BIO102-Foundations of Biology (I)

Enzyme kinetics

INDRAPRASTHA INSTITUTE *of*
INFORMATION TECHNOLOGY **DELHI**

Dr. Jaspreet Kaur Dhanjal
Assistant Professor, Center for Computational Biology
Email ID: jaspreet@iiitd.ac.in

February 03, 2025

Why do we need catalysis

- Many of us, for example, consume substantial amounts of sucrose—common table sugar—as a kind of fuel, whether in the form of sweetened foods and drinks or as sugar itself. The conversion of sucrose to CO_2 and H_2O in the presence of oxygen is a highly exergonic process, releasing free energy that we can use to think, move, taste, and see. However, a bag of sugar can remain on the shelf for years without any obvious conversion to CO_2 and H_2O .
- Although this chemical process is thermodynamically favorable, it is very slow!
- Yet when sucrose is consumed by a human (or almost any other organism), it releases its chemical energy in seconds. The difference is catalysis.
- Without catalysis, chemical reactions such as sucrose oxidation could not occur on a useful time scale, and thus could not sustain life.

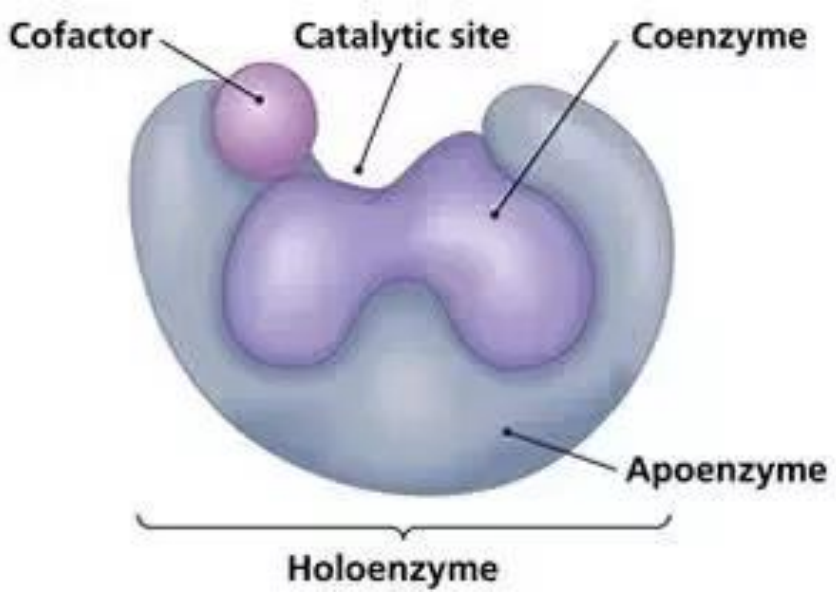
Enzymes are biological catalysts

- The enzymes are the most remarkable and highly specialized proteins, with the exception of a small group of catalytic RNA molecules.
- Enzymes have extraordinary catalytic power, often far greater than that of synthetic or inorganic catalysts.
- They have a high degree of specificity for their substrates.
- They accelerate chemical reactions tremendously, giving increase in speed up to 10 million times or more.
- They function in aqueous solutions under very mild conditions of temperature and pH.
- Only a few non-biological catalysts have all these properties.

Enzymes are biological catalysts

- The study of enzymes has immense practical importance.
- In some diseases, especially inheritable genetic disorders, there may be a deficiency or even a total absence of one or more enzymes.
- For other disease conditions, excessive activity of an enzyme may be the cause. Measurements of the activities of enzymes in blood plasma, erythrocytes, or tissue samples are important in diagnosing certain illnesses.
- Many drugs exert their biological effects through interactions with enzymes.

Enzymes are biological catalysts



- Some enzymes require no chemical groups for activity other than their amino acid residues.
- Others require an additional chemical component called a cofactor—either one or more inorganic ions, such as Fe^{2+}, Mg^{2+}, Mn^{2+}, or Zn^{2+}.
- Others may require a complex organic or metalloorganic molecule called a coenzyme. Example: Biocytin, Coenzyme A, Nicotinamide adenine dinucleotide.

International Classification of Enzymes

No.	*Class*	*Type of reaction catalyzed*
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Addition of groups to double bonds, or formation of double bonds by removal of groups
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to ATP cleavage

International Classification of Enzymes

Each enzyme is assigned a four-part classification number and a systematic name, which identifies the reaction it catalyzes.

$$\text{ATP + D-glucose} \rightarrow \text{ADP + D-glucose 6-phosphate}$$

ATP:glucose phosphotransferase - catalyzes the transfer of a phosphoryl group from ATP to glucose.

Its Enzyme Commission number (E.C. number) is 2.7.1.1.

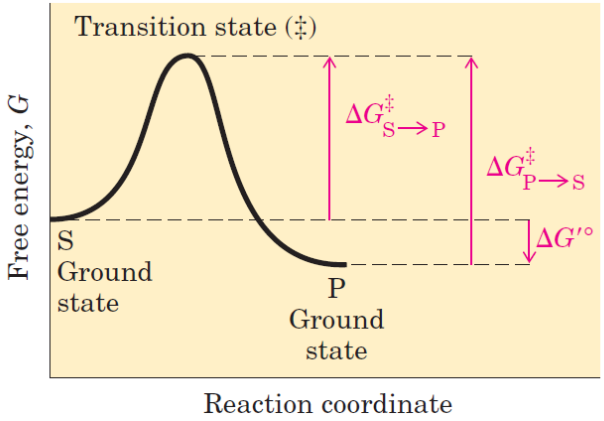
The first number (2) denotes the class name (transferase);

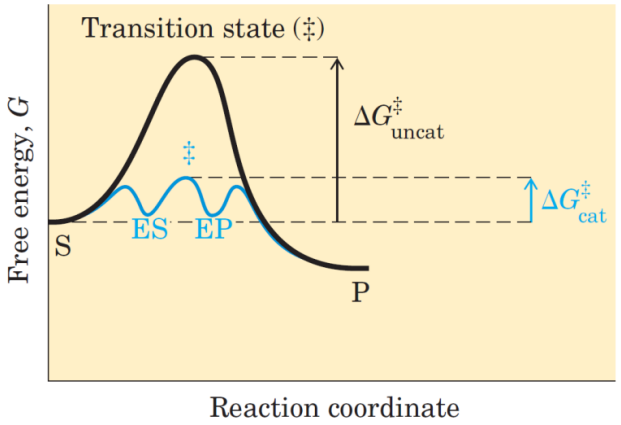
the second number (7) denotes the subclass phosphotransferase;

the third number (1) denotes a phosphotransferase with a hydroxyl group as acceptor;

and the fourth number (1) denotes D-glucose as the phosphoryl group acceptor.

Enzymes lower the activation energy

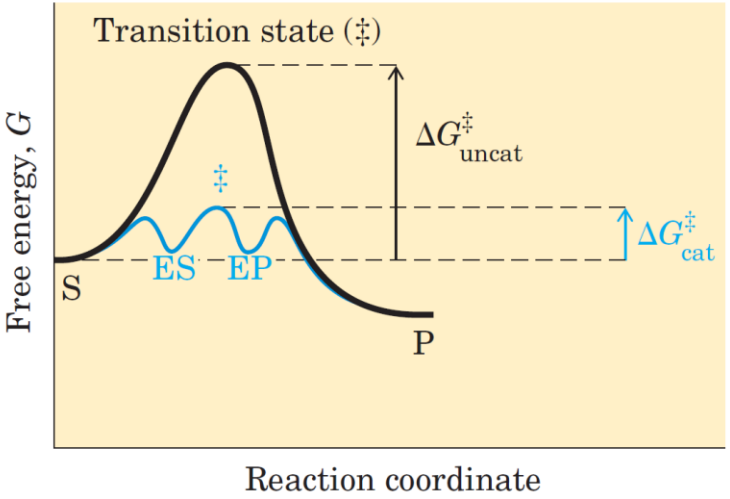




The function of a catalyst is to increase the rate of a reaction. Catalysts do not affect the position and direction of equilibrium.

A favorable equilibrium does not mean that the S → P conversion will occur at a detectable rate. The rate of a reaction is dependent on an entirely different parameter. There is an energy barrier between S and P: the energy required for alignment of reacting groups, formation of transient unstable charges, bond rearrangements, and other transformations required for the reaction to proceed in either direction.

What do enzymes do?



In the above reaction $S \underset{k_{-1}=10^{-5}}{\overset{k_1=10^{-3}}{\rightleftharpoons}} P$ *and* $k_{eq} = \frac{[P]}{[S]} = 100.$

If both k_1 and k_{-1} increase by 10^6 fold by enzyme, still K_{eq} will be

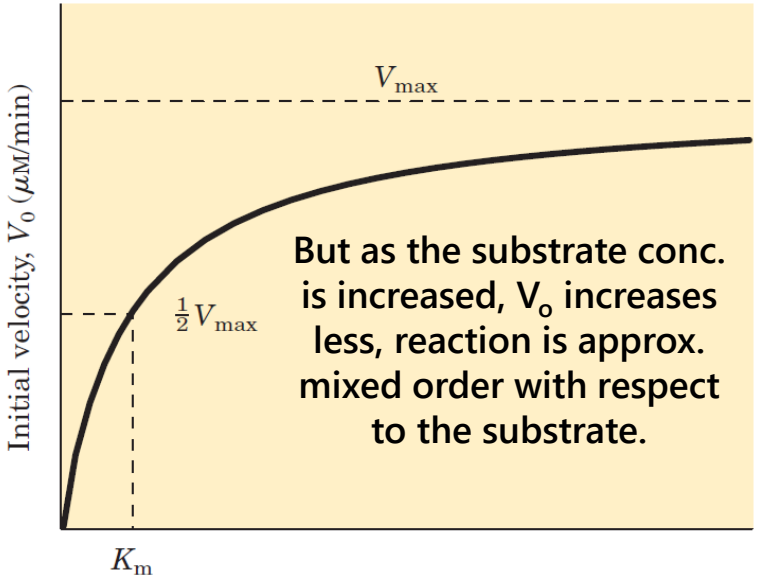
$$k_{eq} = \frac{10^{(-3+6)}}{10^{(-5+6)}} = 100.$$

Enzymes Catalysis – Michaelis-Menten Equation

The central approach to studying the mechanism of an enzyme-catalyzed reaction is to determine the rate of the reaction and how it changes in response to changes in experimental parameters, a discipline known as enzyme kinetics.

Substrate concentration affects the rate of enzyme-catalyzed reactions

At low substrate concentration, V_0 is nearly proportional to the substrate and the reaction is approx. first order with respect to the substrate.



With further increase in substrate conc., the reaction rate becomes independent of substrate conc. (zero order) and asymptomatically approaches a constant rate.

↓

Enzyme is saturated

Derivation of Michaelis-Menten equation

Assumption: enzyme first combines reversibly with its substrate to form an enzyme-substrate complex in a relatively fast reversible step:

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES}$$

The ES complex then breaks down in a slower second step to yield the free enzyme and the reaction product P:

$$\mathrm{ES} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{E} + \mathrm{P}$$

These reactions are assumed to be reversible.

Michaelis-Menten equation expresses the mathematical relationship between the initial rate of an enzyme-catalyzed reaction, the concentration of the substrate, and certain characteristics of the enzyme.

It is the rate equation for the reactions catalyzed by enzymes having a single substrate.

Derivation of Michaelis-Menten equation

Conventions:
[E] = free or uncombined enzyme
[ES] = concentration of enzyme substrate complex
[E_t] = total enzyme concentration (free + combined forms)
[S] = substrate concentration
[S]>>[E], so amount of S bound by E at any given time is negligible compared to total concentration of S

The purpose here is to define a general expression for $V_{0,}$ initial velocity of an enzyme-catalyzed reaction, assuming that it takes place in two steps.

Derivation of Michaelis-Menten equation

The initial velocity is equal to the breakdown of the ES complex, and can be written as a first order rate equation,

$$V_0 = k_2[\mathrm{ES}]$$

But neither k_2 or [ES] can be determined experimentally.

To find alternative variables:

$$\text{Rate of ES formation} = k_1([\mathrm{E_t}] - [\mathrm{ES}])[\mathrm{S}]$$

$$\text{Rate of ES breakdown} = k_{-1}[\mathrm{ES}] + k_2[\mathrm{ES}]$$

ES can also be formed from E and P by reverse reaction, but can be neglected since we are considering the beginning of the reaction in the forward direction, where [S] is very high and [P] is close to zero.

At steady state, rate of formation of ES is equal to rate of breakdown of ES, and thus ES remains constant:

$$k_1([\mathrm{E_t}] - [\mathrm{ES}])[\mathrm{S}] = k_{-1}[\mathrm{ES}] + k_2[\mathrm{ES}]$$

Derivation of Michaelis-Menten equation

The equation is rearranged to solve for [ES]:

$$k_1([\mathrm{E_t}] - [\mathrm{ES}])[\mathrm{S}] = k_{-1}[\mathrm{ES}] + k_2[\mathrm{ES}]$$

$$k_1[\mathrm{E_t}][\mathrm{S}] - k_1[\mathrm{ES}][\mathrm{S}] = (k_{-1} + k_2)[\mathrm{ES}]$$

$$k_1[\mathrm{E_t}][\mathrm{S}] = (k_1[\mathrm{S}] + k_{-1} + k_2)[\mathrm{ES}]$$

$$[\mathrm{ES}] = \frac{k_1[\mathrm{E_t}][\mathrm{S}]}{k_1[\mathrm{S}] + k_{-1} + k_2}$$

$$[\mathrm{ES}] = \frac{[\mathrm{E_t}][\mathrm{S}]}{[\mathrm{S}] + (k_2 + k_{-1})/k_1}$$

The term $(k_2 + k_{-1})/k_1$ is defined as the Michaelis-Menten constant, K_m.

$$[\mathrm{ES}] = \frac{[\mathrm{E_t}][\mathrm{S}]}{K_\mathrm{m} + [\mathrm{S}]}$$

Derivation of Michaelis-Menten equation

We already know, $V_0 = k_2[\mathrm{ES}]$

Substituting in previous equation we get, $$V_0 = \frac{k_2[\mathrm{E_t}][\mathrm{S}]}{K_\mathrm{m} + [\mathrm{S}]}$$

This equation can be further simplified. Because the maximum velocity occurs when the enzyme is saturated (that is, with [ES] = [E_t]) V_{max} can be defined as $k_2[E_t]$.

$$V_0 = \frac{V_{\mathrm{max}}[\mathrm{S}]}{K_\mathrm{m} + [\mathrm{S}]}$$

This is the Michaelis-Menten equation, the rate equation for a one-substrate enzyme-catalyzed reaction. It is a statement of the quantitative relationship between the initial velocity V_0, the maximum velocity V_{max}, and the initial substrate concentration [S], all related through the Michaelis constant K_m.

Derivation of Michaelis-Menten equation

An important numerical relationship emerges from the Michaelis-Menten equation in the special case when V_0 is exactly one-half V_{max}.

$$\frac{V_{max}}{2} = \frac{V_{max}[S]}{K_m + [S]}$$

$$\frac{1}{2} = \frac{[S]}{K_m + [S]}$$

Solving for K_m, we get $K_m + [S] = 2[S]$, or

$$K_m = [S], \text{ when } V_0 = \frac{1}{2}V_{max}$$

K_m is equivalent to the substrate concentration at which V_0 is one-half V_{max}.

Lineweaver-Burk plot to estimate parameters

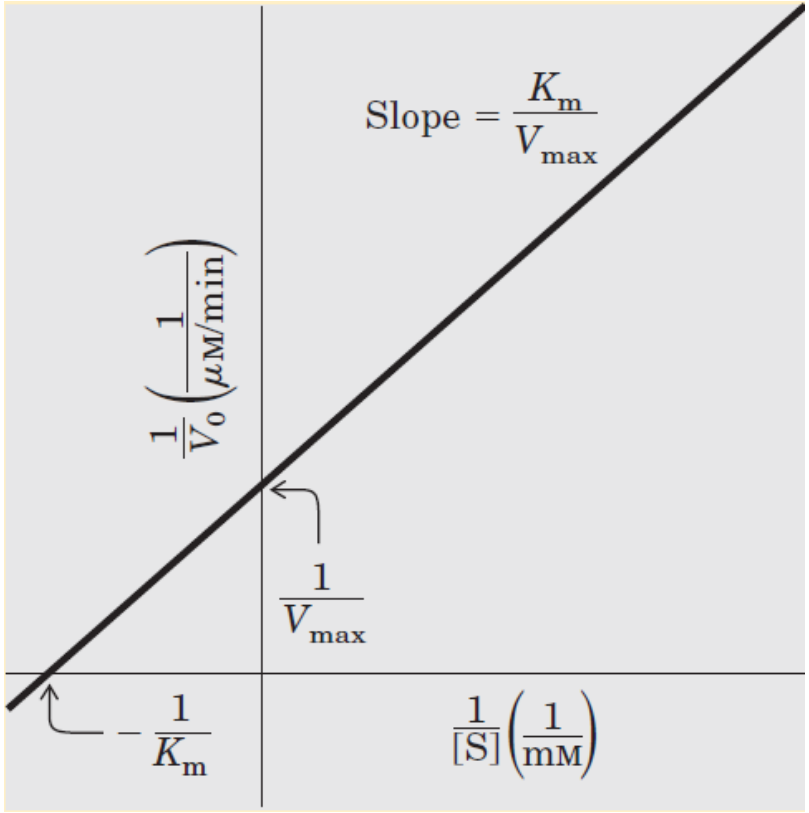
Rewritten in the following manner, the equation gives a straight line, which is useful to determinate the parameters K_m and V_{max} (Lineweaver-Burk representation):

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

$$\frac{1}{V_0} = \frac{K_m + [S]}{V_{max}[S]}$$

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$



Importance of K_m

Example:

Leukemia was found to get suppressed by intravenous administration of the enzyme asparaginase catalyzing the following reaction,

$$\text{Asparagine} + H_2O \rightarrow \text{aspartate} + NH_4^+ \text{ (reversible reaction)}$$

Next, was to find the source of asparagase for treatment.
Not all asparaginases were found to be effective.

Explanation:

Asparaginases from different animals, plants or bacterial sources differ in their K_m for asparagine.

Since the concentration of asparagine in blood is very low. The administration of an asparagine from another species can be effective only if its K_m value is low enough to hydrolyze asparagine rapidly at low concentration at which it is present in the blood.

K_m tells about the enzyme's affinity for its substrate.

Problems to solve

1. Which of the following effects would be brought about by any enzyme catalyzing the simple reaction

$$\mathrm{S} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{P} \quad \text{where} \quad K'_{\mathrm{eq}} = \frac{[\mathrm{P}]}{[\mathrm{S}]}?$$

(a) Decreased K_{eq} (b)Increased k_1

(c) Increased K_{eq} (d) Increased $\Delta G^{\ddagger}$

(e) Decreased $\Delta G^{\ddagger}$ (f) More negative $\Delta G'^{o}$

(g) Increased k_2

Problems to solve

2. The enzyme urease enhances the rate of urea hydrolysis at pH 8.0 and 20°C by a factor of 10^{14}. If a given quantity of urease can completely hydrolyze a given quantity of urea in 5.0 min at 20°C and pH 8.0, how long would it take for this amount of urea to be hydrolyzed under the same conditions in the absence of urease? Assume that both reactions take place in sterile systems so that bacteria cannot attack the urea.

Problems to solve

3. Determine the fraction of V_{max} that would be obtained at the following substrate concentrations [S]: ½K_m and 10K_m.

Problems to solve

4. Neutral sphingomyelinase is an enzyme that converts sphingomyelin into ceramide and phosphocholine. A student studying the kinetics of this enzyme added 3 X 10-5 M of sphingomyelin to the reaction and observed an initial velocity of 6.0 μM/min. Calculate the KM for this reaction, provided the Vmax is 35 μM/min.