REB, The Book
Example 4.8.3 Solution

Suppose an enzyme, E, catalyzes the conversion of substrate, S, into product, P, as indicated in the apparent, non-elementary reaction (1). If the mechanism consists of reactions (2) and (3), derive a mechanistic rate expression for the apparent generation of P via reaction (1) assuming step (3) is effectively irreversible. The resulting rate expression should be suitable for reaction engineering purposes. That is, it should not contain concentrations of reactive intermediates.

$$S \rightleftarrows P \tag{1}$$

$$E + S \rightleftarrows E\text{–}S \tag{2}$$

$$E\text{–}S \rightleftarrows E + P \tag{3}$$

Assignment Summary

Apparent Reaction:

$$S \rightleftarrows P \tag{1}$$

Mechanistic Steps:

$$E + S \rightleftarrows E\text{–}S \tag{2}$$

$$E\text{–}S \rightleftarrows E + P \qquad \text{(irrev.) (3)}$$

Quantifiable Reagents: S and P

Reactive Intermediates: E and E–S

Deliverables: simplified expression for $r_{P,1}$

Equations for Generating the Mechanistic Rate Expression

Rates of the Mechanistic Steps

$$r_2 = k_{2,f}\,[E]\,\left[S\right] - k_{2,r}\left[E-S\right] \tag{4}$$

$$r_3 = k_{3,f}\left[E-S\right] \tag{5}$$

<u>Apparent Rate of the Non-Elementary Reaction</u>

$$r_{P,1} = r_3 \tag{6}$$

<u>Bodenstein Steady-State Approximations or Quasi-Equilibrium Expressions</u>

$$0 = r_2 - r_3 \tag{7}$$

$$0 = -r_2 + r_3 \tag{8}$$

<u>Enzyme Conservation Equation</u>

$$E_0 = [E] + \left[E-S\right] \tag{9}$$

Calculations

The algebraic details for the calculations in this section are presented in the file, Example 4.8.1 Calculations.pdf. The calculations proceed as follows:

1. Substitute the rate expressions for the mechanistic steps, equations (4) and (5) into equations (6) through (8).
2. Solve the version of equation (7) (or equation (8)) from step 1 and equation (9) to get expressions for the concentrations of the reactive intermediates.

$$[E] = E_0 \frac{k_{2,r} + k_{3,f}}{k_{2,r} + k_{3,f} + k_{2,f}\left[S\right]} \tag{10}$$

$$\left[E-S\right] = \frac{k_{2,f}E_0\left[S\right]}{k_{2,r} + k_{3,f} + k_{2,f}\left[S\right]} \tag{11}$$

3. Substitute the expressions for the concentrations of the reactive intermediates, equations (10) and (11), in the version of equation (6) from step 1 to get the mechanistic rate expression.

$$r_{P,1} = \frac{k_{2,f}k_{3,f}E_0\left[S\right]}{k_{2,r} + k_{3,f} + k_{2,f}\left[S\right]} \tag{12}$$

Simplification and Discussion

The rate expression contains three rate coefficients. It might be difficult to estimate their values using experimental data, and for this reason, it is useful to define the minimum number of effective rate coefficients to replace them. Michaelis and Menten[1] studied enzymatic conversion of a substrate to product and were the first to propose a mechanistic rate expression of the form of equation (12). They defined two kinetics parameters, V_{max} and K_m, as shown in equations (13) and (14). Substitution of those definitions in equation (12) yields the so-called Michaelis-Menten rate expression, equation (15).

$$V_{max} = k_{3,f} E_0 \tag{13}$$

$$K_m = \frac{k_{2,r} + k_{3,f}}{k_{2,f}} \tag{14}$$

$$r_{P,1} = \frac{V_{max}\left[S\right]}{K_m + \left[S\right]} \tag{15}$$

The parameter, V_{max}, is simply a constant, E_0, times a true rate coefficient, so it will exhibit Arrhenius temperature dependence. In contrast, the parameter, K_m, includes the sum of two rate coefficients. As such, it will not exhibit Arrhenius temperature dependence. However, enzymes are often only stable over a narrow range of temperatures. As a consequence, assuming Arrhenius temperature dependence may not introduce too much error.

The Michaelis-Menten rate expression will exhibit asymptotic behavior as the concentration of substrate approaches zero and becomes negligible compared to K_m. That is, the rate will asymptotically approach the first order behavior shown in equation (16) when $K_m \gg \left[S\right]$ in the denominator of equation (15). Indeed, if K_m is always very large compared to the substrate concentration, the rate will be first order at all substrate concentrations.

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[1] Michaelis, L., and Menten, M.L. 1913. “Die Kinetik Der Invertinwirkung.” Biochem Z. 49: 333–69.

$$r_{P,1} = \frac{V_{max}}{K_m}\left[S\right] \tag{16}$$

The Michaelis-Menten rate expression also will exhibit asymptotic behavior as the concentration of substrate increases. That is, the rate will asymptotically approach V_{max} as $\left[S\right]$ increases such that $\left[S\right] \gg K_m$ in the denominator of equation (15).

Deliverables

If the mechanism is as described, the kinetics will be described by the Michaelis-Menten rate expression, below. The parameter, K_m, will not display Arrhenius temperature dependence. Experimental studies will need to be performed to determine whether this rate expression captures the composition and temperature dependence of the rate with sufficient accuracy.

$$r_{P,1} = \frac{V_{max}}{K_m}\left[S\right]$$