

## Asymptotic Properties of Enzymatic Rate Equations of the Wong-Hanes Type

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It is shown that steady-state reciprocal rate equations derived from the general Wong-Hanes eqn. (1) may become asymptotically linear with respect to any reactant for any higher-degree enzymatic reaction mechanism. This means that it may be difficult or practically impossible to distinguish between rate equations of different degree by means of reciprocal rate plots of experimental observations, and that the exhibition of linear reciprocal plots cannot be taken as evidence for elimination of higher-degree mechanisms. An equation which relates coefficients in the linear asymptote to coefficients in the non-linear theoretical rate equations is derived.

In their theoretical analysis of a general mechanism for enzyme reactions, Wong and Hanes introduced the concept of the degree of a mechanism and its steady-state rate equation, and classified enzyme mechanisms with respect to the degree of the corresponding rate equations.<sup>1</sup> The diagnostic value of such a classification is, evidently, dependent upon the possibility to distinguish experimentally between rate equations of different degree. The present investigation shows that theoretical steady-state rate equations of different degree under certain conditions may be practically indistinguishable, and thus emphasizes the importance of making a clear distinction between theoretical and experimentally estimated rate equations.

### THEORETICAL

Almost all enzymatic reaction mechanisms hitherto described in the literature are included in the general case discussed by Wong and Hanes, who showed that the steady-state rate equation with respect to a certain reactant R (substrate, product, or modifier) is given by

$$v = \frac{\sum_{k=0}^d \alpha_k' [R]^k}{\sum_{k=0}^d \beta_k' [R]^k} \quad (1)$$

where  $v$  stands for the reaction rate per enzyme unit, and where the coefficients  $\alpha_k'$  and  $\beta_k'$  can be expressed in terms of concentrations of other reactants and of rate constants for individual steps in the reaction mechanism.<sup>1</sup> The exponent  $d$  of the highest power of  $[R]$  in eqn. (1) defines the degree of the mechanism (and the rate equation) with respect to  $R$ , and usually represents the number of enzyme-containing species (including free enzyme) in the mechanism which react with  $R$ .

Experimental determinations of the steady-state reaction velocity  $v$  as a function of  $[R]$  can obviously (see eqn. (1)) only give  $2d+1$  independent relationships between ratios of the different  $\alpha_k'$  and  $\beta_k'$ , and for practical purposes any one of the non-vanishing coefficients in eqn. (1) may be put equal to unity.  $\beta_0'$  is the only coefficient which *a priori* is known to be non-vanishing for mechanisms of any degree, and putting

$$\alpha_k = \frac{\alpha_k'}{\beta_0'}; \quad k=0, 1, \dots, d \quad (2)$$

$$\beta_k = \frac{\beta_k'}{\beta_0'}; \quad k=1, \dots, d \quad (3)$$

the general Wong-Hanes eqn. (1) may be written as

$$v = \frac{\sum_{k=0}^d \alpha_k [R]^k}{1 + \sum_{k=1}^d \beta_k [R]^k} \quad (4)$$

In the most frequently encountered practical applications  $R$  stands for a substrate, and no reaction takes place in absence of the substrate ( $v=0$  when  $[R]=0$ ). According to eqn. (4) this implies that  $\alpha_0=0$ . It may be observed that when  $d=1$  and  $\alpha_0=0$  eqn. (4) reduces to

$$v = \frac{\alpha_1 [R]}{1 + \beta_1 [R]} \quad (5)$$

which is an alternate form of the classical Michaelis-Menten equation

$$v = \frac{V[R]}{K_m + [R]} \quad (6)$$

Results from steady-state experiments are usually analyzed by means of reciprocal rate plots, and when  $\alpha_0=0$  the reciprocal rate equation ( $y=1/v$  as a function of  $z=1/[R]$ ) corresponding to eqn. (4) becomes

$$y = \frac{z^d + \sum_{k=1}^d \beta_k z^{d-k}}{\sum_{k=1}^d \alpha_k z^{d-k}} \quad (7)$$

For  $d=1$  eqn. (7) reduces to the linear relationship

$$y = \frac{\beta_1}{\alpha_1} + \frac{1}{\alpha_1} z \quad (8)$$

For  $d=2$  eqn. (7) becomes

$$y = \frac{\beta_2 + \beta_1 z + z^2}{\alpha_2 + \alpha_1 z} \quad (9)$$

Defining  $y_{as}$  as (cf. Ref. 2)

$$y_{as} = \frac{\alpha_1 \beta_1 - \alpha_2}{\alpha_1^2} + \frac{1}{\alpha_1} z \quad (10)$$

we get

$$y - y_{as} = \frac{\alpha_2^2 + \alpha_1^2 \beta_2 - \alpha_1 \alpha_2 \beta_1}{\alpha_1^2 (\alpha_2 + \alpha_1 z)} \quad (11)$$

Eqn. (11) shows that the difference  $y - y_{as}$  becomes negligibly small for sufficiently large values of  $z$ . This means that the function  $y$  defined by eqn. (9) approaches the linear asymptote  $y_{as}$  when  $z$  approaches infinity, *i.e.* at low reactant concentrations.

Similarly, eqns. (7) and (10) may be combined for any  $d \geq 3$  to give

$$y - y_{as} = \frac{\sum_{k=2}^d \beta_k z^{d-k} - y_{as} \sum_{k=3}^d \alpha_k z^{d-k} - \frac{\alpha_2 (\alpha_1 \beta_1 - \alpha_2)}{\alpha_1^2} z^{d-2}}{\sum_{k=1}^d \alpha_k z^{d-k}} \quad (12)$$

Examination of the expression on the right hand side in eqn. (12) shows that the polynomial in the denominator is of a higher degree ( $d-1$ ) than the polynomial in the numerator ( $d-2$ ), which means that the difference  $y - y_{as}$  approaches zero when  $z$  approaches infinity. It follows that the general reciprocal rate eqn. (7) for any value of  $d > 1$  asymptotically approaches the linear relationship (10) at low reactant concentrations.

## DISCUSSION

The only restrictions imposed on enzymatic reaction mechanisms included in the general case described by Wong and Hanes are that all intermediates in the enzyme reaction are enzyme-containing species which do not react with one another, and that interconversions of these species do not involve reactions which are more complex than bimolecular.<sup>1</sup> Most enzyme systems will, therefore, conform to a theoretical steady-state rate equation of the Wong-Hanes type, and experimental results should primarily be discussed in view of this general relationship.

For the practical purpose of determination of kinetic coefficients eqn. (1) may be written in the form of eqn. (4), which is obtained by putting  $\beta_0'$  equal to unity. Such a mode of notation is clearly advantageous to one where  $\beta_1'$  is put equal to unity. The latter mode of notation is customary for first degree rate equations when  $\alpha_0 = 0$ , as the rate equation then assumes the classical Michaelis-Menten form (eqn. (6)). The coefficients  $\alpha_1$  and  $\beta_1$  in eqn. (5), however, are equally well fitted as  $V$  and  $K_m$  for characterization of first degree equations, and the use of eqn. (5) prevents mis-interpretation of the kinetic coefficients in terms of equilibrium constants or affinities.

The modern definition of  $K_m$  as the reactant concentration at which the reaction rate equals  $V/2$ , in fact, eliminates all reasons for choosing notations based on  $V$ - and  $K_m$ -values, which become rather impractical in the cases of higher-degree rate equations and of combined rate equations including several concentration variables (several reactants).<sup>3</sup>

As was stated in the theoretical section, steady-state enzyme kinetic data are frequently analyzed by means of reciprocal rate plots. The diagnostic value of such plots is based upon the fact that the reciprocal rate eqn. (7) contains non-linear terms as soon as  $d$  exceeds unity, while the linear relationship (8) is obtained for  $d=1$ . This means that curved reciprocal plots always must be interpreted in terms of a higher-degree mechanism. On the other hand, the reverse is not necessarily true. Eqn. (7) will, for instance, be linear for any  $d > 1$  when the numerator contains the denominator as a factor. This may, however, only be the case under certain very restrictive conditions,<sup>2</sup> and the asymptotic properties of eqn. (7) are of much greater practical importance. As was shown in the theoretical section eqn. (7) becomes asymptotically linear for any value of  $d > 1$  at low concentrations of any reactant R. This means that any enzyme system which conforms to a higher-degree rate equation, may exhibit a kinetic behaviour which is indistinguishable from the behaviour predicted by the simple Michaelis-Menten equation, or where deviations from the latter relationship may be very difficult to detect. Consequently, the mere exhibition of linear reciprocal plots cannot be taken as evidence for elimination of possible higher-degree reaction mechanisms.

It will, in general, not be possible to tell whether the reactant concentrations at which enzymatic velocity determinations have been performed should be considered as low or not. Kinetic coefficients which have been estimated from apparently linear reciprocal rate plots should, therefore, always be discussed in view of the possibility that the theoretical relationship is given by eqn. (10) obtained for higher-degree mechanisms, rather than by eqn. (8) which is obtained for a first degree equation of the Michaelis-Menten type. It may in this connection be mentioned that the method for determination of asymptote equations used by Reiner<sup>4,5</sup> is mathematically incorrect. The present investigation proves unequivocally that the true asymptote is given by eqn. (10) for any higher-degree rate equation.

The facts discussed above indicate that a clear distinction must be made between theoretical and experimentally estimated rate equations, which cannot always be assumed to be identical. Such problems will be discussed in detail in the following paper, which concerns methods for determination of empirical rate equations.

#### REFERENCES

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