Review of: "Unlocking Complexity: The Versatility of Substrate Modulation Equations in Enzyme Analysis"

S. O. Karakhim[1]

1 Palladin Institute of Biochemistry

Potential competing interests: No potential competing interests to declare.

Manuscript Review

Title: Unlocking Complexity: The Versatility of Substrate Modulation Equations in Enzyme Analysis

Author: Ryan Walsh

Reviewer: S. Karakhim

The theory of enzyme-catalyzed reactions is well developed for cases where Michaelis-Menten type kinetics is observed, i.e., the dependence of the initial rate on substrate (or inhibitor, activator, modifier, effector) concentration is hyperbolic. In such cases, various methods of linearization of kinetic curves have long been used, which allows us to calculate kinetic constants such as the Michaelis constant and limiting rate (in most cases).

However, not all enzyme-catalyzed reactions are characterized by Michaelis-Menten kinetics. Various deviations from “classical” Michaelis-Menten kinetics are often observed, so that Hill, for example, was motivated to develop an alternative model for allosteric enzymes. In this model, just as in the Michaelis-Menten model, the initial rate reaches saturation at high substrate concentrations, but in a certain range of substrate concentrations, there is a much faster increase in the initial rate than in the Michaelis-Menten model.

Complex multistep enzyme-catalyzed reactions are usually characterized by even more complicated kinetics, when a maximum is formed on the kinetic curves. To treat such kinetics, it is no longer possible to use standard linearization methods to calculate kinetic constants (only for some models is the theory well developed, for example, for substrate inhibition and pH-dependence of the initial rate). Often for such reactions, it is not possible to derive the initial rate equation in an analytical form suitable for the treatment of experimental data. Naturally, in the absence of suitable processing methods, analysis of the kinetics of such reactions is practically not available. Therefore, in my opinion, any research that aims to enhance the understanding of such complex enzymatic processes should be welcomed.

In this article, the author presents a theoretical basis for the study of a rather complex mechanism of substrate modulation, which was presented and investigated in the article by Rodic et al. [1] and is analogous to the general modifier mechanism proposed by Botts and Morales (in particular, the mechanism of partial noncompetitive inhibition, for which α = 1), in which the substrate acts as a modifier.

For this particular mechanism, an initial velocity equation consisting of three factors was derived. This case is characterized by the fact that in these factors, there is a complete separation of the contributions determined by k_S and k_{SS}. The author denotes these constants as k_S and k_{SS}, but from my point of view, it is better to denote them as K_S and K_{SS} to emphasize that they are equilibrium constants (since equation (1) is derived in the equilibrium approximation (or under the assumption of fast equilibrium), the constants k_M (k_S) and k_{SS} have the meaning of equilibrium constants of the corresponding steps: in the general modifier mechanism, they correspond to the steps of substrate and modifier binding, respectively). Although they can be interpreted as Michaelis constants when the experimental data obtained under quasi-steady-state conditions are treated, k_M, k_S, and k_{SS} will be presented hereafter as K_M, K_S, and K_{SS}, respectively.

Note that it is possible to separate the contributions of K_S and K_{SS} into three different multipliers only for the mechanism with α = 1, but not for the general case (when α ≠ 1). For the general modifier mechanism, a similar separation has been made ([2], see equation (30)), but this required the application of other kinetic constants. The dependence of the initial rate (v_0^m) on the modifier concentration ([*M*]) is described by the following equation [2]:

$$v_0^m = v_0 \frac{1+\frac{[M]}{Q_M}}{1+\frac{[M]}{M_{50}}} = \frac{[S]}{K_s+[S]} V_{max} \frac{1+\frac{[M]}{Q_M}}{1+\frac{[M]}{M_{50}}} \qquad \text{(R1)}$$

where v_0 is the initial rate in the absence of modifier (a constant at a given substrate concentration), M_{50} is a constant (at a given substrate concentration) that corresponds to the modifier concentration such that the initial reaction rate takes the average value between the initial rate in the absence of modifier and the initial rate at a saturating modifier concentration; Q_M is a constant that is independent of substrate and modifier concentrations.

Since in the general case,

$$Q_M = \frac{\alpha K_i}{\beta}, \text{ and } M_{50} = \alpha K_i \left(\frac{K_s+[S]}{\alpha K_s+[S]} \right)$$

equation (R1) can be rewritten:

$$v_0^m = \frac{[S]}{K_S + [S]} V_{max} \frac{1 + \beta \frac{[M]}{\alpha K_i}}{1 + \frac{[M]}{\alpha K_i \left(\frac{K_s + [S]}{\alpha K_s + [S]} \right)}} \qquad \text{(R2)}$$

If α = 1 (as in the model studied by Rodic et al. [1]) and taking into account that in this model the substrate acts as a modifier, equation (R2) turns into equation (1). Note, however, that in equation (R2), $V_{max}[S]/(K_S + [S])$ is a constant, whereas in equation (1), this factor is not a constant. This is the essential difference between equation (1) and (R2).

From my point of view, in the general case (when α ≠ 1), it is impossible to derive an equation similar to equation (1) with the separation of constants K_S and K_{SS} in three different factors, and even more so with an unlimited number of substrate modulation terms. This possibility exists only for mechanisms with α = 1. However, new theoretical results obtained for one particular mechanism may also be important if they facilitate experimental measurements, simplify calculations, or clarify or refine the understanding of the processes under investigation.

It should be noted that the author has tried to show how equation (14) can be easily derived, but this target equation (equation (14)) is not equivalent to equation (1). If we compare equations (13) and (14), we can conclude that equation (14) should be different, namely:

$$v = V_S \frac{[S]}{([S] + K_S)} - V_S \frac{[S]}{([S] + K_S)} \frac{[S]}{([S] + K_{SS})} + V_{SS} \frac{[S]}{([S] + K_S)} \frac{[S]}{([S] + K_{SS})} \qquad \text{(R3)}$$

To verify this, we can perform calculations using equations (13), (14), and (R3) with the following parameters: V_{max} (V_S) = $3 \cdot 10^{-7}$; bV_{max} (V_{SS}) = $1 \cdot 10^{-8}$; K_S = $1 \cdot 10^{-4}$; K_{SS} = $1 \cdot 10^{-5}$. According to equation (14), at low substrate concentrations (up to 1 mM), negative values of the initial rate are obtained, while according to equation (13), there are no negative values and the calculated values of the initial rate coincide with the values calculated according to equation (R3). Therefore, it is necessary to change the derivation of the equation given under the heading "Derivation of an iteratively expandable substrate modulation equation" in order to obtain equation (R3) as a result.

Regarding equation (R3), it can be said that it is impossible to consider the processes occurring at the regulatory sites without taking into account the processes occurring at the catalytic sites.

Equation (1) can describe both saturation kinetics and kinetic curves that have a maximum point. Therefore, there may be some discussion that Michaelis constants cannot be used to describe such complex kinetic curves. However, this case is an example of how a complex kinetic curve can be successfully described using several hyperbolic functions. As stated earlier, equation (1) is derived in the equilibrium approximation by analyzing a kinetic scheme analogous to the general modifier mechanism with α = 1 and a substrate that behaves similarly to the modifier. In this model, the constants K_S and K_{SS} have the meaning of equilibrium constants. Therefore, they can be used to describe the complex kinetics of a bell-

shaped type.

Conclusion: The paper should be revised because the target equation (equation (14)), to whose derivation the author's efforts were directed, was found to be inaccurate.

References

1. Radic Z, Pickering NA, Vellom DC, Camp S, Taylor P. Three distinct domains in the cholinesterase molecule confer selectivity for acetyl- and butyrylcholinesterase inhibitors. Biochemistry. 1993, 32(45):12074-84. https://doi.org/10.1021/bi00096a018

2. Karakhim S.O. Kinetics of the enzyme titration process by reversible modifiers. Biochimie. 2023. 214(Part B): 11–26. https://doi.org/10.1016/j.biochi.2023.06.001