Enzyme Kinetics

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Kinetic Experiments:

- **Enzymatic Hydrolysis of Urea**
- **Decomposition of Hydrogen Peroxide catalyzed by Catalase**

Enzymatic decomposition of a substrate can be described as follows:

$$\mathbf{E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} ES \xrightarrow{k_{+2}} E + P} \qquad (1)$$

Enzyme **E** combines with substrate **S** to form an enzyme substrate complex **ES**, with rate constant k_1. The **ES** complex has two possible fates. It can dissociate back to form **E** + **S**, with a rate constant $\mathbf{k_{-1}}$, or it can proceed to form product P with a rate constant $\mathbf{k_{+2}}$.

In the **steady state** the rates of formation and disappearance of **ES** cancel out**:**

$$k_{+1} \cdot c_E \cdot c_S = (k_{-1} + k_{+2}) \cdot c_{ES} \qquad (2)$$

$$\frac{c_E \cdot c_S}{c_{ES}} = \frac{(k_{-1} + k_{+2})}{k_{+1}} = K_m \qquad (3)$$

$\mathbf{K_m}$ is called **Michaelis constant**. In order to determine this constant from simply measurable parameters, the following considerations are taken as basis:

The rate **v** (formation of the product) is directly proportional to the concentration of **ES**:

$$v = k_{+2} \cdot c_{ES} \qquad (4)$$

The total of the concentrations of **E** and **ES** is independent of the substrate concentration. $\mathbf{c_{ET}}$ is the total enzyme concentration. Substituting $\mathbf{c_E} = \mathbf{c_{ET}} - \mathbf{c_{ES}}$ into equation **(3)** and solving for $\mathbf{c_{ES}}$ leads to:

$$c_{ES} = \frac{c_{ET} \cdot c_S}{K_m + c_S} \qquad (5)$$

Combining equations **(4)** with **(5)** yields:

$$\mathbf{v = k_{+2} \cdot c_{ET} \cdot \frac{c_S}{K_m + c_S}} \quad \textbf{(6)}$$

From equation **(6)** is obtained:
At very low substrate concentration, where $\mathbf{c_S << K_m}$ the initial rate is linearly proportional to the substrate concentration $\mathbf{c_S}$. At high substrate concentration $\mathbf{c_S >> K_m}$ the reaction rate is constant and independent of the substrate concentration $\mathbf{c_S}$ (saturation of the enzyme with substrate).

Therefore:

$$\mathbf{v_{max} = k_{+2} \cdot c_{ET}} \quad \textbf{(7)}$$

Substituting **(7)** into equation **(6)** results in the **Michaelis-Menten-Equation**:

$$\mathbf{v = v_{max} \cdot \frac{c_S}{K_m + c_S}} \quad \textbf{(8)}$$

In practice one maintains the enzyme concentration constant, measures the reaction rates with different substrate concentrations and draws the diagram **v** as function of **[S]**.

At the half of the maximal reaction rate half of the enzyme must be present as reactive enzyme substrate complex.($\mathbf{c_E = c_{ES}}$). From equation **(3)** is given:

$$\mathbf{K_m = c_S} \quad \textbf{(9)}$$

The meaning of $\mathbf{K_m}$ is evident from equation **(8)**. When $\mathbf{K_m = c_S}$

$$\text{then } \mathbf{v} = \frac{\mathbf{v_{max}}}{2} \quad \textbf{(10)}$$

Thus $\mathbf{K_m}$ corresponds to the substrate concentration where the reaction rate is half of its maximal value.

The recording of a saturation curve and the sufficiently exact determination of the maximum value require many measuring points. If additionally with larger substrate concentrations an enzyme inhibition occurs, the max. reaction rate does not correspond to the status of the saturation of the enzyme.

Both disadvantages are avoided by taking the reciprocal of both sides of the equation **(8)** to give:

$$\frac{1}{v} = \frac{K_m}{v_{max}} \cdot \frac{1}{c_S} + \frac{1}{v_{max}} \qquad \textbf{(11)}$$

Thus a plot of **1 / v** versus **1 / c_S** will give a straight-line plot with a slope of **K_m / v_{max}** and an intercept on the ordinate of **1 / v_{max}**. This double reciprocal plot is known as a Lineweaver-Burk plot.

When $\frac{1}{v} = 0$ (intersection of the straight line with the abscissa)

then $\frac{1}{K_m} = -\frac{1}{c_S}$

and when $\frac{1}{c_S} = 0$ (intersection of the straight line with the ordinate)

then $\frac{1}{v_{max}} = \frac{1}{v}$

In order to determine a straight line only few data points are required

The system of equations can be applied accurately only to a one-substrate enzyme-catalyzed reaction. Because the practically constant water concentration hydrolyses in diluted, aqueous solutions can be treated as such.