GENERALIZED DEFINITIONS OF INHIBITION PATTERNS FOR NON-MICHAELIAN ENZYME STEADY-STATE KINETICS

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1. Introduction

The finding that the steady-state kinetics of many enzymes studied in our laboratory [1–5] and elsewhere (cf. [6,7]) cannot be described by the Michaelis-Menten equation has called for a generalization of the nomenclature of inhibition patterns of reversible inhibitors. The problem has been whether the competitive, noncompetitive and uncompetitive (or anticompetitive) inhibition can be defined for cases in which rate equations contain second- or higher-degree terms in substrate concentration. The present paper gives such definitions which yield the inhibition patterns for the simple Michaelis-Menten equation (as defined [8]) as limiting cases. These generalized definitions have been applied in the analysis of the kinetics of, e.g., glutathione *S*-transferase A from rat liver [5,9]. They can also be used to describe the interaction of two ligands in an equation of ligand binding.

2. Theory

2.1. *Rate equations*

Rate equations for the steady state can be derived by the structural rule [10,11] and analyzed in coefficient form. The coefficients are composed of rate constants and (in some terms) nonvaried reactants. When the values of the coefficients are not subject to study the coefficients for reactant-containing terms can, for simplicity, be omitted from the equations. Thus, the classical Michaelis-Menten equation could be presented:

$$v = \frac{A}{K + A}$$

where A symbolizes a term in substrate concentration and K a constant term. Rate equations have to be used in complete form in regression analysis, the coefficients being used as parameters in the computer program (see [5]), but the above shorthand notation emphasized more clearly the algebraic structure in terms of reactant concentrations considered.

2.2. *Inhibition patterns for Michaelian kinetics*

Three distinct inhibition patterns can mathematically be defined for enzymes obeying Michaelis–Menten kinetics: competitive, noncompetitive, and uncompetitive (or anticompetitive). They can all be written as:

$$v = \frac{V\,[A]}{K_m \cdot f_1 + [A] \cdot f_2}$$

where f_1 and f_2 are functions of inhibitor concentration, $f([I])$, which at zero inhibitor concentration are equal to unity, $f(0) = 1$. The factor $f([I])$ can be any rational function of $[I]$ which can be derived from steady-state rate equations corresponding to reaction schemes considered for the enzymatic catalysis, for example:

$$\left(1 + \frac{[I]}{K_1}\right)$$

$$\left(1 + \frac{[I]}{K_1} + \frac{[I]^2}{K_2}\right)$$

$$\text{or} \left(\frac{1 + K_1 [I]}{1 + K_2 [I]}\right)$$

where K_i ($i = 1,2$) are constants. Competitive inhibition obtains when $f_2 \equiv 1$ (for all values of $[I]$), uncompetitive (anticompetitive) inhibition when $f_1 \equiv 1$, and noncompetitive inhibition when both f_1 and f_2 change with $[I]$. 'Classical' ('pure') noncompetitive inhibition obtains when $f_1 = f_2$. Thus, the three kinds of inhibition, defined [8], can be described as effects on the denominator of the Michaelis-Menten equation. In competitive inhibition the constant term (consisting of constants and non-varied substrate concentrations) is affected, in uncompetitive inhibition the varied substrate term is affected, and in noncompetitive inhibition both terms are affected. These formulations of the inhibition types are independent of graphical representation of the experimental data, whereas the definitions [8] are based on the double-reciprocal plot [12], which several investigators normally prefer not to use [13–15].

2.3. *Inhibition patterns for non-Michaelian kinetics*

The literature does not seem to contain any previous generalization of the nomenclature of inhibition to rate equations which contain second- or higher-degree terms in substrate concentration.

(i) The principle of competitive inhibition, viz., cancellation of the effect of the inhibitor at high substrate concentrations, is most readily generalized as expressed in the following equation:

$$v = \frac{\left(\sum_{j=0}^{h} \sum_{i=1}^{m-1} A^i I^j\right) + A^m}{\left(\sum_{j=0}^{k} \sum_{i=0}^{n-1} A^i I^j\right) + A^n}; \quad \begin{matrix}(m \leq n)\\(h \leq k)\end{matrix}$$

The coefficients (not written out) for some of the $A^i I^j$ terms may be zero and in this case these terms vanish. A simple example which is second-degree in substrate concentration is:

$$v = \frac{A + A^2 + AI}{K + A + A^2 + I + I^2 + AI}$$

(ii) Noncompetitive inhibition in the generalized sense implies that the inhibition cannot be overcome by substrate in any concentration range:

$$v = \frac{\sum_{j=0}^{h} \sum_{i=1}^{m} A^i I^j}{\sum_{j=0}^{k} \sum_{i=0}^{n} A^i I^j}; \quad \begin{matrix}(m \leq n)\\(h \leq k)\end{matrix}$$

In this equation at least one of the highest-degree terms $A^m I^j$ ($j = 1, \ldots, h$) or one of $A^n I^j$ ($j = 1, \ldots, k$) must be nonvanishing, that is:

$$\sum_{j=1}^{h} A^m I^j \neq 0$$

$$\text{or} \sum_{j=1}^{k} A^n I^j \neq 0$$

and the sum of substrate-free terms:

$$\sum_{j=1}^{k} A^0 I^j \neq 0$$

A simple example which is second degree in substrate concentration is:

$$v = \frac{A + A^2 + AI + A^2 I}{K + A + A^2 + I + I^2 + AI + A^2 I}$$

(iii) The principle of uncompetitive inhibition is that the inhibition can be overcome by lowering the substrate concentration. Thus, I-containing terms must always contain the substrate concentration as a factor and the A-terms of lowest degree (i.e., normally A terms of the numerator and constant terms of the denominator) must not contain I as a factor:

$$v = \frac{\left(\sum_{j=0}^{h} \sum_{i=2}^{m} A^i I^j\right) + A}{\left(\sum_{j=0}^{k} \sum_{i=1}^{n} A^i I^j\right) + K}; \quad \begin{matrix}(m \leq n)\\(h \leq k)\end{matrix}$$

an example is:

$$v = \frac{A + A^2 + A^2 I}{K + A + A^2 + AI + A^2 I}$$

3. Discussion

The generalized definitions proposed in the present paper are intuitively consonant with the previous nomenclature for Michaelian enzyme kinetics and contain the latter as a special case. It should also be noted that the nomenclature is generally applicable for description of the interaction of any two ligands A and I (substrates, inhibitors, activators etc.) which can be described by a rate or binding equation. Illustrative cases from our laboratory of generalized interaction (inhibition) patterns are: competitive – fig.1 in [2], fig.3,4 in [3] (alternative-substrates kinetics), fig.1 in [9] (two inhibitors); noncompetitive – fig.4 in [5]. From the experimental point of view it is clear that the distinction of competitive and uncompetitive from noncompetitive interactions is based on the asymptotic properties of the equations at high and low concentrations, respectively, of the varied reactant. Therefore the concentrations should be varied over as wide ranges as possible. The analysis of experimental data can in many cases be made graphically, even if the curves are nonlinear, but for an objective discrimination between alternative mathematical models the use of regression analysis [16,17] is of great assistance.

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