The Time Dimension in Steady-state Kinetics: A Simplified Representation of Control Coefficients

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Introduction

In the study of kinetic transients, the importance of time as a dimension is well understood and widely used. For example, first-order rate constants are frequently represented by their reciprocals, the corresponding 'relaxation times'. However, in steady-state kinetics the rate is commonly treated as if it were in itself a dimension, the fact that it is a concentration divided by time being, if not forgotten, then at least ignored. However, the ratio of the Michaelis constant to the limiting rate of an enzyme obeying Michaelis-Menten kinetics, usually represented as K_m/V, has the dimensions of time, and various aspects of enzyme kinetics, especially in relation to control theory, can be much more transparently presented if this is made explicit. This interpretation of K_m/V has been noted by Easterby,[1] but in a discussion of the time required to reach a steady state or to switch from one steady state to another, rather than of the steady state itself.

It is now widely accepted, particularly as a result of cogent and persuasive arguments advanced by Fersht,[2] that the proper parameter for discussing enzyme specificity is k_o/K_m, the catalytic constant divided by the Michaelis constant. The International Union of Biochemistry has accordingly proposed[3] that this quantity should be given the name 'specificity constant' and the symbol k_A (where the subscript is not fixed but defines the substrate referred to), making it clear that it is a major parameter in its own right and should not be regarded just as the ratio of two other quantities. However, from the experimental point of view it is often V/K_m that is measured rather than k_o/K_m, sometimes as the only quantity that can be measured,[4] and this ratio is equally satisfactory as a measure of specificity, because the enzyme concentration cancels when the value for one substrate is compared with that for another. Moreover, in estimating the values of the parameters of the Michaelis-Menten equation, whether from the common straight-line plots or from non-linear regression, it is often convenient to obtain values of K_m/V and $1/V$ first, calculating K_m etc, from them.[5] Despite this, neither V/K_m nor its reciprocal, K_m/V, have accepted names or symbols. As K_m/V is a time and is directly relevant to enzyme specificity, it seems appropriate to call it the 'specificity time' and to use the symbol τ_A, where the subscript defines which substrate is referred to.

Definition

In this section the proposal outlined in the **Introduction** is set out more formally. For an enzyme obeying Michaelis-Menten kinetics, the rate v may be expressed in terms of the total concentration e_o of the enzyme and the concentration a of a substrate A in any of the following equivalent ways:

$$\begin{aligned} v &= k_o e_o a/(K_m + a) = Va/(K_m + a) \\ &= k_A e_o a/(1 + a/K_m) \end{aligned} \quad (1)$$

where K_m is the Michaelis constant, k_o is the catalytic constant, k_A is the specificity constant and $V = k_o e_o$ is the limiting rate. If τ_A is given the name 'specificity time' and defined as $\tau_A = 1/k_A e_o = K_m/V$, then Eqn (1) may also be written as follows:

$$v = (a/\tau_A)/(1 + a/K_m) \quad (2)$$

What time is it?

At first sight it may appear a disadvantage of the proposed definition that it represents K_m/V rather than V/K_m, so that it appears in Eqn (2) as a divisor rather than as a multiplier. However, the algebraic inconvenience of this is more than compensated for by the fact that it is easier to visualize the physical meaning of a time than of a reciprocal time (just as most biochemists have found it easier to attach a physical meaning to K_m, a concentration, than to its reciprocal). Moreover, for processes in series, properly defined times are often found to be additive,[1] whereas reciprocal times (first-order rate constants) are additive only for processes in parallel, which need to be considered much less often.

However, before we can examine the usefulness of a symbol τ_A we need to consider what physical time it represents. It is, in fact, the time that would be required to exhaust the substrate if the enzyme were operating under first-order conditions and if the initial rate were maintained. For considering most reactions under assay conditions this is a somewhat abstract interpretation, given that enzymes are not usually assayed under first-order conditions and, even when they are, the initial rate is never maintained until the substrate is exhausted. For enzymes in the cell, however, it becomes much less abstract: when a pathway is in a steady state the concentration of the substrate for an enzyme within the pathway remains constant because it is replenished as fast as it reacts. Moreover, many enzymes exist in the cell 'in excess', ie well removed from saturation, which has been variously attributed to the need for safety factors,[6] catalytic efficiency[7,8] or the summation theorem.[9] As the last of these derives from mathematical necessity it might be considered to make evolutionary explanations redundant, but recent studies[10] have shown that pathways in which a near-proportionate decrease in flux results from appreciable decrease in any enzyme concentration are not impossible. In any case it is not unreasonable to suppose that near-first-order conditions are more common in the cell than they are in kinetic studies on isolated enzymes.

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Binding time and catalysis time

The ordinate in a plot of a/v against a represents the time that would be required to turn over all of the substrate if the initial rate v were maintained (without any assumption about order of reaction). The equation for this plot,

$$a/v = \tau_A + a/V \tag{3}$$

shows that it is composed of two parts, the specificity time τ_A, representing the time required by the enzyme for binding the substrate, and the time a/V required for converting it into products, ie the time for catalysis. Thus an alternative name for the specificity time might be the 'binding time'. Formulated in this way it is evident that it must be the specificity time that is responsible for enzyme specificity, because competition between substrates is only possible when the binding site is vacant. Differences in catalysis time may result in inhibition of one reaction by a competing one, but they cannot alter the discriminating power, ie the specificity, of the enzyme.

It is interesting to note that very early in the development of enzyme kinetics, Van Slyke and Cullen[11] interpreted the time required for one catalytic cycle of an enzyme as composed of two parts conceptually very similar to those considered here, but their interpretation was not taken up by later authors.

Control coefficients for a linear pathway of first-order enzymes

The advantages in clarity of presentation that result from the use of specificity times are especially apparent when considering the control analysis of linear pathways of enzymes, such as the following:

$$\mathrm{A} \underset{k_{-1}e_1}{\overset{k_1e_1}{\rightleftharpoons}} \mathrm{M}_1 \underset{k_{-2}e_2}{\overset{k_2e_2}{\rightleftharpoons}} \mathrm{M}_2 \underset{k_{-3}e_3}{\overset{k_3e_3}{\rightleftharpoons}} \cdots \underset{k_{-j}e_j}{\overset{k_je_j}{\rightleftharpoons}} \mathrm{M}_j \rightleftharpoons \cdots \underset{k_{-n}e_n}{\overset{k_ne_n}{\rightleftharpoons}} \mathrm{Z} \tag{4}$$

in which a reactant A is converted into an end-product Z by means of a series of n enzyme-catalysed reactions, each of which is sufficiently below saturation that its rate can be expressed as a reversible first-order reaction. Although this is clearly an over-simplification of a real pathway, it is valuable as a starting point because it is simple to obtain an analytical expression for the pathway flux. A scheme of this kind has been used for this purpose by Kacser and Burns,[9] and a slightly different one, with an irreversible last step, by Heinrich and Rapoport.[12] However, the expressions that they give for the pathway flux and the flux control coefficients are unnecessarily complicated and obscure the essential simplicity of the properties of this pathway.

Each pseudo-first-order rate constant k_je_j may be written in terms of the corresponding specificity time as $1/\tau_j$, and one may also use the fact that each ratio k_j/k_{-j} of specificity constants may be replaced by the equilibrium constant K_j for the step. Then at any instant the rates of all of the steps may be expressed as follows:

$$\begin{aligned} v_1 &= (a - m_1/K_1)/\tau_1 \\ v_2 &= (m_1 - m_2/K_2)/\tau_2 \\ &\vdots \\ v_n &= (m_{n-1} - z/K_n)/\tau_n \end{aligned} \tag{5}$$

etc, where a, m_j and z represent the concentrations of A, M_j and Z respectively. In the steady state, all of these rates are equal to the pathway flux J, and hence

$$\begin{aligned} J\tau_1K_1K_2K_3\ldots K_n &= aK_1K_2K_3\ldots K_n - m_1K_2K_3K_4\ldots K_n \\ J\tau_2K_2K_3K_4\ldots K_n &= m_1K_2K_3K_4\ldots K_n - m_2K_3K_4K_5\ldots K_n \\ &\vdots \\ J\tau_nK_n &= m_{n-1}K_n - z \end{aligned} \tag{6}$$

in which each equation is multiplied by the appropriate product of equilibrium constants to ensure that the unknown concentrations of intermediates are eliminated when the whole set of equations is added together:

$$J(\tau_1K_{1n} + \tau_2K_{2n} + \ldots + \tau_nK_{nn}) = aK_{1n} - z \tag{7}$$

where the products of equilibrium constants are concisely expressed as K_{1n}, etc.: in general $K_{jn} = K_jK_{j+1}K_{j+2}\ldots K_n$ represents the equilibrium constant for the whole process represented by reactions j to n. One can then write a simple expression for the pathway flux:

$$J = (aK_{1n} - z)/\Sigma\tau_jK_{jn} \tag{8}$$

in which the summation is over all the enzymes in the pathway.

Each term in the denominator of this expression represents the time that would be required by an enzyme operating in isolation to turn over an amount of its substrate equal to the concentration of the final product of the pathway that would be in equilibrium with it. This time is an essential characteristic of each enzyme for considering its role in controlling flux, and will be referred to as the 'completion time' in the remainder of this paper. Thus the pathway flux is simply the increase in concentration of the final product Z needed to bring it into equilibrium with the first substrate A, divided by the sum of the completion times for all of the enzymes in the pathway.

Expressing the flux in this way it is a simple matter to show that the flux control coefficient[13] for each enzyme is proportional to its completion time:

$$C^J_{e_j} = \frac{\partial \ln J}{\partial \ln e_j} = \tau_jK_{jn}/\Sigma\tau_jK_{jn} \tag{9}$$

Eqns (8–9) are exactly equivalent to Eqns (C2–C3) of Kacser and Burns,[9] but now their physical meanings are obvious from inspection, and in consequence they can be memorized very easily and their properties are trans-

parent. It is obvious from inspection, for example, that the sum of all the flux control coefficients defined by Eqn (9) is equal to unity, as required by the summation theorem.[9]

As Heinrich and Rapoport[12] analysed their system in terms of times, one might expect that Eqns (8–9) might resemble Eqns (12) and (23) of these authors rather more closely than they do. Part of the difference is due to their analysis of a different system, with an irreversible last step, but a more important difference, resulting in equations of greater apparent complexity, is their defining the 'characteristic time' of each enzyme not as $1/k_j$, the specificity time, but as $1/(k_j + k_{-j})$. This illustrates an important point made by Easterby:[1] that for the times associated with the different steps of a sequential process to be additive they need to be defined appropriately. In addition, Eqns (12) and (23) of Heinrich and Rapoport[12] contain an undefined quantity, q_{n+1} in their symbols, but the difficulty can be resolved by taking the products containing this quantity to be unity by definition.

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References

[1] Easterby, J S (1981) *Biochem J* **199**, 155–161

[2] Fersht, A R (1977) *Enzyme Structure and Mechanism*, 1st edition, pp 274–289 (also in 2nd edition (1985) pp 347–354), Freeman, New York

[3] International Union of Biochemistry, Nomenclature Committee (1982) *Eur J Biochem* **128**, 281–291

[4] Crompton, I E and Waley, S G (1986) *Biochem J* **239**, 221–224

[5] Cornish-Bowden, A (1979) *Fundamentals of Enzyme Kinetics*, Butterworths, London and Boston

[6] Atkinson, D E (1969) *Current Topics Cell Reg* **1**, 29–43

[7] Fersht, A R (1974) *Proc Roy Soc ser B* **187**, 397–407

[8] Cornish-Bowden, A (1976) *J Mol Biol* **101**, 1–9

[9] Kacser, H and Burns, J A (1973) *Rate Control of Biological Processes* (edited by Davies, D D) pp 65–104, Cambridge University Press, London

[10] Cornish-Bowden, A (1987) *J Theor Biol* **125**, 333–338

[11] Van Slyke, D D and Cullen, G E (1914) *J Biol Chem* **19**, 141–180

[12] Heinrich, R and Rapoport, T A (1974) *Eur J Biochem* **42**, 89–95

[13] Burns, J A, Cornish-Bowden, A, Groen, A K, Heinrich, R, Kacser, H, Porteous, J W, Rapoport, S M, Rapoport, T A, Stucki, J W, Tager, J M, Wanders, R J A and Westerhoff, H V (1985) *Trends Biochem Sci* **10**, 16