The Nuts and Bolts of Michaelis−Menten Enzyme Kinetics: Suggestions and Clarifications

Todd Silverstein†

Chemistry Department, Willamette University, Salem, Oregon 97301-3922, United States
tsilvers@willamette.edu
†Current address: School of Chemistry, National University of Ireland, Galway, Ireland.

Matthew Junker recently contributed to this *Journal (1)* a wonderful addition to the select literature on enzyme kinetics applications and analogies (see for example ref *2*). In Junker's classroom exercise, students simulate enzyme activity by unscrewing nut−bolt "substrate molecules", thus, converting them into separate nuts and bolts "products". Those who teach biochemistry will wish to consider this simple and fun student activity for their own classrooms.

I have a few suggestions and interpretations that I think improve the clarity and accuracy of this classroom activity as an analogy for Michaelis−Menten kinetics. In his first paragraph, Junker gives the classic Michaelis−Menten equation for the hyperbolic saturation of enzyme activity under steady-state conditions:

$$v_0 = \frac{V_{max}[S]_0}{[S]_0 + K_M}$$

sometimes written as

$$v_0 = \frac{V_{max}}{1 + \frac{K_M}{[S]_0}}$$

where $K_M = (k_{-1} + k_2)/k_1$, and $V_{max} = k_2[E]_{total}$.[1]

Junker writes that as the student-enzyme grabs nut−bolts and unscrews them, the class can fill in the missing steps in the mechanism: S → ??? → $P_1 + P_2$. Junker fills in a single step, E + S → ES, followed by a second step, the production *and* release of product, ES → E + P_1 + P_2. In fact, students easily observe the student-enzyme grabbing a nut−bolt (ES formation), unscrewing it (catalysis with bound products), and then dropping the separated nuts and bolts (product release), before reaching for the next nut−bolt "substrate". So students can infer an even more complete mechanism in which enzyme-bound substrate is first converted to enzyme-bound products [ES → E(P_1 + P_2)], after which products are released (EP → E + P_1 + P_2). This is important because, although Junker's statement that "k_2 is k_{cat}, the intrinsic catalytic rate constant for the enzyme" is true for many enzymes, there are exceptions. In fact, k_{cat} is more accurately described as the rate constant for the rate-determining step(s) in the reaction mechanism, k_{rds}.[2] For enzymes like alcohol dehydrogenase for which product release is the slowest step, $k_{cat} \approx k_3$, not k_2 *(4)*.

In Junker's "Condition 1: Limiting Substrate Concentration", 15 nut−bolt substrates are scattered about the room, and the student-enzyme must walk to one and pick it up, unscrew it, put down the separated products, then walk to the next nut−bolt substrate. Here the initial substrate concentration, $[S]_0$ is low.[3] Junker has his students "predict how changing k_1 (using a faster or slower traveling student) or changing $[S]_0$ would change the rate for forming ES and the overall rate of the reaction." I would add to this that envisioning the effect of changing $[E]_{tot}$ (i.e., increasing the number of student-enzymes in the room) is a further useful exercise, as is the realization that k_1 involves not just the rate of travel of the student-enzyme, but also how quickly she or he picks up the nut−bolt from the counter.

Junker also has his students "deduce that the overall rate of the reaction under condition 1 cannot be used to determine k_2 for the student-enzyme because step 1 limits the overall rate of the reaction." This is not exactly true. What is true is that from a single determination of v_0 at a single $[S]_0$, one cannot determine k_2. However, neither can you determine k_1, K_M, or any other kinetic parameter from a single datum. At the very least, to determine the value of two kinetic parameters, k_{cat} and K_M, we must know v_0 for at least two different $[S]_0$ values. For example, if $[S]_0$ is very low, the enzyme efficiency parameter, k_{cat}/K_M (see note 3), can be calculated from $v_0/([E]_{tot}[S]_0)$; in order to calculate k_{cat}, one must already know K_M. Below, I will return to this calculation of k_{cat} ($\approx k_2$) from condition 1 results.

Junker's "Condition 2: Saturating Substrate Concentration" has all of the nut−bolt assemblies gathered into a single pile. This resembles the more typical situation for an enzyme in vivo, where step 2 is rate determining and almost all of the enzyme is tied up in the steady state as ES. In describing this regime, Junker makes the odd statement that "the measured velocity is greater than that observed in condition 1 because the rate-limiting first step is bypassed." This is, of course, not the case. The first step cannot be "bypassed": Enzyme must bind substrate before the reaction can be catalyzed. What Junker meant to state, I believe, is that under conditions of saturating substrate concentration, first step does not affect the reaction rate: [ES] is maximal ($\approx [E]_{total}$), the reaction velocity is also maximal, and $v_0 = k_2[ES] \approx k_2[E]_{total}$. Another way to think about this is that, under these conditions, $[S]_0 \gg K_M$, and the Michaelis−Menten equation reduces to

$$v_0 = V_{max} = k_{cat}[E]_{total} \approx k_2[E]_{total}$$

Junker argues that "the enzyme concentration is 1 for a single student-enzyme [in the room]", in which case $k_{cat} \approx v_0/1 = 18\ min^{-1}$. Once we know k_{cat}, we can calculate K_M from the Michaelis−Menten equation:

$$K_M = [S]_0\left(\frac{k_{cat}[E]_{total}}{v_0} - 1\right)$$

However, first we must have values for the substrate (and enzyme) concentration. What follows here is a series of calculations

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that clearly lie beyond the scope of Junker's original simple classroom activity. This further application could be useful as a homework problem or bonus problem, based on the results obtained in the classroom activity. Assume that the room is 20 ft × 20 ft (400 sq ft), and the pile of nuts and bolts covers about 6 in. × 6 in. (0.25 sq ft). Given that the student-enzyme's hands and the bolts are in roughly the same plane, concentration can be calculated as number of objects per area. Under these conditions, the initial concentration of piled nut−bolt assemblies is $[S]_{0,pile} = 15/0.25\ ft^2 = 60\ ft^{-2}$ (and for the scattered bolts, $[S]_{0,room} = 15/400 = 0.0375\ ft^{-2}$). Using this concentration value, $[S]_{0,pile} = 60\ ft^{-2}$, and Junker's definition of $[E]_{total}$ as one, we can calculate:

$$K_M = (60\ ft^{-2})\left[\frac{(18)(1)}{(18)} - 1\right] = 0$$

Clearly there is a rounding error here, because K_M cannot be zero. This error occurs because, under conditions of saturating substrate, $v_0 \approx V_{max}$, and thus, $(V_{max}/v_0) - 1 \approx 0$. We thus learn that K_M cannot be calculated accurately under conditions of saturating substrate. From Junker's condition 1 results at low concentration (i.e., scattered nut-bolts), we can calculate:

$$K_M = (0.0375\ ft^{-2})\left[\frac{(18)(1)}{(10)} - 1\right] = 0.030\ ft^{-2}$$

Here, we calculated k_{cat} from v_0(high substrate) and K_M from v_0(low substrate). For the very advanced student, it might be worth pointing out that v_0(high substrate)/v_0(low substrate) = 18/10 = 1.8, and K_M may be calculated from this ratio of two rates and the Michaelis−Menten equation[4]:

$$K_M = \frac{(0.8)(60\ ft^{-2})(0.0375\ ft^{-2})}{(60\ ft^{-2}) - 1.8(0.0375\ ft^{-2})} = 0.030\ ft^{-2}$$

This value of K_M can then be used, along with condition 1's v_0(low substrate), the enzyme and substrate concentrations, and the Michaelis−Menten equation, to calculate k_{cat} ($\approx k_2$) = 18 min^{-1}. Thus, the low substrate rate (condition 1) can in fact "be used to determine k_2 for the student-enzyme."

Junker concludes his classroom exercise and analogy with condition 3 (competitive inhibition) and condition 4 (noncompetitive inhibition). He simulates noncompetitive inhibition "by describing how a second student could act as an inhibitor by grasping the student-enzyme by the wrists, outside the active site, to hold the enzyme's hands apart." One must be careful here, because competitive, mixed (noncompetitive), and uncompetitive inhibitors are reversible; they bind noncovalently to the enzyme. The notion of grasping the enzyme-student's wrist may suggest, in the mind of students observing the activity, covalent binding and irreversible inhibition.

With a bit of modification and clarification, this analogy for noncompetitive inhibition can actually be quite apt. First, keep in mind that noncompetitive inhibition is the special case of mixed inhibition for which the substrate binding step is completely unaffected by inhibitor binding at a regulatory site some distance away from the substrate binding site. Hence, the binding of a noncompetitive inhibitor hinders catalysis [k_2 (+ inh) $\ll k_2$ (control)] while leaving substrate binding unaltered (k_1, k_{-1} unchanged).

If the enzyme-student picks up the nut−bolt units with a single hand, say the right-hand, then this hand represents the substrate-binding site. Both hands coming together to unscrew the nut from the bolt then represents the enzyme active site. A noncompetitive inhibitor must leave the student-enzyme's right-hand free to grasp each nut−bolt, but hinder the process of the two hands coming together and unscrewing the nut. Accordingly, instead of grasping the enzyme-student's wrists, the inhibitor-student should place her two arms in between the enzyme-student's two arms and, thus, hinder the process of two-handed unscrewing at the enzyme's active site. Here the inhibitor-student does not grasp, but rather simply places her arms in a critical position, thus, deforming the active site, while still leaving substrate binding with the right-hand unaltered. (Alternatively, the inhibitor could grab the enzyme's *left* hand, keeping it away from the right-hand with the nut−bolt assembly, but then let go after a specific time set by the inherent "affinity" of the inhibitor for the enzyme.) In addition to this being a more accurate analogy to true noncompetitive inhibition, it also allows the undertaking of an actual simulation with the collection of quantitative results (as opposed to the zero activity recorded by Junker in his table).

I believe that with these corrections and suggestions, Junker's nuts-and-bolts analogy becomes a very attractive and instructive classroom exercise in the teaching of enzyme kinetics.

Notes

1. As presented in any biochemistry textbook (see for example refs *3*, *4*), k_1 is the forward rate constant for the first step, the fast binding of substrate (S) to enzyme (E) to make the noncovalent enzyme−substrate complex (ES); k_{-1} is the reverse rate constant for the dissociation of ES back into E + S; and k_2 is the rate constant for the subsequent slow catalytic step converting S to product. For the classic Michaelis−Menten enzyme, $k_1 > k_{-1} \gg k_2$.
2. For a simple Michaelis−Menten enzyme, the mechanism comprises three steps: (i) noncovalent substrate binding; (ii) catalysis: conversion of bound substrate(s) to bound product(s); and (iii) release of product(s). These steps are characterized respectively by rate constants k_1, k_2, and k_3. Typically, the binding and release steps are much faster than the catalytic step, but this is by no means always the case.
3. If $[S]_0 \ll K_M$, then $v_0 \approx V_{max}[S]_0/K_M = (k_{cat}/K_M)[E]_{tot}[S]_0$. k_{cat}/K_M, the enzyme efficiency (or specificity) parameter, has units of L mol^{-1} $time^{-1}$; it is essentially the second-order rate constant for forming the E + S collision complex. For very efficient enzymes, this parameter can approach diffusion-controlled values of 10^8−10^{10} L mol^{-1} s^{-1}.
4. $v_{hi\ S}/v_{lo\ S} = 18/10 = \{k_2[E]_{total}[S]_{hi}/([S]_{hi} + K_M)\}/\{k_2[E]_{total}[S]_{lo}/([S]_{lo} + K_M)\}$, or $1.8 = \{[S]_{hi}/([S]_{hi} + K_M)\}/\{[S]_{lo}/([S]_{lo} + K_M)\}$, which reduces to $K_M = 0.8[S]_{hi}[S]_{lo}/\{[S]_{hi} - 1.8[S]_{lo}\}$.

Literature Cited

1. Junker, M. *J. Chem. Educ.* **2010**, *87* (3), 294–295.
2. Silverstein, T. P. *J. Chem. Educ.* **1995**, *72* (7), 645–646.
3. Voet, D.; Voet, J. G. *Biochemistry*, 3rd ed.; J. Wiley & Sons: New York, 2004; pp 477−481.
4. Nelson, D. L.; Cox, M. M. *Lehninger Principles of Biochemsitry*, 5th ed.; W. H. Freeman: New York, 2008; pp 194−199.

