A thermal-variation method for analysing the rate constants of the Michaelis–Menten mechanism

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By analysing the variations of saturation velocity and Michaelis constant with temperature and invoking the mathematical constraint represented by the Arrhenius equation, it becomes possible to estimate k_{+2} and indistinguishably k_{+1} and k_{-1} for the Michaelis–Menten mechanism of one-substrate enzyme reactions. Distinction between k_{+1} and k_{-1} may be obtained through the determination of isotopic rate effects. This procedure thus provides a basis for evaluating all three rate constants of the one-substrate mechanism, and disproves the suggestion that k_{+1} and k_{-1} are intrinsically unobtainable from steady-state kinetic measurements.

The Michaelis–Menten mechanism for basic one-substrate enzyme reactions consists of three elementary reactions steps:

Under steady-state conditions, its reaction velocity, v , is determined by the saturation velocity, V , and Michaelis constant, K_m :

$$v = \frac{V[S]}{[S] + K_m} \quad (1)$$

$$V = k_{+2}[E]_0 \quad (2)$$

$$K_m = \frac{k_{+2} + k_{-1}}{k_{+1}} \quad (3)$$

where $[S]$ and $[E]_0$ represent substrate and enzyme concentrations respectively. Of the three elementary rate constants, k_{+2} is obtainable from V and concentration of enzyme active site on the basis of eqn. (2). Hitherto, it has not been possible to evaluate k_{+1} and k_{-1} for this mechanism from steady-state kinetic measurements. This deficiency in fact gave rise to the notion that the steady-state method cannot be employed to estimate enzymic rate constants. Although this notion has been dispelled for more complex mechanisms with multiple concentration variables (see Wong, 1975), even today it remains to be disproven for the simple Michaelis–Menten mechanism, over half a century after the investigation by Michaelis & Menten (1913) and Briggs & Haldane (1925).

In view of this, the object of the present study is to propose an analytical approach that permits the estimation of, not only k_{+2} , but also k_{+1} and k_{-1} , on the basis of steady-state measurements.

Theory

The failure of eqns. (1)–(3) to yield k_{+1} and k_{-1} indicates that the parameters V and K_m do not sufficiently constrain these two rate constants with respect to their numerical values. Therefore, to estimate these constants some additional constraint must be introduced. The thermal variation of rate constants conforms to the Arrhenius equation:

$$k = Ae^{-E/RT} \quad (4)$$

where A represents the frequency factor, E the activation energy, R the gas constant and T the absolute temperature. In the case of the Michaelis–Menten mechanism, each of the three rate constants is described by an Arrhenius equation:

$$k_{+1} = A_{+1}e^{-E_{+1}/RT} \quad (5)$$

$$k_{-1} = A_{-1}e^{-E_{-1}/RT} \quad (6)$$

$$k_{+2} = A_{+2}e^{-E_{+2}/RT} \quad (7)$$

Since eqns. (5)–(7) place important constraints on the behaviour of the rate constants as functions of temperature, they can be exploited towards defining k_{+1} and k_{-1} from an analysis of their thermal variations, as follows.

Step 1

By evaluating saturation velocity V at a series of temperatures, a series of k_{+2} values are obtained. They yield, on the basis of eqn. (7), an estimation of the parameters A_{+2} and E_{+2} .

Step 2

By substituting eqns. (5)–(7) into eqn. (3), the Michaelis constant is transformed into an explicit function in the frequency factors and activation energies:

$$K_m = \frac{A_{+2}}{A_{+1}} \cdot e^{-(E_{+2}-E_{+1})/RT} + \frac{A_{-1}}{A_{+1}} \cdot e^{-(E_{-1}-E_{+1})/RT} \quad (8)$$

Recognizing that K_m is a sum of two exponential terms in T , by evaluating K_m at a series of different temperatures the dependence of K_m on T can be fitted to eqn. (9) to yield estimates of the four parameters P_1 , P_2 , P_3 and P_4 :

$$K_m = P_1 e^{P_2/RT} + P_3 e^{P_4/RT} \quad (9)$$

Step 3

Comparison of eqns. (8) and (9) indicates that two sets of solutions to the parameters in the former equation may be obtained from the parameters in the latter equation, as follows.

Solution (a):

If $P_1 = A_{+2}/A_{+1}$, it follows that:

$$A_{+1} = A_{+2}/P_1$$

$$P_2 = E_{+1} - E_{+2}$$

$$E_{+1} = P_2 + E_{+2}$$

and, furthermore:

$$\left. \begin{aligned} P_3 &= A_{-1}/A_{+1} \\ A_{-1} &= P_3 A_{+1} = P_3 A_{+2}/P_1 \\ P_4 &= E_{+1} - E_{-1} \\ E_{-1} &= E_{+1} - P_4 = P_2 + E_{+2} - P_4 \end{aligned} \right\} \quad (10)$$

Solution (b):

If $P_3 = A_{+2}/A_{+1}$, it follows that:

$$\left. \begin{aligned} A_{+1} &= A_{+2}/P_3 \\ P_4 &= E_{+1} - E_{+2} \\ E_{+1} &= P_4 + E_{+2} \end{aligned} \right\} \quad (11)$$

and, furthermore:

$$\begin{aligned} P_1 &= A_{-1}/A_{+1} \\ A_{-1} &= P_1 A_{+1} = P_2 A_{+2}/P_3 \\ P_2 &= E_{+1} - E_{-1} \\ E_{-1} &= E_{+1} - P_2 = P_4 + E_{+2} - P_2 \end{aligned}$$

In either case, because A_{+2} and E_{+2} are already known from the variation of k_{+2} with T as described

by eqn. (7), the parameters A_{+1} , E_{+1} , A_{-1} and E_{-1} can be calculated from the values of A_{+2} and E_{+2} together with P_1 , P_2 , P_3 and P_4 . In turn, k_{+1} may be derived from A_{+1} and E_{+1} , and k_{-1} from A_{-1} and E_{-1} .

The calculations can proceed provided that the two exponential terms in eqn. (8) are not excessively unequal. Otherwise only one of the two terms can be fitted meaningfully to K_m by any algorithm for curve-fitting. If the first term is dominant, A_{+1} may be obtained from A_{+2}/A_{+1} and A_{+2} , and E_{+1} obtained from $E_{+2} - E_{+1}$ and E_{+2} . If the second term is dominant, only A_{-1}/A_{+1} and $E_{-1} - E_{+1}$ are obtained.

In the general case, the calculations of A_{+1} , A_{-1} , E_{+1} and E_{-1} require a choice between Solutions (a) and (b). The most direct way to decide the choice is to determine the kinetic isotopic effects on P_2 and P_4 . Solution (a) predicts a much larger isotopic effect on P_2 than on P_4 , whereas Solution (b) predicts a much larger isotopic effect on P_4 than on P_2 .

Discussion

The present study proposes that an analysis of the thermal variations of V and K_m is capable of yielding estimates for all three rate constants of the Michaelis–Menten mechanism. This is made possible by utilizing the constraint imposed on these rate constants by the Arrhenius equation. Three important factors need to be considered in connection with the approach. First, it requires a choice between Solutions (a) and (b), as described by eqns. (10) and (11). A general solution to this requirement is to measure isotopic rate effects. In Solution (a), the parameter P_2 depends on k_{+2} , a bond-breaking step, and k_{+1} , a non-bond-breaking step; in contrast, the parameter P_4 depends on k_{-1} and k_{+1} , both non-bond-breaking steps. Accordingly, isotopic rate effects on the value of P_2 are expected to be much more pronounced than that on P_4 (see Jencks, 1969). The converse is expected for Solution (b). It should be noted in this regard that isotopic rate effects by themselves do not yield estimates of k_{+1} and k_{-1} , since they do not offer a means for extracting the numerical values of these rate constants from K_m . However, they usefully supplement the present method by distinguishing between the estimates for k_{+1} and k_{-1} .

Secondly, changes in enzyme conformation with temperature or enzyme denaturation may bring about non-linear deviations from the Arrhenius equations for the three rate constants. Although such deviations are not readily detectable for k_{+1} and k_{-1} , they are for k_{+2} . Therefore a linear Arrhenius plot of $\log V$ versus $1/T$, indicating conformance of k_{+2} to the Arrhenius equation, constitutes a minimal prerequisite to application of the present method.

Thirdly, since the calculation of P_1 , P_2 , P_3 and P_4 from eqn. (9) is based on the thermal variation of K_m with T , it is facilitated by a larger variation of K_m with T within the experimental temperature range. In the extreme, if K_m does not vary at all with T , extraction of the four parameters will not be able to proceed.

In conclusion, analysis suggests that the variation of steady-state kinetics with temperature provides a method for estimating all three rate constants of the Michaelis-Menten mechanism. Although the method is by no means devoid of practical limitations, recognition of its principle suffices to disprove the notion that intrinsically steady-state kinetics do not permit the estimation of the rate constants of this simple enzyme mechanism. Earlier investigations (Hanes *et al.*, 1972; Wong, 1975; Cornish-Bowden & Wong, 1978) have already established the sufficiency of steady-state kinetics for

estimating the rate constants of more complex enzyme mechanisms.

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