B.Sc. Botany (Hons) – 2ND SEM

by Dr. Raman Kumar Ravi

Factors affecting enzymatic reaction

The factors that mainly influence any enzyme-catalysed reaction are:

1. Substrate concentration

2. Enzyme concentration

3. Temperature

4. pH

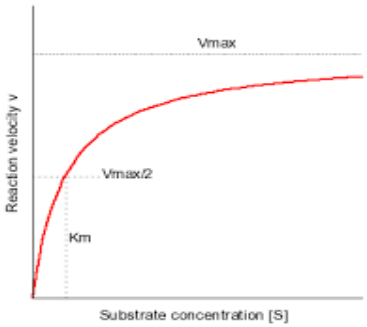
5. Inhibitors

Other factors such as **state of enzyme (oxidation), time and activators** also affect enzyme-catalysed reaction to certain extent.

Substrate concentration

Keeping the factors such as pH, temperature and enzyme concentration at optimum levels, if the **substrate concentration is increased, the velocity of the reaction recorded a rectangular hyperbola.**

- At **very low substrate concentration** the initial reaction velocity (v) is nearly proportional to the substrate concentration (first order kinetics).
- However, if the substrate concentration is increased the rate of increase slows down (mixed order kinetics).
- With a further increase in the substrate concentration the reaction rate approaches a constant (zero order-reaction where velocity is independent of substrate concentration).
- At initial point, eventhough the substrate molecules are present in excess than enzyme on molar basis, not all the enzyme molecules present combine with the substrate.
- Hence, increasing the substrate concentration will increase the amount of enzyme associated with substrate as ES and thus v will depend on [S].
- At Vmax, all the enzyme molecules are saturated with substrate molecules so that further increase in [S] cannot result in increased reaction rate.
- Michaelis-Menten derived an equation to explain this type of behaviour.



$$v = \frac{\text{Vmax [S]}}{\text{Km+[S]}}$$

Where, [S] = Substrate concentration

Vmax = Maximum velocity

v = Velocity of the reaction

At half maximal velocity [S] = Km

i.e Vmax/2 = Vmax [S]/Km + [S]

Km + [S] /2 = Vmax [S]/ Vmax

Km + [S] = 2 [S]

Km = [S]

Hence, Michaelis - Menten constant, **Km, is defined as the substrate concentration at half maximal velocity and is expressed as mole per litre**.

A plot of 1/v versus 1/ [S] (the double reciprocal) yields a straight line.

- This line intercept X-axis at -1/Km and Y-axis at 1/Vmax.
- The slope of the line is **Km/Vmax.**
- The Lineweaver-Burk plot has the great advantage of allowing more accurate determination of Vmax and Km

Significance of Km

i. Km value may vary with substrate.

ii. An enzyme whose Km is very low will have a high degree of affinity for its substrate

Enzyme concentration

- When compared to substrate concentration, the concentration of enzyme is always **very very low** on molar basis.

- Hence, **increasing the enzyme concentration will always increase the reaction rate**

Temperature

- The **velocity of enzyme-catalysed reactions** roughly **doubles with a 10oC** rise in temperature over a limited range of temperature
- Enzymes, being proteins, are **denatured by heat** and become **inactive** as the temperature increases beyond a certain point.
- Most of the enzymes are inactivated at temperatures **above 60ºC.**
- The temperature at which the reaction rate is maximum is known as **optimum temperature**

pH

- Most enzymes have a **characteristic pH** at which their activity is maximum; above or below this pH, the activity declines
- The pH affects the ionic state of the enzyme and frequently that of the substrate also.
- If a negatively charged enzyme (E-) reacts with a positively charged substrate (SH+), ESH is formed.
- At low pH values, E- will be protonated and ESH is not formed.
- Similarly, at very high pH values SH+ will ionize and lose its positive charge.

E- + SH+ ------> ESH

acidic pH

E- + SH+ ------> EH + SH+ --------> No ESH formation

alkaline pH

SH+ ----------> S + H+ + E- --------> No ESH formation

- Another important factor is the **change in conformation (denaturation) of enzyme at extreme pH values.**

Inhibitors

- Compounds that have the **ability to combine with certain enzymes** but **do not serve as substrates** and therefore **block catalysis** are called **inhibitors**.
- The important type of inhibitors are **competitive** and **noncompetitive inhibitors**.

Competitive inhibitor

- Any compound which **possessess a close structural resemblance to a particular**

substrate and which **competes with that of substrate for the same active site on the enzyme** is called as **competitive inhibitor**.

- The inhibitor is not acted upon by the enzyme and so remains bound to the enzyme preventing the substrate to bind.
- This is a **reversible process**.
- It **depends upon the relative concentration of substrate and inhibitor**.
- Competitive inhibition can be completely reversed by addition of large excess of substrate high inhibitor concn.

E + I ----------> E I

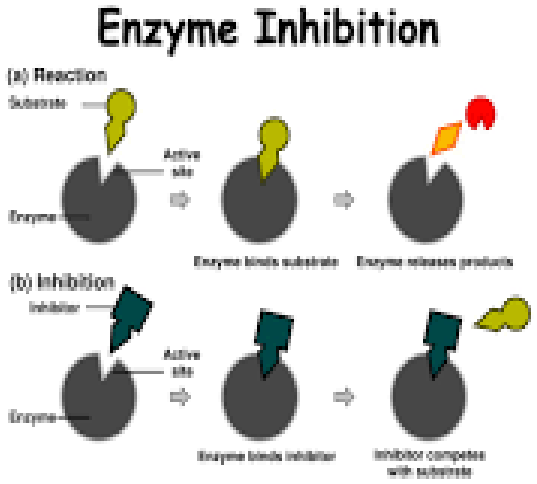
<----------

high substrate concn.

Eg. the enzyme, succinate dehydrogenase converts succinate to fumarate.

For this reaction, **malonic acid** is a **competitive inhibitor** as it structurally resembles that of succinate

- In case of competitive inhibition, **Km is increased** but **Vmax is not altered**.



Non-competitive inhibitor

- Non-competitive inhibitors **bind to a site other than the active site on the enzyme** often to **deform the enzyme**, so that, it does not form the ES complex

at its normal rate.

- Once formed, the ES complex does not decompose at the normal rate to yield products.
- These **effects are not reversed** by increasing the substrate concentration.

E + I -------> EI

ES + I ------> ESI

- Some enzymes possessing an essential -SH group are non-competitively inhibited by heavy metal ions (Hg2+, Pb2+).
- Some **metalloenzymes** are inhibited **non competitively by metal chelating agents like ethylene diamine tetraacetic acid (EDTA).**
- Inhibitors are used as **tools to probe the mechanism of enzyme - catalysed reactions** and as **therapeutic agents**.
- In case of noncompetitive inhibition, Vmax is lowered but Km is not altered

Uncompetitive inhibitor

- In case of uncompetitive inhibition, the inhibitor binds only to free enzyme and not to the enzyme substrate [ES] complex