \text{s}^{-k}$ and mM^{-k} , respectively, for $k \geq 1$ and β_0 is dimensionless [4], so the dimensions of v_{33} and s are s^{-1} and mM , respectively.

The kinetics of lactate dehydrogenase (E.C. 1.1.1.27) have been described by four or five step mechanisms, which requires at least eight rate constants [27, 28], but the data of Bardsley *et al.* [4] were adequately described by (2) using $p = q = 3$ (Figure 5A). In this case, the errors of the coefficient estimates were somewhat smaller (standard deviations were up to 85% of the coefficient estimate) than was the case for chymotrypsin. The estimated ε_v is systematically related to s (Figure

5A) as is also apparent in the Eadie-Hofstee coordinates (Figure 5B). The coefficient elasticities are dominated by α_2 and β_3 for $s > 25$, but the contribution of α_3 is just starting to increase at $s = 50$ (Figure 4C). The other coefficients (α_1 , β_0 and β_2) make little contribution at $s \geq 10$ (Figure 4, C and D).

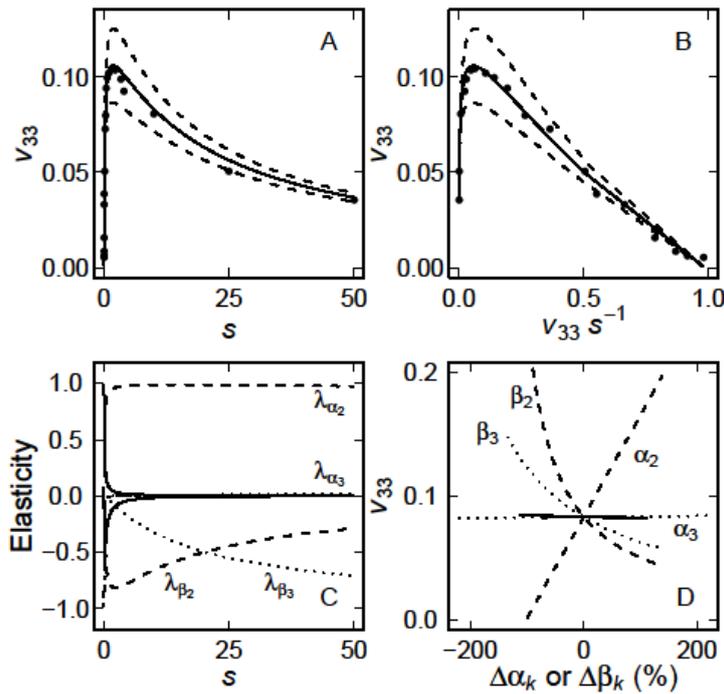


Figure 5. Properties of v_{33} for the muscle isozyme of lactate dehydrogenase based on the coefficient estimates of Bardsley *et al.* [4]. Panels (A) and (B) show the untransformed and Eadie-Hofstee transformed reaction rate estimates (solid curves) with approximate 99% confidence bands (dashed curves) and the data (\bullet) [4]. Panel (C) shows the elasticities of all the coefficients ($\lambda_{\alpha_i} \geq 0$ and $\lambda_{\beta_i} \leq 0$: λ_{β_0} — — —; λ_{α_1} , λ_{β_1} — — —; λ_{α_2} , λ_{β_2} - - -; λ_{α_3} , λ_{β_3} ····; λ_{α_4} , λ_{β_4} - · - · - ·), but not all are labelled. Panel (D) shows the effect on v_{33} of varying each coefficient over the range indicated ($\Delta\alpha_k$ or $\Delta\beta_k$) while holding all other coefficients constant and $s = 10$. The λ_{α_i} and λ_{β_i} were calculated using (14) and (15), respectively, assuming the coefficient values were $\alpha = (33.5, 417, 0.2)$ and $\beta = (36, 845, 3349, 165)$ and the corresponding standard deviations were $\varepsilon_\alpha = (10, 224, 0.17)$ and $\varepsilon_\beta = (10, 366, 1763, 86)$ [4]. The dimensions of α_k and β_k are $\text{mM}^{-k} \text{s}^{-k}$ and mM^{-k} , respectively, for $k \geq 1$ and β_0 is dimensionless [4], so the dimensions of v_{33} and s are s^{-1} and mM , respectively.

The Eadie-Hofstee coordinates emphasise the data for low s and v_{pq} by making them more apparent (Figures 4B and 5B). However, the sensitivity of v_{pq} to changes in s increases as $s \rightarrow 0$ (8) which makes it relatively difficult to measure v_{pq} . In Figures 4B and 5B it is clear that the confidence band obtained by fitting (2) to the untransformed data narrows as $v_{pq} s^{-1}$ increases. In each case data points are more likely to lie outside the confidence band at high $v_{pq} s^{-1}$ than at low $v_{pq} s^{-1}$.

6 DISCUSSION

The kinetics of enzymes catalysing reactions of a single substrate can be characterised using rational functions (2) [4]. While (2) may be consistent with some enzyme mechanisms, it can be used without requiring any implication of mechanism. Nevertheless, some features of (2) are directly useful, for example the asymptotic rate (7) and the kinetic power (8) can be estimated directly from the coefficients [29-33]. In general, however, the arbitrary selection of a single coefficient as a basis of comparison with other enzymes or between different conditions, for example, has no obvious significance. The confidence band (12) is a more reliable means of making such comparisons even in those cases, unlike (2), where parameters do have mechanistic significance [8, 18].

The number of coefficients ($= p + q + 1$) required in (2) can be large [4]. The relative significance of the contribution to v_{pq} associated with particular coefficients can be assessed using the coefficient elasticities (14-15) as we have demonstrated for chymotrypsin (Figure 4C) and lactate dehydrogenase (Figure 5C). A simple graphical approach provides a complementary assessment of the possible contributions of each coefficient at a particular s (Figures 4D and 5D).

For the many enzymes for which no mechanism has been established, (2) is useful providing that only one substrate is considered. However, where more than one substrate is involved, inverse polynomials [5] may provide a means of extending (2). On the other hand, there are at least three other ways of expressing (2). First, the numerator and denominator of (2) can be factored to yield

$$v_{pq} = \frac{\alpha_p s \prod_{i=1}^{p-1} (s + a_i)}{\beta_q \prod_{j=1}^q (s + b_j)} \quad (16)$$

or, second, after introducing a_p to avoid difficulties for $s = 0$,

$$\log(v_{pq}) = \log\left(\frac{\alpha_p}{\beta_q}\right) + \sum_{i=1}^p \log(s + a_i) - \sum_{j=1}^q \log(s + b_j). \quad (17)$$

An expression similar to (17) was obtained by Savageau [34], who suggested that it would be useful in obtaining the initial parameter estimates required for nonlinear regression. The third version of (2) is obtained from the partial fraction expansion of (16), with or without a_p . For $a_p = 0$ this can be written

$$v_{pq} = \frac{\alpha_p}{\beta_q} \frac{s}{s + b_1} \left(1 - \sum_{j=2}^q \frac{c_j}{s + b_j} \right), \quad (18)$$

where the c_j are simple functions of the a_i s and b_j s and $\sum_j (c_j/b_j) = 1$. Equation (18) is interesting because it prompts a different view of (2). For example, the term in (18) outside the brackets is the rate equation of a standard Michaelis-Menten enzyme (3). So (18) might be thought of in terms of a standard Michaelis-Menten enzyme that has $q - 1$ independent s -binding sites in addition to the usual active site in which the substrate binds. The term in brackets in (18) represents the effect of occupation of the extra sites, each of which has a dissociation constant b_j and contributes as much as c_j/b_j to the modulation of the total activity of the enzyme. Naturally, the

biological significance of such an interpretation of (2) requires further consideration, which we will provide in due course.

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