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Biochemistry

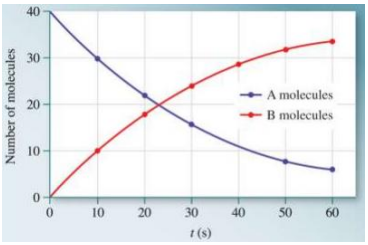
Enzymes 2

❖ Kinetics

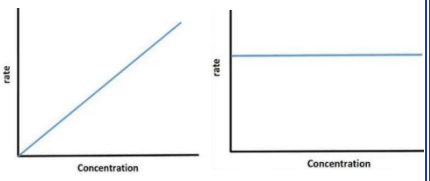
- Kinetics deals with the **rates** of chemical reactions
  - Enzyme kinetics is the study of the rates of enzymatic reactions

$$-\frac{\Delta[A]}{\Delta t} = \frac{\Delta[B]}{\Delta t}$$

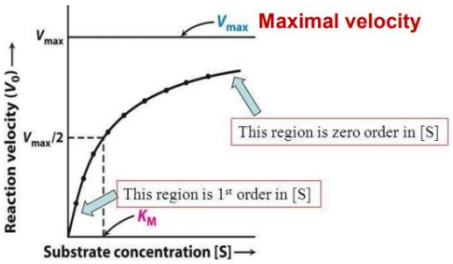
- Velocity (v) or rate of the reaction (A → B) is the <u>amount of products formed</u> or the <u>amount of reactants consumed</u> **per unit time**
  - **V = - k [reactants] = k [products]**
  - K is the rate constant ($time^{-1}$)
  - The negative sign indicates the consumption of reactants
  - This is known as the rate law, which describes how concentrations of reactants affect the rate of the reaction during a certain period



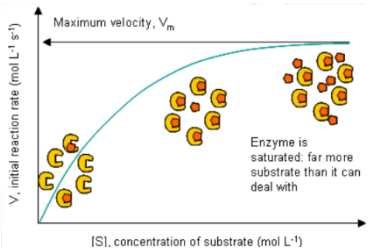
- Uncatalyzed reactions are either:
  - **First ordered reactions:** The rate of a reaction **<u>increases linearly</u>** with increasing substrate concentration → rate = K [reactants]
  - **Zero ordered reactions:** The rate of the reaction is **<u>independent</u>** of substrates → rate = $k[A]^0 = k$



- For enzymes:
  - The plot is a **hyperbolic** (saturation) curve
  - Initial velocity (V_0) varies with the substrate concentration [S] where the rate of catalysis **<u>rises linearly</u>** as the substrate concentration increases and then levels off and approaches a <u>constant</u>, **<u>maximal velocity</u>** (Vmax) at higher substrate concentrations



- V_0 it is the rate of the reaction at a certain substrate concentration
  - It depends mainly on the concentration of substrates and the rate constant (K) which depends on the reaction conditions
  - When S is **small**, they are **linear** proportional
  - When S is **large**, V0 is **independent** to S because it reached Vmax

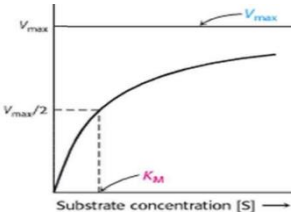


- V_{max} it is the highest rate reached when all enzyme molecules are saturated
  - It is a **constant** value for each reaction with certain conditions
  - It reveals (proportional related to) the turn over number
  - **Turn over number:** It is number of substrate molecules converted into products by an enzyme molecule in a unit time per concentration of enzyme when the enzyme is fully saturated

- **Michaelis-Menten equation:** a quantitative description of the relationship between the rate of an enzyme catalyzed reaction (V_o), substrate concentration [S], a rate constant (K_M) and maximal velocity (Vmax)

$$Vo = \frac{V_{max}[S]}{Km + [S]}$$

- **K_M:** is the concentration of <u>substrate</u> at which <u>half the active sites</u> are filled
  - K_M is [S] when $V_o = ½ V_{max}$
  - It is **<u>inversely</u>** related to the affinity (lower K_M, higher affinity), but it is not an accurate measure for affinity
  - At very low substrate concentration, the rate is directly proportional to the substrate concentration and is affected by how well a substrate binds to an enzyme
  - At high substrate concentration, the rate is maximal, independent of substrate concentration or how well an enzyme binds to the substrate



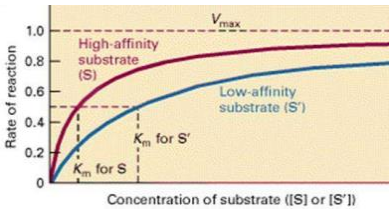
- $K_M = (K_{-1} + K_2) / (K_1)$
  - It is related to the rate of dissociation of a substrate from the enzyme to the rate of enzyme-substrate association
  - The K_M values of enzymes range widely (mostly, 10^{-7} to 10^{-1})

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

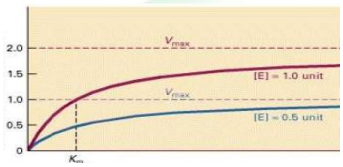
- **Dissociation constant (K_D)** is the actual measure of the affinity
  - $K_D = K_{-1} / K_1$
  - It is **inversely** related to the affinity

The efficiency of an enzyme catalyzed reaction is determined by V_{max}

- Each **substrate** has a unique **K_M** for a given **enzymatic** process
- **V_{max}** is related to the **enzyme** and is the same for the same reaction of more than one substrate
- For an enzyme catalyzed reaction:
  - If the same enzyme is used with different substrates, and gave the same products (similar reaction)
    - ✓ V_{max} is the same, but K_M may be different
  - If the same enzyme is used with different substrates, and gave different products (different reaction)
    - ✓ V_{max} may be different, and K_M may be different
    - ✓ Such as hexokinase phosphorylates glucose, fructose, and mannose at different Vmax values

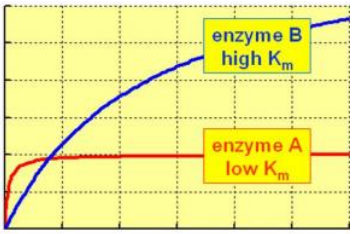


  - For different enzymes which catalyze similar reactions
    - ✓ V_{max} may be different, and K_M may be different
    - ✓ Such as hexokinase and fructokinase phosphorylate fructose



  - If the concentration of the enzyme molecules is increased
    - ✓ V_{max} increases, but K_M is the same
  - If the concentration of the substrate is increased
    - ✓ V_{max} and K_M remain the same, but the rate of the reaction increases until reaching V_{max}

- Example: According to the adjacent figure:
  - Which reaction is favorable when [S] is very low?
  - Which reaction is favorable when [S] is very high?
  - Which enzyme has a higher Affinity toward S?
  - Which enzyme is more efficient?



- Uses of K_M:
  - Determine the substrate preferences of an enzyme
    - ✓ If an enzyme has more than one substrate, the substrate with the lowest K_M is probably the preferred physiological substrate
  - Distinguish isozymes, which are different enzymes catalyzing the same reaction
    - ✓ Isozymes often have different affinities for the same substrate
  - Check for abnormalities in an enzyme

❖ V_{max} & K_{cat}

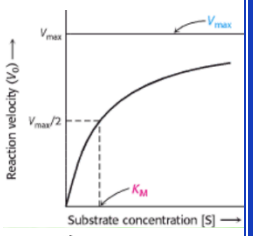
- V_{max} & K_{cat} are a measure of **enzyme efficiency**
  - The maximal rate is equal to the product of K_2 (also called K_{cat}) and the total concentration of the enzyme
  - **$V_{max} = K_{cat} \times [E]_T$**

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

- **K_{cat}:** It is the turnover number which is the concentration (or moles) of substrate molecules converted into product per unit time per enzyme concentration (or moles), when **fully saturated**
  - It is a **constant** for any given enzyme
  - It describes how quickly an enzyme acts (how fast the ES complex proceeds to E + P)
  - Turnover numbers of most enzymes with their physiological substrates in the range from 1 to 10^4 S^{-1}
- Each catalyzed reaction takes place in a **time = $1/k_2$**

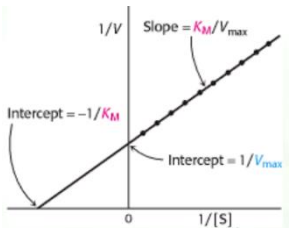
- **Catalytic efficiency = K_{cat} / K_M**
  - Efficiency is **directly** related to K_{cat} and V_{max}, and **inversely** related to K_M
- **Rate of reaction** is calculated as concentration of substrate disappearing (or concentration of product appearing) per unit time ($mol.L^{-1}.sec^{-1}$ or $M.sec^{-1}$)
  - **Rate = ΔM / Time**
- In order to measure **enzyme activity**, we measure the number of moles of substrate disappearing (or products appearing) per unit time ($mol.sec^{-1}$)
  - **Enzyme activity = substrate Moles / time**
  - **Enzyme activity = rate of reaction × reaction volume**
- **Specific activity** is usually a measure of enzyme **purity and quality** in a sample after purification
  - It is described as moles of substrate converted per unit time per unit mass of enzyme ($mol.sec^{-1}.g^{-1}$)
  - **Specific activity = enzyme activity / mass of enzyme (grams)**
- **Turnover number (k_{cat})**
  - **k_{cat} = specific activity × molecular weight of enzyme**
  - **$k_{cat} = V_{max} / [E]_T$**

- Disadvantage of the Michaelis-Menten equation:
  - Determination of K_M from hyperbolic plots is not accurate since a large amount of substrate is required in order to reach V_{max}, preventing the calculation of both K_M and V_{max}



- **Lineweaver-Burk or double-reciprocal plot:** A plot of $1/V_o$ versus 1/[S]
  - It yields a straight line
  - Y intercept of $1/V_0 = 1/V_{max}$
  - X intercept of $1/[S] = -1/K_M$
  - Slope = K_M / V_{max}

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



- Examples:
  - **A biochemist obtains the following set of data for an enzyme that is known to follow Michaelis-Menten kinetics. Approximately, Vmax of this enzyme is … & KM is …?**

    A. 5000 & 699
    B. 699 & 5000
    C. 621 & 50
    D. 94 & 1
    E. 700 & 8

Substrate Concentration (μM)	Initial velocity (μmol/min)
1	49
2	96
8	349
50	621
100	676
1000	698
5000	699

  - **A 10^{-6} M solution of carbonic anhydrase catalyzes the formation of 0.6 M H_2CO_3 per second when it is fully saturated, calculate the turnover number and the time required for the reaction**
    - ✓ Kcat = 6×10^5 S^{-1} , T = 2.7×10^{-6} min / reaction

- **You are working on the enzyme "Medicine" which has a molecular weight of 50,000 g/mol You have used 10 µg of the enzyme in an experiment and the results show that the enzyme at best converts 9.6 µmol of the substrate per min at 25°C. turnover number (k_{cat}) for the enzyme is:**
  A. 960 S^{-1}
  B. 9.6 S^{-1}
  C. 800 S^{-1}
  D. 48 S^{-1}
  E. 1920 S^{-1}

- **A solution of 25×10^{-4} mol.L^{-1} of peptide substrate and 150 µg chymotrypsin in 2.5 mL, after 10 minutes 18.6×10^{-4} mol.L^{-1} of peptide substrate remain (molar mass of chymotrypsin is 25,000 g.mol^{-1}), calculate K_{cat}:**

✓ 45 S^{-1}