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Computer-Based Modeling in the Teaching of Steady-State Enzyme Kinetics

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Received August 15, 1975

Equations are derived for the steady-state treatment of enzyme reactions with one type of inhibitor, consisting of one reaction cycle or two connected cycles. Computerized simulation programs are described which are designed to acquaint the student thoroughly with the behavior of enzyme systems. The user has freedom in the choice of parameters for the system, including up to three product-producing rate constants for the two-cycle system.

INTRODUCTION

Steady-state enzyme kinetics has been known since the original derivations of Michaelis and Menten.[1] The theory was refined later by Haldane.[2] However, some early thoughts on steady-state kinetics go back to studies by Henri.[3] In presentations of steady-state kinetics the educator generally follows rather closely the derivations of Michaelis and Menten, referring to a one-site enzyme with one substrate. The various types of inhibitions are subsequently added, and such treatments frequently terminate presentations in steady-state kinetics. We shall start our presentation with a cyclic scheme of four steps. Although this derivation is somewhat more difficult than that of the simple Michaelis–Menten scheme, the various types of inhibition can quickly be derived as limiting conditions among the various constants in the cyclic scheme. Such a derivation is intellectually much more satisfactory than one starting with a very simple derivation.

To introduce the concepts of steady-state kinetics more effectively, three lessons in Computer-Aided-Instruction were written which may be used by any student with access to a cathode-ray terminal, which allows addressing of coordinate locations. We used the Hazeltine 2000 video terminal. The first lesson introduces basic concepts of steady-state kinetics, the second lesson introduces those of inhibition, and the third one allows simulation of bell-shaped curves and permits input of parameter values by the student.

A special system was designed to produce easily bell-shaped curves of enzyme activity. The system consists of two cycles of four steps each, containing three different enzyme–substrate complexes, all of which may decompose into the same product. A simulation program was written, allowing the student considerable flexibility in the choice of the value of various parameters. This way he should obtain a good understanding of the relationship between changes in various parameters of the system and resulting curves, which may relate to results on specific enzyme systems. Only two types of plots are available, either with substrate concentration as abscissa or with inhibitor concentration as abscissa. The other concentration is then used as the "fixed" parameter allowing one to submit three different values, thus producing a group of three curves.

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I. UNDERLYING THEORY FOR "PLANAR" SCHEMES

Most textbooks on biochemistry now present the original derivations of Michaelis and Menten, sometimes modified in one way or another. Such derivations are treated in a rather thorough manner in a monograph by Reiner.[4] They will not be repeated here, although they are actually utilized in two of the three lessons in Computer-Aided-Instruction. The concepts and equations are directly introduced in these lessons, as will be discussed in the next section.

A comparatively elegant derivation of the simple enzyme system with the various types of inhibitions may be presented by using the cyclic scheme shown in Figure 1 with the additional assumption that $k_0 = 0$. In the presented scheme, E denotes enzyme, I the inhibitor, and S the substrate. Only ES, the binary enzyme–substrate complex, is initially considered to generate product. All even-subscripted rate constants k_i (as well as k_9 and k_0) are monomolecular; all other are bimolecular "constants". With $C_E^0 << C_I^0, S_S^0$, there is also the general assumption

$$k_3 C_I^0, k_7 C_I^0, k_4, k_8 >> k_2, k_9 \quad (1)$$

The adjustment between EI and E does not need to be considered as it is controlled by the fast steps with the inhibitor because of the scheme's cyclic nature. (However, k_2 in the Michaelis constant could become k_6, if $k_6 >> k_2$; also more complex intermediate cases may take place.)

For the general case one needs the equations

$$C_E^0 = [E] + [EI] + [ES] + [EIS] \quad (2)$$

$$C_I^0 = [I] + [EI] + [EIS] \quad (3)$$

$$C_S^0 = [S] + [ES] + [EIS] \quad (4)$$

[EIS]: E binds I and S; no preferred order is implied in this representation. The last two terms in eq 3 and 4 are negligibly

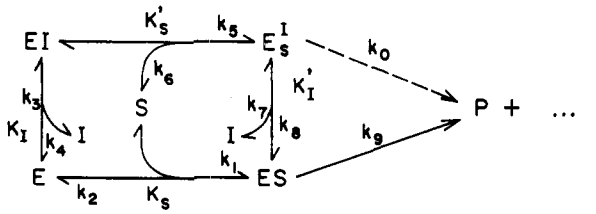

Figure 1. The planar "quadratic" scheme, containing the definitions of the rate constants (lower case letters) and the dissociation constants (upper case letters). Letter S denotes substrate, E enzyme, I inhibitor, and P product.

small because of the condition:

$$C_E^0 << C_I^0, C_S^0 \tag{5}$$

Furthermore, the following differential equations apply:

$$d[ES]/dt = k_1[E][S] + k_8[EIS] - [ES](k_2 + k_9 + k_7[I]) \tag{6}$$

$$d[P]/dt = k_9[ES] \tag{7}$$

The preequilibrium condition[5] yields, very shortly after mixing:

$$\frac{d[E]}{dt} = 0 = k_4[EI] - k_3[E][I] \text{ or } K_I \equiv \frac{k_4}{k_3} = \frac{[E][I]}{[EI]} \tag{8}$$

$$\frac{d[ES]}{dt} = 0 = k_8[EIS] - k_7[ES][I] \text{ or } K_I' \equiv \frac{k_8}{k_7} = \frac{[ES][I]}{[EIS]} \tag{9}$$

Equation 2 simplifies with relations 5, 8, and 9 to:

$$C_E^0 = [E](1 + C_I^0/K_I) + [ES](1 + C_I^0/K_I') \tag{10}$$

Equation 6 may now be combined with eq 10 and eq 9 (to express EIS in terms of ES) giving:

$$\frac{d[ES]}{dt} = k_1 C_S^0 \frac{C_E^0 - [ES](1 + C_I^0 K_I')}{1 + C_I^0/K_I} - [ES](k_9 + k_2 + k_7 C_I^0 - k_8 C_I^0/K_I') \tag{11}$$

$$\frac{d[ES]}{dt} = 0 = k_1 C_S^0 \frac{C_E^0}{1 + C_I^0/K_I} - [ES]\left(k_1 C_S^0 \frac{1 + C_I^0/K_I'}{1 + C_I^0/K_I} + k_9 + k_2\right) \tag{12}$$

or

$$[ES] = \frac{k_1 C_S^0 C_E^0/(1 + C_I^0/K_I)}{k_1 C_S^0 \frac{1 + C_I^0/K_I'}{1 + C_I^0/K_I} + k_2 + k_9} \tag{13}$$

Equation 4 thus becomes:

$$(d[P]/dt)_{t \to 0} \equiv V = k_9 C_E^0 (1 + C_I^0/K_I' + (1 + C_I^0/K_I) K_M/C_S^0)^{-1} \tag{14}$$

with

$$K_M = (k_2 + k_9)/k_1 \tag{15}$$

Inversion and introduction of

$$V_{max} \equiv k_9 C_E^0 \tag{16}$$

give:

$$\frac{1}{V} = \frac{1}{V_{max}}\left(1 + \frac{C_I^0}{K_I'} + \frac{K_M(1 + C_I^0 K_I)}{C_S^0}\right) \tag{17}$$

Other forms of this equation are

$$\frac{1}{V} = \frac{1 + C_I^0/K_I}{V_{max}}\left(1 + \frac{K_M}{C_S^0}\frac{1 + C_I^0/K_I}{1 + C_I^0/K_I'}\right) \tag{18}$$

$$\frac{1}{V} = \frac{1 + C_I^0/K_I}{V_{max}}\left(\frac{1 + C_I^0/K_I}{1 + C_I^0/K_I} + \frac{K_M}{C_S^0}\right) \tag{19}$$

Equilibrium constants are also shown in the scheme of Figure 1 with two definitions given in eq 8 and 9. Furthermore:

$$K_S \equiv k_2/k_1 = [E][S]/[ES] \tag{20}$$

$$K_S' \equiv k_6/k_5 = [EI][S]/[EIS] \tag{21}$$

Before proceeding to special cases, three convenient definitions should be introduced:

$k_1 < k_2$ is to mean k_1 "smaller than" k_2 (within factor 10)

$k_1 << k_2$ is to mean k_1 "much smaller than" k_2 (factor 10 to 100)

$k_1 <<< k_2$ is to mean k_1 "very much smaller than" k_2 (factor ~1000 or more, permitting *neglect* of either k_1 *or* k_2, depending upon treatment)

SPECIAL CASES

(A) $K_I' = K_I$: eq 19 becomes

$$\frac{1}{V} = \frac{1 + C_I^0/K_I}{V_{max}}\left(1 + \frac{K_M}{C_S^0}\right) \tag{22}$$

In a plot of $1/V$ vs. $1/C_S^0$ both slope and intercept change with C_I^0 in the same manner: definition of noncompetitive inhibition, abscissa – *intercept* constants.

(B) $K_S <<< K_S'$ *with* $C_S^0 << K_S'$ for all C_S^0; for "competition" to be competition, also $K_I = K_S$ *or* $K_I < K_S$ *or* $K_I > K_S$ (in other words, these constants are generally not too far apart). It follows then for the preequilibrium cycle: $K_I K_S' = K_S K_I'$, or $K_I <<< K_I'$ *with* $C_I^0 << K_I'$ for all C_I^0.

Result: competitive inhibition with ignoring K_S' and K_I'. Equations 17, 18, and 19 no longer contain K_S' (as it may be expressed in terms of the other three dissociation constants). Equation 17 leads directly to the final result for $K_I' \to \infty$:

$$\frac{1}{V} = \frac{1}{V_{max}}\left[1 + \frac{K_M}{C_S^0}\left(1 + \frac{C_I^0}{K_I}\right)\right] \tag{23}$$

In a plot of $1/V$ vs. $1/C_S^0$ (with C_I^0 as critical parameter), the intercept with the ordinate remains unchanged, while the slope changes (and, thus the intercept with the abscissa does, as well): ideal competitive inhibitor. Figure 4 shows a computer-generated plot, also known as Lineweaver–Burk plot.[6]

(C) One case remains: $K_S >>> K_S'$, now together with $C_S^0 >> K_S'$ for all C_S^0; however, an *equivalent* case is more suitable, which may also be called a subcase of the previous one. There, K_I was in the range of K_S; now, K_I' is considered to be in the range of K_S. One derives from $K_S K_I' = K_I K_S'$ that a *very* large K_I (relative to K_S and K_I') requires a *very* small K_S': [EI] is practically nonexistent! The result $K_I >>> K_S'$, $K_I' >>> K_S'$ means in effect (1) that E practically does not combine with I, and (2) that the ternary complex practically does not dissociate into EI + S (but certainty into ES + I).

Equation 17 becomes for $K_I \to \infty$:

$$\frac{1}{V} = \frac{1}{V_{max}}\left(1 + \frac{C_I^0}{K_I'}\right) + \frac{K_M}{C_S^0} \tag{24}$$

In a plot of $1/V$ vs. $1/C_S^0$ only the intercept with the abscissa (and ordinate as well) changes; the slope remains constant, defining uncompetitive inhibition.

Intermediate cases can easily be derived from eq 17, 18, or 19, optimally visualized by computer simulation (using as key parameters K_I/K_S and K_I'/K_S). The case $k_0 > 0$ may be considered a "subcase" of the "bicyclic" scheme and therefore does not need to be discussed now.

A bell-shaped curve is most easily obtained with the bicyclic reaction scheme, shown in Figure 2. The definitions in the scheme have practically no relation to those of the cyclic

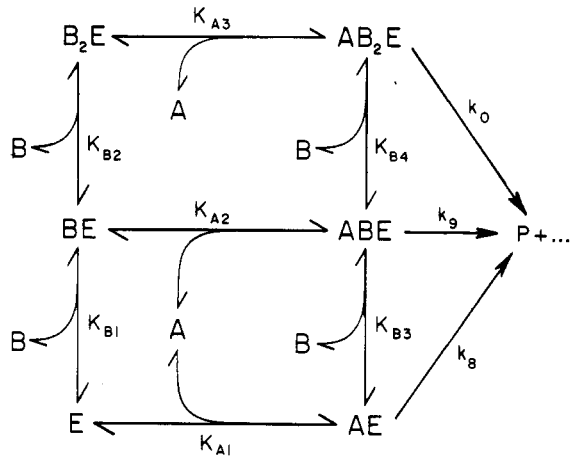

Figure 2. The planar "double-quadratic" scheme, containing the same kinds of definitions, listed for Figure 1, but with A as (generalized) substrate and B as (generalized) effector (may be inhibitor or activator).

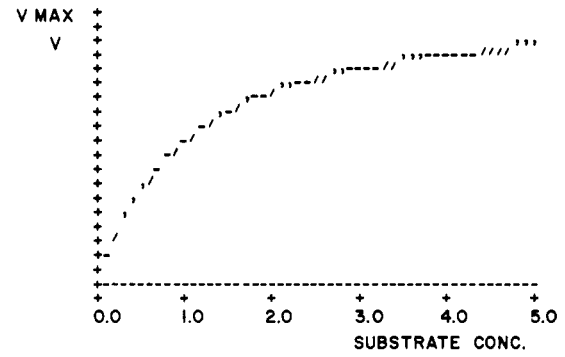

Figure 3. Output from the first CAI lesson, introducing basic concepts of steady-state kinetics. The presented curve is used to introduce V_{max} and also K_M. Values are internally normalized.

scheme. The definitions in Figure 2 were set up in order to relate easily to schemes discussed later in a separate section. In the initial derivation, two of the three product generating rate constants will be set at zero. One then obtains directly the "ideal" bell-shaped curve (when only ABE generates product). This equation is utilized in the Computer-Aided-Instruction lesson for bell-shaped curves. The user is also given the option of eliminating one of the two cycles to thereby treat the much simpler "monocyclic" system of Figure 1.

For the general case, one may derive from the scheme of Figure 2 the following differential equation:

$$d[P]/dt = k_8[AE] + k_9[ABE] + k_0[AB_2E] \quad (25)$$

One may now either proceed directly or first set any two of the three rate constants at zero. The latter derivation leads to a result, which is already similar in structure to the general solution. The derivation implies that the B-binding steps are all in preequilibrium and the A-binding steps are adequately rapid. One may write for the total concentration of enzyme an expression containing six terms. There are four equations given by the dissociation constants K_B's. The expression for the total enzyme concentration may then be reduced to two terms, both containing only one concentration of an enzyme-containing component. Next, one writes the equation of the time change of the complex with enzyme which generates product (compare eq 25). This equation contains three kinetic terms and is zero under the conditions of the steady state. This equation allows the solution of the system, leading to an expression for the concentration of the product-generating enzyme complex, which may then directly be utilized in the equation for the appearance of product. One arrives easily

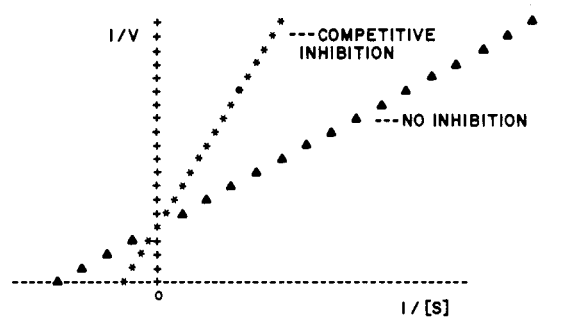

Figure 4. Double reciprocal plot of $1/V$ vs. $1/[S]$, introducing the concept of competitive inhibition.

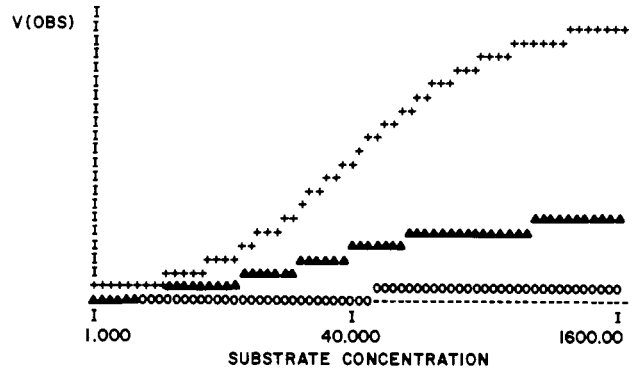

Figure 5. A plot of V(obsd) vs. substrate concentration (normalized coordinates) for the "double-quadratic" scheme of Figure 2. Equation 26 applies with $k_8 = k_0 = 0$ and $k_9 = 10$ (reciprocal time units); furthermore, $K_{B_1} = 5.0$ μM; $K_{B_2} = 50.0$ μM; $K_{B_3} = 10.0$ μM; $K_{B_4} = 100.0$ μM; $K_{A_2} = 40.0$ μM. The parameter $C_B{}^0$ (initial inhibitor concentration) assumes the values [MAX]/100, [MAX]/10, [MAX] concentration units, denoted by +, 0, ▲, respectively. [MAX] = 20 μM was chosen.

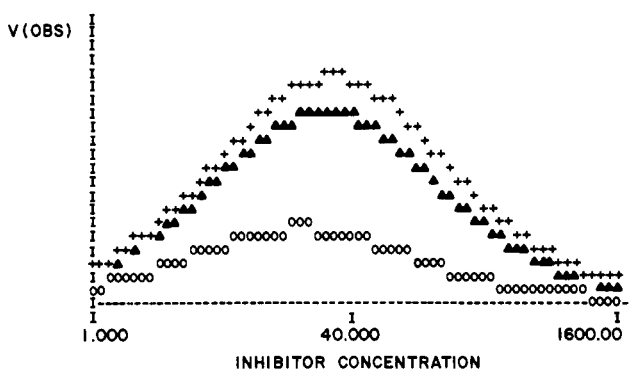

Figure 6. A plot of V(obsd) vs. inhibitor concentration (normalized coordinates) for the "double-quadratic" scheme with the parameter $C_A{}^0$ (initial substrate concentration) assuming the values [MAX]/100, [MAX]/10, [MAX] concentration units, denoted by +, 0, ▲, respectively. The various constants have the values, already given in Figure 5. [MAX] = 2000 μM was chosen.

at the final result for the initial rate of the appearance of product, V (eq 26). This equation may be utilized as is or

$$V = k_8 C_E{}^0 \times \left[\left(1 + \frac{K_{B_3}}{C_B{}^0} + \frac{C_B{}^0}{K_{B_4}}\right)\frac{C_B{}^0}{K_{B_3}} + \left(1 + \frac{K_{B_1}}{C_B{}^0} + \frac{C_B{}^0}{K_B}\right)\frac{C_B{}^0}{K_B}\frac{K_{A_1}}{C_A{}^0}\right]^{-1} +$$
$$k_9 C_E{}^0\left[\left(1 + \frac{K_{B_3}}{C_B{}^0} + \frac{C_B{}^0}{K_{B_4}}\right) + \left(1 + \frac{K_{B_1}}{C_B{}^0} + \frac{C_B{}^0}{K_{B_2}}\right)\frac{K_{A_2}}{C_A{}^0}\right]^{-1} +$$
$$k_0 C_E{}^0\left[\left(1 + \frac{K_{B_3}}{C_B{}^0} + \frac{C_B{}^0}{K_{B_4}}\right)\frac{K_{B_4}}{C_B{}^0} + \left(1 + \frac{K_{B_1}}{C_B{}^0} + \frac{C_B{}^0}{K_{B_2}}\right)\frac{K_{B_4}}{C_B{}^0}\frac{K_{A_3}}{C_A{}^0}\right]^{-1} \quad (26)$$

simplified in any manner. The greatest simplifications are obtained if two of the three product-generating rate constants

are zero: eq 26 reduces to about one-third of its original length.

II. COMPUTER SIMULATION OF STEADY-STATE KINETICS

Three lessons in Computer-Aided-Instruction are offered to the students. The first lesson introduces the concept of basic steady-state kinetics and shows the student the steady-state treatment of Michaelis and Menten as well as the various forms of the equations. A typical output from this lesson is given in Figure 3. The second "lesson" concentrates on inhibition of steady-state enzyme kinetics and attempts to bring the basic concepts to the full attention of the student. Various plots are produced, allowing student's inference. One such plot is presented in Figure 4.

The use of the bicyclic scheme allows not only the use of a single cycle, but provides also rather extensive freedom in the choice of values for the constants and analytical concentrations. The user may also choose various values for the rate constants, which generate the product. Two types of curves are available to the user, presented in Figures 5 and 6.

ACKNOWLEDGMENT

The technical assistance of Byron Long and Julian Aguda is gratefully acknowledged.

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Wiswesser Line Notation Processing at Chemical Abstracts Service†

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Received October 16, 1975

A WLN data base has been created for 40,000 ring systems, representing 18,000 unique ring graphs identified by the CAS Chemical Registry System. The manual encoding effort is supported by a canonical WLN generation program for complex ring graphs. WLNs are automatically machine edited through syntax checks and by comparison of molecular formulas derived from the WLN with those in Registry Files.

INTRODUCTION

In 1968, Chemical Abstracts Service (CAS) began associating Wiswesser Line Notations[1] (WLNs) with ring systems by publishing a list[2] of WLNs for structures in "The Ring Index".[3] These notations were obtained from files made available by Elbert G. Smith, Professor of Chemistry, Mills College, and from tapes provided by the Dow Chemical Company. Since this initial effort, CAS has continued to generate WLNs for new ring systems being added to the ring file. The ring file has grown since 1968 to include almost 40,000 ring systems, representing 18,000 unique ring graphs identified by the CAS Chemical Registry System.[4] In order to generate WLNs for these ring systems efficiently and accurately, the manual encoding and editing effort is supported by the current version of the Registry System, Registry III, and by two computer programs. One program generates canonical WLNs for complex polycyclic structures, and an editing program automatically checks all WLNs added to the data base. As illustrated by Figure 1, this paper discusses the manual encoding procedure and the relevant computer support for processing WLNs at CAS.

IDENTIFICATION OF RING SYSTEMS

Registry III identifies ring systems within chemical substances to assist in the generation of substance names and to support structure display programs. A unique ring system identifier (RID), which is contained within the structure record for a substance, is assigned to each ring system. The RID is actually a composite identifier, as illustrated by Figure 2. Each unique ring graph (cyclic skeleton) is assigned a basic identifier, to which is appended an identifier for the ring atom variation, and also an identifier for the bond variation. The RID, therefore, uniquely identifies a ring system and can be used to associate related ring systems.

During substance registration, whenever Registry III identifies a ring system with a new ring graph, it assigns a new RID, adds the ring system to the file, and generates a New Ring Graph Alert message. If the ring system has a ring graph that is already in the file, but has a new heteroatom variation, then an RID is constructed using the previously assigned ring graph identifier with a new heteroatom identifier, the ring system is added to the file, and a New Ring System Alert message is generated. These new ring systems, identified by either message, are encoded into WLN by one of the following procedures.

RING SYSTEMS WITH COMPLEX RING GRAPHS

The canonical notation for a ring system is generated by describing the structural features of the ring graph according to the hierarchical requirements of WLN Rule 30.[5] The function of Rule 30 is to determine the preferred locant path that unambiguously describes the ring graph. The most difficult and time-consuming task in generating a WLN for a complex polycyclic structure is finding all the possible locant paths in order to choose the preferred path. Since this task is ideally suited for a computer, and since the number of complex ring graphs in the CAS ring file is large, it was necessary to develop computer support for this manual encoding effort.

Based on a program[6] written at the Dow Chemical

† Presented to the Division of Chemical Information, 170th National Meeting of the American Chemical Society, Chicago, Ill., Aug 25, 1975.

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