REB, The Course
Class 6 Practice Assignment Solution

Suppose that substrate S can be converted to product P in the presence of the enzyme, E. The conversion, reaction (1), is non-elementary. Suppose reactions (2) through (4) are the reaction mechanism, that reaction (3) is effectively irreversible, and that reactions (2) and (4) are reversible. Derive an expression for the apparent rate of reaction (1) if reaction (3) is effectively irreversible. Eliminate concentrations of intermediates from the rate expression and group and rename products of powers of the true rate coefficients to minimize the number of apparent rate coefficients in the rate expression.

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

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

$$E\text{-}S \rightarrow E\text{-}P \qquad (3)$$

$$E\text{-}P \rightleftarrows E + P \qquad (4)$$

Assignment Summary

Apparent Reaction:

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

Mechanistic Steps:

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

$$E\text{-}S \rightarrow E\text{-}P \qquad \text{(irrev.) } (3)$$

$$E\text{-}P \rightleftarrows E + P \qquad (4)$$

Quantifiable Reagents: S, P, and E_0

Reactive Intermediates: E, E-S, and E-P

Deliverable: simplified expression for r_1

Equations for Generating the Mechanistic Rate Expression

Rates of the Mechanistic Steps

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

$$r_3 = k_{3,f}\,\left[E\text{-}S\right] \tag{6}$$

$$r_4 = k_{4,f}\,[E\text{-}P] - k_{4,r}\,[E]\,[P] \tag{7}$$

Apparent Rate of the Non-Elementary Reaction

$$r_1 = r_P = r_4 \tag{8}$$

Bodenstein Steady-State Approximations[1]

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

$$0 = r_2 - r_3 \tag{10}$$

$$0 = r_3 - r_4 \tag{11}$$

Enzyme Conservation Equation

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

Calculations

The algebraic details for the calculations in this section are presented in the file, Practice Assignment 6 Calculations.pdf. The calculations proceed as folllows:

1. Substitute the rate expressions for the mechanistic steps in equations (8) through (11).
2. Solve the enzyme conservation equation, (12), together with two of the Bodenstein steady-state approximations, equations (9) through (11) to get expressions for the concentrations of the reactive intermediates. (An expression for the concentration of E-S could be calculated, but it isn't needed for generating the rate expression.)

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[1] Only two of the three Bodenstein steady-state approximations are mathematically independent.

$$E = \frac{k_{4,f}\left(k_{2,r} + k_{3,f}\right) E_0}{\left(\begin{array}{c} k_{4,f}\left(k_{2,r} + k_{3,f}\right) + k_{2,f}\left(k_{3,f} + k_{4,f}\right)\left[S\right] \\ +k_{4,r}\left(k_{2,r} + k_{3,f}\right)\left[P\right] \end{array}\right)} \tag{13}$$

$$[E\text{-}P] = \frac{k_{2,f}k_{3,f}E_0\left[S\right] + k_{4,r}\left(k_{2,r} + k_{3,f}\right) E_0\left[P\right]}{\left(\begin{array}{c} k_{4,f}\left(k_{2,r} + k_{3,f}\right) + k_{2,f}\left(k_{3,f} + k_{4,f}\right)\left[S\right] \\ +k_{4,r}\left(k_{2,r} + k_{3,f}\right)\left[P\right] \end{array}\right)} \tag{14}$$

3. Substitute the expressions for the concentrations of the reactive intermediates, equations (13), (14), and (15) into the version of equation (8) resulting from step 1 to get the mechanistic rate expression.

$$r_P = \frac{k_{2,f}k_{3,f}k_{4,f}E_0\left[S\right]}{\left(\begin{array}{c} k_{4,f}\left(k_{2,r} + k_{3,f}\right) + k_{2,f}\left(k_{3,f} + k_{4,f}\right)\left[S\right] \\ +k_{4,r}\left(k_{2,r} + k_{3,f}\right)\left[P\right] \end{array}\right)} \tag{15}$$

Simplification and Discussion

Dividing the numerator and denominator of equation (15) by the first term in the denominator yields equation (16).

$$r_P = \frac{\frac{k_{2,f}k_{3,f}k_{4,f}}{k_{4,f}\left(k_{2,r} + k_{3,f}\right)}E_0\left[S\right]}{1 + \frac{k_{2,f}\left(k_{3,f} + k_{4,f}\right)}{k_{4,f}\left(k_{2,r} + k_{3,f}\right)}\left[S\right] + \frac{k_{4,r}\left(k_{2,r} + k_{3,f}\right)}{k_{4,f}\left(k_{2,r} + k_{3,f}\right)}\left[P\right]} \tag{16}$$

Defining V_{max}, K_s, and K_p as in equations (17) through (19) and substitution into equation (16) yields the Michaelis-Menten form of the rate expression shown in equation (20). (Alternatively k, k', and k'' could be used instead of V_{max}, K_s, and K_p.)

$$V_{max} = \frac{k_{2,f} k_{3,f} k_{4,f}}{k_{4,f}\left(k_{2,r} + k_{3,f}\right)} E_0 \tag{17}$$

$$K_s = \frac{k_{2,f}\left(k_{3,f} + k_{4,f}\right)}{k_{4,f}\left(k_{2,r} + k_{3,f}\right)} \tag{18}$$

$$K_p = \frac{k_{4,r}\left(k_{2,r} + k_{3,f}\right)}{k_{4,f}\left(k_{2,r} + k_{3,f}\right)} \tag{19}$$

$$r_P = \frac{V_{max}\left[S\right]}{1 + K_s\left[S\right] + K_p\left[P\right]} \tag{20}$$

The coefficients, V_{max}, K_s, and K_p, will not exhibit Arrhenius temperature dependence because each of them contains at least one sum of rate coefficients. However, enzymatic reactions are often limited to a narrow temperature range, in which case they could display Arrhenius-like temperature dependence over that range. Several limiting forms of the rate expression can be found by setting one or two of the terms in the denominator equal to zero.

It is interesting to compare the results of this assignment to Example 4.8.3 in *REB, the Book*. In that example the [ES] complex decomposed directly to E and P, and the rate expression only had two terms in the denominator, equation (21). The number of terms in the denominator of a mechanistic rate expression for a catalytic or enzymatic reaction is often equal to the number of different complexes the catalyst forms. In the present assignment there are three forms, E, E-S, and E-P, and three terms in the denominator. In Example 4.8.3 there are two forms, E and E-S, and two terms in the denominator.

$$r_{P,1} = \frac{V_{max}[S]}{K_m + [S]} \tag{21}$$

Deliverables

If the proposed mechanism is correct, the rate of generation of P will be given by equation (20). Experimental studies will need to be performed to determine whether it captures the composition and temperature dependence of the rate with sufficient accuracy.

$$r_P = \frac{V_{max}\left[S\right]}{1 + K_s\left[S\right] + K_p[P]} \tag{20}$$