REB, The Book
Example 4.8.4 Solution

Suppose that enzyme E catalyzes the conversion of substrate S to product P, but if the reagent I is present in the system, it inhibits the enzyme. The apparent reaction is shown in equation (1), and the proposed mechanism consists of reactions (2) through (4). Assume that step (4) is effectively irreversible and the concentration of the free inhibitor is easily measured. Derive a Michaelis-Menten type of rate expression for the apparent rate of reaction (1) in terms of easily quantifiable reagents.

Apparent Reaction

$$S \rightleftarrows P \qquad (1)$$

Mechanism

$$E + S \rightleftarrows E\text{-}S \qquad (2)$$

$$E + I \rightleftarrows E\text{-}I \qquad (3)$$

$$E\text{-}S \rightarrow E + P \qquad \text{(irrev.) } (4)$$

Assignment Summary

Apparent Reaction:

$$S \rightleftarrows P \qquad (1)$$

Mechanistic Steps:

$$E + S \rightleftarrows E\text{-}S \qquad (2)$$

$$E + I \rightleftarrows E\text{-}I \qquad (3)$$

$$E\text{-}S \rightarrow E + P \qquad \text{(irrev) } (4)$$

Quantifiable Reagents: S, P, I, E_0

Reactive Intermediates: E, E-S, E-I

Deliverable: Michaelis-Menten type rate expression for reaction (1)

Equations for Generating the Mechanistic Rate Expression

Rates of the Mechanistic Steps

$$r_2 = k_{2,f}[E]\left[S\right] - k_{2,r}\left[E{-}S\right] \tag{5}$$

$$r_3 = k_{3,f}[E][I] - k_{3,r}[E{-}I] \tag{6}$$

$$r_4 = k_{4,f}\left[E{-}S\right] \tag{7}$$

Apparent Rate of the Non-Elementary Reaction

$$r_1 = \frac{r_{P,1}}{\nu_{P,1}} = \frac{r_{P,1}}{1} = r_{P,1} \tag{8}$$

Bodenstein Steady-State Approximations

$$0 = -r_2 - r_3 + r_4 \tag{9}$$

$$0 = r_2 - r_4 \tag{10}$$

$$0 = r_3 \tag{11}$$

Catalyst/Enzyme/Site/Charge Conservation Equation

$$E_0 = [E] + \left[E{-}S\right] + [E{-}I] \tag{12}$$

Calculations

The algebraic details for the calculations in this section are presented in the file, Example 4.8.4 Calculations.pdf. The calculations proceed as follows:

1. Substitute equations (5), (6), and (7) into equations (8), (9),(10), and (11).
2. Solve the versions of equations (10), (11), and (12) from step 1 for the concentrations of the reactive intermediates.

$$[E{-}I] = \frac{k_{3,f}E_0[I]}{k_{3,r} + \frac{k_{2,f}k_{3,r}}{k_{2,r}+k_{4,f}}\left[S\right] + k_{3,f}[I]} \tag{13}$$

$$[E{-}S] = \frac{k_{2,f}k_{3,r}E_0\,[S]}{k_{2,r}k_{3,r} + k_{4,f}k_{3,r} + k_{2,f}k_{3,r}\,[S] + k_{3,f}\left(k_{2,r} + k_{4,f}\right)[I]} \quad (14)$$

$$[E] = \frac{E_0}{1 + \frac{k_{2,f}[S]}{k_{2,r} + k_{4,f}} + \frac{k_{3,f}[I]}{k_{3,r}}} \quad (15)$$

3. Substitute equation (13) into the version of equation (8) resulting from step 1.

$$r_1 = \frac{k_{2,f}k_{4,f}k_{3,r}E_0\,[S]}{k_{2,r}k_{3,r} + k_{4,f}k_{3,r} + k_{2,f}k_{3,r}\,[S] + k_{3,f}\left(k_{2,r} + k_{4,f}\right)[I]} \quad (16)$$

Simplification and Discussion

Dividing numerator and denominator of equation (16) by $k_{2,f}k_{3,r}$ and rearrangement yields equation (17).

$$r_1 = \frac{k_{4,f}E_0\,[S]}{[S] + \frac{k_{3,f}\left(k_{2,r} + k_{4,f}\right)}{k_{2,f}k_{3,r}}[I] + \frac{k_{2,r}k_{3,r} + k_{4,f}k_{3,r}}{k_{2,f}k_{3,r}}}$$

$$r_1 = \frac{k_{4,f}E_0\,[S]}{[S] + \frac{k_{3,f}\left(k_{2,r} + k_{4,f}\right)}{k_{2,f}k_{3,r}}[I] + \frac{k_{2,r} + k_{4,f}}{k_{2,f}}} \quad (17)$$

If V_{max}, K_m, and K_I are defined as shown in equations (18) through (20), the rate expression takes the form shown in equation (21).

$$V_{max} = k_{4,f}E_0 \quad (18)$$

$$K_m = \frac{k_{2,r} + k_{4,f}}{k_{2,f}} \tag{19}$$

$$K_I = \frac{k_{3,f}\left(k_{2,r} + k_{4,f}\right)}{k_{2,f}k_{3,r}} \tag{20}$$

$$r_1 = \frac{V_{max}\left[S\right]}{\left[S\right] + K_I\left[I\right] + K_m} \tag{21}$$

The Michaelis-Menten type expression for the rate of reaction (1) is given in equation (21). V_{max} will exhibit Arrhenius temperature dependence, but K_m and K_I will not because the sum of rate coefficients appear in their defining equations. However, if the range of the temperatures of interest is narrow, the deviation from Arrhenius behavior may be negligibly small.

Note that if $K_I\left[I\right]$ is very small compared to the other terms in the denominator, inhibition is small, and the rate expression reduces to the Michaelis-Menten expression for a reaction without an inhibitor.

Deliverables

If the mechanism is accurate, the apparent rate of reaction (1) is given below. Experimental studies will need to be performed to determine whether it captures the composition and temperature dependence of the rate with sufficient accuracy.

$$r_1 = \frac{V_{max}\left[S\right]}{\left[S\right] + K_I\left[I\right] + K_m}$$