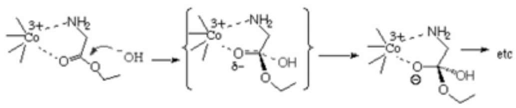
Principles of Catalysis

Metal Ion Catalysis

- A specific type of electrostatic catalysis
- Employs the positively charged metal ion to stabilize negative charges for increased catalysis (also called Electrophilic catalysis)
- Coordination of the cobalt complex increases the ability of a base to catalyze the hydrolysis of glycine ester two million fold



Enzyme Action

- Enzymes are the catalysts which make possible biochemical reactions.
- Consider that biochemistry takes place at about 37 degrees C in water and contrast that to typical reaction conditions in organic chemistry.
  - For example, to hydrolyze (saponify) fats we boil them with concentrated sodium hydroxide solution for a few hours.
  - Enzymes called lipases do the same thing at body temperature in minutes.
  - Without enzymes, our body chemistry would not occur, and life would not exist.
- This illustrates the impressive power of enzymes as catalysts.

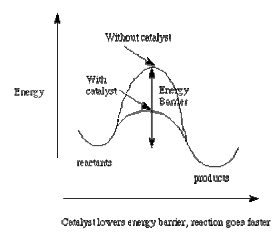
Enzyme Action

Enzymes differ from simple catalysts another very important way.

- Enzymes are much more specific.
  - Sulfuric acid as a source of H^+ will catalyze the formation of any ester from the appropriate alcohol and carboxylic acid,
  - Many enzymes are so specialized that they will catalyze a reaction of one molecule, but will leave untouched a very similar molecule.
    - **Amylase, a digestive enzyme, will hydrolyze starch, but not cellulose. Both molecules are polymers of glucose.**
      - They differ in the orientation of one bond at the junction of glucose units. Other enzymes can work effectively on a broader range of substrates (the molecule whose reaction is being catalyzed.

Mechanism of Action

- **Catalysts increase the rate of a reaction, but are not themselves consumed or produced by the reaction.**
- **They do not change the equilibrium constant of a reaction.**
  - **This means that any catalyst which catalyzes a reaction in one direction (e.g., esterification) also catalyzes the reverse (e.g., ester hydrolysis) reaction.**
- **To say these things another way, catalysts do not change the energy balance between reactants and products; catalysts do lower the energy barrier between reactants and products.**
  - **These statements are true of enzymes as well as other types of catalysts.**

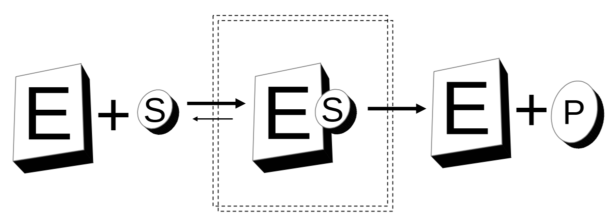


Mode of Action

- **Enzymes can act in several ways, all of which lower ΔG:**
  - **Lowering the activation energy**
    - by creating an environment in which the transition state is stabilized (e.g. straining the shape of a substrate - by binding the transition-state conformation of the substrate/product molecules, the enzyme distorts the bound substrate(s) into their transition state form, thereby reducing the amount of energy required to complete the transition).
  - **Providing an alternative pathway**
    - (e.g. temporarily reacting with the substrate to form an intermediate ES Complex which would be impossible in the absence of the enzyme).
  - **Reducing the reaction entropy change**
    - by bringing substrates together in the correct orientation to react.

Essential of Enzyme Kinetics

Steady State Theory



The reaction ES=E + P determines catalytic rate
The reaction ES=E + P is irreversible
ES is in equilibrium. Formation = Removal

In steady state, the production and consumption of the transition state proceed at the same rate. So the concentration of transition state keeps a constant.

Chemical Reactions and Rates

- Rate of a chemical reaction is described by the number of molecules of reactant(s) that are converted into product(s) in a specified time period.
- Reaction rate is always dependent on the concentration of the chemicals involved in the process and on rate constants that are characteristic of the reaction.
  - For example, the reaction in which A is converted to B is written as follows:

A ------> B

- The rate of this reaction is expressed algebraically as either a decrease in the concentration of reactant A:

$-[A] = k[B]$

- or an increase in the concentration of product B:

$[B] = k[A]$

  - In the second equation the negative sign signifies a decrease in concentration of A as the reaction progresses,
  - Brackets define concentration in molarity
  - *k* is known as a rate constant.
  - Rate constants are simply proportionality constants that provide a quantitative connection between chemical concentrations and reaction rates.

Chemical Reactions and Rates

- **Each chemical reaction has characteristic values for its rate constants.**
- **These in turn directly relate to the equilibrium constant for that reaction.**
- **Thus, reaction can be rewritten as an equilibrium expression in order to show the relationship between reaction rates, rate constants and the equilibrium constant for this simple case.**
- **The rate constant for the forward reaction is denoted as k_{+1} and the reverse as k_{-1}.**

Michaelis-Menten Kinetics

- The **Michaelis-Menten equation** is a quantitative description of the relationship among the
  - rate of an enzyme- catalyzed reaction [v1],
  - the concentration of substrate [S]
  - two constants, Vmax and Km
- The symbols used in the Michaelis-Menton equation refer to the
  - reaction rate [v1],
  - maximum reaction rate (Vmax),
  - substrate concentration [S] Michaelis-Menton constant (Km).

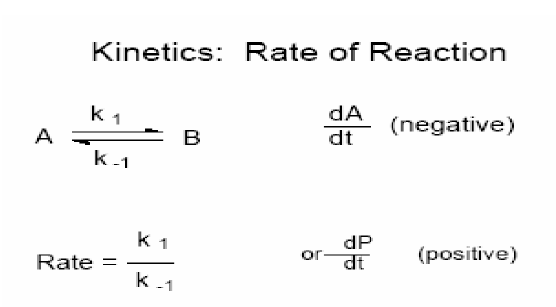
Michaelis-Menten Kinetics

- Michaelis-Menten Equation for Enzyme Kinetics
- Assumptions:

E + S = ES = E + P

  - The reaction ES=E + P determines catalytic rate
  - The reaction ES=E + P is irreversible
  - ES is in equilibrium. Formation = Removal

Michaelis-Menten Kinetics



Michaelis-Menten Kinetics

At Steady State,

$[ES]_{formation} = [ES]_{destruction}$

- $[ES]_{formation} = k_1[E][S]$
- $[ES]_{destruction} = k_{-1}[ES] + k_2[ES]$
- $k_1[E][S] = k_{-1}[ES] + k_2[ES]$
- Algebraic manipulation (and some assumptions) yields…

Michaelis-Menten Kinetics

$$v_0 = \frac{Vmax[S]}{Km + [S]}$$

Where

V_o is the initial velocity

Vmax is the velocity under optimal conditions

[S] is the substrate concentration

Km is the Michaelis constant

Michaelis-Menten Kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E$$

Leonor Michaelis and Maud Menten proposed a model to explain enzyme kinetics in 1913 (developing theories of earlier workers)

- Michaelis-Menten assumptions:
  1. [E] << [S] (formation of [ES] doesn't deplete [S] significantly)
  2. [P] ≈ 0 (product is absent at outset of reaction, the amount formed is so small that the reverse reaction can be ignored)
  3. There is a pre-equilibrium between E, S, and ES, that is $k_{-1} >> k_2$
- in their model an enzyme combines with a substrate to form an ES complex
- the ES complex is necessary for catalysis such that the rate of the reaction can be written as:

$$V_0 = k_2[ES]$$

Michaelis-Menten Kinetics

Michaelis-Menten equation

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E$$

$$V_0 = k_2[ES] \qquad [ES] = [E]_T \frac{[S]}{K_M + [S]}$$

$$V_0 = k_2[E]_T \frac{[S]}{K_M + [S]}$$

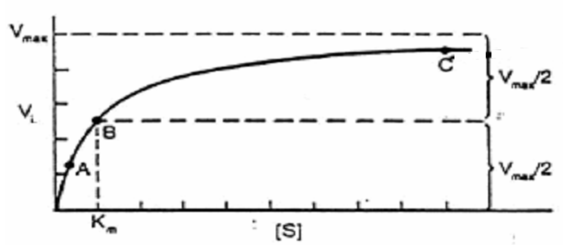
- The Michaelis–Menten equation describes the initial rate of an enzymatic reaction as a function of substrate concentration: yields enzyme parameters

Michaelis-Menten Kinetics

Michaelis-Menton Rate Definitions

$$k_1 = \frac{[ES]}{[E][S]} \qquad k_{-1} = \frac{[E][S]}{[ES]} \qquad k_2 = \frac{[E][P]}{[ES]}$$

Michaelis-Menton Plot



Effect of substrate concentration on the velocity of an enzyme-catalyzed reaction.

Lineweaver-Burk Kinetics

- To avoid dealing with curvilinear plots of enzyme catalyzed reactions, biochemists **Lineweaver and Burk** introduced an analysis of enzyme kinetics based on the following rearrangement of the Michaelis-Menten equation:

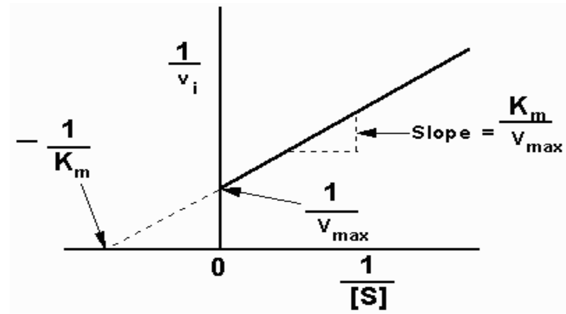
  [1/v] = [Km (1)/ Vmax[S] + (1)/Vmax]

- Plots of 1/v versus 1/[S] yield straight lines having a slope of Km/Vmax and an intercept on the ordinate at 1/Vmax.

Lineweaver-Burk Kinetics

- Is the linear way of looking at Michaelis Menton equation
- **Competative inhibition** = Vmax doesn't change but Km increases
- **Noncompetative inhibtion** = Km doesn't change but Vmax decreases

Lineweaver-Burk Plot



An Example for Enzyme Kinetics

1) Use predefined amount of Enzyme	→ E
2) Add substrate in various concentrations	→ S (x □)
3) Measure Product in fixed Time (P/t)	→ v_o (y □)
4) (x, y) plot get hyperbolic curve, estimate	→ V_{max}
5) When y = 1/2 V_{max} calculate x ([S])	→ K_m