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Notes & Tips

Application of the Van Slyke–Cullen irreversible mechanism in the analysis of enzymatic progress curves

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ABSTRACT

For enzymatic progress curves conforming to the Michaelis–Menten mechanism $E + S \rightleftharpoons ES \rightarrow E + P$, the minimal fitting model cast as a system of numerically integrated differential equations is the simplified, irreversible Van Slyke–Cullen mechanism $E + S \rightarrow ES \rightarrow E + P$. The best-fit value of the bimolecular association rate constant is identical to the specificity constant k_{cat}/K_M. An illustrative example involves a fluorogenic continuous assay of the HIV protease, analyzed by the differential-equation oriented software package DYNAFIT [P. Kuzmic, Anal. Biochem. 237 (1996) 260].

This report proposes a shortcut that has proved very useful in the practical analysis of enzymatic progress curve, using advanced software tools that represent reaction mechanisms in terms of microscopic rate constants. The problem is that the experimental progress curves can never fully support the Michaelis–Menten mechanism, $E + S \rightleftharpoons ES \rightarrow E + P$, which is the "true" reaction mechanism in most cases. The trick is to invoke the *irreversible* Van Slyke–Cullen mechanism, $E + S \rightarrow ES \rightarrow E + P$. We know in advance that this mechanism is physically incorrect (the binding of substrates to enzymes is always *reversible*). It turns out, however, that even with the "wrong" model we can accomplish something very useful in the end.

Several currently available software tools for the analysis of enzyme kinetic data (e.g. KINSIM [1], DYNAFIT [2], or COPASI [3]) allow the user to specify the postulated reaction mechanism in terms of conventional stoichiometric equations. For example, the user can type `E + S <==> ES --> E + P` on the keyboard, to represent the classic Michaelis–Menten mechanism [4] (Scheme 1), and the software automatically derives the underlying system of ordinary differential equations (ODE) as the mathematical model for reaction progress.

When biochemistry students and researchers first encounter software tools that allow model specification in terms of microscopic rate constants, the Michaelis–Menten mechanism often is the first model to experiment with. Frequently, it is also the first source of disappointment, because only two out of three microscopic rate constants in Scheme 1 can be uniquely determined from experimental progress curves. In the realm of traditional steady-state kinetic analysis, this restriction parallels our abilility to determine only two of three possible steady-state kinetic constants: the turnover number k_{cat}, the specificity constant k_{cat}/K_m, and the Michaelis constant K_m.

One obvious solution to this problem would be to set the bimolecular association rate constant k_1 to some arbitrary value that, importantly, must be sufficiently high (k_1 must always be higher than k_{cat}/K_m, because $k_{cat}/K_m = k_1k_3/(k_2 + k_3)$) and keep it fixed in the model. Based on typical experimental values of bimolecular association rate constants [5], the value of $k_1 = 10^8\ M^{-1}\,s^{-1}$ might appear to be safe choice, as has been advocated elsewhere [6,7]. However, for the DinB homologue DNA polymerase from *Sulfolobus solfataricus*, $k_1 = 1.4 \times 10^9\ M^{-1}\,s^{-1}$ [8], and there are other similar examples. Should we then set $k_1 = 10^{10}\ M^{-1}\,s^{-1}$ or even $k_1 = 10^{20}\ M^{-1}\,s^{-1}$? One serious problem with arbitrarily ratchetting up the bimolecular association rate constant is that, at extremely high values of k_1, the underlying system of differential equations becomes numerically unsolvable (a "stiffness" problem [9]).

I propose a different solution—a shortcut, in a way—based on the Van Slyke–Cullen mechanism [10] shown in Scheme 2.

The microscopic rate constant k_1^* in Scheme 2 is now an "apparent" bimolecular association rate constant, exactly equivalent to the specificity constant k_{cat}/K_m. A representative experimental data set, from a continuous fluorogenic assay of the HIV protease [11,12], is shown in Fig. 1. The smooth model curve corresponds to the best-fit values $k_1^* = (4.27 \pm 0.02)\ \mu M^{-1}\,s^{-1}$ and $k_3 = 8.7 \pm 0.2\ s^{-1}$. The full listing of a DYNAFIT [2] input file is shown in the Appendix.

An exactly identical model curve (within eight significant digits) was obtained by fitting the same data to the Michaelis–Menten model (Scheme 1), in which k_1 was held constant at $k_1 = 100\ \mu M^{-1}\,s^{-1}$. The best-fit values of the adjustable rate constants were $k_2 = 193\ s^{-1}$ and $k_3 = 8.7\ s^{-1}$, from which $k_{cat}/K_m = k_1k_3/(k_2 + k_3) = 4.3\ \mu M^{-1}\,s^{-1}$—numerically identical to k_1^* in the Van

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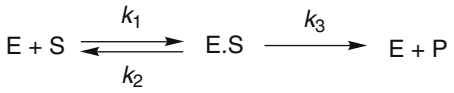

Scheme 1.

Scheme 2.

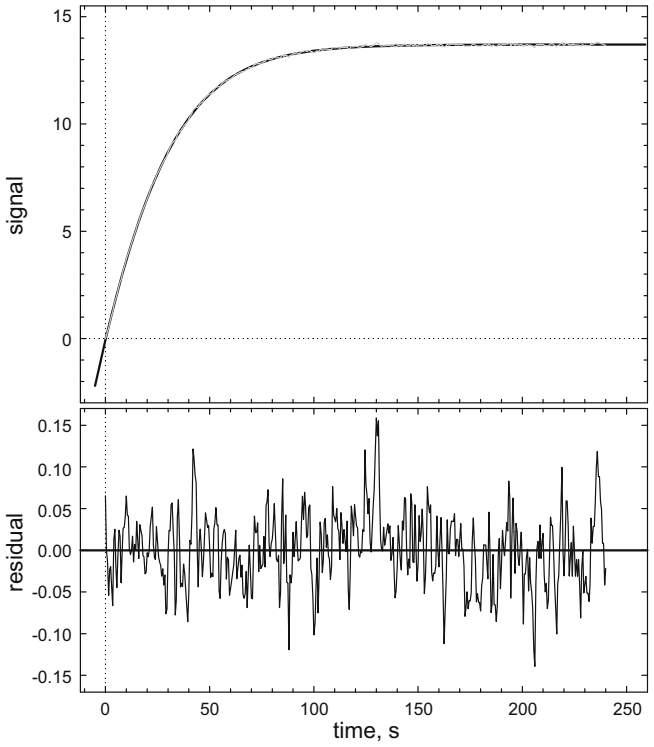

Figure 1. Jagged curve: Experimental data from the fluorogenic assays of HIV protease ($[E]_0 = 10$ nM, $[S]_0 = 10\mu$M; see Refs. [11,12] for details). Smooth curve: Least-squares fit either to the Michaelis–Menten mechanism (Scheme 1) or to the Van Slyke–Cullen mechanism (Scheme 2).

Slyke–Cullen model. Note that $k_3 = k_{cat}$ is also identical in both models.

Most importantly, an exhaustive search in the multidimensional parameter space for all plausible values of k_1, using the *profile-t* algorithm of Bates and Watts [13–15], produced $k_1 = 4.3\ \mu M^{-1} s^{-1}$ as the *lower limit* on k_1 (results now shown). Note that, as is expected based on theoretical considerations (k_{cat}/K_m is necessearily the lowest estimate for k_1), this lowest *limiting value* for k_1 is exactly the same as the *best-fit* value for k_1^*. This exhaustive search for parameter limits is a computationally very intensive procedure. For example, a recently described systematic search method [7] may take up many minutes of computing time even for relatively simple mechanisms.

In a way, we have gained something quite valuable by trading the physically realistic but experimentally underdetermined Michaelis–Menten mechanism for the physically implausible (on account of its irreversibility) Van Slyke–Cullen model. In particular, we do not have to undertake computationally expensive systematic searches for the lowest plausible value of k_1, to make sure that we do not accidentally "freeze" it in the fitting model at some arbitrary value that might be too low. The lowest possible value of k_1 that is consistent with the given set of experimental data is simply the best-fit value of k_1^*.

Experience shows that at least some enzymologists initially hesitate about employing the "wrong" Van Slyke–Cullen mechanism for routine analysis of important laboratory data. However, as was pointed out by a reviewer, the classic Michaelis–Menten mechanism is also physically unrealistic, because the chemical transformation step and the subsequent product dissociation are treated as a single event. To quote a famous statistician, this proves that "essentially, all models are wrong, but some are useful" [16, p. 424].

Appendix A

The following DYNAFIT [2] script is used to fit experimental data in a two-column (time vs fluorescence intensity) text file 16-2d.txt to the Van Slyke–Cullen mechanism in Scheme 2, to generate the best-fit model curve in Fig. 1.

```
[task]
  task = fit | data = progress
[mechanism]
  E+S ⟶ E.S : k1
  E.S ⟶ E+P : k3
[constants]
  k1 = 1 ? | k3 = 10 ?
[concentrations]
  E = 0.01, S = 1
[responses]
  P = 15 ?
[data]
  file ./hiv-protease/data/16-2d.txt
  delay = 5 | offset = 0 ?
[output]
  directory ./hiv-protease/output
[end]
```

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