The steady state approximation is a method used to estimate the overall reaction rate of a multi-step reaction. It assumes that the rate of change of intermediate concentration in a multi-step reaction are constant. This method can only be applied when the first step of the reaction is significantly slower than subsequent step in an intermediate-forming consecutive reaction.

**Introduction**

Before discussing the steady state approximation, it must be understood that the approximation is derived to simplify the kinetic expression for product concentration, \([\text{product}]\). Consider the following sequential reaction:

\[
A \xrightarrow{k_1} B \xrightarrow{k_2} C
\]

Calculating the \([\text{product}]\) depends on all the rate constants in each step. For example, if the kinetic method was used to find the concentration of C, \([C]\), at time \(t\) in the above reaction, the expression would be

\[
[C] = [A]_0 \left(1 + \frac{k_2e^{-k_1t} - k_1e^{-k_2t}}{k_1 - k_2}\right) \quad \text{(1)}
\]

With a more complicated mechanisms, the kinetic expression becomes harder to derive. To simplify this calculation, scientists developed the steady state approximation and the \textit{pre-equilibrium approximation} for determining the overall reaction rates of consecutive reactions. This article concerns the steady state approximation.

**Steady State Approximation**

The steady state approximation is applies to a consecutive reaction with a slow first step and a fast second step (\(k_1<<k_2\)). If the first step is very slow in comparison to the second step, there is no accumulation of intermediate product, such as product B in the above example.

\[
\frac{dB}{dt} = 0 = k_1[A] - k_2[B] \quad \text{(2)}
\]

Thus

\[
[B] = \frac{k_1[A]}{k_2} \quad \text{(3)}
\]

From the mechanism:

\[
\frac{d[C]}{dt} = k_2[B] = \frac{k_2k_1[A]}{k_2} = k_1[A] \quad \text{(4)}
\]

Solving for \([C]\):

\[
[C] = [A]_0 (1 - e^{-k_1t}) \quad \text{(5)}
\]

Equation \((\text{ref}(2))\) is much simpler to derive than Equation \((\text{ref}(1))\), especially with a more complicated reaction mechanisms.

Example \((\text{PageIndex}(1))\)
Consider the reaction:

\[ (A + 2B \xrightarrow{\text{k}} C) \]

A: What is the expected rate law according to the following proposed multi-step mechanism under the steady state approximation with \((k_2 >> k_{-1})\) for the following mechanism:

- \( (A + B \xrightarrow{\text{k}_{-1}} I) \) Slow
- \( (I + B \xrightarrow{\text{k}_2} C) \) Fast

B: If \( x \) is the order of the reaction with respect to \( A \), \( y \) is the order of the reaction with respect to \( B \), and \( n \) is the overall reaction order. What are the values of \( x \), \( y \), and \( n \)?

**SOLUTION**

A:

\[
\frac{d[I]}{dt} = k_1[A][B] - k_{-1}[I] - k_2[I][B] = 0
\]

\[
[I] = \frac{k_1[A][B]}{k_{-1} + k_2[B]}
\]

because \((k_2 >> k_{-1})\) then \(k_{-1} = 0\). Therefore, \([I] = \frac{k_1[A]}{k_2} \)

\[
\frac{d[C]}{dt} = k_2[I][B]
\]

\[
\frac{d[C]}{dt} = \frac{k_1k_2[A][B]}{k_2}
\]

\[
\frac{d[C]}{dt} = k_1[A][B]
\]

B:

- \((x = 1)\)
- \((y = 1)\)
- \((n = 2)\)

**Use of the Steady-State Approximation in Enzyme Kinetics**

In 1925, George E. Briggs and John B. S. Haldane applied the steady state approximation method to determine the rate law of the enzyme-catalyzed reaction (Figure 1). The following assumptions were made:

1. The rate constant of the first step must be slower than the rate constant of the second step (\((k_1 << k_2)\)), hence \(\frac{d[d[ES]]}{dt} = 0\).
2. Enzyme concentration must be significantly lower than the substrate concentration to keep the first step slower than the second step.
Figure 1: Steady state dynamics in enzymes

This gives the following:

\[
\frac{d[P]}{dt} = k_{-2}[ES] \tag{6}
\]

where

\[
\frac{d[ES]}{dt} = 0 = k_{-1}[E][S] - k_{-1}[ES] - k_{2}[ES] \tag{7}
\]

Because

\[[S] >> [E] \tag{8}\]

Using the second assumption and the fact that enzyme concentration equals the initial concentration of enzyme minus the concentration of the enzyme-substrate intermediate,

\[[E] = [E]_o - [ES] \tag{9}\]

The following equation is obtained:

\[k_{1}[E]_o[S] = k_{-1}[ES] + k_{2}[ES] + k_{1}[ES][S] \tag{10}\]

From this equation, the concentration of the ES intermediate can be found:

\[[ES] = \frac{k_{1}[E]_o[S]}{(k_{-1} + k_{2}) + k_{1}[S]} \tag{11}\]

Substitute this into Equation (3) gives,

\[
\frac{d[P]}{dt} = \frac{k_{2}[E]_0[S]}{(k_{1}+k_{2})/k_{1}+[S]} = \frac{k_{2}[E]_0[S]}{(K_M+[S]} \tag{12}\]

\[\]
where
\[ K_M = \frac{k_{-1} + k_2}{k_1} \]

Because in most of the cases, only the initial \( \frac{d[P]}{dt} \) is measured to determine the rate of product formation, (4) can be rewritten as:
\[ v_0 = \frac{d[P]_0}{dt} = \frac{k_2[E]_0[S]}{K_M + [S]} \]

Because \( [E]_0 = \frac{V_{max}}{k_2} \). Equation \((\text{ref}(5))\) becomes the following:
\[ v_0 = \frac{d[P]_0}{dt} = \frac{k_2/k_2)\frac{v_{max}}{k_2)[S]}{(K_M + [S])} \]
\[ = \frac{V_{max}[S]}{K_M + [S]} \]

This equation is a very useful tool to in calculating \( v_{max} \) and \( K_M \) (the Michaelis constant), of an enzyme by using the Lineweaver-Burk plot \((1/[S] \text{ vs. } 1/v_0)\) or the Eadie-Hofstee plot \((v_0/[S] \text{ vs. } v_0)\).

### Problems

Given the reaction \( A \xrightarrow{k_1} B \xrightarrow{k_2} C \)

where \( k_1 = 0.2 \text{ M}^{-1}\text{s}^{-1} \), \( k_2 = 2000 \text{ s}^{-1} \)

1. Write the reaction rates for A, B, and C.
2. Is this a steady-state reaction?
3. Write the expression for \( \frac{d[C]}{dt} \) using the Steady State Approximation
4. Calculate \( \frac{d[C]}{dt} \) if \( [A] = 1 \text{M} \)
5. Calculate \( [C] \) at \( t = 3 \text{ s} \) and \( [A]_0 = 2 \text{M} \)

### Solutions

1) \( \frac{d[A]}{dt} = -k_1[A]; \frac{d[B]}{dt} = k_1[A] - k_2[B]; \frac{d[C]}{dt} = k_2[B] \)

2) Because \( k_1 \) is much larger than \( k_2 \), this is a steady state reaction.

3) \( \frac{d[C]}{dt} = k_2[B] \)

where \( \frac{d[B]}{dt} = k_1[A] - k_2[B] = 0 \)

so, \( [B] = \frac{k_1[A]}{k_2} \)

Substitute this into \( \frac{d[C]}{dt} \)
\[ \frac{d[C]}{dt} = k_1[A] \]
4) \( \frac{d[C]}{dt} = 0.2 \text{M}^{-1} \text{s}^{-1} \times 1 \text{M} = 0.2 \text{ s}^{-1} \)

5) \( [C] = [A]_0 \left(1 - e^{-kt}\right) = 2 \text{M} \times (1 - e^{-0.2 \times 3}) = 0.9 \text{ M} \)

References:


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