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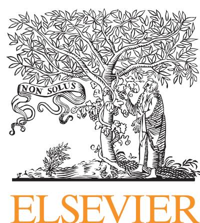

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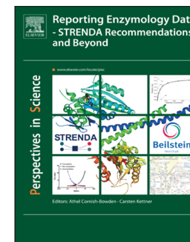

REVIEW

Current IUBMB recommendations on enzyme nomenclature and kinetics☆

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Abstract
The International Union of Biochemistry (IUB, now IUBMB) prepared recommendations for describing the kinetic behaviour of enzymes in 1981. Despite the more than 30 years that have passed since these have not subsequently been revised, though in various respects they do not adequately cover current needs. The IUBMB is also responsible for recommendations on the naming and classification of enzymes. In contrast to the case of kinetics, these recommendations are kept continuously up to date.
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Introduction

The International Union of Biochemistry and Molecular Biology (IUBMB) oversees two areas of nomenclature that are central to the concerns of STRENDA (Tipton et al., 2014), classifying enzyme-catalysed reactions, and recommending symbols and terms used in enzyme kinetics.

Both of these are discussed in this chapter, but in reverse order, as there are more current problems with the kinetics recommendations than with those on enzyme nomenclature. It is worth noting at the outset, incidentally, that although both activities are attributed surprisingly often in the research literature to the International Union of Pure and Applied Chemistry (IUPAC) both are in reality the exclusive responsibility of the IUBMB, though expert chemists are, of course, consulted when appropriate.

The two topics differ in the important respect that one is a matter of continuous revision, whereas the other is not. The list of enzymes is revised continuously, and new activities are typically formalized within months of being reported to the IUBMB, but the recommendations on kinetics have not been revised to take account of developments over the past 30 years.

Kinetics introduction

The IUBMB (then the International Union of Biochemistry, IUB) approved recommendations on the symbolism and terminology of enzyme kinetics in 1981, which were published in three journals of biochemistry (IUB, 1982, 1983a, 1983b), and later in the Compendium of *Biochemical Nomenclature and Related Documents* (IUBMB, 1992a).[1]

30 years have passed since these recommendations were approved, and even at the time they were a compromise between the strict rules that some experts wanted, and complete freedom for authors to proceed as they wished that others wanted. The panel of the time[2] largely avoided topics for which agreement appeared impossible, and also overlooked some that now appear more important than they did then. Irreversible inhibition, for example, is barely mentioned, and is not the subject of any recommendations.

[1]The recommendations can be found at http://www.chem.qmul.ac.uk/iubmb/kinetics/ek1t3.html.

[2]The panel was composed of A. Cornish-Bowden (UK, convenor), H.B.F. Dixon (UK), K.J. Laidler (Canada), I.H. Segel (USA), J. Ricard (France), S.F. Velick (USA) and E.C. Webb (Australia). Many other experts were consulted during preparation of the proposals.

Moreover, genetic engineering was in its infancy, and there is no mention of particular requirements for describing the properties of enzymes cloned in other species, or the treatment of His-tags, or other points that have acquired importance in the intervening years.

In 1981 the International Union of Pure and Applied Chemistry (IUPAC) had just published recommendations on the symbolism and terminology of chemical kinetics (IUPAC, 1981), and K.J. Laidler, the chairman of the IUPAC subcommittee[3] that prepared the recommendations, was also a member of the IUB panel, and, indeed, played a major part in the drafting of the IUB document. Inevitably, therefore, there was a desire to harmonize the two sets of recommendations as far as possible, and the results document bears more similarity with the IUPAC recommendations that it would probably have done if it had been prepared by a panel consisting only of biochemists.

It is clear that the recommendations of 1981 no longer fulfil the needs of modern biochemistry, but it is less obvious what to do about it. As discussed by Tipton et al. elsewhere in this special issue, the STRENDA Commission is in process of developing guidelines for the publication of results, and these have already achieved a substantial degree of acceptance by the main journals in the field, and it may be that a full set of printed recommendations as envisaged in 1981 is no longer appropriate. Nonetheless, it is useful to discuss these to identify points on which they remain appropriate, and points on which they are clearly obsolete. To facilitate cross-referencing I shall discuss items in the same order as they appear in the IUBMB recommendations.

Although the 1981 recommendations are still applicable, in the sense that there has been no formal revision, I shall refer to them in the past tense in this chapter to it make easier to distinguish what was recommended then and what the members of STRENDA think now (Tipton et al., 2014).

Introduction to enzyme nomenclature

This introduction is deferred until after the discussion of kinetics.

Basic definitions

This section contained definitions of standard terms used in biochemistry, most notably *catalyst*, *concentration*, *enzyme*, *substrate*, *inhibitor*, *activator*, *effector* and *modifier*. Most of these require no comment, as they were defined in accordance with ordinary practice in biochemistry, but *concentration* was considered to be an abbreviation for *amount-of-substance concentration*, a term that most biochemists will never have encountered, and which is virtually never used by them as it is normally the only kind of concentration they ever use. Its formal SI unit is mol dm^{-3}, but this is virtually never written in this way in biochemical publications, being (equivalently) written as mol l^{-l}, mol L^{-1} or simply M. Although not stated in the recommendations it is generally accepted that any of these last three units can be prefixed m (milli, 10^{-3}), μ (micro, 10^{-6}), p (pico, 10^{-9}), n (nano, 10^{-12}), as appropriate.

Rates of consumption and formation

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

$$v_A = -\frac{d[A]}{dt} \quad (1)$$

in which t represents time. Square brackets could be used without definition, as here, to represent concentrations. Other symbols, such as a for the concentration of A, were permissible, but needed to be explicitly defined. The *rate of formation* of a product[4] of concentration [P] is defined as

$$v_P = \frac{d[P]}{dt} \quad (2)$$

The terms *rate* and *velocity* are synonymous, and these are normally measured in $\mathrm{M\,s^{-1}}$, or one of the obvious variants implicit in the discussion above. Because of the minus sign in Eq. (1) the values of v_A and v_P are equal if A and P have equal stoichiometric coefficients, as is the case in most (but not all) enzyme-catalysed reactions, and if so the subscripts can be omitted from v and the term *rate of reaction* used.

Rate of reaction

The section began by discussing the complications that arise when the stoichiometry is not one-to-one, when, for example, two molecules of the same product are generated when one molecule of substrate is consumed. Reactions of this kind are not common in enzyme kinetics, but they do occur, for example, the hydrolysis of maltose catalysed by α-glucosidase. A second complication, *time-dependent stoichiometry* is much more important: if intermediates in the reaction reach concentrations comparable with those of the reactants there is not a one-to-one relationship between changes in the reactant concentrations, and one cannot then refer to a rate of reaction. This becomes important when the enzyme concentration is large, as is usually the case in studies of fast reactions.

The rate of reaction as defined here is an *intensive quantity*. This means that its value does not change with the total amount of material considered, so a concentration of 1 mM glucose in a solution is the same whether we are concerned with 1 ml or with 1 μl, whereas the amount of glucose, an *extensive quantity* is not. IUPAC recommendations older than those of 1981 defined the rate of reaction as an extensive quantity with dimensions of amount of substance divided by time, but this definition is obsolete in chemistry and has hardly ever been used in biochemistry. Most biochemists, indeed, would be surprised to learn that it had ever been suggested.

[3]E.T. Denisov (USSR), K.H. Homann (Federal Republic of Germany), K.J. Laidler (Canada, chairman) and T.M.Sugden (UK).

[4]For consistency with the IUPAC recommendations, and at Laidler's insistence, the document used Z, Y. X... as symbols for products, but this is almost never done in biochemical documents, and here I shall follow usual practice in biochemistry of writing P, Q, R....

Elementary and composite reactions

An *elementary reaction* was defined as one with no reaction intermediates in the chemical mechanism; such a reaction is said to occur in a *single step*. Few if any complete enzyme catalysed reactions are of this type, but are instead *composite*, consisting of two or more *elementary steps*, which are, however, themselves elementary reactions. This section noted that the term *molecularity* should only be applied to elementary reactions, and then defines *bimolecular* and *unimolecular* in the ways universally used in biochemistry, so no discussion is required here.

Order of reaction, and rate constant

The document stated that "the term *order of reaction* can be applied to any elementary reaction considered in one direction only, and to certain composite reactions". This is certainly the meaning that applies in chemical kinetics, but it is too restrictive for enzyme-catalysed reactions, for which the idea is well established that saturation of an enzyme implies a gradual decrease (through fractional values) of the order of reaction from 1 at zero substrate concentration to 0 at saturation. I see no objection to saying that a reaction has an order i with respect to a concentration a in conditions where the derivative

$$\frac{\mathrm{d} \ln v}{\mathrm{d} \ln a} = i$$

is applicable, with no implication that i is a constant independent of a. In a later paragraph the 1981 recommendations admit this possibility, and suggest the term *apparent order*.

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. When two or more reactants are considered there is an *overall order* for the whole set of reactant, and separate orders with respect to the different reactants. The 1981 recommendations define the orders with respect to the individual reactants as *partial orders*, but this term is virtually unknown in the biochemical literature.

For a reversible process $A \leftrightharpoons B$ with rate constants k_1 for the forward reaction and k_{-1} for the reverse reaction the net rate v is $k_1a - k_{-1}b$, but it is sometimes necessary (especially for discussion of rates of transfer of isotopic labels), to consider the individual unidirectional rates k_1a and $k_{-1}b$ that compose this sum. In chemistry these are called *chemical fluxes* or *chemifluxes*, but it is more usual in biochemistry to call them simply *fluxes*. The shorter term should, however, be avoided when there is any danger of confusion with the quite different use of the same term for discussing metabolic pathways.

Numbering of reactions

An inordinate amount of time was devoted by the panel of 1981 in their preliminary discussions to deciding which system of numbering rate constants to recommend, finishing with the commonsense advice that authors could use any system they wished as long as it was defined explicitly. The preferred system was that of IUPAC:

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

in which the elementary reactions in a composite mechanism are numbered in such a way that reverse processes are easily recognized (i.e. with the use of minus signs). Much earlier the Enzyme Commission (IUB, 1961) had suggested that ambiguity could be avoided by prefixing positive subscripts with plus signs, writing k_1 as k_{+1}, for example. The ambiguity that this was intended to avoid arose in particular for the symbol k_2, which was used without definition by some authors to refer to the forward rate constant for the second step in a sequence, and by others, again without definition, for the reverse rate constant of the first step. It had been felt that if k_{+2} was used with the first meaning then the + sign would make the meaning clear. However, the panel of 1981 took the view that a better solution was to require authors to specify how their rate constants were defined, especially as no single convention could be expected to satisfy all needs, from the simplest to the most complicated mechanisms. In the years since then the use of + signs has largely disappeared from the literature.

As an example of when a different approach might be preferable, the panel noted that 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 number the step from, for example, E_3 to E_4 as 34, and the step from E_4 to E_3 as 43, and so on. With this scheme the numbering of enzyme forms needs to be given explicitly and the rate constants and rates listed above would then become

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

Although this potentially creates a problem if there are more than nine enzyme forms in the mechanism this is easily solved by separating the subscripts by a comma, e.g. $k_{10,11}$ but this can be omitted when it is not required for clarity.

A different scheme, in which odd subscripts refer to forward steps and even subscripts to reverse steps:

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

was regarded as less satisfactory, both because it conflicts with the IUPAC Recommendations (IUPAC, 1981) and because it makes it more difficult to recognize the forward and reverse rate constants for particular steps. At the time the Recommendations were prepared this system was widely used, but in the subsequent years it has become much less common, though it has not completely disappeared. It is still used, for example, in at least one current textbook (Cook and Cleland, 2007), but most others (Bisswanger, 2002; Copeland, 2000; Cornish-Bowden, 2012; Fersht, 1999; Marangoni, 2002) use positive and negative indexes.

Steady-state approximation

Most of this section of the Recommendations was standard textbook material that hardly needs discussion here. The only significant point of terminology or symbolism is the definition of the equilibrium dissociation constant of the enzyme–substrate complex as the *substrate dissociation* constant with the symbol K_{sA} for a complex EA, the qualifier A being unnecessary in contexts where only one substrate is in question. At the time the Recommendations were prepared the identity the substrate was often identified by a

superscript rather than a subscript, i.e. K_s^A, and it was commented that the location of the qualifier was just a matter of typographical convenience. This practice is less common today, but it is still used in some textbooks (Bisswanger, 2002; Copeland, 2000; Marangoni, 2002).

Enzyme reactions involving a single substrate

Limiting kinetics of enzyme-catalysed reactions

Michaelis-Menten kinetics

These two sections also consisted mainly of textbook material, but included the definitions of some important terms and symbols. They will be dealt with together here. Michaelis-Menten kinetics was defined as adherence to an equation of the following form:

$$v = \frac{k_{cat}e_0a}{K_m+a} = \frac{Va}{K_m+a} \tag{3}$$

in which the rate v is expressed as a function of substrate concentration a and total enzyme concentration e_0. For the total enzyme concentration, the symbols $[E]_0$, $[E]_t$ or $[E]_{stoich}$ were suggested: $[E]_0$ is a natural alternative to e_0 for authors who prefer a more explicit way of showing that it is a concentration, whereas $[E]_t$ is little used in practice, and $[E]_{stoich}$ virtually never.

The Panel preferred the symbol k_0 over k_{cat}, but the latter seems overwhelmingly more common in the literature, and was also mentioned as a possibility. Regardless of the symbol, the name *catalytic constant* was recommended. Surprisingly, the term *turnover number* was not mentioned, though whether this was an oversight or an indication that it was deprecated is not clear. The name *limiting rate* and symbol V were suggested for $k_{cat}e_0$, the common terms *maximum rate* and *maximum velocity* being deprecated as misleading for a quantity that is not a maximum in the mathematical sense. Nonetheless, the convenience, especially in speech, of using V_{max} rather than V, was admitted.

The name *Michaelis constant* was given to the quantity shown here as K_m, but used the symbol K_{mA} for it, later indicating that it could be written as K_m when the substrate at issue was obvious, or as K_m^A if preferred. The alternative name *Michaelis concentration* was also suggested, but this appears to have no currency in the literature. My feeling is that readers who have not understood that it is a concentration are also unlikely to realize that it is the same quantity as the one usually called the Michaelis constant.

An alternative way of writing the Michaelis-Menten equation:

$$v = \frac{k_{cat}k_Ae_0a}{k_{cat}+k_Ae_0a}$$

was introduced, with K_m replaced by k_{cat}/k_A. The symbol k_A has achieved almost no currency, but the name specificity constant suggested for it has become widely accepted. This was a new term at the time, but it followed in a natural way from the realisation (Fersht, 1977) that it was the natural parameter for quantifying the ability of an enzyme to discriminate between two or more alternative substrates that are simultaneously available.

Non-Michaelis-Menten kinetics

The section dealing with reactions that do not obey Michaelis-Menten kinetics was essentially confined to a brief mention of an equation for inhibition by excess substrate:

$$v = \frac{V'a}{K'_{mA}+a+a^2/K_{ia}}$$

It was noted that the parameters V' and K'_{mA} are not parameters of the Michaelis-Menten equation because this is not the Michaelis-Menten equation, so a symbol such as $a_{0.5}$ is appropriate to represent the substrate concentration at which $v=0.5V'$, and definitely not K'_{mA}, which is not equal to that concentration.

For more elaborate kinds of departures from Michaelis-Menten kinetics (cooperativity and so on) the document referred to a later section with the same name.

Enzyme reactions involving more than one substrate

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:

$$v = \frac{e_0}{(1/k_{cat})+(1/k_Aa)+(1/k_Bb)+\ldots+(1/k_{AB}ab)+\ldots+(p/k_A^Pa)} \tag{4}$$

which can be regarded as a generalization of the Michaelis-Menten equation for one substrate, and in which p represents the concentration of a product. 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 denominator. Thus a, b, ab, etc., are all acceptable concentrations in the denominator of any individual denominator term, but a^2, for example, would not be; p, q, pq, p^2, etc., are all acceptable concentration factors in the numerator of any denominator term. The constant k_{cat} corresponds to k_{cat} in Eq. (3); 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 its numerator. The constant term $1/k_{cat}$ must be present (because otherwise the rate would increase without limit with increasing concentrations of all substrate concentrations), 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 Eq. (4) with coefficients k_{AB} and k_A^P, may or may not be present. The paragraph concluded by mentioning Dalziel coefficients, which use ϕ_A, for example, as the symbol corresponding to $1/k_A$. However, these have almost disappeared from the current literature, and probably do not need to be mentioned in any future revision of the recommendations.

Eq. (4) 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 the next section.

Michaelis-Menten kinetics of a two-substrate reaction

For a two-substrate reaction in the absence of products Eq. (4) simplifies to

$$v = \frac{e_0}{(1/k_{\mathrm{cat}})+(1/k_{\mathrm{A}}a)+(1/k_{\mathrm{B}}b)+(1/k_{\mathrm{AB}}ab)} \tag{5}$$

It is common practice to vary one substrate concentration at a time, for example a, keeping the other constant. If this is done then terms that do not contain the varied concentration are also concentration, and in this case the rate follows Michaelis–Menten kinetics with respect to varied concentration, because Eq. (5) can be rearranged to

$$v = \frac{k_{\mathrm{cat}}^{\mathrm{app}} e_0 a}{K_{\mathrm{m}}^{\mathrm{app}} + a} \tag{6}$$

in which $k_{\mathrm{cat}}^{\mathrm{app}}$ and $K_{\mathrm{m}}^{\mathrm{app}}$ are the apparent values of k_{cat} and K_{m}, which means that they are the values that these values appear to have when certain specified conditions (the concentration b in this case) are held constant. The Recommendations also defined $k_{\mathrm{A}}^{\mathrm{app}}$ as the apparent specificity constant, but this term and symbol have been very little used.

A difficulty that still exists is the way to treat the other constants with dimensions of concentrations in addition to the Michaelis constants. These arise because Eq. (5) can also be arranged in a way that resembles Eq. (3), and this representation is very commonly used:

$$v = \frac{Vab}{K_{\mathrm{iA}}K_{\mathrm{mB}} + K_{\mathrm{mB}}a + K_{\mathrm{ma}}b + ab} \tag{7}$$

In this equation most of the symbols and the names for them present no particular problem, but what about K_{iA}? Everyone agrees, of course, that there is a constant term in the denominator independent of a and b, but how to write it and what to call it? When the subject was being developed in the 1950s and 1960s there were several variants for the term that appears as $K_{\mathrm{iA}}K_{\mathrm{mB}}$ in Eq. (7), (Alberty, 1956) wrote K_{AB}, Dalziel (1957) wrote ϕ_{12}, Cleland (1963) wrote $K_{\mathrm{ia}}K_{\mathrm{b}}$, Mahler and Cordes (1966) wrote $\overline{K}_{\mathrm{a}}K_{\mathrm{b}}$, Dixon and Webb (1958) initially wrote $K_{\mathrm{a}}K_{\mathrm{b}}'$, but later they changed this to $K_{\mathrm{s}}^{\mathrm{A}}K_{\mathrm{m}}^{\mathrm{B}}$ (Dixon and Webb, 1979). It is worth mentioning this variability as it reflects a real uncertainty about how best to write the equation. The subscript i in some of these reflects the fact that in some conditions the constant is the same as an inhibition constant, and the subscript s in others reflects the fact that under simple conditions it is a true substrate dissociation constant. The Recommendations of 1981 chose $K_{\mathrm{iA}}K_{\mathrm{mB}}$, as in Eq. (7), in part for typographical reasons — $K_{\mathrm{iA}}K_{\mathrm{mB}}$ is easier to typeset than $K_{\mathrm{i}}^{\mathrm{A}}K_{\mathrm{m}}^{\mathrm{B}}$, but made it clear that not everyone was happy:

> However, the relationships are not always simple and quantities such as K_{iA}... 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.

That is still the case today. The change from italic to roman subscripts (and superscripts, when relevant) was adopted but not explained in the Recommendations. It was probably done to agree with the IUPAC recommendations, and because of the mathematical convention that italics are used to denote algebraic variables: K may be an algebraic variable, but its subscripts i, m, A, B and so on are not. In such cases A, for example, refers to the chemical entity A, which is not an algebraic variable, not to its concentration [A] or a, which is.

Inhibition

Reversible and irreversible inhibition

This section of the Recommendations was essentially textbook material that requires no particular discussion here.

Linear and non-linear inhibition

This section was (and remains), more contentious, because of uncertainty about what "linear" means. The word has well-defined (but different) meanings in mathematics, physics and statistics, and in other usages it sometimes means a relationship that can be plotted as a straight line, and it sometimes means that one variable depends only on the first power of another. In the context of the recommendations it had this last meaning, but the variables in question are not the rate v and inhibitor concentration i (which would be logical but not very useful for describing inhibition, because inhibition is never linear in this sense), but the reciprocal rate $1/v$ and 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.

Degree of inhibition

The *degree of inhibition*, defined as $\varepsilon_{\mathrm{i}} = (v_0 - v_{\mathrm{i}})/v_0$, where v_0 is the rate in the absence of inhibitor and v_{i} is the rate in the presence of inhibitor, was included at the insistence of a member of the panel who thought it was important, but this term is very little used by biochemists (though it is common in papers in related fields but not written by biochemists). As far as I can detect it is not defined or used in any of the current textbooks on enzyme kinetics (Bisswanger, 2002; Cook and Cleland, 2007; Cornish-Bowden, 2012; Marangoni, 2002). Although its utility might seem to be obvious — and doubtless does seem to be obvious to the non-biochemists who use it — it is generally much more informative to characterize inhibition in terms of *inhibition constants*.

An important illustration of this is the *concentration for half-inhibition*, variously symbolized as $i_{0.5}$, I_{50} and other

similar ways, which is the inhibitor concentration for $\varepsilon=0.5$. This is very commonly found in the pharmacology literature, but it has very little mechanistic meaning, because it has no straightforward relationship to inhibition constants. In my view any future recommendations of the IUBMB should do nothing to encourage what is effectively bad terminology, and should either avoid all mention of the degree of inhibition or mention it only to deprecate it.

Classification of inhibition types

This section again consisted mainly of textbook material, and defined *competitive inhibition* as a decrease in the apparent value of k_A with increases in the inhibitor concentration i,

$$\frac{1}{k_A^{app}} = \frac{K_m^{app}}{k_{cat}^{app}} = \frac{K_m}{k_{cat}}\left(1+\frac{i}{K_{ic}}\right) \quad (8)$$

and K_i is the *competitive inhibition constant*. Uncompetitive inhibition was defined as the analogous effect decrease in the apparent value of k_{cat},

$$\frac{1}{k_{cat}^{app}} = \frac{1}{k_{cat}}\left(1+\frac{i}{K_{iu}}\right) \quad (9)$$

and *mixed inhibition* as decreases (not necessarily equal) in both. The use of the term *non-competitive inhibition* as a synonym for *mixed inhibition* was deprecated, as it is also used for the special case of mixed inhibition in which the two inhibition constants are equal, $K_{ic}=K_{iu}$.

At the time of when the recommendations were made the symbol K_i was widely used for the competitive inhibition constant (as it still is), but there were considerable variation in the symbol for the uncompetitive inhibition constant, K_i, K_i' and K_{ii} all having some currency. It was felt that ambiguity could be avoided with second subscripts c (for "competitive") and u (for "uncompetitive"), but they could be omitted when it was clear which sort of inhibition was at issue. An alternative system (now less common than it was in 1981) in which K_{is} was used instead of K_{ic}, and K_{ii} was used instead of K_{iu}, was deprecated, because the second subscripts s (for "slope") and i (for "intercept") have meaning only in relation to a particular graphical method of analysing data, and are the wrong way round or completely meaningless for others. Although not mentioned in the recommendations, the fact that they have the same initial letters as "substrate" and "inhibitor" could also be a source of misunderstanding.

In reactions with more than one substrate the type of inhibition varies for a given inhibitor according which substrate concentration is varied. One therefore needs to specify the substrate, using terminology such as "competitive with respect to glucose, but mixed with respect to ATP".

A point that was made in the Introduction to the recommendations, but which applies particularly to terminology for inhibition, is that the definitions of kinetic constants are *operational*, in other words they describe what is observed, not how it is interpreted mechanistically. Inhibition according to Eq. (8) is competitive regardless of whether there is competition between substrate and inhibitor for a binding site, and inhibition in which such competition does occur is not necessarily competitive.

Product inhibition

This section noted that nearly all products of enzyme-catalysed reactions can act as inhibitors.

Activation

This section began by defining *degree of activation* in an analogous way to the definition of degree of inhibition above. However, this terminology is open to the same objections as those discussed there, and it should probably be dropped.

The remainder of the section noted that activation had been less studied than inhibition, and had no universally recognized system of terminology or symbolism. *Linear activation* was suggested for cases where the dependences are analogous to Eqs. (8 and 9) with terms of the form $1+i/K_i$ replaced by terms of the form $1+K/$[activator]. The term *specific activation* was suggested for increases in the apparent specificity constant (and *catalytic activation* for the opposite case), because although specific activation is algebraically analogous to competitive inhibition it does not correspond to any meaningful idea of competition even for the simplest mechanisms. None of these terms have become widely accepted in the biochemical literature.

pH effects

This section was rather superficial, contenting itself with saying, for example, that "the pH dependence of the Michaelis constant is often too complex to be readily interpretable", which seems excessively pessimistic. However, it is not really necessary to present a different view, as this would essentially be a textbook topic that would not raise any particular questions of symbolism or terminology.

The basic Michaelis equation for a bell-shaped profile,

$$k = \frac{\tilde{k}}{1+[H^+]/K_1+K_2/[H^+]} \quad (10)$$

was introduced, defining $\tilde{k}$ as the "parameter that would be observed if the enzyme existed entirely in the optimal state of protonation", and suggesting the name *pH-independent value* for it, but was not discussed in any detail.

Pre-steady-state kinetics

This section was even more superficial, and would clearly be regarded as completely inadequate by anyone concerned with pre-steady-state kinetics. Apart from brief mention of some techniques — barely relevant in nomenclature recommendations — the term *relaxation time* was defined as "the time it takes for the extent of reaction to change by a proportion $1-e^{-1}$". Any future recommendations will need to be drafted by an expert panel.

Non-Michaelis–Menten kinetics

The first part of the section dealt with the representation of non-Michaelis–Menten kinetics in terms of *rational functions* of the substrate concentration, i.e. the ratio of two polynomial expressions. As this type of representation is hardly ever used except in the most theoretical comparisons of different models of cooperativity it seems unnecessary to discuss it. The term *Michaelis constant* and K_m were not mentioned, though they should have been, if only to point out that they refer explicitly to the Michaelis–Menten equation and should not be used in the context of non-Michaelis–Menten kinetics. The *limiting rate V* may have meaning, however, when the rate shows a monotonic dependence on substrate concentration.

Cooperativity was discussed in the context of the Hill plot of $\log[v/(V-v)]$ against $\log v$.[5] The slope of such a plot was defined as the *Hill coefficient* and the symbol h suggested. This symbol was relatively unknown at the time, but has become well accepted.

The older symbol n was deprecated as it conveys the wrong suggestion that it is equal to the number of binding sites on the enzyme, and n_H was regarded as acceptable but typographically inconvenient for a symbol that often needs to be used as a superscript to a concentration. In the context of the Hill coefficient, the kinetic behaviour was defined as *cooperative* (or *positively cooperative* in contexts where ambiguity might otherwise be likely) for $h>1$, *negatively cooperative* for $h<1$, and *non-cooperative* for $h=1$.

Types of mechanisms for enzymatic catalysis

This section introduced the terms *free enzyme*, *enzyme-substrate complex*, *enzyme-product complex*, *enzyme-inhibitor complex*, etc., all of them in an obvious way that does not require discussion. Complexes between two entities were defined as *binary complexes*, and higher-order complexes as *ternary complexes* and *quaternary complexes*. The term *substituted enzyme* was suggested for a second form of free enzyme differing from the first by the presence of a covalently bound group that is transferred in the reaction. The panel seems to have avoided the question of whether such a mechanism should be called a *substituted-enzyme mechanism* or a *ping pong mechanism*, as neither name was mentioned.

Other terms defined essentially as one would find in a textbook were *dead-end complex, dead-end reaction, abortive complex,* and *non-productive complex, compulsory-order mechanism, random-order mechanism, branched mechanism, preferred-order mechanism, binding step, release step*, *isomerization* and *allosteric effector*.

Mechanisms as the term would be understood by an organic chemist were not considered.

Enzyme activity

The *catalytic activity* of an enzyme was defined as the property measured by the increase in the rate of conversion (i.e. the rate of reaction multiplied by the volume: see above) of a specified chemical reaction that the enzyme produces in a specified assay system.

Note that this is an extensive quantity, because it needs to increase with the total amount of enzyme activity. Derived quantities are the *specific catalytic activity*, the *catalytic activity* divided by the mass of protein, and the *molar catalytic activity*, the catalytic activity divided by the number of moles of enzyme catalytic centres or of multi-centre molecules.

Summary of recommended symbols and units

This consisted mainly of a Table of symbols and units, with no important information not already dealt with.

Discussion of the situation in 2014

In most respects the earlier sections of the original recommendations, and also the discussion of enzyme activity at the end, have survived well, and that although some revision might be desirable this could easily be done by a new Commission set up by the IUBMB. The later sections are another matter, however. It is not obvious that there is a very strong demand for new recommendations on activation terminology, but this could likewise be done without great difficulty by a Commission of experts in this field. However, pH effects are very extensively studied, and it is difficult to believe that the 1981 recommendations can be considered adequate, especially as they took an excessively pessimistic view of what information could be deduced from measurements of pH activity.

The section on pre-steady-state kinetics was another example, in this case because the earlier panel had no experts in this area. This would seem to be an important area for the IUBMB to consider, but any new recommendations would need to be prepared by specialists, not simply as part of the task of a group responsible for enzyme kinetics as a whole.

Non-Michaelis–Menten kinetics has become a far less active area of current research than it was in the 1970s, and although the 1981 recommendations were not at all detailed they may be sufficient for present needs.

The discussion of types of mechanism seems only peripherally linked to the main topic of the recommendations. If updated this section should be dealt with separately, and should take account of the terms used by organic chemists to classify mechanisms.

Enzyme nomenclature: introduction

As long as only a few enzymes were known to biochemists it mattered very little if these were named in an ad hoc fashion by their discoverers as invertase, Zwischenferment, malic enzyme and so on, but by the middle of the 1950s it was clear that this unsystematic approach could not continue without producing utter confusion. Two proposals of ways of classifying enzyme-catalysed reactions later became the basis of the classification scheme adopted by the IUBMB (Dixon and Webb, 1958; Hoffmann-Ostenhof,

[5] Today it has become more usual to write $\ln[v/(V-v)]$ against $\ln v$, which has the same slope, as common logarithms have lost much of their usefulness in the age of electronic calculators.

1953). Already in 1958 the first edition of *Enzymes* (Dixon and Webb, 1958) listed 659 enzymes, far too many for unsystematic names to be intelligible. When the last printed edition of *Enzyme Nomenclature* (IUBMB, 1992b) appeared in 1992 this number had grown to 3196, and at the time of writing this introduction it is 5588, and continues to increase.

To overcome the risk of imminent chaos, the IUB set up the *Enzyme Commission* in 1956[6] which presented its Report in 1961 (IUB, 1961), in which a classification of enzyme-catalysed reactions into six groups. The Enzyme Commission itself was replaced in 1961 by the IUB *Standing Committee on Enzymes*, and its work is now the responsibility of the *Nomenclature Committee* of the IUBMB. Despite these changes in responsibility, however, the original classification has been maintained, and the system today is the same as that of 1961. In part for that reason, and also because the prefix EC is still used in enzyme numbers, the term "*Enzyme Commission*" is still often used, though the commission it refers to ceased to exist more than half a century ago.

Other topics covered in the original report

I shall be primarily concerned in this chapter with the classication of enzyme-catalysed reactions, which was the major emphasis of the original Report (IUB, 1961), but it is worth noting that the original commission also considered several other topics, as follows:

- units of enzyme activity: these are now covered by separate recommendations IUB, 1979; IUB, 1980) and briefly in (see above) in the recommendations on enzyme kinetics[1-4] (IUB, 1982, 1983a, 1983b; IUBMB, 1992a);
- symbols of enzyme kinetics: these are now covered by separate recommendations (IUB, 1982, 1983a, 1983b, 1992), as discussed in Sections 2-14 of this chapter;
- classification of cytochromes, extended in the last printed edition of *Enzyme Nomenclature* (IUBMB, 1992b) to cover electron-transport proteins in general;[7]
- The terminology of enzyme formation. This chapter, written at a time when the study of protein synthesis was in its infancy, is essentially obsolete.

Principles of enzyme classification

The enzyme list is a classification of *enzyme-catalysed* reactions; it is not a classification of protein structures. A single protein may have two or more EC numbers if it catalyses two or more reactions. This is the case, for example, for two proteins in *Escherichia coli*, each of which catalyses the reactions both of aspartate kinase (EC 2.7.2.4) and of homoserine dehydrogenase (EC 1.1.1.3). It may also happen that two or more proteins with no detectable evidence of homology[8] catalyse the same reaction. For example, various different proteins catalyse the superoxide dismutase reaction, and share a single EC number, EC 1.15.1.1. This latter case is relatively rare, but it is almost universal that proteins catalysing the same reaction in different organisms, or sets of isoenzymes in one organism, are homologous, with easily recognisable similarities in sequence. The Nomenclature Committee of IUBMB discussed ways of incorporating structural information in the enzyme list in a systematic way, i.e. going beyond what are little more than anecdotal notes in the Comments. Nothing was ever agreed or implemented, however, but fortunately the web-based list includes links to databases such as EXPASY, thus allowing structural information to be combined with reaction information.

The original classification scheme remains very satisfactory for the enzymes of central metabolism, but there have always been some problem groups, most notably the peptidases, and the wholesale reorganization of group 3.4 in 1972 reflected the difficulties. The primary problem is that although the enzymes of central metabolism have sufficient specificity for reaction to be defined with some precision, many peptidases have broad and overlapping specificity. In addition, the fact that the peptidases constituted a much higher proportion in 1961 than now of the enzymes that had been studied meant that numerous enzymes that differ mainly in being derived from different organisms have been classified as different enzymes with different EC numbers. For example, papain (now EC 3.4.22.2), ficain (EC 3.4.22.3), asclepain (EC 3.4.22.7), actinidain (EC 3.4.22.14) and stem bromelain (EC 3.4.22.32) all have very similar catalytic properties. Classifying the overlapping specificity of peptidases (many more of which are known today than there were at the time of the original Report (IUB, 1961)) is now more efficiently covered by a dedicated database (Rawlings et al. 2012).[9]

At the other extreme are the enzymes of the restriction-modification systems. For example, EC 1.1.1.113 contains the enzymes collectively known as site-specific DNA-methyltransferase (cytosine-N^4-specific). This is actually a large group of enzymes, each clearly distinct, that recognize specific sequences of DNA. Although it would be technically possible to classify these in the same way as the enzymes of central metabolism, giving each one a separate name and EC number, the purpose is better served by referring to specialized databases such as REBASE (Roberts et al. 2010).[10]

Enzyme classes

There are six main classes of enzymes, as follows (Schomburg et al., 2014):

[6]The Enzyme Commission was composed of M. Dixon (UK, president), E.F. Gale (UK), S.P. Colowick (USA), A.L. Lehninger (USA), A.E. Braunstein (USSR), W.A. Engelhardt (USSR), K. Linderstrøm-Lang, Denmark, P.A.E. Desnuelle, France, F. Lynen (Germany), and O. Hoffmann-Ostenhof (Austria, secretary), with F. Egami (Japan) and L.F. Leloir (Argentina) as corresponding members. E.C. Webb (UK) was appointed to the Commission after the death of K. Linderstrøm-Lang in 1957.

[7]http://www.chem.qmul.ac.uk/iubmb/etp/.

[8]The term homology means "having a common evolutionary origin" (Reeck, et al. 1987); it does not mean "similar in structure". This point is not specific to discussion of enzymes, but its misuse in the literature is so common that it is worthwhile drawing attention to it. It has been estimated (Marabotti and Facchiano, 2009) that as recently as 2007 around 40% of papers continue to misuse the term.

[9]http://merops.sanger.ac.uk.

[10]http://rebase.neb.com/rebase/rebase.html.

EC 1 *Oxidoreductases* catalyse reactions in which a substrate donates one or more electrons to an electron acceptor, becoming oxidized in the process.
EC 2 *Transferases* catalyse reactions in which a chemical group is transferred from a donor substrate to an acceptor substrate.
EC 3 *Hydrolases* catalyse reactions in which a bond in a substrate is hydrolysed to produce two fragments.
EC 4 *Lyases* catalyse non-hydrolytic reactions in which a chemical group is removed from a substrate leaving a double bond.
EC 5 *Isomerases* catalyse one-substrate one-product reactions that can be regarded as isomerization reactions.
EC 6 *Ligases* catalyse the joining together of two or more molecules coupled to hydrolysis of ATP or an analogous molecule. These enzymes are also sometimes called *synthetases*, a name that was already in use before creation of the original Enzyme Commission, with unfortunate consequences, as discussed below.

In reality all of the enzymes in classes 1–3 satisfy the definition of transferases. However, as these three classes are all large compared with the other three groups, it is convenient to break them into three classes, and to reserve the name *transferase* for enzymes that are not oxidoreductases or hydrolases.

In addition to the name *synthetase* for ligases, the name *synthase* can be used for any enzyme when it is appropriate to use a name that emphasizes the name of the product synthesizes. Metzler (1980) pointed out that using two such similar names in contrasting ways was a source of confusion.[11] There is also a difference between the way enzymes in EC 6 are named: ligases are named according to the substrates that are joined, whereas synthetases and synthases are named according to the product. In some cases the resulting names may differ very little, as for example *tyrosine-arginine ligase* and *tyrosyl-arginine synthase* are different names for EC 6.3.2.4, but in others they can be quite different, as with L-*histidine*:β-*alanine ligase* and *carnosine synthetase* for EC 6.3.2.11.

Subclasses

Each of the six classes is divided into subclasses on the basis of the salient differences between the enzymes in the class. In EC 1, for example, the subclasses define the type of substrate acted on:

EC 1.1 Acting on the CH–OH group of donors
EC 1.2 Acting on the aldehyde or oxo group of donors
EC 1.3...
EC 1.19 Acting on reduced flavodoxin as donor
EC 1.97 Other oxidoreductases.

[11] He suggested that it was analogous to restricting the word *whale* to cetaceans, but allowing the word *whayle* to be used for fishes.

This last subclass is numbered EC 1.97 because it is provisional. In due course the enzymes it contains may be reclassified more appropriately. The original Report (IUB, 1961) had two subclasses EC 1.99 and EC 1.98 that were removed when sufficient information was available to place the enzymes they contained elsewhere.

Classes EC 3–5 are divided into subclasses on the basis of types of substrate, in much the same way as in EC 1. In EC 2, however, it was more useful to emphasize the nature of the transferred group. So, for example, we have

EC 2.1 Transferring one-carbon groups
EC 2.2 Transferring aldehyde or ketone residues
EC 2.3 Acyltransferases
EC 2.4...
EC 2.8 CoA-transferases

In EC 6 the division into subclasses is made on the basis of the type of product:

EC 6.1 Forming carbon–oxygen bonds
EC 6.2 Forming carbon–sulphur bonds
EC 6.3 Forming carbon–nitrogen bonds
EC 6.4 Forming carbon–carbon bonds
EC 6.5 Forming phosphoric ester bonds.

Sub-subclasses

The subclasses are divided into sub-subclasses in much the same way as the way the subclasses themselves are defined. For example, EC 1.16 (oxidoreductases oxidizing metal ions) contains two sub-subclasses:

EC 1.16.1 With NAD^+ or $NADP^+$ as acceptor
EC 1.16.2 With oxygen as acceptor

As with the numbering of subclasses, 99 (or a smaller number if necessary) is used for sub-subclasses containing a miscellaneous group of enzymes. For example, subsection EC 1.6 contains oxidoreductases acting on NADH or NADPH, and within this there is EC 1.6.99 for miscellaneous acceptors.

Assignment of newly discovered enzymes to sub-subclasses

It follows from the preceding discussion that with the aid of the complete list of sub-subclasses it is usually easy to decide where a newly discovered enzyme activity should be classified, except on the rare occasions where it is a new type of reaction unlike any of those known previously. When authors report new activities to the Nomenclature Committee of IUBMB, therefore, they can suggest in which sub-subclass of Enzyme Nomenclature it should appear, and the Nomenclature Committee will normally accept such suggestions unless they are obviously inappropriate.

What authors should *not* do, however, is to propose a complete four-part EC number, and in particular they *should not use any complete number in a publication* until it has been assigned by the Committee.[12] One reason for that is obvious: in a rapidly expanding area of research it will often happen that new activities in the same sub-subclass will be discovered in parallel by different groups, who might then choose the same number for different activities, or different numbers for the same activity. In either case this would create ambiguity that would be subsequently difficult to eliminate.

A less obvious difficulty may arise with apparent "gaps" in the enzyme list. For example, there is no EC 1.5.3.8, though EC 1.5.3.7 (L-pipecolate oxidase) and EC 1.5.3.9 (reticuline oxidase) exist. Such a gap is not an indication of a number that is still available to be assigned; it is an indication of an entry that has been reclassified, in this case to EC 1.3.3.8, tetrahydroberine oxidase. Once a number is removed it is never reassigned,[13] as this would create difficulties for reading the older literature. On occasion whole sub-subclasses are reclassified: for example, EC 3.4.1 to 3.4.10 do not exist, as wholesale reclassification of the peptidases has been necessary.

Individual entries

As should be obvious from the preceding discussion, the complete four-part EC number specifies a particular enzyme activity. In some cases this will be very precise, and that is the ideal for all entries. For example, the listing of EC 2.7.2.12 is as follows:

	EC 2.7.2.12
Accepted name:	acetate kinase (diphosphate)
Reaction:	diphosphate + acetate = phosphate + acetyl phosphate
Other name(s):	pyrophosphate-acetate phosphotransferase
Systematic name:	diphosphate:acetate phosphotransferase
Links to other databases:	BRENDA, EXPASY, IUBMB, KEGG, METACYC, CAS registry number: 57657-58-6
References:	1. Reeves, R.E. and Guthrie, J.D. Acetate kinase (pyrophosphate). A fourth pyrophosphate-dependent kinase from *Entamoeba histolytic*a. Biochem. *Biophys. Res. Commun.* **66** (1975) 1389–1395. [PMID: 172079]

In this case there is no line for Comments, so one can conclude that this enzyme catalyses the reaction specified and no other. What do the other lines mean? The *Accepted name* is the recognized name that ought to appear at least once in any publication about the enzyme. *Other name(s)* are names that have sometimes been used for the same enzyme. In this example the other name given is harmless and unlikely to cause any ambiguity. However, among the other names given for EC 1.1.1.49 (glucose 6-phosphate dehydrogenase) are "Zwischenferment" and "GPD", of which the former will be unintelligible to many modern readers, and the latter easy to confuse with the names of other enzymes with the same initials, such as EC 1.2.1.9 (glyceraldehyde 3-phosphate dehydrogenase). The *Systematic name* is formed in accordance with definite rules, and defines the activity of the enzyme as precisely as possible. In some cases application of the rules produces a cumbersome name that is hardly suitable for everyday use. The recommendation is that where a particular is mentioned in a paper but is not the principal focus the EC number is sufficient to define it, but when it is the main focus either the systematic name or the reaction catalysed should be specified as well.

Since the original Report (IUB, 1961) appeared the status of the *accepted name* has undergone various changes, which reflect controversy over exactly what it means and how important it is. Originally it was called the *Trivial name*, and appeared only in the third column of the table, by implication having lower status than the *Systematic name*. In 1972 it was renamed *Recommended name* and listed in column 2, after the number. At the same time the *Other names* appeared. The same arrangement was used in 1984, but in 1992, in the last complete printed version of *Enzyme Nomenclature* (International Union of Biochemistry and Molecular Biology, 1992b) it appeared first but was not given any particular name. The current web-based list[14] uses the term *Common name* when setting out the rules, but in the list itself it uses *Accepted name*, a term that does not appear to be defined anywhere.

Notice in the above example that the reaction is written as

diphosphate + acetate = phosphate + acetyl phosphate

and not, say, as

$$P_2O_7^{4-} + CH_3CO_2^- = PO_4^{3-} + CH_3CO_2PO_3^{3-} + H^+$$

In general, charges should not be shown in the reactions, and in particular H^+ should be not shown as a reactant or product. The reasons for this are discussed elsewhere (Alberty et al., 2011), and by Goldberg in his contribution to this special issue. Briefly, it is not appropriate to write specific ionic forms for species that exist as equilibrium mixtures of different ions, especially as one may sometimes not know which ionic forms actually participate in a reaction. This principle was followed scrupulously in the original Report (IUB, 1961), which showed no charges at all but over the years it became increasingly diluted. Taking alcohol dehydrogenase (EC 1.1.1.1) as an example, this was listed in the Report as

Alcohol + NAD = aldehyde or ketone + $NADH_2$

By the time of the last printed edition of Enzyme Nomenclature[15] this had become

An alcohol + NAD^+ = an aldehyde or ketone + NADH

[12]Most of the usual journals that publish information about newly discovered enzymes would not allow that anyway, but authors should themselves be conscious of the prohibition.

[13]If an activity is reinstated after having previously been deleted, as happened, for example, with EC 1.1.1.149, the original number is usually also reinstated.

[14]http://www.enzyme-database.org/index.php.

[15]These are the addresses in use in 2012. They may change in the future, but it will probably be possible to find the new ones by searching for "enzyme nomenclature".

and in the present list it is

(1) A primary alcohol + NAD^+ = an aldehyde + NADH + H^+
(2) A secondary alcohol + NAD^+ = a ketone + NADH + H^+

Some of the changes reflect increased knowledge of the specificity of the enzyme. In addition, although the original Report used and recommended the symbols NAD and $NADH_2$ for the oxidized and reduced forms respectively of the coenzyme, they also suggested NAD^+ and NADH respectively as alternatives. This latter system has the advantage that it allows the plain symbol NAD to refer to the two forms collectively, but it has the disadvantage that it assigns a + superscript to what is in reality an anion. In practice the system with NAD^+ and NADH has become overwhelmingly the most used, and when it became adopted in Enzyme Nomenclature there was a feeling that the equation looked unbalanced with unequal charges on the left and right-hand sides. In what Alberty in particular considered as a misguided move, this was then "corrected" by including protons in equations. A suggested way to avoid the problem (Alberty and Cornish-Bowden, 1993), in which the two forms of coenzyme were to be written as NAD_{ox} and NAD_{red} has received no significant adoption in the literature.

As the entry for acetate kinase considered above is one of the simpler examples, with no comments or specificity information (with the implication that the enzyme catalyses that one reaction only) it is useful to examine a more typical entry:

	EC 2.7.1.1
Accepted name:	hexokinase
Reaction:	ATP + D-hexose = ADP + D-hexose 6-phosphate
Other name(s):	hexokinase type IV, glucokinase; hexokinase D; hexokinase type IV; hexokinase (phosphorylating); ATP-dependent hexokinase; glucose ATP phosphotransferase
Comments:	D-Glucose, D-mannose, D-fructose, sorbitol and D-glucosamine can act as acceptors; ITP and dATP can act as donors. The liver isoenzyme has sometimes been called glucokinase.
Systematic name:	ATP:D-hexose 6-phosphotransferase
Links to other databases:	BRENDA, EXPASY, GTD, IUBMB, KEGG, METACYC, PDB, UM-BBD, CAS registry number: 9001-51-8
References:	1. Bailey, K. andWebb, E.C. Purification of yeast hexokinase and its reaction with ββ′-dichlorodiethyl sulphide. *Biochem. J.* **42** (1948) 60–68. [PMID: 16748250]. 2. Berger, L., Slein, M.W., Colowick, S.P. and Cori, C.F. Isolation of hexokinase from baker's yeast. *J. Gen. Physiol.* **29** (1946) 379–391. 3. Kunitz, M. and McDonald, M.R. Crystalline hexokinase (heterophosphatase). Method of isolation and properties. *J. Gen. Physiol.* **29** (1946) 393–412. 4. Pollard-Knight, D. and Cornish-Bowden, A. Mechanism of liver glucokinase. *Mol. Cell.* Biochem. **44** (1982) 71–80. [PMID: 7048063]. 5. Ureta, T., Radojković, J., Lagos, R., Guixé, V. and Núñez, L. Phylogenetic and ontogenetic studies of glucose phosphorylating isozymes of vertebrates. *Arch. Biol. Med. Exp.* **12** (1979) 587–604. [PMID: 233226]. 6. Cárdenas, M.L., Rabajille, E. and Niemeyer, H. Fructose: A good substrate for rat-liver 'glucokinase' (hexokinase D). *Biochem. J.* **222** (1984) 363–370. [PMID: 6477520]

In this example the purpose of the comments and additional references is to provide information about the specificity of hexokinase, and the other names illustrate the variety of names that have been used. In other cases the comments may indicate, for example, that a particular enzyme is a flavoprotein or that it requires Zn^+, or they may mention the variations in specificity found in different organisms. The list of other names of hexokinase hints ("type IV") at the variety of isoenzymes known. However, it is hardly practical to deal with isoenzymes in any systematic way, not only because of the great increase in complexity of the list as a whole that it would entail, but also because nature itself is not systematic. Although all vertebrates contain hexokinase, and all known vertebrates contain isoenzymes of hexokinase, there is great variation, even between similar species, in the particular isoenzymes present. This type of complexity is best dealt with by supplying a suitable reference, in this case to Ref. 5 of the list.

Reporting a new enzyme activity

As already noted, classification and definitive naming of new enzyme activities is the task of the Nomenclature Committee of the IUBMB. A certain proportion of new entries result from searches of the literature by the members of the Committee or by people involved in compiling databases such as BRENDA (Scheer et al., 2011). However, it is obviously more efficient if new activities are directly reported by the researchers who discover and publish them, using the form at http://www.enzyme-database.org/newform.php. Likewise researchers who find errors or omissions in existing entries can report them on the form at http://www.enzyme-database.org/updateform.php.[16]

The information requested for a new enzyme activity is as follows:

- *Proposed sub-subclass* (e.g. EC 1.2.3.-). Note that there is no field for the fourth number, which should not be suggested.

[16]In 2014 the Nomenclature Committee of the IUBMB has the following members: K. Axelsen (Switzerland), R. Cammack (UK), A. McDonald (Ireland), G. P. Moss (UK, Chairman), I. Schomburg (Germany), K. F. Tipton (Ireland). It always meets jointly and works closely with the IUPAC-IUBMB Joint Commission on Biochemical Nomenclature, which provides the following additional members: R. Caspi (USA), M. Ennis (UK). Associate members of one or other committee are as follows: A. Cornish-Bowden (France), T. Damhus (Denmark), K.-H. Hellwich (Germany), D. Horton (USA), A. P. Rauter (Portugal), J. F. G. Vliegenthart (The Netherlands). Coordination with database curators is provided by R. Apweiler (UNIPROT, UK), H. Berman (wwPDB, USA), M. Kotera (KEGG, Japan), D. Schomburg (Germany, BRENDA).

- *Accepted name*, i.e. the name used by the authors in their publications.
- *Synonyms*, i.e. names used by other authors, if any.
- *Reaction catalysed* (**required**). This should be written as a chemically balanced equation if possible.
- *Cofactor requirements*.
- *Brief comment on specificity*.
- *Other comment*.
- *References*. These should include the details required for most journal articles today (title of paper; names of all authors, with initials; name or standard abbreviation of the journal; volume number; start and end pages; year; PMID number, if available).

Although the reaction catalysed is the only required item, in practice at least one reference should be given, and suggested entries are only likely to be accepted if they are supported by at least one paper that is published or in press ("in preparation", "submitted for publication", "personal communication", etc. are unlikely to be acceptable). Cofactor and specificity information should also be included if they are appropriate. In addition to information about the enzyme, contact details for the person suggesting the entry are also required:

- Name (**required**)
- Address (**required**)
- Telephone
- Fax
- E-mail (**required**)

Published work cited should be submitted with the suggested entry. This can be done either by sending hard copies by post, or by attaching PDF files to e-mail messages. The addresses are given on the form, and at present are Andrew McDonald, Department of Biochemistry, Trinity College, Dublin 2, Ireland; fax: +353-1-6772400; e-mail: amcdonld@tcd.ie.

Reporting errors or omissions in existing entries

The form for reporting an error or suggesting a revision in an existing entry asks for the following information:

(*EC number*, e.g. EC 1.2.3.4). In this case the complete four-part number is to be given as the change refers to an enzyme that has already been listed.

Description of the error or update. This is required, because without this information the Committee cannot proceed.

References. These should include the details required for most journal articles today (title of paper; names of **all** authors, with initials; name or standard abbreviation of the journal; volume number; start and end pages; year; PMID number, if available).

Supporting publications and contact information are required exactly as for a new enzyme.

General remarks on enzyme classification

As the system for classifying enzymes has been continuously revised and updated since it was first set up in 1960, it has remained far more in tune with current research than the recommendations on enzyme kinetics have done, and the present web-based system for proposing new entries works very smoothly at present. Some hundreds of new entries are added every year. Nonetheless, researchers should be conscious that any expert on a particular enzyme is likely to know far more about it than any member of the Nomenclature Committee can know, and is therefore well placed to notice and correct errors and omissions in the list. The future health of the classification system must depend in part on the willingness of biochemists to communicate new information and to correct errors in old information.

Conflict of interest statement

The author has no conflict of interest.

References

Alberty, R.A., 1956. Enzyme kinetics. Adv. Enzymol. 17, 1–64.

Alberty, R.A., Cornish-Bowden, A., 1993. The pH dependence of the apparent equilibrium constant, K′, of a biochemical reaction. Trends Biochem. Sci. 18, 288–289.

Alberty, R.A., Cornish-Bowden, A., Goldberg, R.N., Hammes, G.G., Tipton, K., Westerhoff, H.V., 2011. Recommendations for terminology and databases for biochemical thermodynamics. Biophys. Chem. 155 (2–3), 89–103.

Bisswanger, H., 2002. Enzyme Kinetics: Principles and Methods. Wiley-VCH, New York.

Cleland, W.W., 1963. The kinetics of enzyme-catalyzed reactions with two or more substrates or products. I. Nomenclature and rate equations. Biochim. Biophys. Acta 67, 104–137.

Cook, P.F., Cleland, W.W., 2007. Enzyme Kinetics and Mechanism. Garland Science, London.

Copeland, R.A., 2000. Enzymes: A Practical Introduction to Structure, Mechanism and Data Analysis, 2 edition Wiley-VCH, New York.

Cornish-Bowden, A., 2012. Fundamentals of Enzyme Kinetics, 4 edition Wiley–VCH, Weinheim.

Dalziel, K., 1957. Initial steady state velocities in the evaluation of enzyme-substrate reaction mechanisms. Acta Chem. Scand. 11, 1706–1723.

Dixon, M., Webb, E.C., 1958. Enzymes. Longmans, Green & Co, London.

Dixon, M., Webb, E.C., 1979. Enzymes, 3 edition Longmans, Green & Co, London.

Fersht, A.R., 1977. Enzyme Structure and Mechanism. Freeman, Reading and San Francisco.

Fersht, A., 1999. Structure and Mechanism in Protein Science. Freeman, New York.

Hoffmann-Ostenhof, O., 1953. Suggestions for a more rational classification and nomenclature of enzymes. Adv. Enzymol. Relat. Subj. Biochem. 14, 219–260.

International Union of Biochemistry, 1982. Symbolism and terminology in enzyme kinetics. Recommendations 1981. Eur. J. Biochem. 128, 281–291.

International Union of Biochemistry, 1983a. Symbolism and terminology in enzyme kinetics. Recommendations 1981. Biochem. J. 213, 561–571.

International Union of Biochemistry, 1983b. Symbolism and terminology in enzyme kinetics. Recommendations 1981. Arch. Biochem. Biophys. 224, 732–740.
International Union of Biochemistry, 1961. Report of the Commission on Enzymes. Pergamon Press, Oxford.
International Union of Biochemistry and Molecular Biology, 1992a. Biochemical nomenclature and related documents. Recommendations 1981. Portland Press, London.
International Union of Biochemistry and Molecular Biology, 1992b. Enzyme Nomenclature. Academic Press, San Diego.
International Union of Pure and Applied Chemistry, 1981. Symbolism and terminology in chemical kinetics, 1980. Pure Appl. Chem. 53, 753–771.
International Union of Biochemistry, 1979. Units of enzyme activity, Recommendations 1978. Eur. J. Biochem. 97, 319–320.
International Union of Biochemistry, 1980. Units of enzyme activity, Recommendations 1978. Corrections and editorial comment. Eur. J. Biochem. 104, 1.
Mahler, H.R., Cordes, E.H., 1966. Biological Chemistry. Harper and Row, New York.
Marabotti, A., Facchiano, A., 2009. When it comes to homology, bad habits die hard. Trends Biochem. Sci. 34, 98–99.
Marangoni, A.G., 2002. Enzyme Kinetics: A Modern Approach. John Wiley and Sons, Hoboken, NJ.
Metzler, E.D., 1980. A reply to P. Karlson's editorial in Biochemical Education. Biochem. Educ. 8, 118.
Rawlings, N.D., Barrett, A.J., Batemanm, A., 2012. MEROPS: the database of proteolytic enzymes, their substrates and inhibitors. Nucleic Acids Res. 40, D343–D350.
Reeck, G.R, de Haën, C., Teller, D.C, Doolittle, R.F., Fitch, W.M., Dickerson, R.E., Chambon, P., McLachlan, A.D., Margoliash, E., Jukes, T.H., Zuckerkandl, E, 1987. 'Homology' in proteins and nucleic acids: a terminology muddle and a way out of it. Cell 50, 667.
Roberts, R.J., Vincze, T., Posfai, J., Macelis, D., 2010. REBASE—a database for DNA restriction and modification: enzymes, genes and genomes. Nucleic Acids Res. 38, D234–D236.
Scheer, M., Grote, A., Chang, A., Schomburg, I., Munaretto, C., Rother, M., Soehngen, C., Stelzer, M., Thiele, J., Schomburg, D., 2011. BRENDA, the enzyme information system in 2011. Nucleic Acids Res. 39, D670–D676.
Schomburg, I., Chang, A., Schomburg, D., 2014. Standardization in enzymology-Data integration in the world's enzyme iInformation system BRENDA. Perspec. Sci. 1, 15–23.
Tipton, Keith, Armstrong, Richard N., Bakker, Barbara, Bairoch, Amos, Cornish-Bowden, Athel, Halling, Peter, Hofmeyr, Jan-Hendrik, Leyh, Thomas S., Kettner, Carsten, Raushel, Frank M., Rohwer, Johann, Schomburg, Dietmar, Steinbeck, Christoph, 2014. Standards for reporting enzyme data: the STRENDA consortium: what it aims to do and why it should be helpful. Perspect. Sci. 1, 131–137.