A First Course on Kinetics and Reaction Engineering

Activity 9.1

Problem Purpose

This problem will help you determine whether you have mastered the learning objectives for this unit. It also introduces the concept of an enzyme co-factor and its effect upon a Michaelis-Menten rate expression.

Problem Statement

Consider the macroscopically observed reaction (1) which is catalyzed by the enzyme E. This enzyme will not complex with the substrate, S, unless it first complexes with the co-factor, C. The complete reaction mechanism is given by reactions (2) through (4), where step (4) is effectively irreversible. Derive a Michaelis-Menten type rate expression based upon this mechanism. You may assume that the concentration of the non-complexed cofactor is easy to measure, and therefore the concentration of non-complexed cofactor may appear in the rate expression.

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

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

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

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

Problem Analysis

The approach to solving this problem will be the same as that used in other Michaelis-Menten mechanism problems. An expression for the overall rate of formation of the product P will first be written. The Bodenstein steady state equations for all but one of the reactive intermediates will then be solved along with an equation for the conservation of enzyme to obtain expressions for the concentrations of the reactive intermediates. These will the be substituted back into the rate expression, thereby eliminating all concentrations of reactive intermediates.

Problem Solution

The net rate of production of P is equal to the sum of its rates of production in each of the mechanistic steps. In this case, P is only produced in reaction (4), and that reaction is effectively irreversible. Thus, the rate of production of P is given by equation (5).

$$r_{P,1} = r_4 = k_{4,f}\left[\text{S-E-C}\right] \tag{5}$$

The substrate-enzyme-cofactor complex is a reactive intermediate, and its concentration may be difficult to measure, so it is desirable to eliminate it from the rate expression. To do so, the Bodenstein steady state approximation, equation (6), can be written for all but one of the reactive intermediates. (It could be written for all the reactive intermediates, but experience shows that one of the resulting equations will not be mathematically independent.) In this case the reactive intermediates are E, E-C, and S-E-C. The Bodenstein steady state equations for E and S-E-C are given in equations (7) and (8). The term corresponding to the rate of step (4) in the reverse direction was dropped since the problem statement says step (4) is effectively irreversible.

$$0 = \sum_{\substack{s=\text{ all}\\ \text{steps}}} \nu_{i,s} \left(k_{s,f} \prod_{\substack{m=\text{all}\\ \text{reactants}}} \left[m\right]^{-\nu_{m,s}} - k_{s,r} \prod_{\substack{n=\text{all}\\ \text{products}}} \left[n\right]^{\nu_{n,s}} \right) \quad i = \text{reactive intermediate} \tag{6}$$

$$0 = -k_{2,f}\left[\text{E}\right]\left[\text{C}\right] + k_{2,r}\left[\text{E-C}\right] \tag{7}$$

$$0 = k_{3,f}\left[\text{E-C}\right]\left[\text{S}\right] - k_{3,r}\left[\text{S-E-C}\right] - k_{4,f}\left[\text{S-E-C}\right] \tag{8}$$

There are two equations, but three unknowns (E, E-C, and S-E-C). The enzyme (in all of its forms) is conserved, however, and that provides a third relationship, equation (9). The quantity E^0 is the equivalent concentration of enzyme before it is added to the system.

$$E^0 = \left[\text{E}\right] + \left[\text{E-C}\right] + \left[\text{S-E-C}\right] \tag{9}$$

Equations (7), (8) and (9) can be solved to obtain expressions for the concentrations of the three reactive intermediates, equations (10), (11) and (12).

$$\left[\text{E-C}\right] = \frac{E^0\left[\text{C}\right]}{\frac{k_{2,r}}{k_{2,f}} + \left[\text{C}\right] + \frac{k_{3,f}}{\left(k_{3,r}+k_{4,f}\right)}\left[\text{S}\right]\left[\text{C}\right]} \tag{10}$$

$$\left[\text{E}\right] = \frac{\frac{k_{2,r}}{k_{2,f}}E^0}{\frac{k_{2,r}}{k_{2,f}} + \left[\text{C}\right] + \frac{k_{3,f}}{\left(k_{3,r}+k_{4,f}\right)}\left[\text{S}\right]\left[\text{C}\right]} \tag{11}$$

$$\left[\text{S-E-C}\right] = \frac{\left(\frac{k_{3,f}}{\left(k_{3,r}+k_{4,f}\right)}\left[\text{S}\right]\left[\text{C}\right]\right)E^0}{\frac{k_{2,r}}{k_{2,f}} + \left[\text{C}\right] + \frac{k_{3,f}}{\left(k_{3,r}+k_{4,f}\right)}\left[\text{S}\right]\left[\text{C}\right]} \tag{12}$$

Equation (12) can then be substituted into equation (5), leading to the rate expression given in equation (14). If V_{max}, K_m and K_c are defined according to equations (15) through (17), the resulting rate expression, equation (18), looks quite similar to the Michaelis-Menten expressions derived in Examples 9.1 and 9.3.

$$r_{P,1} = \frac{\left(\frac{k_{3,f}k_{4,f}}{\left(k_{3,r}+k_{4,f}\right)}[\mathrm{S}][\mathrm{C}]\right)E^0}{\frac{k_{2,r}}{k_{2,f}}+[\mathrm{C}]+\frac{k_{3,f}}{\left(k_{3,r}+k_{4,f}\right)}[\mathrm{S}][\mathrm{C}]} \tag{13}$$

$$r_{P,1} = \frac{k_{4,f}E^0[\mathrm{S}][\mathrm{C}]}{\frac{k_{2,r}\left(k_{3,r}+k_{4,f}\right)}{k_{2,f}k_{3,f}}+\frac{\left(k_{3,r}+k_{4,f}\right)}{k_{3,f}}[\mathrm{C}]+[\mathrm{S}][\mathrm{C}]} \tag{14}$$

$$V_{\max} = k_{4,f}E^0 \tag{15}$$

$$K_m = \frac{k_{2,r}\left(k_{3,r}+k_{4,f}\right)}{k_{2,f}k_{3,f}} \tag{16}$$

$$K_c = \frac{\left(k_{3,r}+k_{4,f}\right)}{k_{3,f}} \tag{17}$$

$$r_{P,1} = \frac{V_{\max}[\mathrm{S}][\mathrm{C}]}{K_m+K_c[\mathrm{C}]+[\mathrm{S}][\mathrm{C}]} = \frac{V_{\max}[\mathrm{S}]}{\frac{K_m}{[\mathrm{C}]}+K_c+[\mathrm{S}]} \tag{18}$$