REB, The Course
Class 7 Practice Assignment Solution

Suppose that the non-elementary conversion of substrate to product, equation (1) is enzymatic and requires a cofactor, C. The concentration of the cofactor is easy to measure. The mechanism of the reaction is given by equations (2) through (4), where step (4) is effectively irreversible. Derive an expression for the apparent rate of enzymatic conversion of substrate to product, and show how the apparent rate expression changes if the enzyme-cofactor complex (E-C) is the most abundant intermediate.

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

$$E + C \rightleftarrows E\text{-}C \tag{2}$$

$$E\text{-}C + S \rightleftarrows S\text{-}E\text{-}C \tag{3}$$

$$S\text{-}E\text{-}C \rightarrow E\text{-}C + P \tag{4}$$

Assignment Summary

Apparent Reaction:

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

Mechanistic Steps:

$$E + C \rightleftarrows E\text{-}C \tag{2}$$

$$E\text{-}C + S \rightleftarrows S\text{-}E\text{-}C \tag{3}$$

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

Quantifiable Reagents: S, P, and C

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

Deliverables: Simplified expressions for $r_{1,P}$ when there is no most-abundant intermediate and when E-C is the most-abundant intermediate.

Equations for Generating the Mechanistic Rate Expression

Rates of the Mechanistic Steps

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

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

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

Apparent Rate of the Non-Elementary Reaction

$$r_{P,1} = r_4 \tag{8}$$

Bodenstein Steady-State Approximations[1]

$$0 = -r_2 \tag{9}$$

$$0 = r_2 - r_3 + r_4 \tag{10}$$

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

Enzyme Conservation Equation

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

Most Abundant Intermediate Inequalities:

$$\left[E\text{-}C\right] \gg [E] \tag{13}$$

$$\left[E\text{-}C\right] \gg \left[S\text{-}E\text{-}C\right] \tag{14}$$

Calculations

The algebraic details for the calculations in this section are presented in the file, Practice Assignment 7 Calculations.pdf. The calculations proceed as follows:

1. Substitute the rate expressions for the mechanistic steps, equations (5), (6), and (7), in equations (8) through (11).

---

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

2. Solve the enzyme conservation equation (12) together with two of the Bodenstein steady-state expressions, equations (9) through (11), to get expressions for the concentrations of the reactive intermediates.

$$[E] = \frac{E_0}{\left(1 + \frac{k_{2,f}}{k_{2,r}}[C] + \frac{k_{2,f}k_{3,f}}{k_{2,r}\left(k_{3,r} + k_{4,f}\right)}[C][S]\right)} \tag{17}$$

$$[E\text{-}C] = \frac{\frac{k_{2,f}}{k_{2,r}}E_0[C]}{1 + \frac{k_{2,f}}{k_{2,r}}[C] + \frac{k_{2,f}k_{3,f}}{k_{2,r}\left(k_{3,r} + k_{4,f}\right)}[C][S]} \tag{18}$$

$$[S\text{-}E\text{-}C] = \frac{\frac{k_{2,f}k_{3,f}}{k_{2,r}\left(k_{3,r} + k_{4,f}\right)}E_0[C][S]}{\left(1 + \frac{k_{2,f}}{k_{2,r}}[C] + \frac{k_{2,f}k_{3,f}}{k_{2,r}\left(k_{3,r} + k_{4,f}\right)}[C][S]\right)} \tag{19}$$

3. Substitute the expressions for the reactive intermediates, equations (15) through (17) into the version of equation (8) resulting from the first step of the calculations to get the mechanistic rate expression.

$$r_{P,1} = \frac{\frac{k_{2,f}k_{3,f}k_{4,f}}{k_{2,r}\left(k_{3,r} + k_{4,f}\right)}E_0[C][S]}{\left(1 + \frac{k_{2,f}}{k_{2,r}}[C] + \frac{k_{2,f}k_{3,f}}{k_{2,r}\left(k_{3,r} + k_{4,f}\right)}[C][S]\right)} \tag{20}$$

4. Substitute equations (17) and (19) in equation (14) and simplify.

$$\frac{k_{2,f}}{k_{2,r}}[C] \gg 1$$

5. Drop the 1 in the denominator of equation (20).
6. Substitute (17) and (19) in (14) and simplify.

$$\frac{k_{2,f}}{k_{2,r}}[C] \gg \frac{k_{2,f}k_{3,f}}{k_{2,r}\left(k_{3,r}+k_{4,f}\right)}[C]\,[S]$$

7. Drop the last term in the de denominator of equation (20).

$$r_{P,1} = \frac{k_{3,f}k_{4,f}}{\left(k_{3,r}+k_{4,f}\right)}E_0\ [S] \tag{21}$$

Simplification and Discussion

Defining the apparent rate coefficient and equilibrium constants shown in equations (22), (23), and (24), the mechanistic rate expression shows the simplified form of equation (25). Of those three parameters, only K will exhibit Arrhenius temperature dependence.

$$k = \frac{k_{2,f}k_{3,f}k_{4,f}}{k_{2,r}\left(k_{3,r}+k_{4,f}\right)} \tag{22}$$

$$K = \frac{k_{2,f}}{k_{2,r}} \tag{23}$$

$$K' = \frac{k_{2,f}k_{3,f}}{k_{2,r}\left(k_{3,r}+k_{4,f}\right)} \tag{24}$$

$$r_{P,1} = \frac{kE_0 \left[C\right] \left[S\right]}{\left(1 + K\left[C\right] + K'\left[C\right]\left[S\right]\right)} \tag{25}$$

Defining the effective rate coefficient shown in equation (26), the rate expression simplifies to equations (27) if the enzyme-cofactor complex (E-C) is the most abundant intermediate. Again, the effective rate coefficient, k, will not display Arrhenius temperature dependence.

$$k = \frac{k_{3,f}k_{4,f}}{\left(k_{3,r} + k_{4,f}\right)} \tag{26}$$

$$r_{P,1} = kE_0 \left[S\right] \tag{27}$$

Deliverables

The apparent rate of generation of P via reaction (1) is given by equation (25) if the proposed mechanism is correct, and by equation (27) if additionally the enzyme-cofactor complex (E-C) is the most abundant intermediate. Experimental studies will need to be performed to determine whether either of these rate expressions capture the composition and temperature dependence of the rate with sufficient accuracy.

$$r_{P,1} = \frac{kE_0 \left[C\right] \left[S\right]}{\left(1 + K\left[C\right] + K'\left[C\right]\left[S\right]\right)} \tag{25}$$

$$r_{P,1} = kE_0 \left[S\right] \tag{27}$$