Enzymes

Definition: Enzymes are biological catalysts that increase the rate of a reaction and are chemically unaltered at the end of the reaction and can thus be reused, and are effective in small amounts.

Characteristics

- Globular proteins → Soluble in water
- Structure:
    - Active site with **specific 3D conformation** that is **complementary in shape and charge** to its specific substrate
        - Contact amino acid residues
            - Interact reversibly with the substrate via weak hydrogen bonds, ionic bonds and hydrophobic interactions, holding the substrate in the correct orientation
        - Catalytic amino acid residues
            - Catalyse the conversion of the substrate to its product by acting on bonds in the substrate
    - Structural amino acid residues maintain specific 3D conformation of the enzyme

Mechanism of Action
Interaction/Binding

- Enzyme has specific active site with **specific 3D conformation** that is **complementary in shape and charge** to the substrate
- Effective collisions between enzyme and substrate form a temporary **enzyme-substrate complex** held together by weak interactions such as hydrogen bonds, ionic bonds and hydrophobic interactions between the contact residues and the substrate
- Based on induced fit hypothesis, binding of substrate induces a change in shape in the enzyme active site so that the active site is a more precise fit for substrate for effective catalysis
- Based on lock and key model, substrate is the key and enzyme is the lock

Catalysis

- R groups of catalytic residues help to catalyse the reaction
- Enzyme **lowers the activation energy** of the reaction by (MSOAP):
    1. Microenvironment effects = By providing a **favorable microenvironment**
    2. Strain effects = By **applying strain on the bonds** to be broken/By distorting the substrates hence reducing the activation energy required to achieve transition state
    3. Orientation effects = By holding the substrates in the correct orientation such that its **bonds are exposed to chemical attack**
    4. Acid-base catalysis = Where the R-groups of catalytic acidic and basic amino

acid residues in active site participate in direct catalysis
5. Proximity effects = By aligning substrates next to each other in **close proximity** in the active site, increasing chance of the reaction occurring

- More of the substrate molecules will possess <u>energy exceeding activation energy</u>, allowing the reaction to proceed at a higher rate

<u>Release</u>

- Products are no longer complementary in shape and charge to the enzyme active site and hence, are released. The enzyme remains unchanged and can be used again.

<u>Cofactors</u>

- Inorganic ions
  - Moulds enzyme/substrate to allow enzyme-substrate complex to form more easily
- Prosthetic group
  - Transfers atoms/chemical groups between active site of enzyme and another substance
  - Permanently bound to enzyme
- Coenzymes
  - Bind to active site of the enzyme and participate in catalysis but are not substrates of the reaction
  - Intermediate carriers of electrons/specific atoms that are not transferred in the overall reaction
  - Organic molecules required to carry out catalysis

Product-Time Graph

- At time 0, [substrate] is the highest ⇒ Frequency of effective collisions between enzyme and substrate molecules is the highest ⇒ Rate of formation of enzyme-substrate complexes is the highest ⇒ Rate of reaction is at the maximum
- Over time, enzyme catalyses the formation of product from the substrate ⇒ ↓ [substrate] ⇒ ↓ Frequency of effective collisions between enzyme and substrate molecules ⇒ ↓ Rate of formation of enzyme-substrate complexes ⇒ ↓ Rate of reaction
- Eventually, all substrate molecules are converted to product ⇒ [substrate] is 0 ⇒ Rate of reaction is 0 ⇒ Graph becomes horizontal

Factors affecting rate of reaction

1. Temperature
   - Draw temperature graph
   - At low temperatures, as temperature increases, rate of reaction increases
     - ↑ Temperature ⇒ ↑ <u>KE</u> of the enzyme and substrate

molecules ⇒ ↑ Frequency of effective collisions between the substrate and enzyme active site ⇒ ↑ Rate of formation of enzyme-substrate complexes
        - Also, ↑ KE ⇒ ↑ No. of molecules with sufficient energy to overcome the activation energy barrier ⇒ ↑ Rate of reaction
    - Reaction rate increases up to optimum temperature
        - Maximum rate of reaction with the highest amount of product formed is achieved
    - Beyond optimum temperature, as temperature increases, rate of reaction decreases
        - ↑ temperature ⇒ ↑ Intramolecular vibrations of the molecules ⇒ Weak interactions such as hydrogen bonds, ionic bonds and hydrophobic interactions that stabilise the specific 3D conformation of the enzyme break ⇒ Specific conformation of the enzyme active site is altered ⇒ Enzyme denatured ⇒ Substrate is no longer complementary in shape to the enzyme active site ⇒ Fewer enzyme-substrate complexes formed ⇒ Lowering rate of reaction
    - Within physiological temperatures, the rate of reaction doubles for every 10°C rise in temperature
2. pH
    - Draw pH graph
    - Each enzyme has optimum pH at which it is most active and rate of reaction is maximum
    - Rate of reaction decreases as pH deviates from optimum pH
    - Excess H^+ and OH^- ions may affect ionisation of the R-groups of acidic and basic amino acids
    - Excess H^+ ions results in $-COO^-$ groups becoming -COOH and excess OH^- results in $-NH_3^+$ groups becoming NH_2 groups
    - Change in specific charge of R groups of structural residues in enzyme ⇒ Ionic bonds and hydrogen bonds that stabilise the specific 3D conformation of the active site are disrupted ⇒ Denaturation of the enzyme
    - Change in specific charge of R groups of catalytic residues in active site ⇒ Loss of catalytic activity of the enzyme
    - Change in specific charge of R groups of contact residues in active site ⇒ Temporary binding between the enzyme and substrate affected ⇒ No enzyme-substrate complexes formed
3. Enzyme concentration
    - At low [enzyme] ⇒ [enzyme] limiting, (linear portion of the graph)
        - ↑ [enzyme] ⇒ ↑ Frequency of effective collisions between enzyme and substrate molecules ⇒ ↑ Rate of formation of enzyme-substrate complexes ⇒ Proportional ↑ rate of reaction

- As enzyme concentration increases ⇒ [enzyme] no longer the only limiting factor, (curved portion of the graph)
  - Rate of reaction increases at a decreasing rate
- When [enzyme] no longer the limiting factor, (plateau)
  - Rate of reaction reaches maximum, ↑ [enzyme] ⇏ ↑ Rate of reaction

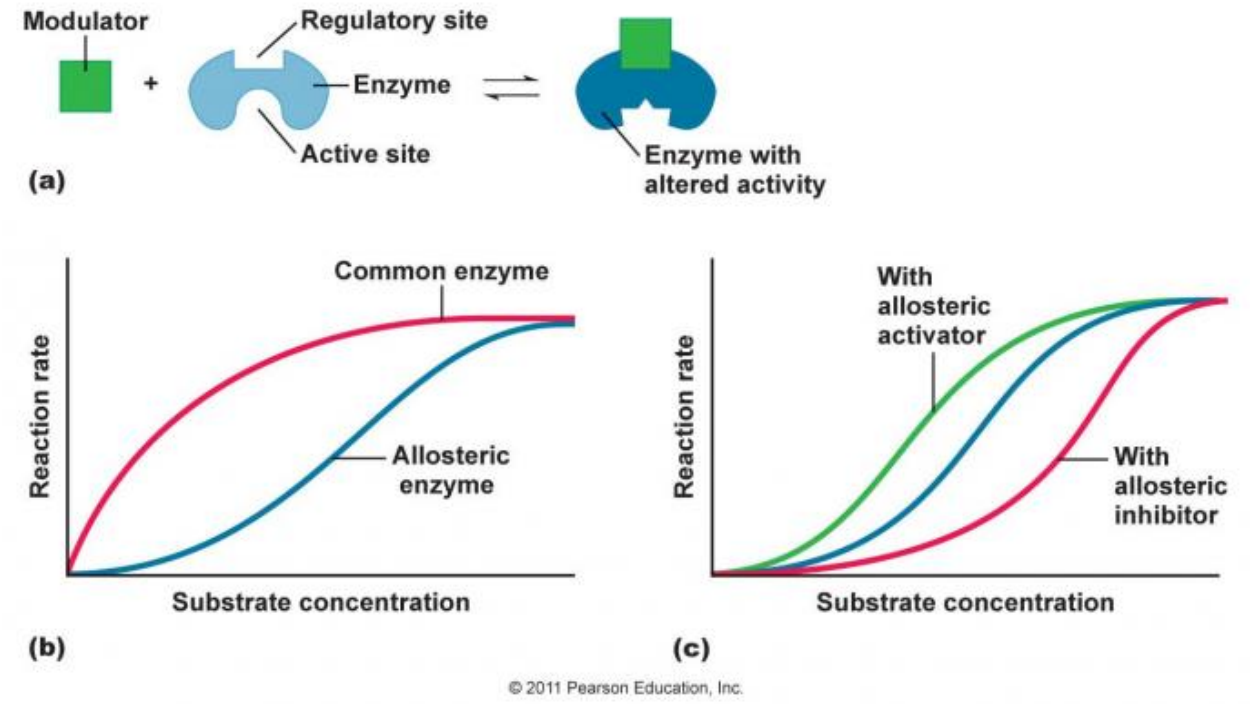
4. Substrate concentration
   - At low [substrate] ⇒ Enzyme active sites readily available, [substrate] is limiting,
     - ↑ [substrate] ⇒ ↑ Frequency of effective collisions between enzyme and substrate molecules ⇒ ↑ Rate of formation of enzyme-substrate complexes ⇒ ↑ Rate of reaction
   - As [substrate] increases ⇒ [substrate] no longer the only limiting factor ⇒ Enzyme active sites start to get saturated, limiting the rate of reaction
     - Rate of reaction increases at a decreasing rate
   - Above a certain [substrate],
     - All available enzyme active sites saturated with substrate ⇒ [substrate] no longer limiting and instead, [enzyme] limiting ⇒ Maximum velocity reached, rate of reaction remains constant as [substrate] increases

Enzyme Inhibition

1. Competitive inhibitors
   - Bind to enzyme active site
   - Effect:
     - Similar in shape and charge to the substrate ⇒ Binds reversibly to enzyme active site ⇒ Competes with the substrate for the enzyme active site ⇒ ↓ Availability of active sites for substrate binding ⇒ ↓ rate of reaction
   - Can be overcome by ↑ [substrate]
     - ↑ [substrate] ⇒ ↑ Chance of substrate binding to enzyme active site instead of inhibitor to form enzyme-substrate complex ⇒ Same maximum velocity observed in the absence of inhibitor can be reached at sufficiently high [substrate] ⇒ ∴ Effects can be overcome by ↑ [substrate]
2. Non-competitive inhibitor
   - Binds to site other than active site
   - Some bind reversibly via weak bonds such as hydrogen bonds whilst others bind irreversibly via strong covalent bonds
   - Effect:
     - Binding to site other than the enzyme active site ⇒ Conformational change in enzyme active site such that it is no longer complementary in shape to the substrate ⇒ Substrate cannot bind to the active site ⇒ ↓ Rate of reaction
     - ↑ concentration of inhibitors ⇒ ↓ rate of reaction

- Cannot be overcome by high [substrate]

Allosteric Regulation



- Allosteric enzyme
  - Has an allosteric site
  - 2 conformational states: Active and Inactive
  - Binding of substrates exhibit cooperative binding
  - i.e. Binding of substrate to first subunit changes the conformation of the other subunits such that it becomes easier to accept subsequent substrates
  - [Substrate]-Rate graph is an S-shaped sigmoid curve
- Allosteric inhibitor/activator
  - Not structurally similar to enzyme substrate
  - Binds to allosteric site
  - Effect:
    - Binding to allosteric site ⇒ Conformational change in enzyme
      - Binding of allosteric activator at allosteric site stabilises the functionally active conformation
      - Binding of an allosteric inhibitor stabilises the inactive conformation of the enzyme
    - Rate vs [substrate] is an S-shaped sigmoid curve which indicates cooperative binding of substrate to active site
    - Allosteric inhibition is overcome with high [substrate]
- Feedback inhibition/End-product inhibition
  - End product acts as an allosteric inhibitor of an enzyme that acts early in the

biochemical pathway, preventing further synthesis of the product
- e.g. Amino acid isoleucine is produced from threonine. As isoleucine accumulates, it inhibits the enzyme threonine deaminase in the first step of the reaction by binding to the site other than active site of the enzyme. Hence, the end product alters the shape of the specific enzyme active site, thus the substrate cannot bind to the active site in the correct orientation, causing the rate of reaction to be decreased.