**Chapter 21. Enzymes and Vitamins**

**21.1 General Characteristics of Enzymes**

**21.2 Enzyme Structure**

**21.3 Nomenclature and Classification of Enzymes**

**21.4 Models of Enzyme Action**

**21.5 Enzyme Specificity**

**21.6 Factors That Affect Enzyme Activity**

**21.7 Enzyme Inhibition**

**21.8 Regulation of Enzyme Activity**

**21.9 Antibiotics That Inhibit Enzyme Activity**

**21.10 Medical Uses of Enzymes**

**21.11 General Characteristics of Vitamins**

**21.12 Water-Soluble Vitamins**

**21.13 Fat-Soluble Vitamins**

Students should be able to:

1. Characterize the function of a catalyst in a chemical reaction.

2. List some characteristics of enzymes.

3. Draw energy diagrams for uncatalyzed and catalyzed reactions.

4. Distinguish between absolute, relative, and stereochemical specificity.

5. Explain the function of cofactors and coenzymes in enzyme catalysis.

6. Distinguish between the six classes of enzymes.

7. Identify the class of an enzyme given the chemical reaction it catalyzes.

8. Interpret a systematic enzyme name.

9. Distinguish between the lock-and-key model and the induced-fit model of

enzyme catalysis.

10. Describe several mechanisms of catalysis.

11. Explain why enzyme assays can be diagnostically useful. Give some

examples.

12. Predict the effects of an increase in the concentration of substrate or enzyme

on the rate of an enzyme catalyzed reaction.

13. Describe the effects of temperature and pH on an enzyme catalyzed reaction.

14. Characterize several modes of enzyme regulation.

15. Distinguish between a positive allosteric regulator and a negative allosteric

regulator.

16. Explain the role of zymogens in enzyme regulation.

17. Distinguish between a competitive enzyme inhibitor and a non-competitive

enzyme inhibitor. Give some examples.

18. Explain the role of genetics in the regulation of enzymatic activity.

19. Explain the connection between enzyme activity and nutrition (specifically

minerals and vitamins).

**21.1 General Characteristics of Enzymes**

**21.2 Enzyme Structure**

**21.3 Nomenclature and Classification of Enzymes**

**21.4 Models of Enzyme Action**

**21.5 Enzyme Specificity**

**21.6 Factors That Affect Enzyme Activity**

**21.7 Enzyme Inhibition**

**21.8 Regulation of Enzyme Activity**

**21.9 Antibiotics That Inhibit Enzyme Activity**

**21.10 Medical Uses of Enzymes**

**21.11 General Characteristics of Vitamins**

**21.12 Water-Soluble Vitamins**

**21.13 Fat-Soluble Vitamins**

**1) What are Enzymes?**   
**Enzymes** are proteins that are capable of speeding up the chemical reactions.   
General features of enzymes:   
1) Most of the **enzymes are proteins**, however, recent work has shown that there are RNA molecules which show catalytic   
    activity (RNA enzymes)   
2) Enzymes increase the rate of reactions but **do not influence the equilibrium**.   
3) Enzymes exhibit high degree of specificity for their **substrates**.   
4) Enzymic catalysis involves formation of an **intermediate complex (ES)** between the enzyme and its substrate(s).   
5) There is a specific site on enzyme where substrate binds (**active site**).   
6) Enzymes lower the **activation energy** required for a chemical reaction.   
7) Enzymes **regulate the reaction rates**.   
8) Some enzymes are **multienzyme** or multifunctional complexes.

**Some terminolgy asscocaited with enzyme reactivity**   
**Catalyst**: Any substance that increases the rate of the chemical reaction. It is neither consumed nor irreversibly   
altered during the reaction.   
Why catalyzed reactions are very important:   
      1. require less time to reach equilibrium.   
      2. occur at lower temperatures.   
      3. occur under milder reaction conditions (pH 7.4 and 37 C).   
      4. reaction occurs at relatively low reagent concentrations.   
Enzymes are some of the most efficient catalysts known:   
      carbonic anhydrase of RBC.   
      CO2 + H2O <===> H2CO3   600,000 CO2 molecules consumed/second.   
**Metabolism**: The chemical reactions that occur in the body, enabling cells to release energy from foods, transport   
various chemicals, synthesize new biomolecules from precursor building blocks, ove the cell or organism, replicate   
the parent cell to yield a new generation, and prepare end products for excretion.   
**Anabolism**: Synthesis of biomolecules from small precursors.   
**Catabolism**: Breakdown of large molecules to their precursors generating the energy.   
**Substrate**: The molecule on which the enzyme acts. Chemical bonds are either made or broken within the substrate molecule.   
**Active site**: The polypeptide chain of the enzyme is folded in such a way that a small, three dimensional pocket or cleft appears on the enzyme. This pocket is called active site where substrate binds to the enzyme to form an enzyme substrate-complex (ES).   
**Lock-and-key theory**: One can think of an enzyme as a lock and the substrate as a key, and the active site as the keyhole of the lock.   
**Specificity**: Enzymes only bind a very specific type of substrate molecule which is capable of fitting into active site. There are four types of specificity:   
1. Absolute specificity: Enzyme acts on only one substrate and catalyzes only one reaction.   
2. Group specificity: Enzyme acts on a class of substrates that posses a common functional group. Eg Phosphatases.   
3. Linkage specificity: Enzymes are specific only for a particular type of chemical bond. Eg. esterases.   
4. Stereospecificity: Enzymes discriminate between D- and L-stereoisomers and use only one of them as substrate.

**2) Nomenclature and Classification of Enzymes**

      Enzymes are usually named in terms of the reactions they catalyze. It is also customary to add suffix -ase to the name of the reaction catalyzed or to the name of the substrate acted on. eg. an enzyme which catalyses an oxidation-reduction reaction is called as oxidoreductase; an enzyme acting on urea is urease. Some trivial names, however, persist such as trypsin or pepsin.  An International Enzyme Commission has devised a rather complex nomenclature and classification of all enzymes.

1. The Classification of the enzymes **based suffix** or word ending: -ase  or -in (older term)

                hexokinase    trypsin

2. The Classification of the enzymes **based on reaction identification**:

a. Oxidoreductases: catalyze oxidation-reduction reactions.Transfer of H atoms, O atoms or electrons.   
b. Transferases: catalyze the transfer of functional groups.   
c. Hydrolases: catalyze hydrolysis reactions; breaking of chemical bonds by adding water. Catalyse   
     hydrolysis   
    (addition of water). AX + H2O -> AH + XOH   
d. Lyases: catalyse the removal of chemical groups, with double bond formation. Catalyse splitting bonds   
    (C-N, C-O, C-C, C-S)   
e. Isomerases: catalyze isomerization reactions. Catalyse structural rearrangements of molecules.   
f. Ligases: catalyze formation of chemical bonds, with ATP cleavage.

3. The Classification of the enzymes **based on substrate identification**:  glucose oxidase,    hexokinase   
4. **International Enzyme Commission**:   enzyme commission numbers:

First number indicates the enzyme's major class. Second number denotes its subclass (acting on peptide bonds). Third number designates its subsubclass. Fourth number is the enzyme's arbitrarily assigned serial number in its subsubclass.

      EC 3.1.3.1  alkaline phosphatase   
      EC 3.1.3.5  5'-nucleotidase

**3) Types of reactions catalyzed by enzymes and their specificity.**   
**Types of reactions**   
a. Oxidoreductases: catalyze oxidation-reduction reactions.Transfer of H atoms, O atoms or electrons.

b. Transferases: catalyze the transfer of functional groups.

c. Hydrolases: catalyze hydrolysis reactions; breaking of chemical bonds by adding water. Catalyse   
     hydrolysis  (addition of water). AX + H2O -> AH + XOH

d. Lyases: catalyse the removal of chemical groups, with double bond formation. Catalyse splitting bonds   
    (C-N, C-O, C-C, C-S)

e. Isomerases: catalyze isomerization reactions. Catalyse structural rearrangements of molecules.

f. Ligases: catalyze formation of chemical bonds, with ATP cleavage.

Enzymes catalyze reaction of only one or a few related molecules or substrates.   Enzyme activity can vary with tissue, cell, physiological conditions.   
**Enzyme Specificity**: Enzymes only bind a very specific type of substrate molecule which is capable of fitting into active site. There are four types of specificity:   
1. Absolute specificity: Enzyme acts on only one substrate and catalyzes only one reaction.   
2. Group specificity: Enzyme acts on a class of substrates that posses a common functional group. Eg Phosphatases.   
3. Linkage specificity: Enzymes are specific only for a particular type of chemical bond. Eg. esterases.   
4. Stereospecificity: Enzymes discriminate between D- and L-stereoisomers and use only one of them as substrate.

**4) Discribe the effect that enzymes have on the activation energy of a reaction.**

**How Does an Enzyme Work?**   
The enzyme structure play a very important role in the catalytic activity.  It binds to the substrate and weakns the bond that needed to be broken.  These effects are summarized below:   
**Five themes that recur during enzymatic reactions:**

1. **The proximity effect**: An enzyme can accelerate a reaction between A and B reactants by holding the tworeactants close together in appropriate orientation.

2. **Electrostatic effect:** Enzymes stabilizes the distribution of electrical charges.

3. **Acid-base catalysis**: Enzymes potentiate the reactive group by increasing its electrophilic or nucleophilic   
character by adding or extracting a proton.

4. **Nucleophilic or electrophilic catalysis by enzymatic functional groups**: Enzyme may provide a functional group, such as the -amino group of lysine, carboxyl group of aspartic acid, hydroxyl group of serine, sulfhydryl group of cysteine; to which substrate can covalently bind.

5. **Structural flexibility**: In order to bind the substrates appropriately, some enzymes change their configuration to provide a better orientation of the substrate (induced-fit hypothesis) so that enzyme closes like a set of jaws around the substrate. This way, the substrate is forced to respond the electrostatic environment provided by the enzyme active site functional groups only, instead of environment.

**Whichever the scenario is enzymes seem to follow a pattern:**   
a) Substrate (S) binds the enzyme (E) active site,   
b) Enzyme-substrate complex (ES) forms   
c) Chemical bonds are made or broken during which ES complex achieves a **transition state** (ES)\* at a significantly  lower   
    activation energy than that of uncatalyzed reaction.   
d)The ES transition state is broken down to an enzyme -product complex (EP)   
e) EP complex finally yields the products from the active site,   
f) Enzyme is regenerated.

S + E --> ES ---> (ES)\* --> EP --> E + P

**How does a catalyst increase rates of a chemical reaction?**   
Enzymes lower the **activation energy** required for a chemical reaction.

                                        A + B ---->  C + D

**Activation energy**: If reactants A and B are to react, they must collide with enough kinetic energy (energy of   
movement) to make or to break a chemical bond during the reaction. Thus, the reactants A and B must have   
enough energy (activation energy) to reach transition state.

**Transition state**: High energy intermediate in which A and B have enough energy to be converted into the products C and D.

**5) The effect of substrate concentration on enzyme-catalyzed reactions.**   
The reaction rate generally increases linearily in proportion to enzyme concentration.  Enzyme concentration affects velocity:   
      Plot of reaction velocity versus [E] is linear.   
  
The reaction rate initially increases proportionally with increasing substrate concentrations but levels off to a flat rate as substrate concentrations get very high. Substrate concentration affects velocity:  Plot of reaction velocity versus [S] for catalyzed reaction is hyperbolic.   
  
**Turnover Number of the enzyme.**   
Turnover Number refers to the efficiency of the enzyme and is expressed as the number of molecules of substrate converted to product per second. The Turnover Number of enzymes can range from 10 to 100,000 molecules per               second......demonstrating the effective catalytic nature of some enzymes.

Reaction velocities or reaction rates:   Expressed as amount of S consumed/time unit. or as amount of P produced/time unit.

The Rate Equation which describes **rate of reaction** (v) as a function of substrate  concentration ([S]) is the **Michaelis-Menton** Equation.

          v = Vmax [S]/(Km + [S])

          Vmax is the a function of the concentration of the enzyme [E] and the   
NOTE: a plot of the Michaelis-Menten equation yields a "hyperbolic curve".   
      Vmax = maximum reaction velocity at saturating substrate concentration.   
 Why can enzymes be saturated?

      E + S <==>  ES --->  E + P

Normally, very few E molecules are present and many more S molecules are present. If enough S is added, all E can be driven into the ES form.   
      Km = [S] giving 1/2 Vmax (formal definition of Km).   
      The Km is a consistent characteristic of an enzyme for a given substrate.   
      Practical application of Km:   
**Km tells the affinity of an enzyme for it substrate.**   
      A small Km value signifies high affinity of enzyme for substrate.   
      A large Km value signifies low affinity of enzyme for substrate.   
      Example:   
      reaction:                                               Km values:   
      glucose + Pi ---> glucose-6-P                0.10 mM   
      galactose + Pi ---> galactose-6-P           0.12 mM   
      fructose + Pi ---> fructose-6-P               0.30 mM   
      ribose + Pi ---> ribose-5-P                    0.60 mM   
      glycerol + Pi ---> glycerol-6-P              no reaction

      Km values give information about:   
      1. how enzyme may work metabolism scheme of the cell.   
      2. how enzyme active site works.   
      3. what functional groups are located at the enzyme active site.   
      4. what functional groups are required on the enzyme substrate.

**6) Discuss the role of the active site and the importance of enzyme specificity.**   
Enzymes are specific for a very limited number of substrates (often just one). They not only insist that their substrate has the correct covalent structure, but each atom must be in the correct three-dimensional geometrical arrangement in space.

A. Enzymes show high specificity for the substrates with which they interact    eg. Lactate dehydrogenase can distinguish L-lactate from D-lactate and will only react with the L-isomer.

B. One theory suggests that the enzyme has a preformed pocket with a specific shape into which the substrate fits like a lock and key interaction. **Lock and Key model:** One model for enzyme specificity is the lock and key, where an enzyme active site and substrate fit snugly together. Here you can see that the first substrate fits in the enzyme active site, as it is the correct shape, but the second does not.   
C. Another theory suggests that the enzyme is more flexible in absence of the substrate and then changes conformation in an **induced-fit model** when it binds with the substrate.

**7) Describe the difference between the lock and-key model and the induced fit model of enzyme-substrate complex formation.**   
**Lock and Key model:**   
One model for enzyme specificity is the lock and key, where an enzyme active site and substrate fit snugly together. Here you can see that the first substrate fits in the enzyme active site, as it is the correct shape, but the second does not.

**The Induced Fit model**: Another is the induced fit model where enzyme changes shape to fit snugly around its substrate.   
Enzyme specificity is absolutely vital to life. Cells are mixtures of thousands of different molecules, all potentially enzyme targets. It is essential that an enzyme works only its correct substrate and ignores everything else.

**8)Discuss the roles of cofactors and coenzymes in enzyme activity.**

**Cofactors and Coenzymes**:   
Many enzymes posses **prosthetic groups** that are non-amino acid in their nature.  **Prosthetic group**  is name reserved for a permanently attached cofactor or coenzyme group.Those **conjugated proteins** (apoenzyme and cofactor/coenzyme) are called as **holoenzymes**. A holoenzyme is dissociated into a protein component **apoenzyme**, and its nonprotein prosthetic group which is called as **cofactor**. Cofactor is tightly bound to protein and not readily dissociated unless the enzyme is denatured. Eg. metals such as cupper or zinc.  Some prosthetic groups are organic compounds and are not tightly bound to enzyme molecules. They can be easily dissociated from the enzyme. Those are referred as coenzymes. Actually, they may be regarded as cosubstrates, since they participate stoichiometrically in the reaction and are consumed with the substrates. They act as acceptors or donors of atoms or of functional groups that are removed or added to the substrate of an enzyme. Mammals do not synthesize most of them and are essential nutritive factors called vitamins.

 Some enzymes are simple proteins: active enzymes composed only of amino acids.   Other enzymes are not catalysts unless other ions or molecules (cofactors or coenzymes) reversibly associate with the enzyme protein:

      apoenzyme + cofactor      <===> holoenzyme.   
                             metal ion   
  
      apoenzyme + coenzymes    <===> holoenzyme.   
                        organic molecule   
                             (vitamins)   
  
Some coenzymes are water soluble vitamins   
      micronutrients = vitamins and minerals.   
      consume milligram and microgram quantities --- use as coenzymes.  Micronutrients are  vitamins and minerals.

The carbohydrates, proteins, lipids are considered as macronutrients which are consumed in gram quantities. They provide building blocks and energy.   
Water-soluble vitamins: vitamins in the human diet that are coenzyme precursors.   
The water soluble vitamins and minerals are considered as micronutrients which are consumed in milligram and microgram quantities. They are used as coenzymes.

**Vitamin form:  riboflavin  (B2)**   
**Examples of coenzymes from vitamin:  riboflavin  (B2):** coenzyme forms:  FAD or FMN   
**FAD:  flavin adenine dinucleotide**.   
        riboflavin-pentose-P-P-ribose-adenine  =  FAD

**.FMN:  flavin adenine mononucleotide**   
riboflavin-pentose-P-P  = FMN.  The structure of FMN is similar to FAD but does not have adinosine unit(ribose-adenine)   
FAD partcipates in oxidation of C-C single bonds to C=C double bonds.

            H H                      H   
FAD     +  -C-C-  --->    FADH2  +  -C=C-   
            H H                        H   
oxidized                 reduced   
          reduced                  oxidized

The FADH2 molecule can carry two electrons and two protons.   
These 2 e- and 2 H+ represent energy and are sometimes referred to as a reducing equivalent and represent stored energy.

ATP is not the only energy currency in the cell, FADH2 also represents energy currency because they they produce ATP ultimately.   
**Vitamin:  niacin(B3)**   
Coenzymes   NAD+  or  NADP+   
Carrier of hydride ions   
NAD+ = nicotinamide adenine dinucleotide.   
nicotinamide-ribose-P-P-ribose-adenine  =  NAD+

NADP+= nicotinamide adenine dinucleotide phosphate.   
nicotinamide-ribose-P-P-ribose-adenine  =  NADP+

             H                        O   
           H O                      H !!   
NAD+    + -C-C-      NADH + H+  +  -C-C-   
           H H                      H   
oxidized            reduced   
         reduced                 oxidized   
The NADH + H+ can carry two electrons and one proton with one H+ free in solution.   
These 2 e- and 2 H+ represent energy and are sometimes referred to as a **reducing equivalent** and represent stored energy.

ATP is not the only energy currency in the cell, NADH + H+  also represents energy currency.   
**Vitamin:  pantothenic acid**   
**coenzyme form: Coenzyme A** acyl group transfer

HS-organic group (pantothenic acid) -P-P-pentose-adenine

  O                      O   
R-C-OH  + HS-CoA  <==> R-C-S-CoA  + H2O   
carrier of acyl groups.   
acetyl CoA   
fatty acyl CoA.

**Other water soluble vitamins:**   
**Vitamin form: thiamine (B1)**   
**coenzyme: thiamine** pyrophosphate involved  in carbohydrate metabolism.  Important in decarboxylation and transfer of activated aldehyde groups.

**Vitamin form: pyridoxine (B6)**   
**coenzyme:** pyridoxal phosphate  involved  in amino acid metabolism.  Important in transamination reactions.

**Vitamin form: folic acid**   
**coenzyme : biotin**  involved in transfer of one carbon units in biosynthesis.   
biotin  carboxylation reactions    R-CH2-H   + CO2  --->  R-CH2-COOH

**Vitamin form: cyanocobalamin (B12)**   
**coenzyme : deoxyadenosyl** cobalamin involved in amino acid metabolism

**9) Discuss the mechanisms by which certain chemicals inhibit enzyme activity.**   
**Competitive and Non-Competitive Inhibition of Enzymes**

**Competitive Inhibition** occurs when a bogus molecule that is close enough to the shape of the true substrate will fit into   
     the active site. Once locked into position, the blocker molecule prevents the true substrate molecule from getting into   
     position. This effectively blocks the active site. The bogus molecule competes for the active site with the true substrate   
     molecule. Many toxic substances owe their toxic properties to their ability to act as inhibitors to important enzymes   
     responsible for catalyzing important biochemical processes. Once the enzyme is inhibited the process cannot take place,   
     and a toxicological symptom occurs that often leads to paralysis, coma or even death of the organism. For example,   
     cyanide poisoning is due to the cyanide ion competitively inhibiting the active site of the cytochromases enzymes   
     responsible for catalyzing the Oxidation and Reduction processes of the Electron Transport System which is responsible   
     for cellular respiration.   
  
     Other inhibitors latch themselves not to the active site itself but to some portion of the enzyme molecule close to the   
     active site which results in the changing of the shape of the active site. This is referred to as non-competitive inhibition.   
     Many heavy metals like Lead, Mercury,and Chromium will function as non-competitive inhibitors. Toxicology is the   
     study of how toxicological substances can interfere with life sustaining enzymes via inhibition.

     The pesticide and herbicide industries make use of competitive and Non-Competitive Inhibitors   
  
     Biological warfare owes its success to enzyme inhibition but so does the life giving chemotherapeutic treatment of   
     cancerous tumor growths with agents that inhibit important cancel cell enzymes. All in all the use of inhibitors can be used   
     for the benefit of mankind or its destruction.   
An enzyme molecule will be inactivated if the substrate can no longer bind to the active site. This may be effected by an inhibitor   
covalently bonding to the site, or binding very tightly so that its dissociation is very slow. This is irreversible inhibition (see the   
example of chymotrypsin below). In reversible inhibition, there is a rapid equilibrium of the enzyme and inhibitor.   
Competitive inhibitors bind to the active site. Non-competitive inhibitors bind elsewhere, but reduce the rate constant (the   
"turnover number") of the formation of enzyme E and product P from the ES complex. Effector molecules may act by having an   
opposite effect. In oligomeric enzymes with several active sites, allosteric inhibitors and effectors associating with one site   
affect the binding capabilities of others (see above on allosteric interactions).   
ENZYME INHIBITION. [CDT289].   
**Positive Allosteric Inhibition**   
  
Presence of afftor molecule alters enzyme  structure and the substrate binds to the enzyme easily   
**Negaive  Allosteric Inhibition**   
Presence of afftor molecule alters enzyme  structure and the substrate find it difficult to bind  to the enzyme.   
      inhibitor -  decreases velocity of enzyme catalyzed reaction.   
      irreversible inhibitor - covalently bound to enzymes.  Does NOT come off.

      E + I  ---->  EI   
      active    inactive   
                irreversible

      nerve gases, insecticides.   
      Nerve gas chemical group becomes covalently attached to a reactive serine at active site of acetylcholinesterase.   
 

      reversible inhibitor - I binds to E by weak noncovalent association (reversible).   
      Activity returns when I dissociates.

      TWO TYPES OF REVERSIBLE INHIBITORS.  [CDT289].

      competitive inhibitor:  The competitive inhibitor and the substrate compete for the active site.   
      The competitive inhibitor resembles the substrate structurally.   
      [CDT290].

      E + S <==>  ES   E + P   
      +   
      I

      EI   
 

      Beneficial applications of competitive inhibition:   
      Example I:   
           THF   DHF

      uracil --->  thymine --->  dTMP --->  cell division (DNA)   
              x   
      methotrexate resembles DHF.   
      used as anticancer agent.   
 

      Example II of a competitive inhibitor:   
      sulfa drugs   
      bacteria make folic acid

      p-aminobenzoic acid   folic acid   DHF   THF   
                          x sulfa drug contains sulfanilamide   
                            sulfanilamide resembles p-aminobenzoic   
                            acid.   
      Host does not make DHF; gets from diet; host is not affected by treatment.

      noncompetitive inhibitor - binds somewhere other than active site and inhibits the enzyme.   
      Active site is open.   
      Noncompetitive inhibitor binds to E and causes conformational change in E.   
      S binds poorly to active site of E after noncompetitive inhibitor binds.

      E + S <==>  ES   E + P   
      +           +   
      I           I

      EI          ESI   
**10) Recognize how pH and temperature affect the rate of an enzyme-catalyzed reaction.**   
**pH Effects on Enzyme Activity**   
Changes in the pH or acidity of the environment can take place that would alter or totally inhibit the enzyme from catalyzing a reaction. This change in the pH will affect the polar and non-polar intramolecular attractive and repulsive forces and alter the shape of the enzyme and the active site as well to the point where the substrate molecule could no longer fit, and the chemical change would be inhibited from taking place as efficiently or not at all. In an acid solution any basic groups such as the Nitrogen groups in the protein would be protonated. If the environment was too basic the acid groups would be deprotonated. This would alter the electrical attractions between polar groups. Every enzyme has an optimum pH range outside of which the enzyme is inhibited. Some enzymes like many of the hydrolytic enzymes in the stomach such as Pepsin and Chymotrypsin effective operate at a very low acidic pH. Other enzymes like alpha amylase found in the saliva of the mouth operate most effectively at near neutrality. Still other enzymes like the lipases will function most effectively at basic pH values. If the pH drops in the blood called acidosis then enzymes in the blood will be inhibited outside their optimal pH range. If the pH climbs to an unacceptably high value called alkalosis then enzymes cease to function effectively. Normally, these conditions do not take place because of the highly efficient buffers found in the blood that restrict the pH of the blood to a very narrow range. Buffers are a substance or mixtures of substances that resist any change in the pH. There are many buffer systems found in the body to adjust the pH so that enzymes might continue to catalyze their reactions.   
Correcting pH or temperature imbalances will usually allow the enzyme to resume its original shape or conformation.  Some substances when added to the system will irreversably break bonds disrupting the primary structure so that the  enzyme is inhibited permanently. The enzyme is said to be irreversably denatured. Many toxic substances will break co-valent bonds and cause the unraveling of the protein enzyme. Other toxic substances will precipitate enzymes effectively removing them from the solution thus preventing them from catalyzing the reaction. This is also called denaturation.   
**Temperature Effects on Enzyme Activity**   
Every enzyme has a temperature range of optimum activity. Outside that temperature range the enzyme is rendered inactive and is said to be totally inhibited. This occurs because as the temperature changes this supplies enough energy to  break some of the intramolecular attractions between polar groups (Hydrogen bonding, dipole-dipole attractions) as well   as the Hydrophobic forces between non-polar groups within the protein structure. When these forces are disturbed and  changed, this causes a change in the secondary and tertiary levels of protein structure, and the active site is altered in its  conformation beyond its ability to accomodate the substrate molecules it was intended to catalyze. Most enzymes (and  there are hundreds within the human organism) within the human cells will shut down at a body temperature below a certain value which varies according to each individual. This can happen if body temperature gets too low (hypothermia) or too high (hyperthermia).

pH EFFECTS ON ENZYME CATALYZED REACTIONS.   
      [CDT286].   
      plot of velocity versus pH for:   
      pepsin (pH 2 optimum)   
      trypsin (pH 7 optimum)

      Note bell shaped curve.   
      Each enzyme has a unique "optimum" pH profile.   
      Activity drops off both above and below the optimum.

      At pH above/below the optimum, the "charges" on functional groups on the enzyme or substrate are changed.   
      The enzyme does not bind the substrate as well and less activity is noted.   
TEMPERATURE EFFECTS ON ENZYME CATALYZED REACTIONS.

      [CDT287].   
      Plot of velocity versus temp for uncatalyzed reaction.

      Plot of velocity versus temp for enzyme catalyzed reaction.   
      increase temp; increase rate.   
      At high temp, denature enzyme and rate drops off.

      S has higher speed and increased energy as temp increases.   
      Increasing temp gives more frequent substrate collisions; also more violent collisions.   
      Outcome:  larger proportion of S goes to P.

**11) Discuss the role of the enzyme chymotrypsin and other serine proteases.**   
The serine proteases hydrolyze the peptide bonds of proteins. Chymotrypsin, trypsin, and elastase are the major enzymes produced by the mammalian pancreas. They are similar in structure and function; the main chain backbone of all 3 can be superimposed on each other very closely. The similarity of chymotrypsin and elastase is shown below:

However, the sequences of the 3 proteins are only 50% homologous. Residues on the surface of the enzyme are only about 10% homologous, whereas the figure for buried residues, ie including the functionally important ones which contribute to the active site, is approximately 60%.

These three proteases are the result of divergent evolution. They differ in their specificity: chymotrypsin has a large pocket which accommodates the large hydrophobic side chains of Phenylalanine, Tyrosine and Tryptophan, and so catalyses the cleavage of peptides and esters of these amino acids. Trypsin has an Aspartate residue (189) at the bottom of the pocket (instead of Ser-189 as in chymotrypsin), and this Asp forms a salt bridge with the positively charged group at the end of the substrate Lysine and Arginine side chains, on which this enzyme acts. Elastase only accommodates small hydrophobic side chains eg Alanine, as the mouth of the pocket is partially blocked by the side chains of Val-216 and Thr-226 (these residues are both Gly in chymotrypsin). Examine Andrew Wallace and Roman Laskowski's LIGPLOT diagram of a tryptophan residue in the active site of chymotrypsin, and Manuel Peitsch's diagram of the enzyme highlighting the active site. There is a similar picture of elastase.

The serine proteases were so named as they have a highly reactive serine residue, Ser-195, which attacks the carboxyl group of the substrate.This results in an **acylenzyme** intermediate consisting of the substrate covalently bound to the enzyme at this serine.

However, the reactivity is dependent upon the arrangement of the serine side chain with two other polar side chains, approximately in a straight line, which is characteristic of all serine proteases. The Ser-195 is positioned at one end of this line, while at the other end is Asp-102, with His-57 in the middle. This is called the **catalytic triad**. Notice how far apart these three residues are in the sequence- the tertiary structure of the polypeptide chain brings them together in the required arrangement.

The catalytic triad is indicated in this diagram.

The residues of the catalytic triad form a **charge relay network**. His-57, polarized by Asp-102, acts as a **proton shuttle** which accepts the hydrogen ion from Ser-195 as it makes a nucleophilic attack on the substrate.

The inactive precursor (the **zymogen**) of chymotrypsin is the 245-residue protein **chymotrypsinogen** . A cleavage is made (by trypsin) between residues 15 and 16, to form an active form of the enzyme called **pi-chymotrypsin**. Further cleavages are made (by another pi-chymotrypsin molecule) to remove residues 14, 15, 147 and 148, to give the stable form of the enzyme, **alpha-chymotrypsin**. Note that trypsin also undergoes similar activation by means of cleavage. This is therefore a positive feedback mechanism, which activates the pancreatic enzymes in the intestine (the zymogens are secreted by the pancreatic cells).   
  
The activation of the zymogen by cleavage involves highly localized conformational changes. Cleavage between the 15th and 16th residues forms an amino-terminal group on Ile-16, which turns inwards and interacts with Asp-194 in the interior of the molecule; this stabilizes the protein. This electrostatic interaction triggers other alterations in conformation, which result in the correct arrangement of the residues forming the cavity for the substrate; this cavity is not fully formed in chymotrypsinogen.

Differences between the structure of alpha-chymotrypsin and chymotrypsinogen are indicated in the   
  
previous diagram. Also examine Manuel Peitsch's diagram highlighting the different position of Ile-16 in the enzyme and zymogen.

Some non-mammalian serine proteases have been found to have a very similar tertiary structure to their mammalian counterparts, and are 20-50% homologous with them; for example see trypsin from *Streptomyces* in a previous diagram. However, other non-mammalian examples have no homology to the mammalian enzymes, and have completely different tertiary structure; yet the same catalytic triad, and arrangement of hydrogen bonding groups to the substrate, has evolved independently (convergent evolution). In the bacterial serine protease **subtilisin**, the triad consists of Ser-221, His-64 and Asp-32 as shown below:

The molecular basis of the function of chymotrypsin will be examined in more detail in a future chapter.   
**12) Describe the process of blood coagulation and the role of vitamin K in the formation of blood clots.**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Normal Blood Clotting**  **Hemostasis - the sequence of local events which culminates in arrest of bleeding from an injured vessel.**  **Four phases of hemostasis:**   * **Constriction of injured blood vessel** * **Formation of platelet plug** * **Formation of fibrin clot** * **Dissolution of clot**   **For this class, we are primarily interested in the formation of the fibrin clot. However, you need some general background on all four phases of hemostasis. The first three phases of hemostasis are depicted in the following set of four diagrams.**  **Hemostasis in Response to Injury to a Blood Vessel**   |  |  | | --- | --- | |  | **In the blood of a healthy person, platelets and many clotting factors, such as fibrinogen, circulate in the inactive form.** | |  | **When a blood vessel is injured, the immediate results are: (1) the vessel constricts, (2) tissue factor is released and (3) collagen in the vessel wall is exposed. Platelets bind to collagen which results in their activation. Activated platelets change shape, activate other platelets and aggregate.** | |  | **The aggregated platelets form a platelet plug which is relatively loose and temporary.** | |  | **Exposed collagen and release of tissue factor into blood initiate the coagulation cascade. In a late step in the coagulation cascade, fibrinogen is cleaved to form fibrin which forms a clot which entraps the platelet plug and other cells. The fibrin clot is relatively stable.** |   **Formation of a platelet plug and a fibrin clot is desirable if the plug and clot are formed at the site of a perforation in a blood vessel. However, formation of a platelet plug and fibrin clot is undesirable if the plug and clot are formed when the blood vessel is intact. The undesirable clot is referred to as a thrombus if it is stationary and an embolus if it moves. The formation of a thrombus can occur if the endothelium of the blood vessel is damaged by atherosclerosis or chemicals or if the coagulation system, anticoagulation system or fibrinolytic system is not functioning properly. The coagulation cascade which culminates in the formation of the fibrin clot is diagrammed below.** |  |

|  |  |  |
| --- | --- | --- |
|  | **Blood Clotting and Vitamin K**  **The formation of the secondary clot, or the fibrin clot, requires vitamin K.**  **Active vitamin K is a cofactor in a step in the activation of four of the coagulation factors in the coagulation pathway (Factors VII, IX, X and II).**  **Details of the Activation of Vitamin K Dependent Coagulation Factors**  **The liver is the site of synthesis of the four vitamin K dependent clotting factors (proteins) and of their posttranslational modification, a reaction requiring vitamin K.**  **When the four vitamin K dependent clotting proteins are synthesized on hepatic ribsomes, they contain many molecules of the amino acid glutamic acid (glu). After translation and before secretion of these clotting proteins into plasma , many of the glutamic acid molecules of the proteins are modified by the addition of another carboxyl group (-COOH). The resulting amino acid is called gamma carboxyglutamic acid (gla) and is negatively charged (-COO-). After secretion of the clotting proteins into plasma, the negatively charged gla molecule binds Ca++. These modified proteins circulate in plasma.**  **If the coagulation cascade is initiated, the clotting factor which precedes each vitamin K dependent clotting factor in the cascade will catalyze the removal of a few amino acids from the end of the factor to produce an active clotting factor.**  **The posttranslational modification and subsequent activation of a vitamin K dependent blood clotting protein is depicted in the top line of the following diagram:**  **Vitamin K Cycle**  **In the diagram above, the vitamin K cycle is depicted in the lower part of the diagram. Active vitamin K serves as a cofactor for the carboxylation of glutamic acid residues in vitamin K dependent clotting proteins. In the process, vitamin K is oxidized to its epoxide form. The vitamin K-2,3 epoxide is reactivated by two reduction reactions.** |  |

**13) Explain the role of acetylcholinesterase in nerve transmission.**   
Acetylcholinesterase (AChE) functions to terminate the action of acetylcholine (ACh) bound to the two types of  cholinergic receptors, which are located at the synapse. The most prevalent form of acetylcholinesterase in  Torpedo californica, an electric fish, is a 75 kD  homodimer, fast-acting enzyme generally localized in the  synaptic cleft. The catalytic efficiency of the enzyme  (kcat/KM = 1.5 X 108 M-1 s-1) in catalyzing ACh to  acetate and choline suggests that AChE is a near perfect  catalyst with a remarkably high turnover rate ( kcat =  14,000 s-1). These observations suggest that AChE  functions at a rate approaching that of a  diffusion-controlled reaction. The catalysis of ACh thus  allows for the proper transmission of nerve impulses  from one neuron to another. Inactivation of AChE with  antagonistic compounds, like organophosphates, results  in the accumulation of ACh at the synaptic junction which leads to the overstimulation of the neuron. This  state can precipitate death in the intoxicated organism.   
**14) Provide examples of medical uses of enzymes.**   
**Galactosemia**   
Milk products which contain lactose are conversted to galctose and glucose in the digestive track. Both glucose and galactose are absorbed by the body and used to produce energy.However, galactose has to be converted phosphorylated glucose before it can be used by the body. Galactosemia is a genetic desease caused by a lack of  enzymes necessary for the conversion of galactose to phosphorylated glucose which is used in the cellular metabolism or glycolysis. A toxic compound formed from galactose accumulates in people with galactosemia. Treatment for galactosemia is the use of non dairy products which does not contain lactose.  Enzyme supplements are also helpful in converting lactose consumed to glucose and galactose.   
**Enzyme replacement therapy**   
The treatment of enzyme deficiency states represents an obvious use of enzymes. For example, oral   
pancreatic extracts have commonly been used in the treatment of cystic fibrosis and pancreatic   
insufficiency. The most commonly available preparations are of three types: (1) pancreatin an alcoholic   
extract of pig pancreas containing amylase and trypsin activity, (2) pancrealipase, a lipase-enriched   
extract, and (3) Viokase also enriched in lipase.

More intriguing is the treatment of the inborn errors of metabolism in which deficiency of a single enzyme   
leads to accumulation of abnormal amounts of substrate. With the recognition that many of these errors   
are owing to inadequacies of lysosomal enzymatic catabolism, it was reasoned that exogenously   
administered enzyme might react with and dispose of such accumulations.

The infusion of crude glucosidase from Aspergillus niger into patients with type 11 glycogenolysis, a   
condition attributed to a deficiency of this enzyme, was reported in the mid 1960s. Infusion of normal   
plasma, which contains galactosidase, the enzyme missing in Fabry's disease, reduced the amount of   
abnormal substrate in the plasma and tissues of two patients heterozygous for this disorder.