Letters

Understanding Enzyme Inhibition

Having published "An Introduction to Enzyme Kinetics" in this *Journal* (*1*), I turned quickly to Raymond S. Ochs's recent paper entitled Understanding Enzyme Inhibition (*2*). While there was much of interest to me in Ochs's paper, I found myself in disagreement with him at several points.

In contrast to Ochs, I believe inhibition is best characterized by the linear double reciprocal plots, since the three possible effects on a straight line correspond exactly to the three possible modes of inhibition: a slope effect (competitive inhibition), an intercept effect (uncompetitive inhibition), and both slope and intercept effects (noncompetitive, combined, or "mixed" inhibition). It is clear that there can be no other possibilities.

Furthermore, whereas Ochs presents the initial velocity equations as eqs 5, 6, and 7, I believe that an intuitive understanding of the initial velocity equations is more easily obtained from equations in the forms shown in eqs 6 and 8 and the unnumbered equation at the bottom of the left column of page 385 in my paper. The reason I believe this is that the terms in the denominators of the fractions in the equations in my paper correspond exactly to the forms in which the enzyme can be present, and the kinetics are completely determined by the relative amounts of the forms.

Also, when the inhibitor can bind to both E and ES (noncompetitive, combined, or "mixed" inhibition), the K_m in the presence of the inhibitor can be either greater or smaller than in its absence. Ochs's assertion that "the fact that K_m is unchanged in the mixed case" can be true only if the affinity of the inhibitor for E and for ES is exactly the same—an unlikely possibility.

Additionally, while it may be surprising or even counterintuitive that K_m is *smaller* in the presence of a purely uncompetitive inhibitor than in its absence, further analysis provides the needed insight, as I explained in my paper starting with the last paragraph on page 384 (*1*).

Finally, I do not believe that the concern for data analysis is relevant. A series of experiments will contain a certain amount of information, and a mathematically and statistically valid analysis of either a "direct" plot or a "double reciprocal" plot will extract the same information.

Literature Cited

1. Ault, Addison. *J. Chem. Educ.* **1974,** *51*, 381–386.
2. Ochs, Raymond S. *J. Chem. Educ.* **2000,** *77*, 1453–1456.

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The author replies:

I agree that presentation of the double reciprocal forms and examination of the terms in equations add to an appreciation of enzyme inhibition—once you already understand the topic. The point of my article is that most people don't. Those who specialize in kinetics may feel the need to keep the flame of orthodox kinetics alive by stressing precision in definition, and naturally gravitate toward double reciprocal plots and the behavior evident in equations. The idea that an intuition can be developed through these practices is another matter. Personally, I favor mental pictures such as the sting operation for uncompetitive inhibition. I disagree that we needn't discuss ideal cases of mixed inhibition where both binding constants are equal on the grounds that they are only occasionally observed: what we are talking about is the concept. Unless the extreme cases of the types of inhibition are clearly understood, we will remain ignorant of the central ideas. Even if the extreme case had an occurrence of zero, it would still be worthy of study. Students, professors, and even textbooks currently struggle with the notion of an uncompetitive inhibitor and therefore of any type of inhibition apart from competitive. I don't believe data transformations or deep examination of equations themselves will lead us out of the morass. I am not advocating ignoring more elaborate treatments: I am merely suggesting that, as a first step, a basic understanding is more important than detail.

One final point must be stressed. To retain the emphasis on K_m itself rather than V/K is the wrong step, even if we are looking to more advanced treatments. This is because K_m is not really a fundamental kinetic constant. Thus, its use, while a simplification and sometimes a useful benchmark, is not a way to more deeply understand kinetics. For a more advanced treatment than my own, I strongly suggest the reader look into Northrop's discussion (*1*).

Literature Cited

1. Northrop, D. B. *J. Chem. Educ*. **1998,** *75*, 1153–1157.

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