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Nomenclature Committee of the International Union of Biochemistry (NC-IUB)

Symbolism and Terminology in Enzyme Kinetics

Recommendations 1981

CONTENTS

1. INTRODUCTION

Recommendations on the symbols and terminology of enzyme kinetics were previously made in conjunction with the recommendations of the Commission on Biochemical Nomenclature and Classification of Enzymes, and were published in *Enzyme Nomenclature (1972)* [1] and in earlier editions. During preparation of *Enzyme Nomenclature (1978)* [2] by NC-IUB it was agreed that the symbols and terminology of enzyme kinetics were properly distinct from the nomenclature of enzymes and should therefore be omitted from a compilation of enzyme names. In consequence the 1972 recommendations [1] have remained in operation even though they take little account of the developments of the subject in the past two decades, particularly the increased interest in reactions with two or more substrates. Accordingly, during 1978–1979 the views of numerous biochemists active in enzyme kinetics were solicited by NC-IUB and subsequently a panel was set up to make new recommendations.

To minimize differences between chemical and biochemical practice, the Report on Symbolism and Terminology in Chemical Kinetics [3] produced by IUPAC in 1980 has been followed where appropriate. Such differences as remain are the consequence of long-standing biochemical practice or of the fact that enzyme-catalysed reactions usually have simple overall stoichiometries and take place at constant pressure in the liquid phase and so require little attention to some details that may be crucial in chemical kinetics.

Certain conflicts that have greatly exercised some biochemists now seem less important than they may have done in the past. For example, because of the great diversity of enzyme mechanisms it is unlikely that any one system of numbering rate constants can satisfy all possible needs. It is much more important that rate constants be clearly defined in the context in which they are used than that they should satisfy any universal system. Thus although this report follows the IUPAC recommendations [3,4] in its examples for illustration it is not implied that these should be used without definition or in all circumstances.

We have adopted the general policy of taking an operational approach to definitions, terms and symbols, rather than basing them on assumed mechanisms. In other words, definitions refer to what one observes, not to the way it is interpreted. This seems to be the safest course in kinetics, and particularly in enzyme kinetics, as conclusions about mechanisms often have to be revised in the light of further investigations, whereas the observations, if they are properly carried out, do not. Definitions based on what is actually observed are therefore on a sounder and more lasting basis than those that depend on an assumed mechanism. In Section 11 we do, however, consider mechanisms briefly.

2. BASIC DEFINITIONS

A *catalyst* is a substance that increases the rate of a reaction without modifying the overall standard Gibbs-energy change in the reaction. This definition is equivalent to the statement that the catalyst does not appear in the stoichiometric expression of the complete reaction. Catalysts are

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said to exert a *catalytic action*, and a reaction in which a catalyst is involved is called a *catalysed reaction*.

Kinetic equations are commonly expressed in terms of the *amount-of-substance concentrations* of the chemical species involved. The *amount-of-substance concentration* is the amount of substance (for which the SI unit is the mole, symbol mol) divided by the volume. As it is the only kind of concentration commonly used in biochemistry it is usually abbreviated to *concentration* and this shorter form will be used in the remainder of this document without further discussion. The unit almost invariably used for concentration is mol dm^{-3}, which is alternatively written as to mol L^{-1}, mol l^{-1}, or simply M (molar).

An *enzyme* is a protein that acts as a catalyst.

A *substrate* is a reactant (other than a catalyst) in a catalysed reaction.

An *inhibitor* is a substance that diminishes the rate of a chemical reaction and the process is called *inhibition*. In enzyme-catalysed reactions an inhibitor frequently acts by binding to the enzyme, in which case it may be called an *enzyme inhibitor*. An *activator* is a substance, other than the catalyst or one of the substrates, that increases the rate of a catalysed reaction. An activator of an enzyme-catalysed reaction may be called an *enzyme activator* if it acts by binding to the enzyme.

The terms *effector* and *modifier* are general terms that apply to substances that interact with enzymes and either increase or decrease their catalytic action. Enzyme inhibitors and enzyme activators are therefore special cases of effectors and modifiers. The term *effector* is more commonly used when the substance produces effects of physiological significance, whereas the term *modifier* is more appropriate for a substance that is artificially added to an enzyme system being studied *in vitro*.

Sometimes added substances increase or decrease the rate of an enzyme-catalysed reaction without interacting with the enzyme itself; they may interact with substrates or with modifiers or effectors that are already present in the system. Such substances may be referred to as *activators* or *inhibitors*, but should not be referred to as enzyme activators, enzyme inhibitors, modifiers or effectors.

A typical overall enzyme-catalysed reaction involving a single substrate and a single product may be written as

$$E + A \rightleftharpoons E + Z$$

where E is the enzyme. A the substrate and Z the product. The double arrows indicate that the reaction occurs in both directions. When two or more substrates and two or more products are involved, the overall reaction may be written as

$$E + A + B + \ldots \rightleftharpoons E + Z + Y + \ldots$$

It is convenient to use early letters of the alphabet for substrates and late letters for products. However, the letter S is frequently used for the substrate of a one-substrate reaction, and P, Q, R are often used for products. In general, any consistent system may be used and the symbols should always be defined. Although the enzyme E is included for completeness in these two examples, it is usually omitted because it is not necessary for specifying the overall stoichiometry of a reaction.

Many of the terms defined in this document, especially in this section, such as *catalyst*, *substrate*, *order of reaction*, *steady state*, etc., are not unique to enzyme kinetics, but have a wide currency in chemistry. The definitions given here are not intended to conflict with those recommended for use by chemists [5], but because of the special needs of enzyme kinetics they are not necessarily identical.

2.1. *Rates of Consumption and Formation*

The rate of consumption of a reactant of concentration [A] is defined as

$$v_A = -\frac{d[A]}{dt} \tag{1}$$

in which t represents time. Square brackets may be used without definition to indicate concentrations, e.g. [A] is the concentration of A. Other symbols, such as a for the concentration of A, may be used for typographical convenience, but these should be defined. The *rate of formation* of a substance of concentration [Z] is defined as

$$v_Z = \frac{d[Z]}{dt}. \tag{2}$$

The usual unit of rate is mol $dm^{-3}\ s^{-1}$ or mol $L^{-1}\ s^{-1}$ (where L = liter = dm^3) or M s^{-1} (where M, molarity, = mol dm^{-3}). In all contexts the term *velocity* may be used as a synonym for *rate*. The alternative will only be mentioned in these Recommendations, however, when it appears to be the more common usage.

In a given reaction the rates of consumption and rates of formation are equal to one another only if there is a one-to-one stoichiometric relationship between the species consumed and formed. For example, in a reaction with stoichiometry

$$A + 3\,B \longrightarrow 2\,Z$$

the rate of formation of Z is twice the rate of consumption of A, and the rate of consumption of B is three times that of A. In most enzyme-catalysed reactions, however, there is a one-to-one stoichiometric ratio between all substrates and products. It is then permissible to omit the subscript from v and use the term *rate of reaction*.

2.2 *Rate of Reaction*

For a reaction of stoichiometry

$$E + A \longrightarrow E + Z$$

at a given time the rate of formation of Z is equal to the rate of consumption of A, and can be called the rate of reaction, v:

$$v = -\frac{d[A]}{dt} = \frac{d[Z]}{dt}. \tag{3}$$

A similar set of relationships applies when there are several reactants in one-to-one stoichiometry. Complications arise if a one-to-one stoichiometry between all substrates and all products does not exist. This can arise in two ways:

a) The overall stoichiometry may be, for example,

$$A + 3\,B \longrightarrow 2\,Z.$$

The numbers -1, -3 and 2 are the *stoichiometric coefficients of* A, B and Z respectively in this reaction; by convention they are positive for products and negative for reactants. For a reaction of this kind the *rate of reaction* is the rate of consumption or formation of any reactant divided by the appropriate stoichiometric coefficient, i.e. (in this example):

$$v = -\frac{d[A]}{dt} = -\frac{1}{3}\frac{d[B]}{dt} = \frac{1}{2}\frac{d[Z]}{dt}. \tag{4}$$

This situation rarely arises in enzyme kinetics.

b) Intermediates may be formed in amounts comparable with those of reactants, for example

$$E + A + B \rightleftharpoons L \rightarrow E + Z + Y .$$

This gives rise to *time-dependent stoichiometry* and there is not a one-to-one relationship between the amounts of reactants. It is not then permissible to speak of rate of reaction for the complete reaction, though it may be meaningful to define rates of reaction for the individual steps. Time-dependent stoichiometry occurs in the pre-steady-state phase of enzyme-catalysed reactions (Section 9), during which the amount of intermediate formed is comparable with the amounts of product.

The rate of reaction as defined here is an intensive[1] quantity with dimensions of concentration divided by time. It conforms with almost universal usage in kinetics and with the current (1980) recommendations of IUPAC [3], but differs from earlier IUPAC recommendations, in which the term *rate of reaction* referred to the corresponding extensive quantity with dimensions of amount of substance divided by time. There is little evidence that this latter recommendation has ever been widely adopted and in the context of enzyme kinetics it is recommended that the term *rate of reaction* be used exclusively for the intensive quantity as defined above. If the extensive quantity is required it may be termed the *rate of conversion* and symbolized $\dot{\xi}$ [3,5].

2.3. *Elementary and Composite Reactions*

An *elementary reaction* is one in which no reaction intermediates have been detected or need be postulated to describe the chemical reaction on a molecular scale. Such reactions are said to occur in a *single step*.

The term *molecularity*, which applies only to an elementary reaction, refers to the number of molecular particles involved in the microscopic chemical event. With reactions in solution, solvent molecules are counted in the molecularity if they enter into the overall process, but not if they merely exert an environmental or solvent effect. For example, the formation of an enzyme-substrate complex in aqueous solution:

$$E + A \longrightarrow EA$$

has a molecularity of two and is said to be bimolecular. The reverse process in solution,

$$EA \longrightarrow E + A$$

has a molecularity of unity and is said to be *unimolecular*. The hydrolysis of an acyl-enzyme, in which water enters into the process,

$$R{-}CO{-}E + H_2O \longrightarrow E{-}H + R{-}COOH$$

is bimolecular.

Virtually all enzyme-catalysed overall reactions occur in more than one elementary step and are described as composite reactions. The terms complex reaction may also be used with the same meaning. Simple examples in enzyme kinetics are as follows:

$$E + A \rightleftharpoons EA \rightleftharpoons EZ \longrightarrow E + Z$$

$$E + A + B \rightleftharpoons EA + B \rightleftharpoons EAB \rightleftharpoons EYZ \longrightarrow EY + Z \longrightarrow E + Y + Z$$

[1] An *intensive quantity* is one like temperature or density that does not depend on the amount of material being considered and may be contrasted with an *extensive quantity*, such as heat or mass, which does.

3. ORDER OF REACTION, AND RATE CONSTANT

The term *order of reaction* can be applied to any elementary reaction considered in one direction only, and to certain composite reactions.

For an elementary reaction occurring in one direction the order of reaction is equal to the molecularity, but it describes the kinetics not the mechanism. Thus, for the unimolecular elementary process

$$EA \longrightarrow E + A$$

the rate of reaction is proportional to the concentration of the reactant EA:

$$v = k[EA] . \qquad (5)$$

As the concentration [EA] is raised to the first power the order is unity and the reaction is said to be *first order*. The constant k is known as the *rate constant* of the reaction.

For the bimolecular elementary process

$$E + A \longrightarrow EA$$

the rate is proportional to the product of the reactant concentrations:

$$v = k[E][A] . \qquad (6)$$

The reaction is first-order in E, first-order in A, but *second-order overall*, and k is again the rate constant. The orders for the individual reactants, unity for E and unity for A, are known as *partial orders* and the sum of all the partial orders of a reaction is the *overall order*.

Second-order rate constants, such as k in Eqn (6), have the dimensions of reciprocal concentration multiplied by reciprocal time, whereas first-order rate constants, such as k in Eqn (5), have the dimensions of reciprocal time. This difference in dimensions is not normally evident from the symbols used to represent rate constants, and care must therefore be taken to avoid making improper comparisons between rate constants of different orders. It is sometimes convenient to multiply a second-order rate constant by an appropriate concentration to produce a quantity with the dimensions of a first-order rate constant, e.g. k[A] from Eqn (6); such a product is called a *pseudo-first-order rate constant*. The units of rate constants vary with the order of reaction in the same way that their dimensions vary. For a first-order rate constant, such as k in Eqn (5), the units are s^{-1}. For a second-order rate constant, such as k in Eqn (6), the units are $dm^3\ mol^{-1}\ s^{-1}$ or $L\ mol^{-1}\ s^{-1}$ or $M^{-1}\ s^{-1}$.

The concept of order of reaction (but not molecularity) can also be applied to certain reactions that occur by composite mechanisms, provided that the rate is proportional to reactant concentrations raised to powers (which need not be integral). However, this is rarely the case with enzyme-catalysed reactions and the concept of order cannot therefore be applied strictly to such reactions overall. Nonetheless the individual steps of composite reactions have orders when considered in one direction. For processes that do not have a true order it is sometimes convenient to define an *apparent order* with respect to a reactant A as $\frac{[A]}{v} \cdot \frac{dv}{d[A]}$ (or as $\frac{d \ln v}{d \ln [A]}$, which is equivalent). For many enzyme-catalysed reactions the true order with respect to any substrate approximates to unity at very low concentrations and to zero at very high concentrations, but is not defined at intermediate concentrations. The apparent order, on the other hand, exists at any concentration.

It is sometimes useful to consider the rates of unidirectional elementary steps of a composite reaction in isolation. When the use of the term *rate of reaction* for such rates would cause ambiguity the term *chemical flux* or *chemiflux* may be used instead. For a full discussion of this terminology, see the IUPAC recommendations [3]. In enzyme kinetics the need for an unambiguous terminology occurs mainly in discussions of the use of rates of transfer of isotopic labels as probes of the chemical fluxes in different parts of the composite reaction.

3.1 *Numbering of Reactions*

The elementary reactions in a composite mechanism should be numbered as systematically as possible and in such a way that reverse processes are easily recognized. The reaction numbers should be used as subscripts to k, for rate constants, or v, for the individual rates (chemical fluxes). The preferred scheme for ordinary use is:

1) $k_1, k_{-1}, k_2, k_{-2}, \ldots; v_1, v_{-1}, v_2, v_{-2}, \ldots$

In this scheme positive subscripts may be prefixed by a + sign, i.e. k_1 may be written as k_{+1}, if it helps avoid ambiguity resulting from confusion with scheme 3 (below), or to emphasize that it refers to a step in the forward direction of the reaction. For some kinds of computer application and for theoretical discussions of enzyme mechanisms it is sometimes convenient to number the different forms of the enzyme rather than the elementary steps and then to denote the step from (e.g.) E_3 to E_4 as 34, etc. With this scheme the numbering of enzyme forms must be given explicity and the rate constants and rates listed above might become

2) $k_{12}, k_{21}, k_{23}, k_{32}, \ldots; v_{12}, v_{21}, v_{23}, v_{32}, \ldots$

If there are more than nine enzyme forms in the mechanism the subscripts should be separated by a comma, e.g. $k_{10,11}$, but this can be omitted when it is not required for clarity.

The following scheme, in which odd subscripts refer to forward steps and even subscripts to reverse steps:

3) $k_1, k_2, k_3, k_4, \ldots; v_1, v_2, v_3, v_4, \ldots$

is less satisfactory, both because it conflicts with IUPAC Recommendations [3] and because it makes it more difficult to recognize the forward and reverse rate constants for particular steps.

It is unrealistic to expect any universal system of numbering rate constants to be equally satisfactory in all circumstances. For example, in a mechanism where enzyme forms in different states of protonation can undergo analogous reactions it may be clearer to assign the same numbers to the analogous steps and distinguish between them by the use of primes, etc., rather than try to apply any of the above schemes in a rigid way. Regardless of what system is used, rate constants should never be referred to except in explicit relation to a mechanism or to a kinetic equation.

3.2. *Steady-State Approximation*

If an intermediate is always present in amounts much less than those of the reactants (other than the enzyme) the rate of change of its concentration is much smaller than that of the reactants. This condition is ensured whenever, as is usually the case in enzyme-catalysed reactions, the concentration of substrate is much higher than that of the enzyme; it is not necessary for the amount of intermediate to be small compared with the amount of enzyme. For example, in the scheme

$$E + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EA \xrightarrow{k_2} E + Z$$

if [EA] is always much less than [A] the following equation is obeyed to a good approximation:

$$\frac{d[EA]}{dt} = k_1[E][A] - k_{-1}[EA] - k_2[EA] = 0 . \quad (7)$$

The intermediate EA is said to be in a steady state. The use of this approximation to obtain an overall rate expression is known as the *steady-state treatment* or the *steady-state approximation*.

At the very beginning of the reaction the concentration of EA in the above scheme is rising from zero to its steady-state value. The steady-state approximation is not valid during these early times and the kinetics are known as *pre-steady-state kinetics* or *transient-phase kinetics*. The transient phase of an enzyme-catalysed reaction usually occupies a very brief period of time (usually a small fraction of a second), and special techniques must be used for investigating this phase of the reaction (Section 9).

The *rate of reaction* of an enzyme-catalysed reaction is not defined during the transient phase, because there is not a one-to-one stoichiometry between the reactants (see section 2.2). In the steady state a one-to-one stoichiometry is established and the rate of reaction can be defined. This rate, extrapolated back to zero time, is called the *initial rate* and given the symbol v_0. The subscript 0 is normally omitted when no other kinds of rate are at issue, i.e. when extended time courses are not being analysed.

The ratio [E][A]/[EA] at equilibrium is called the *substrate dissociation constant* and given the symbol K_{sA}. When only one substrate is in question the qualifier A may be omitted, and when it is included its location is a matter of typographical convenience. The substrate dissociation constant should not be confused with the Michaelis constant K_{mA} (see section 4.1, below), to which it bears no necessary relationship in general. In the example given above K_{sA} is equal to the ratio k_{-1}/k_1.

4. ENZYME REACTIONS INVOLVING A SINGLE SUBSTRATE

4.1. *Limiting Kinetics of Enzyme-Catalysed Reactions*

At very low concentrations of substrate many enzyme-catalysed reactions display approximately second-order kinetics, with rate given by the following equation:

$$v = k_A[E]_0[A] \quad (8)$$

in which the symbol k_A (or, in general, k_R for a reactant R) is the apparent second-order rate constant or *specificity constant* and $[E]_0$, which may also be written as $[E]_t$ or $[E]_{stoich}$, is the total or stoichiometric concentration of catalytic centres. (This corresponds to the total enzyme concentration only if there is a single catalytic centre per molecule.) The rationale for the subscript 0 is that the total enzyme concentration is normally the concentration at the instant of mixing, i.e. at time zero. Conversely, at very high substrate concentrations the same reactions commonly display approximately first-order kinetics (zero-order with respect to substrate):

$$v = k_0[E]_0 , \quad (9)$$

in which k_0, which may also be written as k_{cat}, is the apparent first-order rate constant. Although these limiting types of

behaviour are not universally observed, they are more common than *Michaelis-Menten kinetics* (Section 4.2) and provide a basis for classifying inhibitory and other effects (Section 5) independently of the need for Michaelis-Menten kinetics.

The apparent second-order rate constants k_A and k_B of competing substrates A and B determine the partitioning between competing reactions, regardless of whether the substrate concentrations are very small or not, and it is for this reason that the name *specificity constant* is proposed for this parameter of enzymic catalysis. The apparent first-order rate constant k_0 is a measure of the catalytic potential of the enzyme and is called the *catalytic constant*.

The quantity $k_0[E]_0$ is given the symbol V and the name *limiting rate*. It is particularly useful when k_0 cannot be calculated because the total catalytic-centre concentration is unknown, as in studies of enzymes of unknown purity, subunit structure and molecular mass. The symbol V_{max} and the names *maximum rate* and *maximum velocity* are also in widespread use although under normal circumstances there is no finite substrate concentration at which $v = V$ and hence no maximum in the mathematical sense. The form V_{max} is convenient in speech as it avoids the need for a cumbersome distinction between 'capital V' and 'lower case v'. When a true maximum does occur (as insubstrate inhibition; Section 4.3) the symbol v_{max} (not V_{max}) and the name maximum rate may be used for the true maximum value of v but care should be taken to avoid confusion with the limiting rate.

4.2. *Michaelis-Menten Kinetics*

Sometimes the relationship between the rate of an enzyme-catalysed reaction and the substrate concentration takes the form

$$v = \frac{V[A]}{K_{mA} + [A]} \qquad (10)$$

where V and K_{mA} are constants at a given temperature and a given enzyme concentration. The reaction is then said to display *Michaelis-Menten kinetics*. (The term *hyperbolic kinetics* is also sometimes used because a plot of v against [A] has the form of a rectangular hyperbola through the origin with asymptotes $v = V$ and $[A] = -K_{mA}$. This term, and others that imply the use of particular kinds of plot, should be used with care to avoid ambiguity, as they can be misleading if used out of context.) The constant V is the *limiting rate*, with the same meaning as in Section 4.1. The second constant K_{mA} is known as the *Michaelis constant* for A; the alternative name *Michaelis concentration* may also be used and has the advantage of emphasizing that the quantity concerned has the dimensions of a concentration and is not, in general, an equilibrium constant. When only one substrate is being considered the qualifier A may be omitted, so that the symbol becomes K_m. When the qualifier is included its location is a matter of typographical convenience; no particular significance attaches to such variants as K_m^A or K_{mA}. The Michaelis constant (or Michaelis concentration) is the substrate concentration at which $v = 0.5\ V$, and its usual unit is mol dm^{-3}, which may be written as mol L^{-1} or M. The term Michaelis constant and the symbol K_m should not be used when Michaelis-Menten kinetics are not obeyed (see Section 4.3).

For a reaction obeying Michaelis-Menten kinetics the rate in the limit of very low substrate concentrations is $v = V[A]/K_{mA}$, and comparison with Eqn (8) shows that $V/K_{mA} = k_A[E]_0$. In the limit of very high substrate concentrations $v = V$, and comparison with Eqn (9) gives $V = k_0[E]_0$. The Michaelis constant K_{mA} is therefore k_0/k_A, and Eqn (10) can be written as

$$v = \frac{k_0 k_A [E]_0 [A]}{k_0 + k_A [A]} . \qquad (11)$$

An indefinitely large number of mechanisms generate Michaelis-Menten kinetics, and still more generate limiting behaviour of the kind described in Section 4.1. Consequently there is no general definition of any of the kinetic parameters k_A, k_0, V and K_{mA} in terms of the rate constants for the elementary steps of a particular mechanism.

4.3. *Non-Michaelis-Menten Kinetics*

When the kinetic behaviour does not conform to Eqn (10) or Eqn (11) the reaction is said to exhibit *non-Michaelis-Menten kinetics*. If the Michaelis-Menten equation is obeyed approximately over a restricted range of substrate concentrations it may be convenient to regard this behaviour as a deviation from this equation rather than as an unrelated phenomenon. For example, a reaction may obey an equation of the following form

$$v = \frac{V'[A]}{K'_{mA} + [A] + [A]^2/K_{iA}} \qquad (12)$$

in which the constants V', K'_{mA} and K_{iA} are used for illustration without any implication of universal or standard definitions. If K_{iA} is large compared with K'_{mA} the behaviour predicted by Eqn (12) will approximate to that predicted by Eqn (11), with V and K_{mA} replaced by V' and K'_{mA}, in the lower range of substrate concentrations. However, with Eqn (12) the rate passes through a maximum as the concentration increases, and there is said to be *inhibition by substrate*, and the constant K_{iA}, which has the dimensions of a concentration, is called the *substrate inhibition constant*.

When more complex kinds of non-Michaelis-Menten behaviour occur it is usually unhelpful to use terminology and symbolism suggestive of the Michaelis-Menten equation; instead the approach discussed in Section 10 is appropriate. In all cases it is advisable to avoid the term Michaelis constant and the symbol K_m when the Michaelis-Menten equation is not obeyed, because it is defined as a parameter of that equation. The symbol $[A]_{0.5}$ or $[A]_{1/2}$, not K_{mA}, may be used for the value of [A] at which $v = 0.5\ V$.

5. ENZYME REACTIONS INVOLVING MORE THAN ONE SUBSTRATE

5.1. *Michaelis-Menten Kinetics*

Regardless of the number of substrates, a reaction is said to obey Michaelis-Menten kinetics if the rate equation can be expressed in the following form:

$$v = \frac{[E]_0}{\frac{1}{k_0} + \frac{1}{k_A[A]} + \frac{1}{k_B[B]} + \ldots + \frac{1}{k_{AB}[A][B]} + \ldots + \frac{[Z]}{k_A^Z[A]} + \ldots} \qquad (13)$$

which can be regarded as a generalization of Eqn (11). (Z is used here as an example of a product as suggested in Section 2.) Each term in the denominator of the rate expression contains unity or any number of product concentrations in its numerator, and a coefficient k and any number of substrate concentrations raised to no higher than the first power in its deno-

minator. The constant k_0 corresponds to k_0 in Eqn (11); each other coefficient is assigned a subscript for each substrate concentration in the denominator of the term concerned and a superscript for each product concentration in the numerator. The term $1/k_0$ must be present, together with one term for each substrate of the form $1/k_A[A]$, but the terms in products of concentrations, such as those shown in Eqn (13) with coefficients k_{AB} and k^Z_A, may or may not be present. It is sometimes convenient to write the equation in a form in which each k is replaced by its reciprocal, symbolized by φ with the same same subscripts and superscripts, i.e. $\varphi_0 = 1/k_0$, $\varphi_A = 1/k_A$, $\varphi_{AB} = 1/k_{AB}$, $\varphi^Z_A = 1/k^Z_A$, etc. These reciprocal coefficients are called *Dalziel*[2] *coefficients*.

Eqn (13) can be applied to reactions with any number of substrates and products and can also be extended to some kinds of inhibition by substrate, i.e. to the simpler kinds of non-Michaelis-Menten kinetics. It is thus an equation of considerable generality. It is simplest, however, to consider terminology in the context of a two-substrate reaction, and this will be done in Section 5.2.

5.2. *Michaelis-Menten Kinetics of a Two-Substrate Reaction*

For a two-substrate reaction in the absence of products Eqn (13) simplifies to the following equation:

$$v = \frac{[E]_0}{\frac{1}{k_0} + \frac{1}{k_A[A]} + \frac{1}{k_B[B]} + \frac{1}{k_{AB}[A][B]}}. \quad (14)$$

If the concentration of one substrate, known as the *constant substrate*, is held constant, while that of the other, known as the *variable substrate*, is varied, the rate is of the form of the Michaelis-Menten equation in terms of the variable substrate, because Eqn (14) can be rearranged to

$$v = \frac{k_0^{app} k_A^{app} [E]_0 [A]}{k_0^{app} + k_A^{app} [A]} \quad (15)$$

(cf. Eqn 10), where

$$k_0^{app} = \frac{k_0 k_B [B]}{k_0 + k_B [B]} \quad (16)$$

is known as the apparent *catalytic constant*, and

$$k_A^{app} = \frac{k_A k_{AB} [B]}{k_A + k_{AB} [B]} \quad (17)$$

is known as the *apparent specificity constant* for A. It follows from Eqns (16) and (17) that $k_0^{app} = k_0$ and $k_A^{app} = k_A$ when [B] is extrapolated to an infinite value. This relationship provides the basis for defining the catalytic constant and the specificity constants in reactions with more than one substrate: in general, the *catalytic constant* of an enzyme is the value of $v/[E]_0$ obtained by extrapolating all substrate concentrations to infinity; for any substrate A the *specificity constant* is the apparent value when all other substrate concentrations are extrapolated to infinity.

Eqn (14) may also be rearranged into a form resembling Eqn (11), as follows:

$$v = \frac{V[A][B]}{K_{iA}K_{mB} + K_{mB}[A] + K_{mA}[B] + [A][B]} \quad (18)$$

[2] The conventional Scottish pronunciation of this name may be expressed in the International Phonetic Alphabet as [di:'jel], with only slightly more stress on the second syllable than the first.

in which $V = k_0[E]_0$ is the *limiting rate*, which may also, subject to the reservations noted in section 4.1, be called the maximum rate or maximum velocity and symbolized as V_{max}; $K_{mA} = k_0/k_A$ is the Michaelis constant for A; $K_{mB} = k_0/k_B$ is the Michaelis constant for B; and $K_{iA} = k_B/k_{AB}$ is the *inhibition constant* for A. In some mechanisms K_{iA} is equal to the true dissociation constant for the EA complex: when this is the case the alternative symbol K_{sA} and the name *substrate-dissociation constant* for A (cf. section 3.2) may be used. If Eqn (18) is interpreted operationally rather than as the equation for a particular mechanism it is arbitrary whether the constant in the denominator is written with $K_{iA}K_{mB}$ (as shown) or as $K_{mA}K_{iB}$, where $K_{iB} = k_A/k_{AB}$. However, for some mechanisms only one of the two ratios k_B/k_{AB} and k_A/k_{AB} has a simple mechanistic interpretation and this may dictate which inhibition constant it is appropriate to define.

The term inhibition constant and the symbol K_{iA} derive from the fact that the quantity concerned is related to (and in the limiting cases equal to) the inhibition constant K_{ic} or K_{iu} (as defined below in Section 6.4) measured in experiments where the substrate is treated as an inhibitor of the reverse reaction. However, the relationships are not always simple and quantities such as K_{iA} in Eqn (18) can be and nearly always are defined and measured without any reference to inhibition experiments. For these reasons some members of the panel feel that the symbolism and terminology suggested are not completely satisfactory. No alternative system has so far gained wide support, however.

An *apparent Michaelis constant* for A (and similarly for B) may be defined by dividing Eqn (16) by Eqn (17):

$$K_{mA}^{app} = k_0^{app}/k_A^{app} = \frac{K_{iA}K_{mB} + K_{mA}[B]}{K_{mB} + [B]}. \quad (19)$$

This equation provides the basis for defining the Michaelis constant for any substrate in a reaction with more than one substrate: the *Michaelis constant* for A, K_{mA}, is the value of the apparent Michaelis constant for A when the concentrations of all substrates except A are extrapolated to infinity. This definition applies to reactions with any numbers of substrates, as also does the definition of the limiting rate V as $k_0[E]_0$, but in other respects it becomes very cumbersome to define constants resembling K_{iA} for reactions with more than two substrates. The symbolism of Eqn (13) (or the alternative in terms of Dalziel coefficients) is readily extended to reactions with three or more substrates, however.

6. INHIBITION

6.1. *Reversible and Irreversible Inhibitions*

Sometimes the effect of an inhibitor can be reversed by decreasing the concentration of inhibitor (e.g. by dilution or dialysis). The inhibition is then said to be *reversible*. If, once inhibition has occurred, there is no reversal of inhibition on decreasing the inhibitor concentration the inhibition is said to be *irreversible*; irreversible inhibition is an example of *enzyme inactivation*. The distinction between reversible and irreversible inhibition is not absolute and may be difficult to make if the inhibitor binds very tightly to the enzyme and is released very slowly. Reversible inhibitors that behave in a way that is difficult to distinguish from irreversible inhibition are called *tight-binding inhibitors*.

6.2. *Linear and Non-Linear Inhibition*

Sometimes the effect of an inhibitor I can be expressed by multiplying one or more of the terms in the denominator of the general rate expression (Eqn 13) by factors of the form $(1 + [I]/K_i)$. The inhibition is then said to be *linear* and K_i which has the dimensions of a concentration, is called an *inhibition constant* for the inhibitor I. The word linear in this definition refers to the fact that the inhibition is fully specified by terms in the denominator of the rate expression that are linear in inhibitor concentration, not to the straightness of any plots that may be used to characterize the inhibition experimentally.

If the inhibition cannot be fully expressed by means of linear factors in the denominator the inhibition is said to be *non-linear*.

Linear inhibition is sometimes called *complete inhibition*, and the contrasting term *partial inhibition* is sometimes used for a type of non-linear inhibition in which saturation with inhibitor does not decrease the rate to zero. These latter terms are discouraged because they can be misleading, implying, for example, that the rate may indeed be decreased to zero in 'complete inhibition' at non-saturating concentrations of inhibitor.

6.3. *Degree of Inhibition*

If a reaction occurs in the absence of inhibitor with rate v_0 and in the presence of inhibitor with rate v_i, the *degree of inhibition* is defined as

$$\varepsilon_i = \frac{v_0 - v_i}{v_0} . \qquad (20)$$

As this quantity is a ratio of rates it is dimensionless. The subscripts 'o' and 'i' are useful for distinguishing between uninhibited and inhibited reactions respectively when they are required together, but are usually omitted when no confusion is likely.

6.4. *Classification of Inhibition Types*

Provided that an enzyme behaves in accordance with the limiting behaviour described in Section 4.1 both in the absence of inhibitor (which is always true if Michaelis-Menten kinetics are obeyed and is also true more generally), the type of inhibition may be classified according to whether it affects the apparent value of k_A, the apparent value of k_0, or both.

If the apparent value of k_A is decreased by the inhibitor the inhibition is said to have a *competitive component*, and if the inhibitor has no effect on the apparent value of k_0 the inhibition is said to be *competitive*. In linear inhibition there is a linear effect on $1/k_A^{app}$:

$$\frac{1}{k_A^{app}} = \frac{1}{k_A}(1 + [I]/K_{ic}) \qquad (21)$$

and the constant K_{ic} is called the *competitive inhibition constant* for I.

Conversely, if there is an effect on the apparent value of k_0 the inhibition has an *uncompetitive component*, and if the innibitor has no effect on the apparent value of k_A the inhibition is said to be *uncompetitive*. In linear inhibition there is a linear effect on $1/k_0^{app}$:

$$\frac{1}{k_0^{app}} = \frac{1}{k_0}(1 + [I]/K_{iu}) \qquad (22)$$

and constant K_{iu} is called *uncompetitive inhibition constant* for I.

If both competitive and uncompetitive components are present in the inhibition it is said to be *mixed*. The term *non-competitive inhibition* is sometimes used instead of *mixed inhibition*, but this usage is discouraged, first because the same term is often used for the special case of mixed inhibition in which $K_{ic} = K_{iu}$, second because it suggests that mixed inhibition is the antithesis of competitive inhibition whereas this description actually applies more accurately to uncompetitive inhibition, and third because the shorter word *mixed* expresses clearly the fact that both competitive and uncompetitive components are present.

Mixed inhibition as defined here encompasses such a broad range of behaviour that it may sometimes be helpful to subdivide it further. The case in which $K_{ic} < K_{iu}$ may then be called *predominantly competitive inhibition*, the case with $K_{ic} = K_{iu}$ may be called *pure non-competitive inhibition*, and the case with $K_{ic} > K_{iu}$ may be called *predominantly uncompetitive inhibition*. The classical term for *pure non-competitive inhibition* was simply *non-competitive inhibition*, but this term has become ambiguous because of its widespread use for all kinds of mixed inhibition and because of this ambiguity it is discouraged for all purposes.

Both K_{ic} and K_{iu} have the dimensions of concentrations and may therefore be expressed in mol dm^{-3}, mol L^{-1} or M. In contexts where distinction between K_{ic} and K_{iu} is unnecessary or inappropriate the general symbol K_i may be used for either. In the past there has been no generally understood symbol for the uncompetitive inhibition constant, which has been variously represented as K_i, K_i', K_{ii}, etc. A new and unambiguous symbol seems required, therefore, and K_{iu} is proposed. Although the competitive inhibition constant has much more uniformly been expressed as K_i, the occasional use of the same symbol for the uncompetitive inhibition constant, together with the view that a logical and symmetrical symbolism is desirable, has suggested that the symbol K_{ic} should be used for the competitive inhibition constant whenever any ambiguity might attend the use of the more general symbol K_i.

As K_{ic} and K_{iu} can in principle be determined by measuring the effects of inhibitor on the slopes and ordinate intercepts respectively of plots of $1/v$ against $1/[A]$ they have sometimes been symbolized as K_{is} (for $K_{i\,slope}$) and K_{ii} (for $K_{i\,intercept}$) respectively. Slopes and intercepts are not consistent from one kind of plot to another, however; for example, the slope and intercept in a plot of $[A]/v$ against $[A]$ correspond, respectively, to the intercept and slope of a plot of $1/v$ against $1/[A]$. Such symbols are therefore ambiguous and should not be used except in explicit reference to particular plots.

In reactions with more than one substrate the classification of inhibitors as competitive, uncompetitive or mixed is not absolute but depends on which substrate is variable (in the sense of Section 5.2). For example, a particular inhibitor may cause variation in k_A^{app} without any variation in k_0^{app} when A is the variable substrate, but cause variation in both k_B^{app} and k_0^{app} when B is the variable substrate: it is then said to be a *competitive inhibitor with respect to A* but a *mixed inhibitor with respect to B*. In such systems the inhibition constants K_{ic} and K_{iu} refer to the limiting behaviour for saturating concentrations of all substrates except for the variable substrate. Inhibition constants observed at non-saturating concentrations of the constant substrates are *apparent* values and may be symbolized as K_{ic}^{app} and K_{iu}^{app}.

For some mechanisms some inhibition constants may be true dissociation constants. Whether this is true or not it does not form part of the definitions of the inhibition types and

inhibition constants given above, which are purely operational, in keeping with the policy set out in Section 1. When symbols are required for the dissociation constants of particular species they should be explicitly defined in a way that avoids confusion with the operationally defined inhibition constants. A system of the following kind may be appropriate, but if used it should be explicitly defined in context. For a binary complex, e.g. EI, the dissociation constant may be symbolized as K with the name of the complex as subscript, e.g. K_{EI}. For higher complexes where the nature of the dissociation needs to be specified, a full stop (period) may be used to separate the parts of the complex that dissociate from one another; for example, $K_{EI.S}$ may be used for the dissociation of EIS into EI + S, whereas $K_{ES.I}$ may be used for the dissociation of the same complex into ES + I.

6.5. *Product Inhibition*

The products of nearly all enzyme-catalyzed reactions behave as inhibitors when they are present in the reaction mixture. When considered in this light they are called *product inhibitors* and the phenomenon is known as *product inhibition*. Product inhibition is always reversible (at least in principle) but in other respect occurs in the same varieties as other kinds of inhibition and requires no special discussion or definitions.

7. ACTIVATION

An activator is a substance, other than the catalyst itself or one of the substrates, that increases the rate of an enzyme-catalysed reaction, and it is said to bring about *activation*. If a reaction in the absence of an activator occurs with rate v_0, the *degree of activation* is given by

$$\varepsilon_a = \frac{v - v_0}{v_0} . \qquad (23)$$

This equation is analogous to the definition of *degree of inhibition* (Eqn 20) and ε_a is, like ε_i, dimensionless. In general, the equations for activation closely resemble those for inhibition: wherever a term of the form $(1 + [I]/K_i)$ appears in an inhibition equation, a corresponding term with $(1 + K/[Q])$ can be written for activation of the same kind by an activator Q.

Activation has been studied much less than inhibition, and at present no system of terminology enjoys wide currency. Nonetheless, the rather unsatisfactory term *competitive activation* is occasionally used (see below), and the following classification is offered in the hope of providing a more appropriate terminology. The fundamental division is between *linear activation* which can be fully defined by terms in the denominator of the rate expression that are linear in the reciprocal concentration of activator, i.e. terms of the form $(1 + K/[Q])$, and non-linear activation, which cannot. An alternative division is between *essential activation*, in which the rate is zero in the absence of activator, and *non-essential activation*, in which it is finite. These two classifications overlap to a large extent but are not identical: linear activation must be essential, and non-essential activation must be non-linear, but essential activation can in principle be non-linear. Linear activation can, like linear inhibition, be usefully classified further according to whether the activation affects the apparent value of the specificity constant, in which case it is called *specific activation*, the apparent value of the catalytic constant, in which case it is called *catalytic activation*, the apparent value of the Michaelis constant, in which case it is called *binding activation*, or some combination of these, in which case it is called *mixed activation*. There is no sense in which specific activation can be regarded as resulting from competition between activator and substrate; consequently the term *competitive activation* is meaningless and to be avoided; the terms *uncompetitive* and *non-competitive* are also best avoided in the context of activation.

8. pH EFFECTS

The rates of enzyme-catalysed reactions vary with pH and often pass through a maximum as the pH is varied. If the enzyme obeys Michaelis-Menten kinetics the kinetic parameters k_0 and k_A often behave similarly. The pH at which the rate or a suitable parameter is a maximum is called the *pH optimum* and the plot of rate or parameter against pH is called a *pH profile*. Neither the pH optimum nor the pH profile of an enzyme has any absolute significance and both may vary according to which parameter is plotted and according to the conditions of the measurements.

If the pH is changed and then brought back to its original value, the behaviour is said to be *reversible* if the original properties of the enzyme are restored; otherwise it is *irreversible*. Reversible pH behaviour may occur over a narrow range of pH, but effects of large changes in pH are in most cases irreversible.

The diminution in rate as the pH is taken to the acid side of the optimum can be regarded as inhibition by hydrogen ions. The diminution in rate on the alkaline side can be regarded as inhibition by hydroxide ions. The equations describing pH effects are therefore analogous to inhibition equations. For single-substrate reactions the pH behaviour of the parameters k_0 and k_A can sometimes be represented by an equation of the form

$$k = \frac{\tilde{k}}{1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}} \qquad (24)$$

in which k represents k_0 or k_A, and $\tilde{k}$ is the value of the same parameter that would be observed if the enzyme existed enitrely in the optimal state of protonation; it may be called the *pH-independent* value of the parameter. The constants K_1 and K_2 can sometimes be identified as acid dissociation constants for the enzyme, substrates or other species in the reaction mixture. The identification is, however, never straightforward and has to be justified by independent evidence. The behaviour is frequently much more complicated than represented by Eqn (24).

It is not accidental that this section has referred exclusively to pH dependences of k_0 and k_A. The pH dependence of the initial rate or, worse, the extent of reaction after a given time is rarely meaningful; the pH dependence of the Michaelis constant is often too complex to be readily interpretable.

9. PRE-STEADY-STATE KINETICS

The *pre-steady-state* or *transient* phase in enzyme-catalysed reactions occupies very short periods (usually fractions of a second) and very low product concentrations. Special techniques therefore have to be used. For reactions that are not too fast the *stopped-flow technique*, in which the enzyme and reactants are rapidly mixed and the flow stopped, is commonly used.

For reactions that have to be studied over periods of less than 1 ms *relaxation techniques* are used. In these techniques the system is disturbed, usually but not necessarily from a state of equilibrium, after which it *relaxes* to equilibrium or a new steady state. In the *temperature-jump* (*T jump*) technique the temperature is increased rapidly and the system *relaxes* to a new state of equilibrium or a new steady state at the final temperature. In the *pressure-jump* technique the pressure is rapidly changed.

The *relaxation time* τ of a reaction is the time it takes for the extent of reaction to change by a proportion $(1 - e^{-1})$ of the total change during the relaxation process ($e = 2.71828\ldots$ is the base of natural logarithms). For composite mechanisms, such as those that occur with enzyme-catalysed reactions, relaxation experiments usually reveal more than one relaxation time. These relaxation times can be related to the rate constants of the elementary steps in the mechanism, but the relationships are usually complicated.

10. NON-MICHAELIS-MENTEN KINETICS

Some enzymes display non-Michaelis-Menten kinetics that do not approximate to Michaelis-Menten kinetics to any useful extent. In such case there is little value in retaining the terminology and symbolism of Michaelis-Menten kinetics. Instead it is often possible to express the rate as a *rational function* of the substrate concentration:

$$v = \frac{\alpha_1[A] + \alpha_2[A]^2 + \alpha_3[A]^3 + \ldots + \alpha_n[A]^n}{1 + \beta_1[A] + \beta_2[A]^2 + \beta_3[A]^3 + \ldots + \beta_m[A]^m} \quad (25)$$

(In principle this kind of equation can be generalized to accommodate more than a single substrate but it then becomes highly complicated and only the single-substrate case will be considered here.) A rational function is a ratio of two *polynomials*. The degree of a polynomial is the largest exponent: thus, the degree of the numerator of the expression in Eqn (25) is n and that of the denominator is m. The rational function as a whole may be described as a '$n:m$ function'. In general the degree of the numerator of a rate equation does not exceed the degree of the denominator for enzyme-catalysed reactions, but there is no other necessary relationship between n and m and neither bears any necessary relationship to the number of catalytic centres per molecule of enzyme. In the terminology of Eqn (25) any rate equation obeying *Michaelis-Menten kinetics* can be defined as a 1:1 function.

Any coefficient α_i in the numerator of the right-hand side of Eqn (25) has units $(\text{mol dm}^{-3})^{1-i}\,\text{s}^{-1}$, and any coefficient β_i in the denominator has units $(\text{mol}^{-1}\,\text{dm}^3)^i$. Similar equations are sometimes written in which the constant 1 in the denominator is replaced with a constant β_0. This practice is discouraged, because the equation then contains a redundant parameter and all of the coefficients α_i, β_i have undefined dimensions.

Under some conditions, which cannot be expressed simply and are not normally obvious from inspection of the coefficients of Eqn (25), the equation may generate a plot of v against [A] that shows a monotonic increase in v towards a limiting value at all positive finite values of [A]. A necessary, but not sufficient, condition is that the degrees of the numerator and denominator be equal, i.e. $n = m$. It is then meaningful to define a *limiting rate* $V = \alpha_n/\beta_n$ with the meaning defined in Section 4.1. Moreover, it may also be useful to describe the kinetics quantitatively in terms of the slope of a plot of $\log[v/(V - v)]$ against $\log[A]$, which is known as a *Hill plot*. This slope is called the *Hill coefficient* and is given the symbol h. In a kinetic context it bears no necessary relationship of any kind to the number of catalytic centres per molecule of enzyme and it should not be given a symbol, such as n, that suggests that it does. The symbol n_H has sometimes been used as an alternative to h, but it should be borne in mind that this may cause difficulties in printing when used as an exponent. At any concentration of substrate at which h is greater than unity, the kinetics are said to display *co-operativity*. In some contexts the more explicit term *positive co-operativity* may be preferable to avoid ambiguity. At any concentration at which $h = 1$, the kinetics are said to be *non-co-operative*, and if h is less than unity they are said to be *negatively co-operative*. In the case of Michaelis-Menten kinetics $h = 1$ over the whole concentration range, but in other cases h is not constant and the sign of co-operativity may change one or more times over the range of concentrations. Thus co-operativity is not absolute and in general can only be defined in relation to a particular concentration.

The term *co-operativity* is sometimes restricted to a purely mechanistic meaning, i.e. it is considered to refer to interactions between distinct sites on the enzyme. In common practice, however, the terms discussed above are frequently applied to enzymes in the absence of clear evidence for such interactions, and the aim therefore has been to legitimize such usage by providing purely operational definitions. In contexts where it is considered necessary to emphasize the operational character of the kind of co-operativity defined in terms of the Hill coefficient it may be qualified as *Hill co-operativity* or *kinetic co-operativity*.

11. TYPES OF MECHANISM FOR ENZYMIC CATALYSIS

The definitions given so far have been operational, i.e. they provide a way of describing what may be observed independently of any interpretation that may been placed on it. Little has been said about *mechanisms*, i.e., the detailed descriptions of the chemical events that make up the catalytic process. Nonetheless, the ultimate objective of most investigations in enzyme kinetics is to propose a mechanism. It is to be emphasized at the outset that a kinetic investigation can disprove a proposed mechanism but can never establish a mechanism beyond doubt. A mechanism may be consistent with all of the known facts, yet it is always possible to propose other mechanisms that are also consistent with the facts.

The procedure that is adopted, and the only one that allows progress to be made, is to accept the simplest mechanism that is consistent with all the known facts. This is the principle of *Occam's razor*. A steady-state study of the effect of substrate concentrations on the rate, leading to an empirical rate equation, can often lead to a proposed mechanism. Such a mechanism can be tested by additional investigations, such as of the pre-steady-state kinetics, and of the effects of inhibitors, pH, temperature and solvent composition.

It is not a practical proposition to institute a consistent system for naming mechanisms, although attempts to do this have been made, because except in the most trivial cases it is always simpler and clearer to specify a mechanism by reference to a scheme. Nonetheless, certain terms occur frequently in the descriptions of mechanisms of enzymic catalysts and will be defined here.

The form of an enzyme that exists in solution in the absence of any substrate or other small molecule that can bind

Table 1. *Recommended Symbols and their Units*

Symbol	Meaning	Customary unit	Section of text	See notes below
[A]	concentration of substrate A	mol dm^{-3}	2.1	1, 2, 3
$[A]_{0.5}$ or $[A]_{1/2}$	value of [A] at which $v = 0.5\ V$	mol dm^{-3}	4.2	2
[B]	concentration of substrate B	mol dm^{-3}	2.1	1, 2, 3
$[E]_0$ (or $[E]_t$ or $[E]_{stoich}$)	stoichiometric concentration of active centres	mol dm^{-3}	4.1	1, 3
h (or n_H)	Hill coefficient	dimensionless	10	
[I]	concentration of inhibitor I	mol dm^{-3}	6.2	1, 2, 3
k	rate constant of any order n	$(mol\ dm^{-3})^{1-n}\ s^{-1}$	3	4
$\tilde{k}$	pH-independent value of k	as k	8	5
k^{app}	apparent value of k	as k	5.2, 6.4	5
k_A, k_B	specificity constants for A, B	$mol^{-1}\ dm^3\ s^{-1}$	4.1	2
k_A^Z (etc.)	reciprocal Dalziel coefficient	(various)	5.1	2
k_{cat} (or k_0)	catalytic constant	s^{-1}	4.1, 5.2	
k_i, k_{-i}	forward and reverse rate constants respectively for ith step	as k	3.1	4, 6
K_i	inhibition constant (inhibition type unspecified)	mol dm^{-3}	6.4	
K_{iA}	inhibition constant for A	mol dm^{-3}	5.2	
K_{ic}	competitive inhibition constant	mol dm^{-3}	6.4	
k_{ij}	rate constant for step from E_i to E_j	as k	3.1	4, 6
K_{iu}	uncompetitive inhibition constant	mol dm^{-3}	6.4	
K_m	Michaelis constant (or Michaelis concentration)	mol dm^{-3}	4.2, 5.2	
K_{mA}	Michaelis constant for A	mol dm^{-3}	4.2, 5.2	2
K_s	substrate dissociation constant	mol dm^{-3}	3.2	
K_{sA}	value of K_s for substrate A	mol dm^{-3}	3.2	2
k_0 (or k_{cat})	catalytic constant	s^{-1}	4.1, 5.2	
n_H (or h)	Hill coefficient	dimensionless	10	
[Q]	concentration of activator Q time	mol dm^{-3}	7	1, 2, 3
t	time	s	2.1	
v	rate (or velocity) of reaction	mol $dm^{-3}\ s^{-1}$	2.2	7
V (or V_{max})	limiting rate (or maximum rate, or maximum velocity)	mol $dm^{-3}\ s^{-1}$	4.1, 5.2	
v_A	rate of consumption of A	mol $dm^{-3}\ s^{-1}$	2.1	2
v_i, v_{-i}, v_{ij}	chemical flux (or chemiflux) through step with rate constant k_i, k_{-i}, k_{ij}	mol $dm^{-3}\ s^{-1}$	3.1	4
v_{max}	true maximum value of v	mol $dm^{-3}\ s^{-1}$	4.1	
v_Z	rate of formation of Z	mol $dm^{-3}\ s^{-1}$	2.1	2
v_0	initial rate of reaction	mol $dm^{-3}\ s^{-1}$	3.2	7
[Y]	concentration of product Y	mol dm^{-3}	2.1	1, 2, 3
[Z]	concentration of product Z	mol dm^{-3}	2.1	1, 2, 3
α_i	coefficient of $[A]^i$ in numerator of generalized rate expression	$(mol\ dm^{-3})^{1-i}\ s^{-1}$	10	
β_i	coefficient of $[A]^i$ in denominator of generalized rate expression	$(mol^{-1}\ dm^3)^i$	10	
ε_a	degree of activation	dimensionless	7	
ε_i	degree of inhibition	dimensionless	6.3	
τ	relaxation time	s	9	
ϕ_0 (etc.)	Dalziel coefficient	(various)	5.1	

Notes:

1. Amount-of-substance concentration is abbreviated to concentration. This usage is acceptable in enzyme kinetics without special definition.
2. This symbolism can be extended to other reactants in an obvious way, e.g. K_{mX} is the Michaelis constant of X.
3. Other ways of indicating concentration, such as a for the concentration of A, are acceptable but must be explicitly defined.
4. Any numbering of rate constants must be in explicit relation to a reaction scheme.
5. Other pH-independent or apparent values may be represented similarly, e.g. $\tilde{V}$ is the pH-independent value of V.
6. The rate constants for the ith step of a reaction may alternatively be represented as k_{2i-1} and k_{2i} instead of k_i and k_{-i} respectively (subject to note 4 above).
7. The initial rate may be represented by v, without subscript, when rates at other times are not in question. This rate is the steady-state rate extrapolated to zero time, not the true rate at zero time, which is normally undefined, because the rate of reaction is undefined during the transient phase of an enzyme-catalysed reaction.

to it is called the *free enzyme*. An intermediate derived from the free enzyme by binding of a substrate molecule is called an *enzyme-substrate complex*, and terms such as *enzyme-product complex*, *enzyme-inhibitor complex*, *EA complex* may also be used by an obvious extension of this definition. A complex derived from the free enzyme and one other molecule is called a *binary complex*; one derived from the free enzyme and two other molecules is called a *ternary complex*; one derived from the free enzyme and three other molecules is called a *quaternary complex*. If the catalytic process proceeds through a second form of free enzyme that differs from the first by the presence of a covalently bound group that is transferred in the reaction this second form of free enzyme is called a *substituted enzyme*.

Complexes that do not undergo further reactions that are part of the catalytic pathway are called *dead-end complexes*, and the reactions producing them are called *dead-end reactions*. Enzyme-substrate complexes that do not lead to reaction, which are often but not necessarily dead-end complexes, are called *abortive* or *non-productive complexes*.

When a reaction proceeds through a series of steps that must occur in a specified order, e.g. the substrates must bind in a particular order and the products are released in a particular order, it is said to obey a *compulsory-order mechanism*. (The alternative term *linear mechanism* is sometimes used, but it is discouraged because it can invite confusion with other uses of the term *linear* in enzyme kinetics.) When this is not the case the reaction is said to follow a *random-order mechanism* or a *branched mechanism*. These terms may also be applied to parts of mechanisms: for example, it may happen that substrates bind in random order but products are released in a compulsory order. The distinction between compulsory-order and random-order mechanisms is rarely absolute in practice. The term *preferred-order mechanism* may be used to emphasize that although more than one pathway exists most of the flux is through one of them. The term *random-order mechanism* does not, however, exclude this intermediate case unless explicitly stated to do so.

Elementary steps in which the enzyme forms complexes with small molecules are called *binding steps* and the reverse steps are called *release steps*, usually with a qualifier to indicate the type of species bound or released: e.g. *substrate binding step* or *product-release step*, etc. Elementary steps in which no binding or release occurs may be called *isomerizations*.

An *allosteric* effector is one that acts by binding to the enzyme at a site different from the active site. There is no necessary connection between allosteric effects and co-operative effects, though they often occur together in real systems.

12. ENZYME ACTIVITY

The catalytic activity of an enzyme is the property measure by the increase in the rate of conversion (i.e. the rate of reaction multiplied by the volume: see Section 2.2) of a specified chemical reaction that the enzyme produces in a specified assay system [6]. This is an extensive quantity, and quantities derived from it include *specific catalytic activity* (used for following enzyme purification), which is the catalytic activity divided by the mass of protein, and *molar catalytic activity*, which is the catalytic activity divided by the amount of substance, either of enzyme catalytic centres or of multi-centre molecules. In the latter case it is important to specify whether the measurements refer to catalytic centres or to molecules. Molar catalytic activity has the dimensions of reciprocal time, like first-order rate constants; if it refers to catalytic centres it will approach k_0, the catalytic constant (Section 4.1), if the conditions in the assay are such that v approaches V.

13. SUMMARY OF RECOMMENDED SYMBOLS AND UNITS

Table 1 lists the symbols recommended in this document, together with their units. Although this table includes most alternatives mentioned in the text, it cannot cover all of the points of detailed explanation and should be taken in conjunction with the text. Although for brevity and convenience all volumes are expressed in dm^3, in all cases dm^3 may be replaced identically with L or l (liter), and amount-of-substance concentrations may be expressed in M (molar), which is identical with mol dm^{-3}. Both L and M may be used as primary units for forming multiples and submultiples such as mL, μM, etc.

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