ADDIS ABABA UNIVERSITY
ADDIS ABABA INSTITUTE OF TECHNOLOGY

Course Name: Biochemistry and Molecular Biology

Course Code: CBEg4191; Total Credit hours: 5 ECTS

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Chapter 1: Biomolecules

1.1. The molecules and Chemical rxn of life

❑Introduction to Biochemistry

- Chemical substances present in living organisms 4 elements (C,H,O,N) makes up approximately 95% of the mass of living matter.

- Small amount of S,P,Ca, K, Mg, Cl, Fe

- Trace amount of other elements such as Cu,Mn,Zn, Co , I

CON'D.................

❑**What is Biochemistry ?**

▪ Biochemistry- is a hybrid Science , that concerned with chemical rxns occurring in living organisms.

▪ Major classes of Biomolecules:

✓ COH

✓ Protein

✓ Lipids

✓ Nucleic Acids

Con'd……..

❑ **The cells and Chromosomes**

- Cells are the building block of living organisms.
- They vary in size, shape and function.
- All cells are either Prokaryotic or Eukaryotic
- Prokaryotic cells- consists of
- ✓ a single closed compartment that is surrounded by the plasma membrane
- ✓ lacks defined nucleus
- ✓ has relatively simple internal organization
- ✓ includes : Bacteria and Cynobacteria .

Con'd……….

- Eukaryotic cells : unlike prokaryotic cells, contain

✓A defined membrane bound nucleus

✓Have extensive internal membranes that encloses other compartments, organelles.

✓Includes all plant kingdom, animal kingdom, fungi kingdom and protistas kingdom

The membrane bounded nucleus of eukaryotic cells contain DNA which is extensively folded into the familiar thread like structures known as chromosomes.

The main difference between DNA and chromosome is regarding the role of genes. DNA stands for deoxyribonucleic acid. The DNA is basically made up of cytosine, adenine, thymine and guanine. When you arrange these four bases to create a particular segment, it is called a gene. When these segments are coiled in a form that can be easily duplicated, they are known as chromosomes.

con'd…………

❑Chromosomes: is a thread like str. In the nucleus.

❑Derived from two Greek words:

Chromos-----color

Soma--------body ⎤ chromosome-stained by dyes

❑In every somatic cell there are two copies of each chromosome known as homologous pairs

❑Their numbers in a cell is vary, it could be

✓ N—haploid chromosome ⎤

✓ 2N—diploid chromosomes ⎬d/t sp. Have d/t number of chromosomes.

Con'd………

organisms	chromosomes
Human	2N= 46
Dog	2N=78
Rat	2N= 42
Frog	2N=26
Potato	2N= 48

CONT……..

❑Cell divisions

1. Mitosis cell division(equational division)
➢ Is a cell division for somatic cells or body cells.
➢ It is a means of cell growth and repairs in Multicellular organisms and a means of reproduction for unicellular organisms.
➢ Has different stages or phases, which are acronymed as IPMAT.

2.Meiosis cell division (reductional division)
➢ Is a cell division for germ cells or sex cells.

Cont……….

➤ It is a means of reproduction for Multicellular organisms and for a few unicellular organisms.

➤ Is divided as

✓ Meiosis I

✓ Meiosis II

➤ It's main different from mitosis is due to the occurrence of genetic recombination-resulted from crossing –over of chromosomes.

Water

❑ Water is the most abundant substance in living systems, making up 70% or more of the weight of most organisms.

❑ Properties of water

✓ Physical properties of water

Water has several unique physical properties. These properties are:

1. Water has a high specific heat. Specific heat is the amount of energy required to change the temperature of a substance. Because water has a high specific heat, it can absorb large amounts of heat energy before it begins to get hot.

2. Water in a pure state has a neutral pH. As a result, pure water is neither acidic nor basic. Water changes its pH when substances are dissolved in it.

Cont.

3. Water has high heat of vaporization

4. Water can exist in three forms:

- liquid state---water
- Solid state----ice
- Gaseous state---vapour

5. Water has less viscosity

6. Water has relatively high boiling point

7. Higher melting point

Cont……….

✓ **Chemical properties of water**
1. **Weak interactions in aqueous system**

- Hydrogen bonds between water molecules provide the cohesive forces that make water a liquid at room temperature and that favor the extreme ordering of molecules that is typical of crystalline water (ice). Polar Biomolecules dissolve readily in water because they can replace water-water interactions with more energetically favorable water-solute interactions. In contrast, non-polar Biomolecules interfere with water-water interactions but are unable to form water-solute interactions—consequently, non-polar molecules are poorly soluble in water. In aqueous solutions, non-polar molecules tend to cluster together.

2. Ionizes into H+ and OH –
3. Buffering against PH in biological systems

- **Buffers are aqueous systems that tend to resist changes** in pH when small amounts of acid (H) or base (OH) are added. A buffer system consists of a weak acid (the proton donor) and its conjugate base (the proton acceptor).

4. H2O is a reactant- universal solvent

Thermodynamics applications in Life

❑First law of Thermodynamics…applications in biological process

❑Second law of Thermodynamics….applications in biological process

❑Third law of Thermodynamics.. applications in biological process

Cont……

1.2. Amino acids of proteins

- All proteins are composed of 20 standard amino aids, which are specified by the genetic code.

- The standard amino acids are called α-amino acids because they have a primary amino group and a carboxyl group bound to the same carbon atom (the **α carbon**). Only proline has a secondary amino group attached to the α carbon, but it is still commonly referred to as an α- amino acids.

Cont………

➢ The generic str. Of an aas. at PH 7 is shown below.

variable

group

α-carbon

R---CH—COOH

Carboxyl group

NH2

amino group
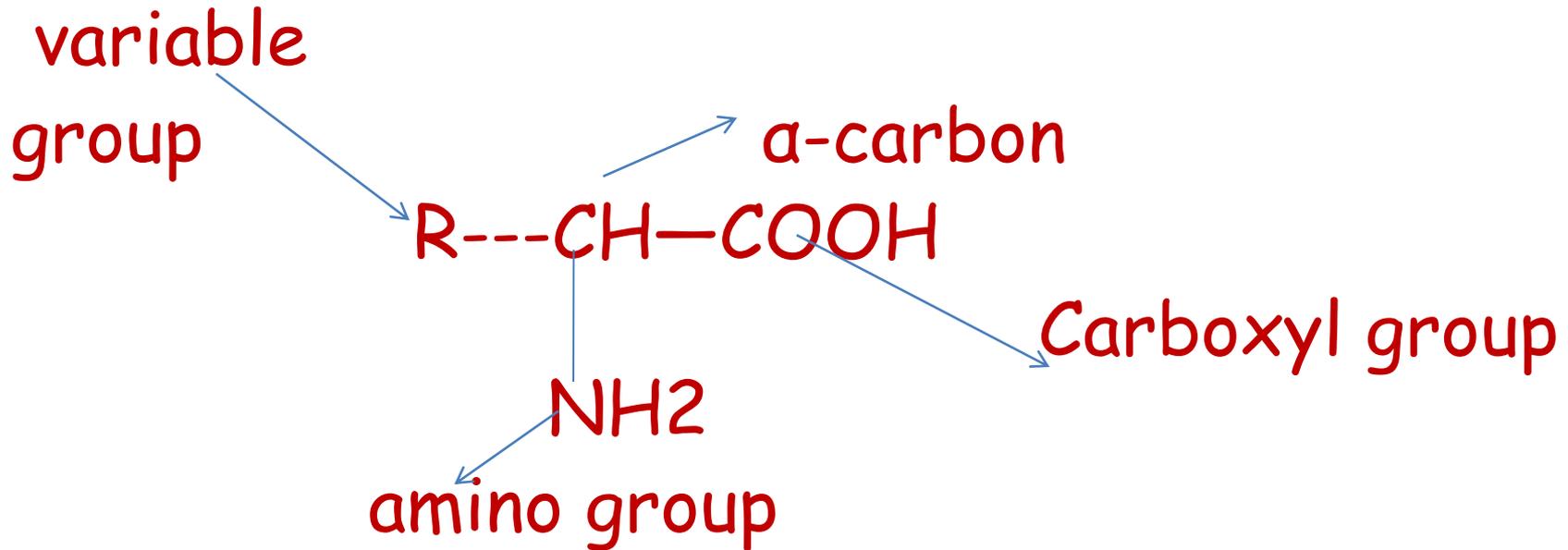
• α-amino acids- carboxylic acids containing an amino (-NH2) group attached to the same carbon, i.e, C-2.

Str. Of a general protein and its constituents aas
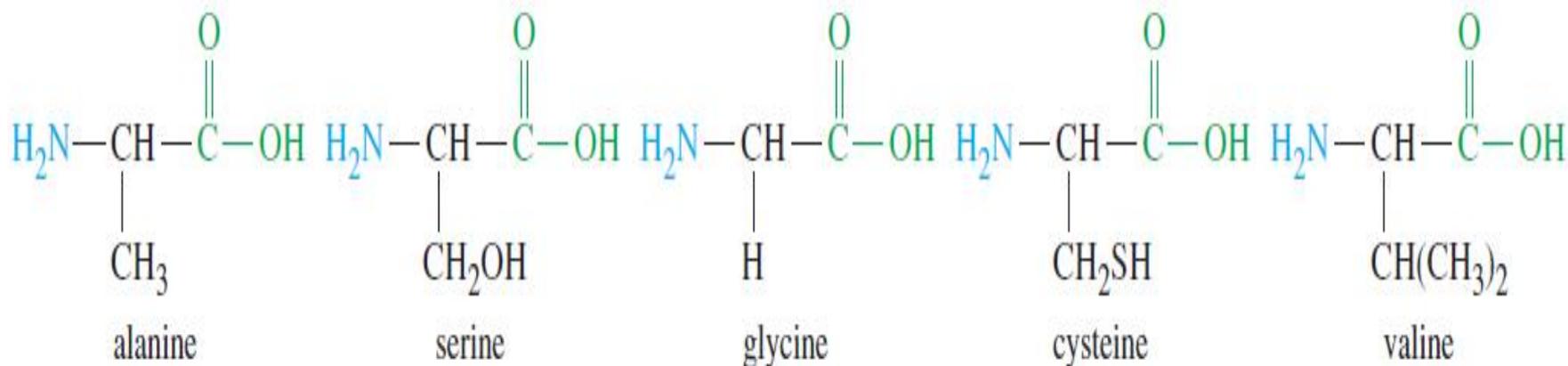
several individual amino acids

$H_2N-CH-\overset{\overset{\displaystyle O}{\|}}{C}-OH$ CH$_3$ alanine

$H_2N-CH-\overset{\overset{\displaystyle O}{\|}}{C}-OH$ CH$_2$OH   serine

$H_2N-CH-\overset{\overset{\displaystyle O}{\|}}{C}-OH$ H   glycine

$H_2N-CH-\overset{\overset{\displaystyle O}{\|}}{C}-OH$ CH$_2$SH   cysteine

$H_2N-CH-\overset{\overset{\displaystyle O}{\|}}{C}-OH$ CH(CH$_3$)$_2$ valine

peptide bonds
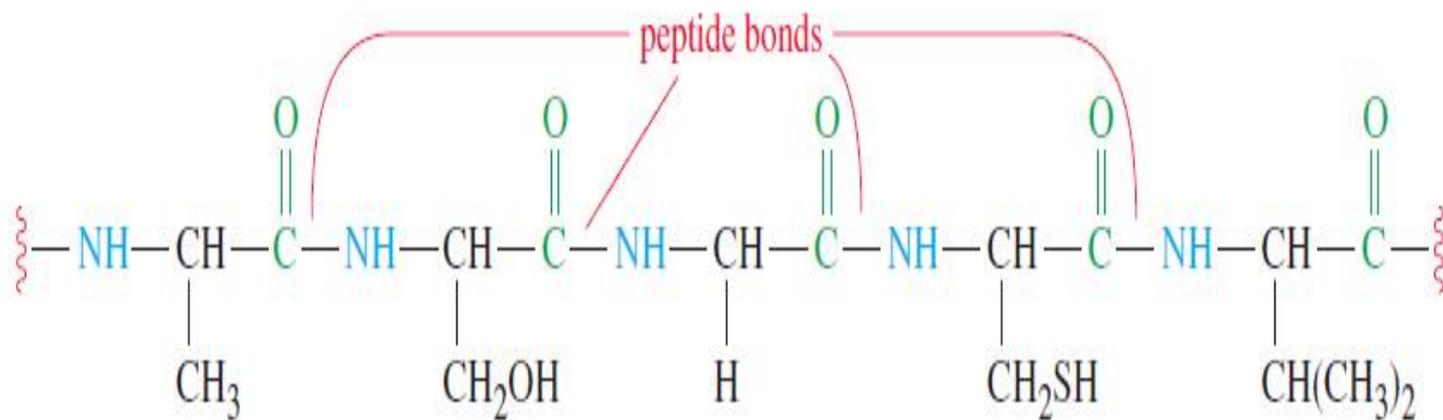
a short section of a protein

Cont………

- Amino acids are polymerized by condensation reactions to form a chain called a polypeptide. Each polypeptide is polarized.

- The **R** groups of the standard 20 aas are classified into **three categories** based on their **polarities** and **charge** at PH 7 :

➢ The non-polar aas

➢ The polar uncharged aas

➢ The charged aas

Cont......

❑ Amino acids with non polar side chains

✓ Glycine, Alanine, Valine, Leucine, Isoleucine, Methionine, Proline, Phenylalanine, Tryptophan

❑ Amino acids with uncharged polar side chains

✓ Serine, Threonine, Aspargine, Glutamine, Tyrosine, cysteine

❑ Amino acids with charged polar side chains

✓ Lysine, Arginine, Histidine, Aspartic acid, Glutamic acid

Covalent str. Of standard aas of proteins

Amino acids with nonpolar side chains

Glycine **Gly** **G**	COO^- $H—C—H$ NH_3^+
Alanine **Ala** **A**	COO^- $H—C—CH_3$ NH_3^+
Valine **Val** **V**	COO^- $\quad CH_3$ $H—C—CH$ $NH_3^+ \quad CH_3$
Leucine **Leu** **L**	COO^- $\quad\quad CH_3$ $H—C—CH_2—CH$ $NH_3^+ \quad\quad CH_3$
Isoleucine **Ile** **I**	$COO^- \quad CH_3$ $H—C—————C^*—CH_2—CH_3$ $NH_3^+ \quad H$
Methionine **Met** **M**	COO^- $H—C—CH_2—CH_2—S—CH_3$ NH_3^+
Proline **Pro** **P**	$COO^- \quad C_3H_2—CH_2$ $C^2 \quad\quad\quad\quad 4$ $H \quad N^{1+} \quad 5CH_2$ H_2
Phenylalanine **Phe** **F**	COO^- $H—C—CH_2—\bigcirc$ NH_3^+
Tryptophan **Trp** **W**	COO^- $H—C—CH_2—$ (indole ring) NH_3^+ N H

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Covalent str. Of standard aas of proteins

Amino acids with uncharged polar side chains

Serine
Ser
S

Threonine
Thr
T

Asparagine'
Asn
N

Glutamine'
Gln
Q

Tyrosine
Tyr
Y

Cysteine
Cys
C

Amino acids with charged polar side chains

Lysine
Lys
K

Arginine
Arg
R

Histidine"
His
H

Aspartic acid'
Asp
D

Glutamic acid'
Glu
E

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Essential AAs

❑Humans can synthesize about half of the aas needed to make proteins. Other aas, called the essential aas, must be provided in the diet. **The ten essential aas are:**

✓ Arginine, Valine, Methionine, Leucine, Threoinne, Phenylalanine, Histidine, Isoleucine, Lysine, and Trytophan

❑Proteins that provide all the essential aas in about the right proportions for human nutrition are called **complete proteins**.

General Properties of amino acids

✓ capacity to polymerize

✓ novel acid-base properties

✓ varied structure and chemical functionality

✓ Optically active

❑ The aas is isolated by the mild hydrolysis of proteins are, with the exception of glycine, all optically active; that is they rotate the plane of plane-polarized light.

❑ Optically active molecules are an asymmetry meaning that they are not superimposable on their morror image, this is said to be property of **chirality**.

Cont……….

❑Molecules that are nonsuperimposable mirror images are known as enantiomers of one another. Look   fluoro-chloro-bromo methane , is it superimposable or not?

Cl

H-C-F

Br

Cl

F-C-H

Br

Mirror plane
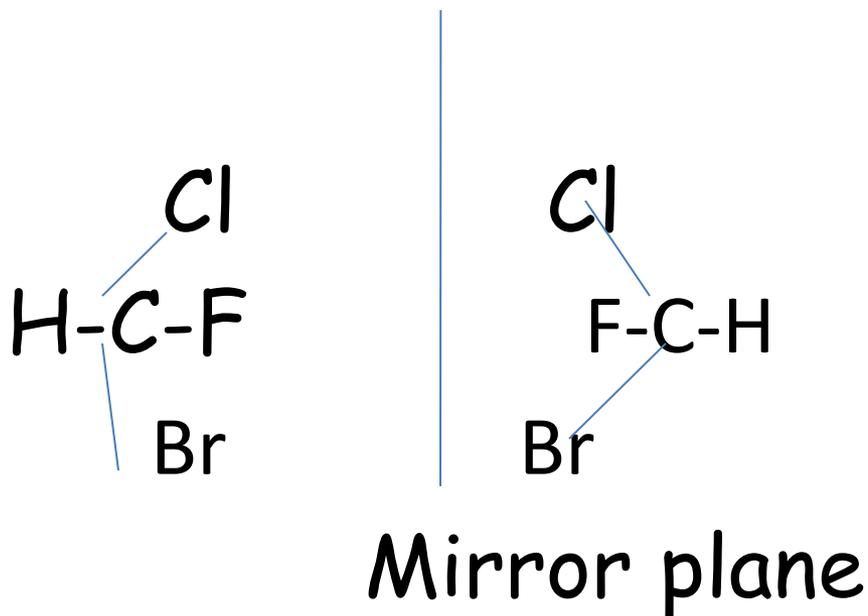
Acid-Base properties of aas

❑Acids- is any molecule that can release a proton and gain a negative charge.

❑Base-is a substance that can accept a proton and gain a positive charge.

❑Negatively charged molecules are called anions and positively charged molecules are called cations.

❑Amino acids have –COOH groups which can release protons, and they have –NH2 groups which can accept protons.

Cont……………

❑ Therefore, depending on the PH of the solution, aas can exist as weak acids or bases.

❑ Since proteins may have unequally balanced numbers of –COOH and –NH2, groups, changes in PH of the solution in which they are dissolved will cause changes in the ratio of charged acidic and basic groups.

❑ For different aas, the **PH at which the COOH and NH2** groups exactly balance each other to create **no net charge** on the molecule is called the **isoelectric PH** of the molecule.

Cont..........

❑In chemical terms, any molecule that has both negatively and positively charged groups is called Zwitterion.

The Henderson-Hasselbalch equation

➢COOH ⟷ COO- + H+ 1

@ equilibrium,

K= [COO-][H+]/[COOH] 2

➢NH3+ ⟷ NH2 + H+..................3

@ equilibrium,

K= [NH2][H+]/ NH3+ 4

CONT..........

❑Using eqn 1 we can derive onewhich enables us to predict the state of ionization of a given aas if we know K and the PH of the solution.

✓Rearranging and take the log of both sides

log K = log [H+] + log [COO-]/[COOH]........5

✓Convert to –log and rearrange

-log [H+] =-log [K] + log [COO-]/[COOH]....6

✓Express in terms of p(-log): the Henderson-Hasselbalch equation equation

pH= pK + log [COO-]/[COOH]…………………………7

CONT……

❑Equation (7) allows us to predict the degree of ionization of the –COOH group of drugs for absorption through biological membranes. Which are lipohpilic and allow only the unionized form of the drug to pass through easily.

❑How could one determines the **predominant** forms of amino acids in solution using pH and pK? (to be discussed in the class).

Cont…………..

❑ Example, consider the isolated L-aa alanine, pK1 for alanine is reported as 2.35 where as pK2 is reported as 9.87. what is the predominant structure of alanine under the pH: 1.0, 3.0 and 10.0 ?

❑ What about amino acids where the side chain also contains a weak acid functional group and has a pK_R ?

✓ Consider the aa lysine having three weak acid functional groups (pK1=2.16, pK2=9.06, and pK_R =10.54). How many possible structures for Lysine are there? Draw the predominant str. For Lysine @ pH= 1.0,5.0,10.0,and 12.0.

TABLE 3-1 Properties and Conventions Associated with the Common Amino Acids Found in Proteins

Amino acid	Abbreviation/ symbol		M_r	pK_a values pK_1 (—COOH)	pK_2 (—NH_3^+)	pK_R (R group)	pI	Hydropathy index*	Occurrence in proteins (%)[†]
Nonpolar, aliphatic R groups									
Glycine	Gly	G	75	2.34	9.60		5.97	−0.4	7.2
Alanine	Ala	A	89	2.34	9.69		6.01	1.8	7.8
Proline	Pro	P	115	1.99	10.96		6.48	1.6	5.2
Valine	Val	V	117	2.32	9.62		5.97	4.2	6.6
Leucine	Leu	L	131	2.36	9.60		5.98	3.8	9.1
Isoleucine	Ile	I	131	2.36	9.68		6.02	4.5	5.3
Methionine	Met	M	149	2.28	9.21		5.74	1.9	2.3
Aromatic R groups									
Phenylalanine	Phe	F	165	1.83	9.13		5.48	2.8	3.9
Tyrosine	Tyr	Y	181	2.20	9.11	10.07	5.66	−1.3	3.2
Tryptophan	Trp	W	204	2.38	9.39		5.89	−0.9	1.4
Polar, uncharged R groups									
Serine	Ser	S	105	2.21	9.15		5.68	−0.8	6.8
Threonine	Thr	T	119	2.11	9.62		5.87	−0.7	5.9
Cysteine	Cys	C	121	1.96	10.28	8.18	5.07	2.5	1.9
Asparagine	Asn	N	132	2.02	8.80		5.41	−3.5	4.3
Glutamine	Gln	Q	146	2.17	9.13		5.65	−3.5	4.2
Positively charged R groups									
Lysine	Lys	K	146	2.18	8.95	10.53	9.74	−3.9	5.9
Histidine	His	H	155	1.82	9.17	6.00	7.59	−3.2	2.3
Arginine	Arg	R	174	2.17	9.04	12.48	10.76	−4.5	5.1
Negatively charged R groups									
Aspartate	Asp	D	133	1.88	9.60	3.65	2.77	−3.5	5.3
Glutamate	Glu	E	147	2.19	9.67	4.25	3.22	−3.5	6.3

Titration curves of Amino acids

❑When an amino acid is dissolved in water it exists predominantly in the isoelectric form.

❑Upon titration with acid, it acts as a base, and upon titration with base, it acts as an acid(a compound that can act as either an acid or a base is known as an amphoteric compound).

❑Example, when glycine titrated with hydrochloric acid, it acts as a base and with sodium hydroxide it acts as acid.

➢ $^+H_3N\text{-}CH_2\text{-}COO^- + HCl \rightarrow {}^+H_3N\text{-}CH_2\text{-}COOH + Cl^-$

      (base)   (acid)
  (1)

➢ $^+H_3N\text{-}CH_2\text{-}COO^- + NaOH \rightarrow H_2N\text{-}CH_2\text{-}COO^- + Na^+ + H_2O$

            (acid)   (base)
  (2)
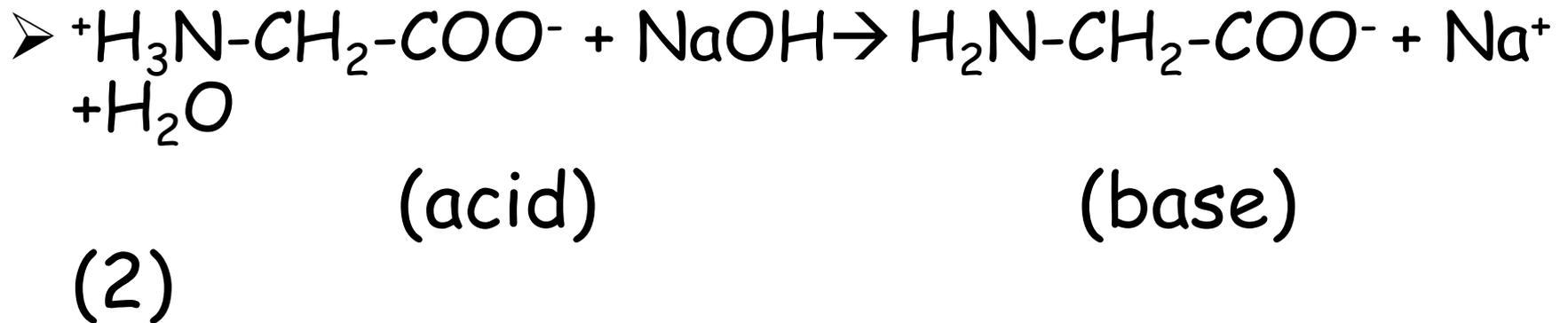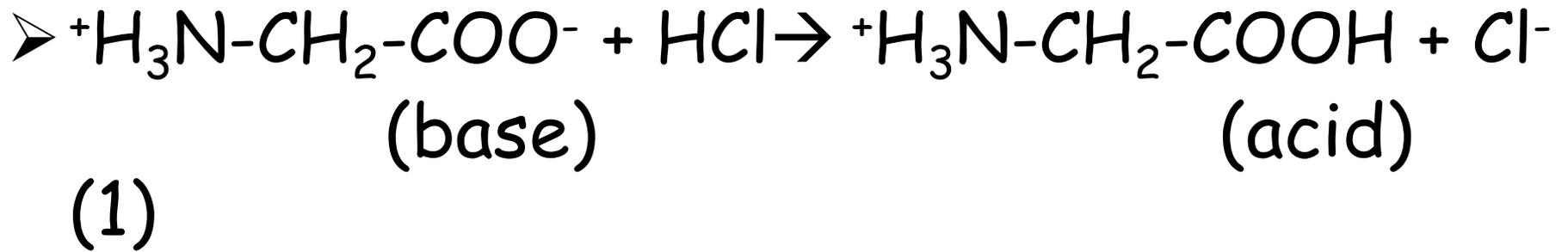
➢ In this experiment, the amino acid represents either the A^- or the HA form in the Henderson-Hasselbalch equation, depending on the titration.

General titration curve of aas
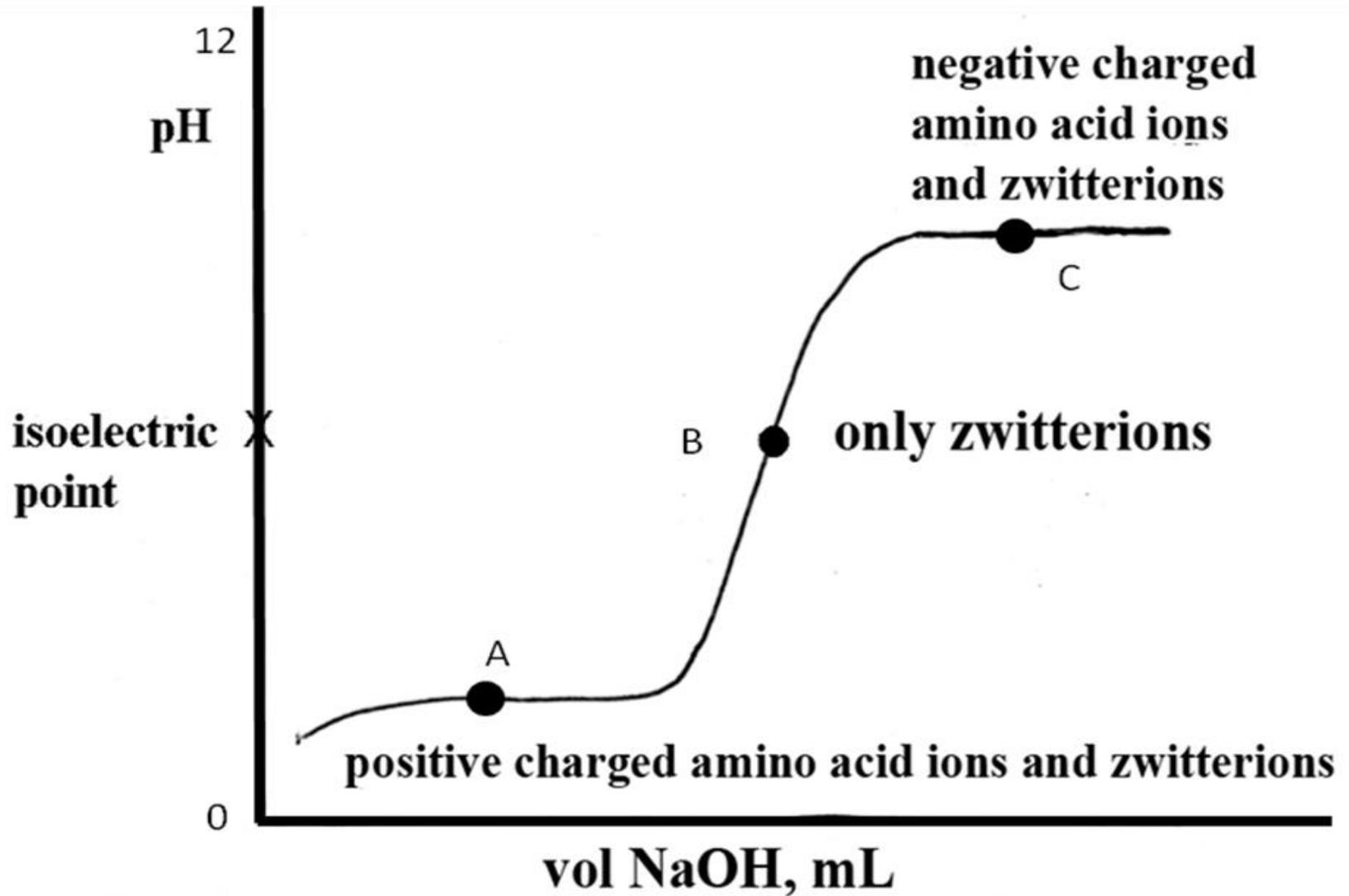
➢ All amino acids contain ionizable groups that act as weak acids or bases, giving off or taking on protons when the pH is altered.

➢ These ionizations follow the Henderson-Hasselbalch equation:

pH=pKa+log [unprotonated form(base)]
                     [protonated form (acid)]

The isoelectric point (pI)

➢ The isoelectric point, **pI**, is the pH of an aqueous solution of an amino acid at which the molecules have no net charge. In other words, the positively charged groups are exactly balanced by the negatively charged groups.

➢ For simple amino acids such as alanine, the pI is an average of the pK_a's of the carboxyl and ammonium groups. If additional acidic or basic groups are present as side-chain functions, the pI is the average of the pK_a's of the two most similar acids.

Glycine

$pK_2 = 9.60$

$pI = 5.97$

$pK_1 = 2.34$

pH

OH$^-$ (equivalents)

Lys²⁺   Lys⁺   Lys⁰   Lys⁻
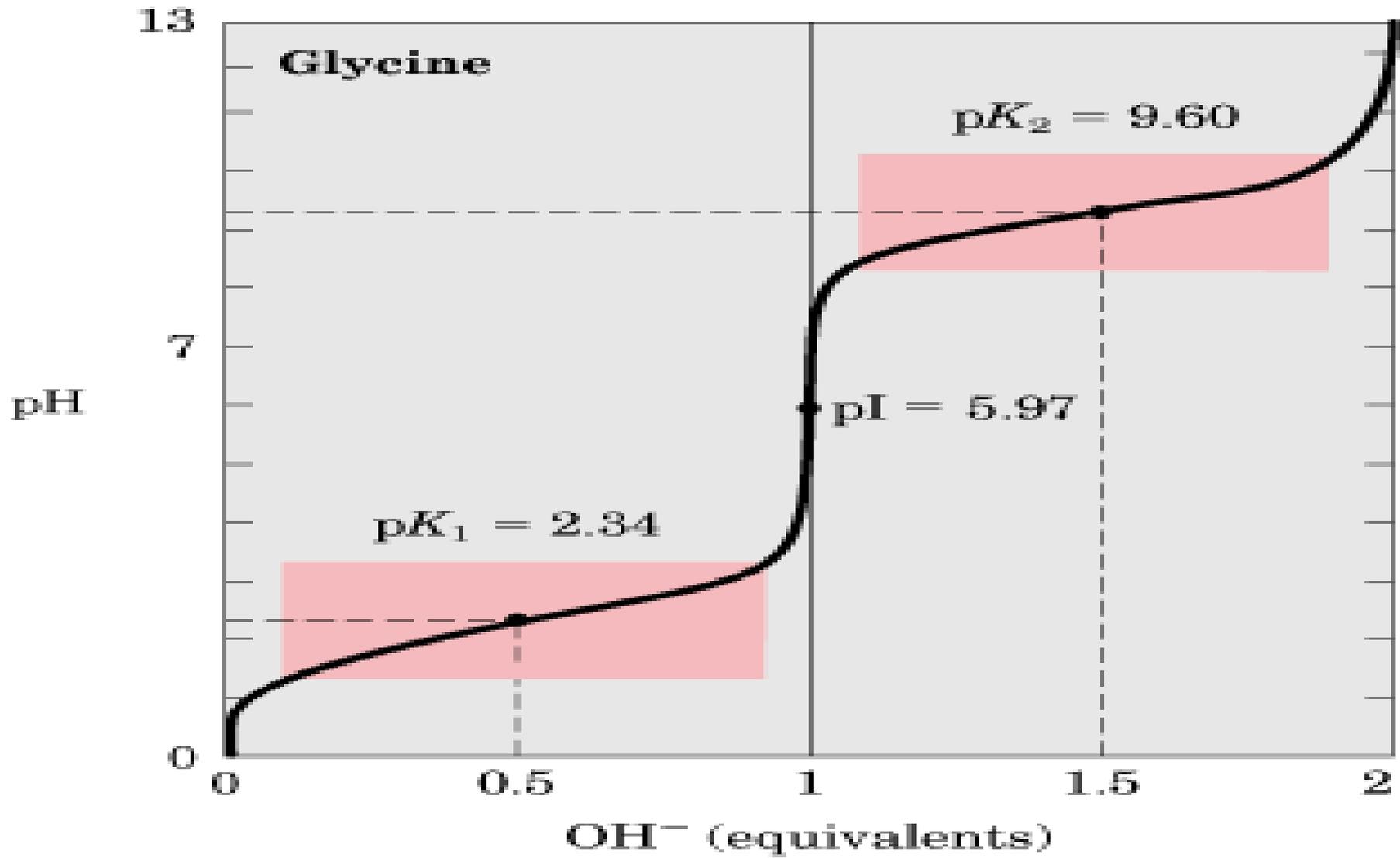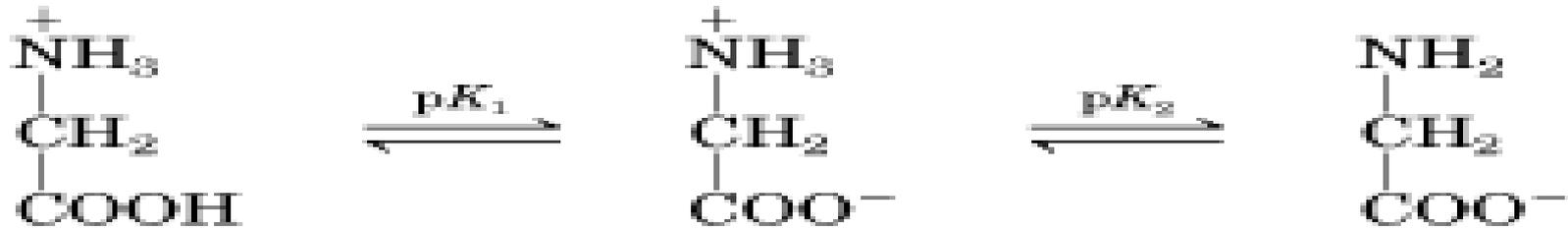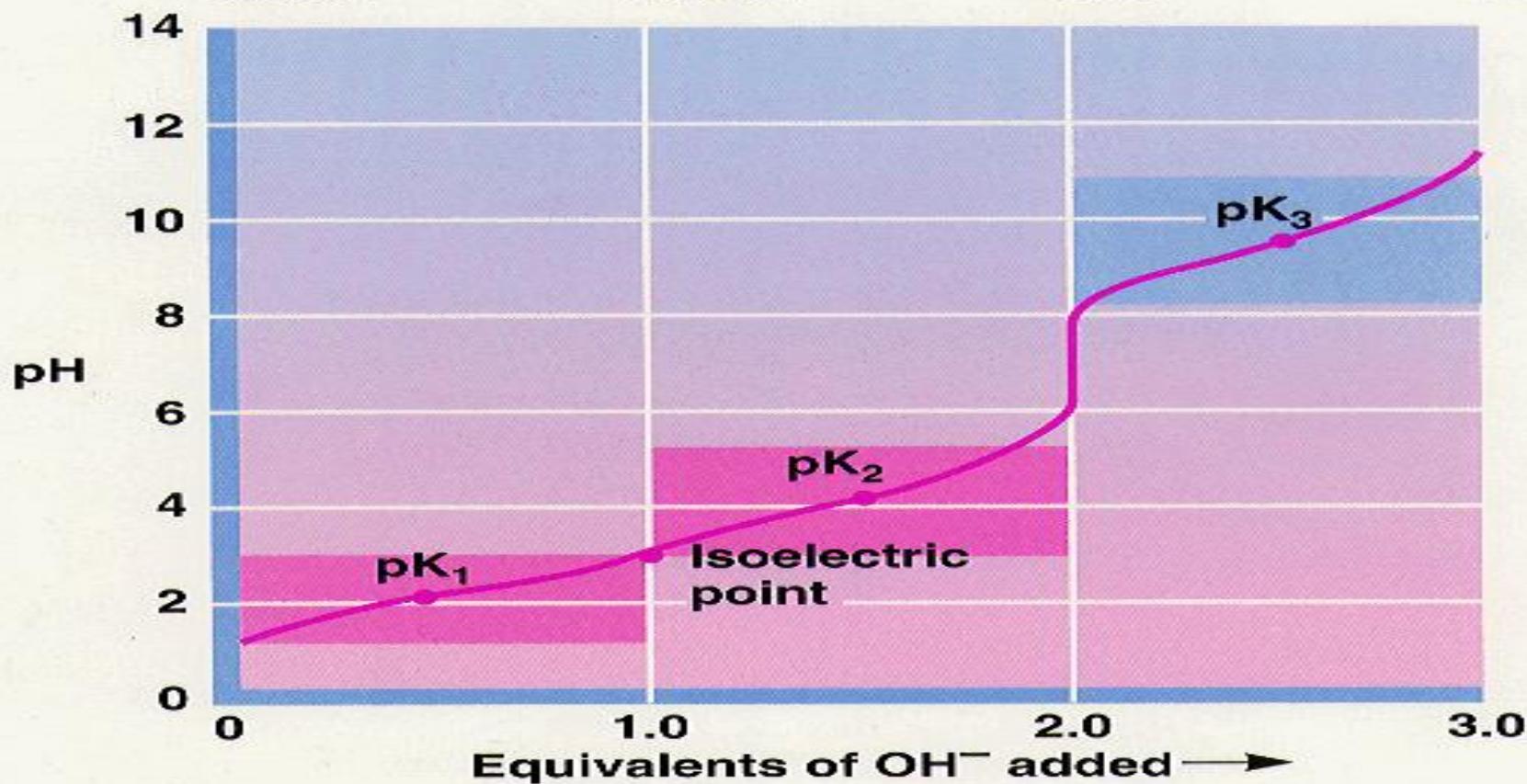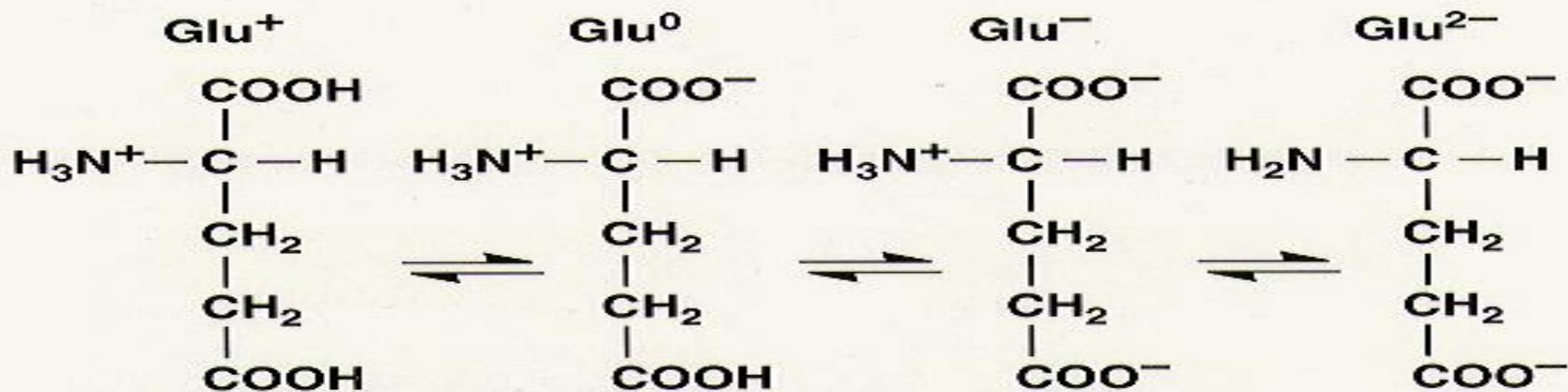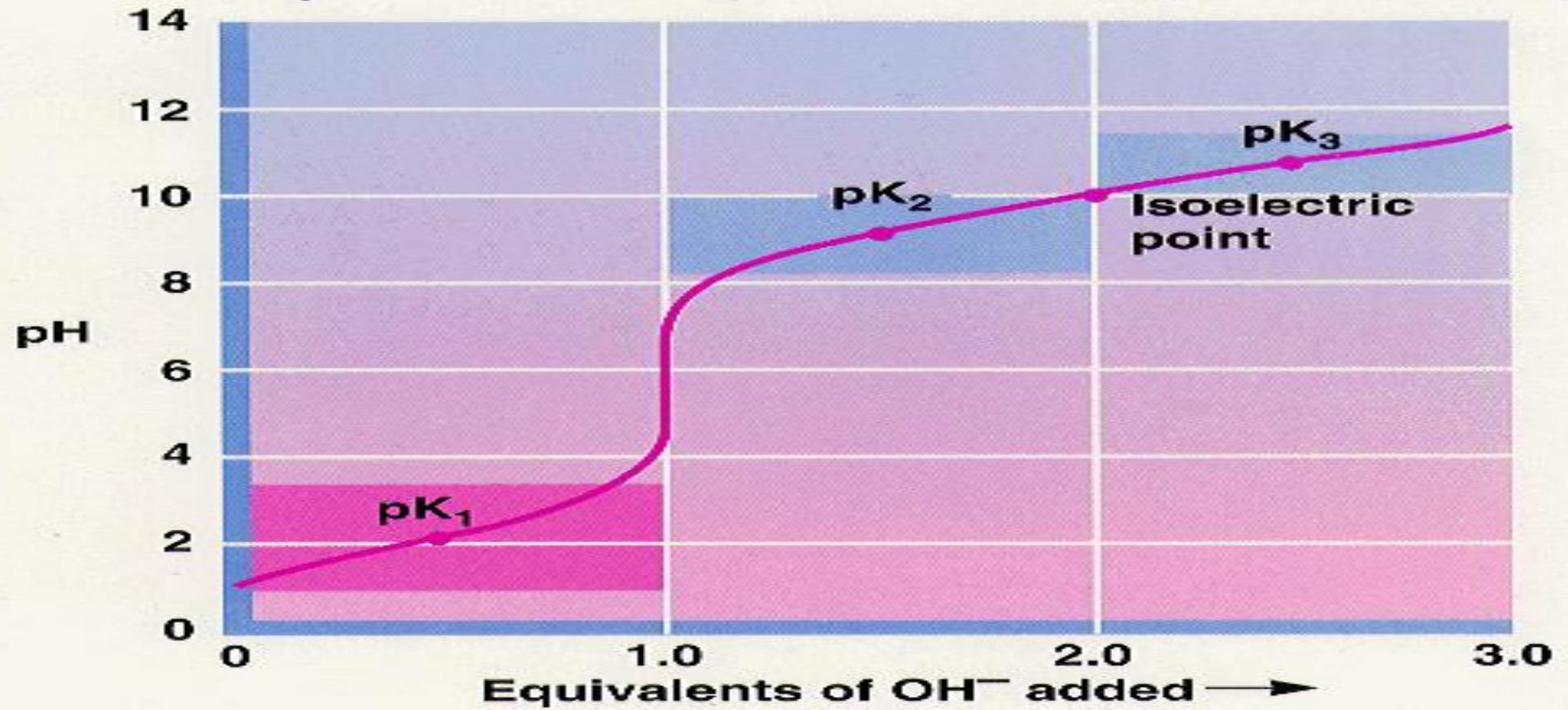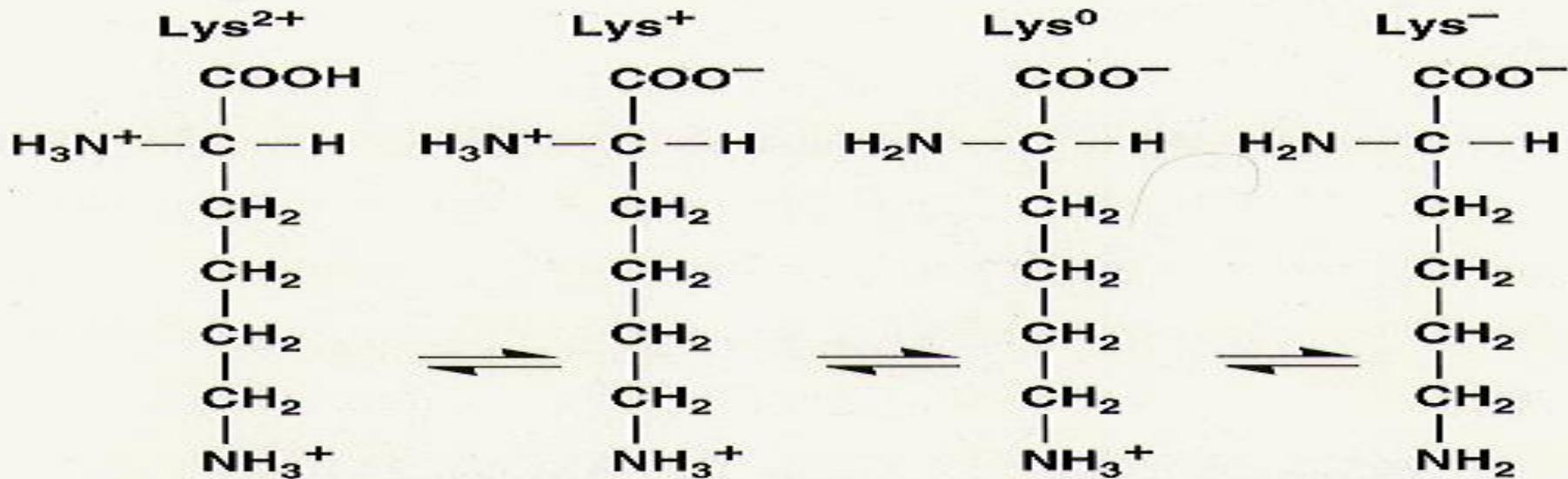
- Example1 A compound has a pka= 7.4. To 100ml of a 1.0 M solution of this compound at pH=8.0 is added 30 ml of 1.0 M HCl. The resulting pH= ?

- Example 2 What percentage of the tyrosine phenol is unprotonated @pH 11 & K_R =3.47*^-11.

Questions

✓ Suppose an aa (HA) has one ionizable group, and the p*Ka of this group is 5.0. If one* has 100 mL of a 0.1 M solution of this compound at an initial pH = 7.0, what is the final pH after 20 mL of 0.1 M HCl are added?

✓ What % age of the histidine imidazole group is protonated @ pH 7.2 ? Pk=6.3

✓ What is the pH of a 0.1 M solution of cacodylic acid (pK= 6.27) ?

Rare and Unusual Amino acids

➤ In addition to the standard aas, other aas are found in protein in smaller quantities.

➤ Some of these amino acids are:

- 4-hydroxyproline-in plant cell wall proteins
- 5-hydroxylysine-collagen
- D-glutamic acid-in cell walls of many bact.
- D-serine-in earthworms
- Γ-Aminobutyric acid (GABA)-neurotrasmiters in brain
- B-alanine-constituents of vit. Pantothenic acid
- Γ-carboxyglutamate-founnd in prothrombin

Cont……
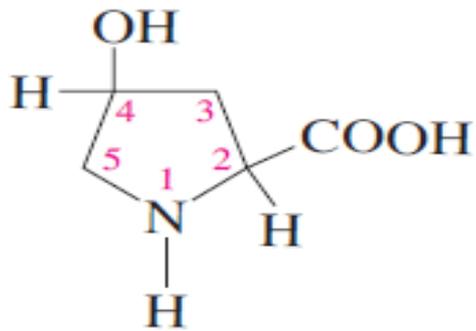
4-hydroxyproline
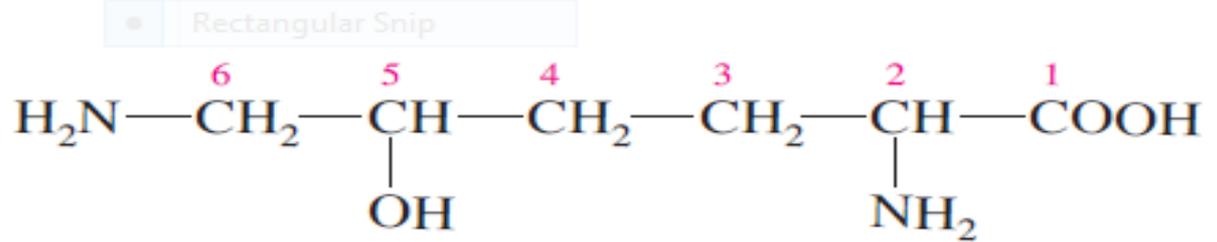
5-hydroxylysine
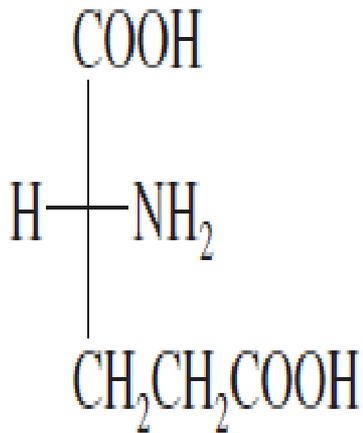
D-glutamic acid
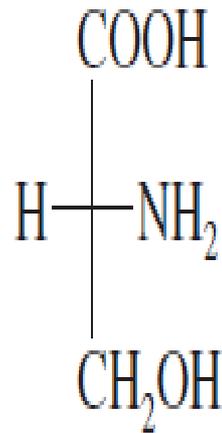
D-serine
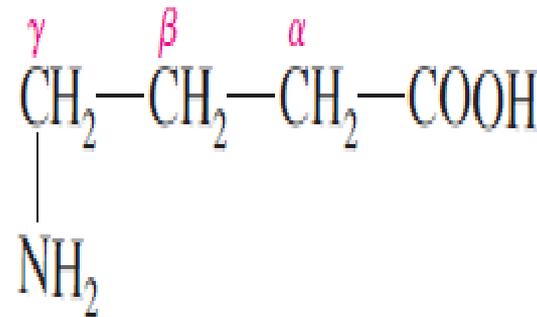
γ-aminobutyric acid

β-alanine

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Cont…….

$$CH_3 \text{—} NH \text{—} CH_2 \text{—} CH_2 \text{—} CH_2 \text{—} CH_2 \text{—} CH \text{—} COO^-$$

with $^+NH_3$ on the CH

6-N-Methyllysine

Found in myosin

$$^-OOC \text{—} CH \text{—} CH_2 \text{—} CH \text{—} COO^-$$

with COO^- above CH and $^+NH_3$ below the second CH
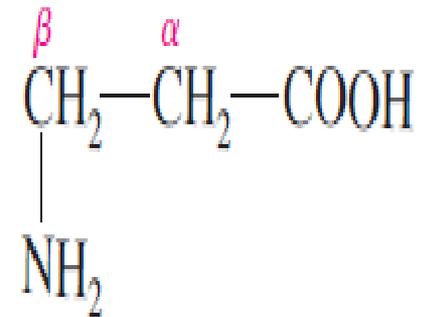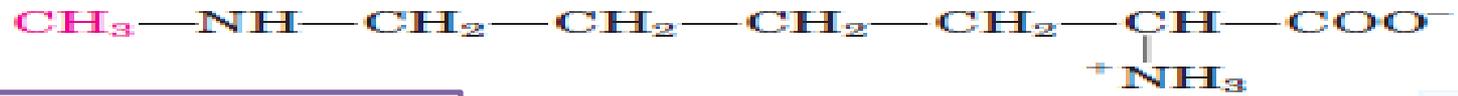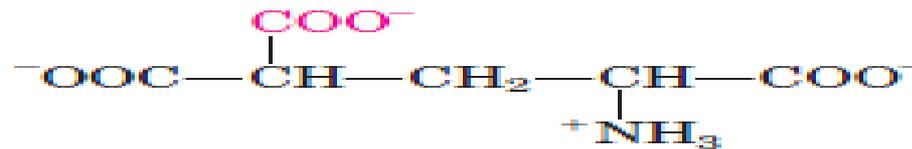
γ-Carboxyglutamate

Desmosine
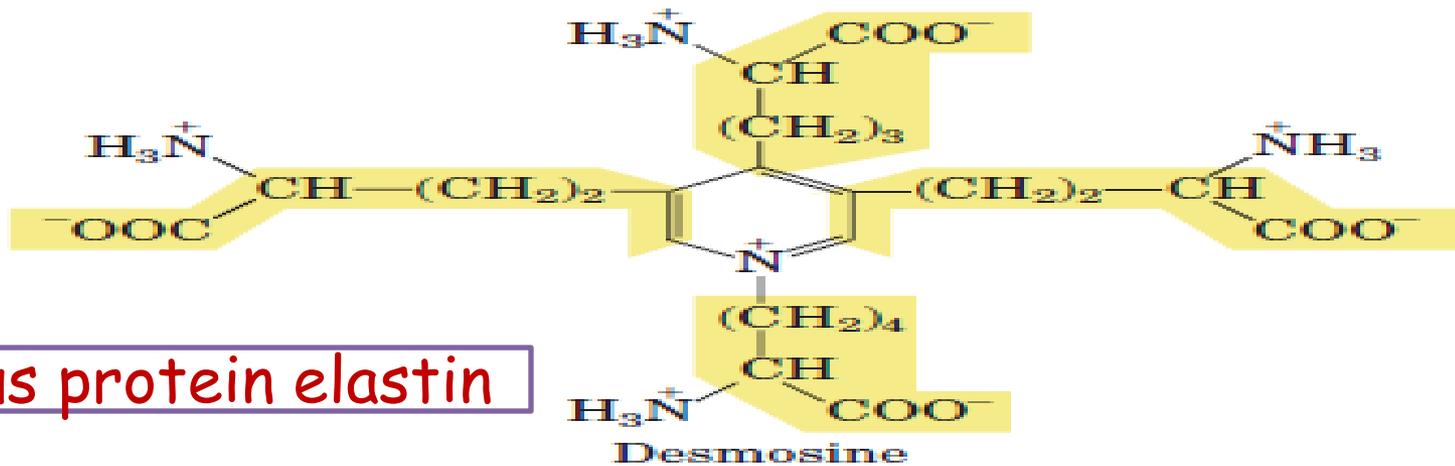
Fibrous protein elastin

$$HSe \text{—} CH_2 \text{—} CH \text{—} COO^-$$

with $^+NH_3$ on the CH
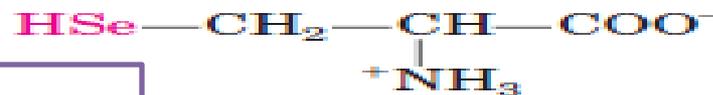
Selenocysteine

Constituent of known proteins

Overview of protein structure

❑ The spatial arrangement of atoms in a protein is called its conformation.

❑ The conformations existing under a given set of conditions are usually the ones that are thermodynamically the most stable, having the lowest Gibbs free energy (G). *Proteins in any of their functional, folded conformations are called **native** proteins.*

❑ In the context of protein structure, the term **stability** can be defined as the tendency to maintain a native conformation.

Level of protein structures

There are four levels of organization in protein structure:

(a) **Primary structure**, which is the amino acid sequence.

(b) **Secondary structure**, which is the local three-dimensional structure of a polypeptide without regard to the conformations of its side chains.

(c) **Tertiary structure**, which refers to the overall three-dimensional structure of an entire polypeptide.

(d) **Quaternary structure**, which refers to the three-dimensional arrangement of polypeptides in a protein composed of multiple polypeptides.

Str. Of protein

Primary structure — Amino acid residues

Secondary structure — α Helix

Tertiary structure — Polypeptide chain

Quaternary structure — Assembled subunits

Lys
Lys
Gly
Gly
Leu
Val
Ala
His

Cot...

Ile → Gln

Tyr   Asn

Cys—S—S—Cys → Pro → Leu → Gly · NH_2

primary structure

N—H⋯O=C ... N—H⋯

O=C   CH

         R   CH   N—H⋯O=C

   R   CH   N—H⋯O=C   CH

         N—H⋯O=C   R
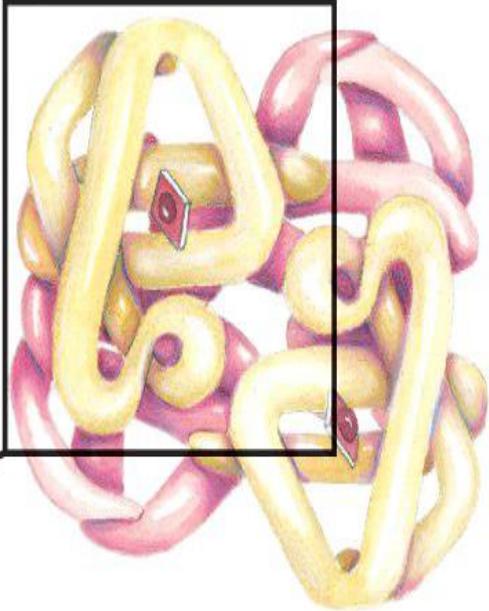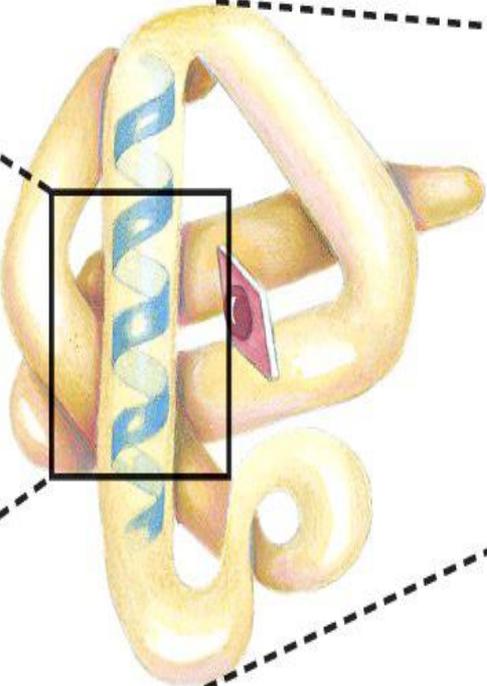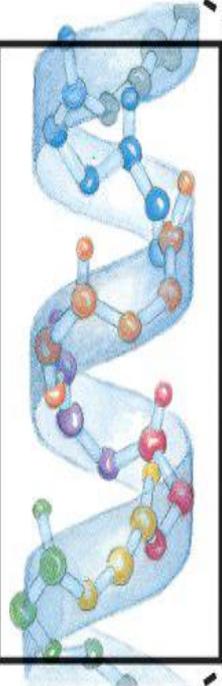
secondary structure
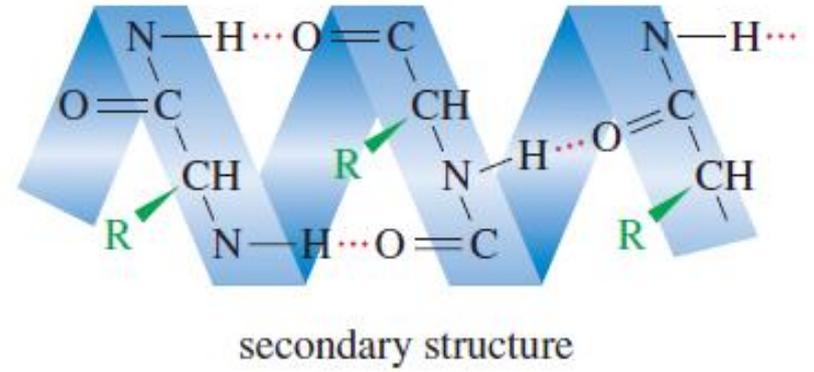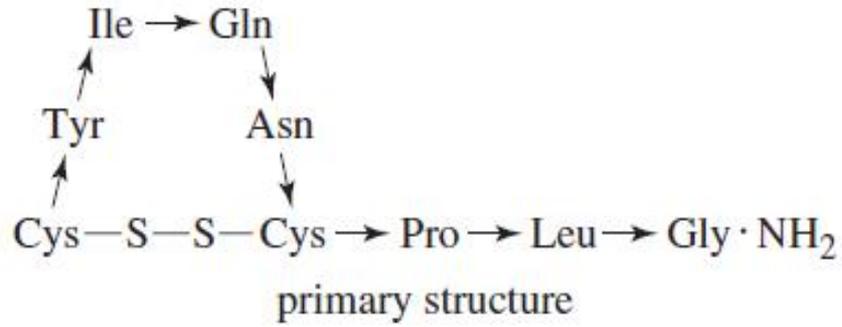
tertiary structure
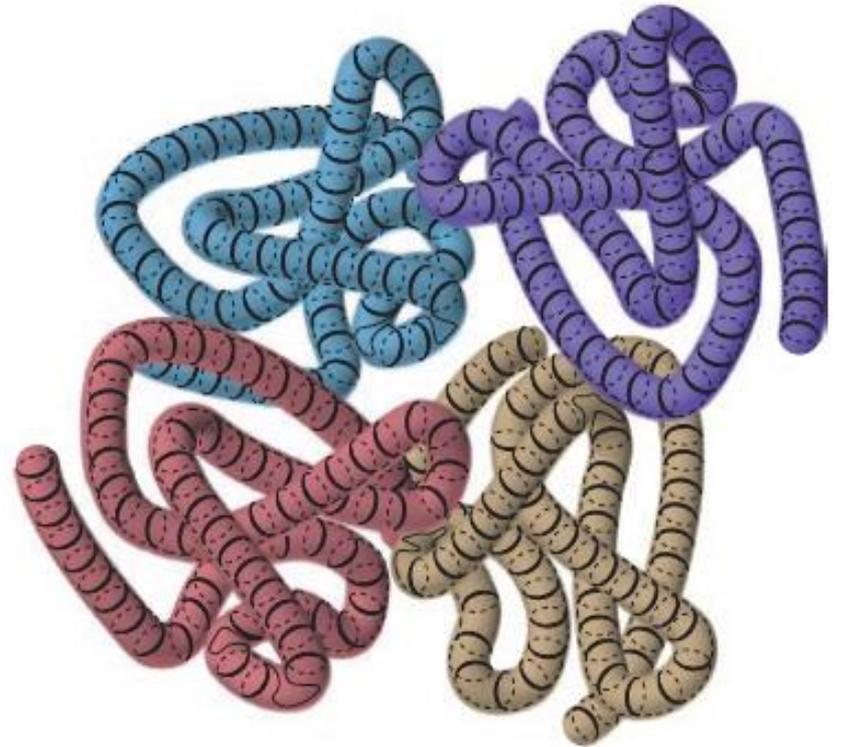
quaternary structure

Cont…

❑For a protein to be biologically active, it must have the correct structure at all levels.

❑The sequence of amino acids must be right, with the correct disulfide bridges linking the cysteines on the chains. The secondary and tertiary structures are important, as well.

❑The protein must be folded into its natural conformation, with the appropriate areas of α-helix and pleated sheet. For an enzyme, the active site must have the right conformation, with the necessary side-chain functional groups in the correct positions.

Cont….

❑Small changes in the environment can cause a chemical or conformational change resulting in **denaturation**: disruption of the normal structure and loss of biological activity. Many factors can cause denaturation, but the most common ones are heat and pH.

❑Denaturation could be irreversible or reversible.

Protein Folding and Protein Dynamics

❑Protein folding : is the physical process by which a protein chain acquires its native **3-D strs**., a conformation that is usually biologically functional, in an expeditious and reproducible manner. It's taken fastelly within microsecond.

❑The aa sequence of a protein determines its native conformation. A protein molecule folds spontaneously during or after biosynthesis, regarded as " folding themselves."

❑Factors for protein folding are the following:

Cont………

➢ Solvent (water or lipid bilayer)

➢ Concentration of salts

➢ pH

➢ Temperature

➢ The possible presence of cofactors.

❑ Protein dynamics

✓ Proteins have well defined 3D structure

✓ Structural hierarchy : from primary – tertiary.

✓ Proteins are not static

✓ The folding – unfolding reaction is a dynamic equilibrium.

Protein Functions

❑A fundamental question in biochemistry is how protein structure influences the functional properties of proteins.

❑Based on this question, best studied strs. **And functions of some proteins in biochemistry are:**

✓ Myoglobin and hemoglobin

-Are globular proteins involved in oxygen storage and transport.

✓Actin and myosin and antibodies.

Examples of protein functions

Class of protein	Example	Function of example
❏Structural proteins	✓Collagen,keratin	✓Strengthen tendons, skin,hair, nails
❏Enzymes	✓DNA polymerase	✓Replicates & repairs DNA
❏Trnsport proteins	✓Hemoglobin	✓Trnsports o2nto the cells
❏Contractile proteins	✓Actin, myosin	✓Cause contraction of muscles
❏Protective proteins	✓Antibodies	✓Cplx with foreign proteins
❏Hormones	✓Insulin	✓Regulates glucose metabolism
❏Toxins	✓Snake venoms	✓Incapacitate prey

Proteins have an amazing range of structural and catalytic properties as a result of their varying amino acid composition. Because of this versatility, proteins serve an astonishing variety of functions in living organisms.

Oxygen Binding on Myoglobin and Haemoglobin

❑ The functions of many proteins involve the reversible binding of other molecules. A molecule bound reversibly by a protein is called a ligand. A ligand may be any kind of molecule, including another protein.

❑ A ligand binds at a site on the protein called the **binding site, which is complementary to the** ligand in size, shape, charge, and hydrophobic or hydrophilic character.

❑ Prosthetic group-is a cpd permanently associated with a protein that contributes to the protein's function

Cont........

❑ The function of myoglobin depends on the protein's ability not only to bind oxygen but also to release it when and where it is needed.

❑ In general, the reversible binding of a protein (P) to a ligand (L) can be described by a simple **equilibrium expression:**

✓ **P +L $\longleftrightarrow$ PL** 1

✓ Ka =[PL]/[P][L] 2

Where Ka is an association constant and is a measure of the affinity of the ligand, L for the protein. Ka has a unit of 1/M.

Cont.......

✓ Ka[L] =[PL]/[P] ……………………….3

-Here the ratio of [PL] to [P] is directly proportional to free ligands, L.

-[L]>>[ligand-binding sites], the binding of the L by the protein does not appreciably change the con. of free (unbound) ligand, i.e., [L] remains constant.

-This condition is broadly applicable to most ligands that bind to **proteins in cells** and simplifies our description of the binding equilibrium.

Cont…

-consider the **binding equilibrium** from the standpoint of the fraction, *θ (theta), of ligand binding* sites on the protein that are occupied by ligand:

✓ θ= Binding site occupied/Total binding sites

$$= [PL]/[PL]+[P]………………..4$$

Substituting Ka[L][P] for [PL] and rearranging

✓ θ= Ka[L][P]/Ka[L][P]+[P] = Ka[L]/Ka[L]+1

✓ = [L]/([L]+ 1/Ka) ……………....5

-Ka is found by plotting θ vs. [L], is a hyperbolic function since any equation in the form of x=y/(y+z) is a hyperbolic function.

Cont……….

❑Now consider the equilibrium constant for the release of ligand   the so called dissociation constant, Kd, which the reciprocal of **Ka (Kd= 1/Ka**) and its unit is molar con. (M).

❑Thus, eq(1) is expressed as

✓ Kd = [P][L]/[PL]……………..……….6

✓[PL] =[P][L]/Kd …………………….7

✓θ= [L]/([L]+Kd) ……………………....8

-when [L]=Kd, **½** of the ligand-binding sites are occupied. As [L] falls below Kd, progressively less of the protein has ligand bound to it. Kd lower, implies a higher affinity of ligand for the protein.

Cont………

❑How ever, since oxygen is a gas and to carry out the exp't conveniently, [L] replaced by [O2], thus eqn(8) slightly changed as

✓θ= [O2]/([O2] +Kd)…………………………….9

❑The con.of O2 is easily measured if it is expressed interms of **partial pressure**, pO2 and when Kd =[O2],1/2 of available ligand –bind sites are occupied, $[O_2]_{0.5} = p_{50}$

✓θ= pO2/(pO2 + p_{50})   …………………….10

Some protein dissociation constants

Protein	Ligand	Kd (M)
Avidin (egg white)	Biotin	1×10^{-15}
Insulin receptor(human)	Insulin	1×10^{-10}
Anti-HIV immunoglobulin (human)	Gp41(HIV-1 surface protein)	4×10^{-10}
Nickel-binding protein (E.coli)	Ni^{2+}	1×10^{-7}
Calmodulin (rat)	Ca^{2+}	3×10^{-6}

Oxygen binding on haemoglobin

❑Nearly all the oxygen carried by whole blood in animals is bound and transported by hemoglobin in erythrocytes (red blood cells). Normal human erythrocytes are small (6 to 9 μm in diameter), biconcave disks.

❑Hemoglobin must bind oxygen efficiently in the lungs, where the pO2 is about **13.3 kPa**, and release oxygen in the tissues, where the pO2 is about 4 kPa.

❑Cooperative binding of oxygen by hemoglobin was first analyzed by Archibald Hill in 1910. From this work came a general approach to the study of cooperative ligand binding to multisubunit proteins.

Cont…….

❑ For a protein with n binding sites, the equilibrium of the reaction:

✓ $P + nL \rightleftharpoons PL_n$ ……………………………..1

✓ $K_a = [PL_n]/[P][L]^n$………………………….2

-The expression for θ is

✓ $\theta = [L]^n/([L]^n + K_d)$ …………………….……3

-Rearranging, then taking the log of both sides, yields

✓ $\theta/(1-\theta) = [L]^n/K_d$…………………………..4

✓ $\log(\theta/(1-\theta)) = n\log[L] - \log K_d$……………….....5

Where $K_d = [L]^n_{0.5}$

Cont….

❑ Equation 5 is the **Hill equation, and a plot of** log $[\theta/(1-\theta)]$ versus log [L] is called a **Hill plot. Based** on the equation, the Hill plot should have a slope of *n*. However, the experimentally determined slope actually reflects not the number of binding sites but the degree of interaction between them.

❑ The slope of a Hill plot is therefore denoted by n_H, *the **Hill coefficient, which*** is a measure of the degree of cooperativity.

❑ If $n_H = 1$ ligand bindinng is not cooperative

$n_H > 1$ +ve cooperative ligand binding

$n_H < 1$ -ve cooperative

$n_H = n$ the binding would be completely cooperative.

Cont…

❑ To adapt the Hill equation to the binding of oxygen to hemoglobin we must again substitute pO2 for [L] and *P^n$_{50}$ for Kd*

✓ **$\log(\theta/(1-\theta)) = n \log pO2 - n \log P^n{}_{50}$ ……6**

Example

1. Protein A has a binding site for ligand X with a Kd of 10^-6 M. Protein B has a binding site for ligand X with a Kd of 10^-9 M. Which protein has a higher affinity for ligand X? Explain your reasoning. Convert the Kd to Ka for both proteins?

2. Examine the plot generated when *Kd=10 μM. How much does increase when [L] increases from 0.2 μ M to 0.4 μM? How much does increase when [L] increases from 40 μ M to 80 μ M?*

Quiz 1(10%)

1. Suppose an α-aa has Pk1=2.4, pK2=8.25 & pKR=9.5 with a pH of 1.5 ,3.5, 8.5 & 9.8

a. Calculate pI

b. How many possible structures can you draw?

c. Determine the predominant groups in the solution and draw it.

2. Protein X and Y has a binding site for ligand Z with dissociation constant

✓ Kdx= 10^-2 M & Kdy= 10^-4 M.

a. Which protein has a higher affinity for ligand Z?

b. Convert the Kd to Ka for both proteins?

1.3.Nucleotides Nucleic acid

➢ **Nucleotides are one of the 4 major biological molecules.**

➢ **They are the most functionally diverse and involved in :**

  - energy transfer

  - catalysis

  - signals within and between the cells

  - stores, decodes and transmission of genetic   information

➢ Nucleotides are composed of nitrogen bases, 5-C sugars and phosphate groups.

Cont..........

➢ Nucleotides are the monomeric units of RNA and DNA.

    * RNA- Ribonucleic Acid

        - composed of

            + Adenine, Guanine, Cytosine and Uracil , 5-C sugar called pentose and phosphate group

      - Pairing: A-U & G-C

    *DNA- Deoxyribonucleic Acid

        - composed of

           +Adenine, Guanine, Cytosine and Thymine, 5-C sugar called deoxyribose and phosphate group

Cont………

- pairing : A-T & G-C

➤ The best known nucleotide the energy transmitter called ATP-adenosine triphosphate, synthesised from ADP-adenosine diphosphate.

$$ATP \xrightleftharpoons{\text{E-required}} ADP + Pi$$

Cont……….

❑Nucleic Acid Structure

➢Nucleic acids are polymers of nucleotides in which phosphate groups link the 3′ and 5′ positions of neighboring ribose residues. This linkage is called a phosphodiester bond because the phosphate is esterified to the two ribose groups. The phosphates are acidic at biological pH and so the polynucleotide is a polyanion.

Cont…..

➤ Nucleic acids are inherently asymmetric, so that one end (with a 5' phosphate) is different form the other end (with a 3' hydroxyl). This asymmetry, or polarity, is critical for the information storage function of nucleic acids. In fact, all linear biological molecules show this kind of polarity.   5'   3'

                3'   5'
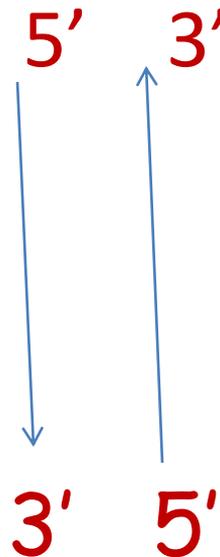
Cont...

➢ The structure of DNA was determined by Francis Crick and James Watson in 1953. Key information used to build their model included the following:

a. DNA has equal no of Adenine and thymine residues and equal no of Guanine and cytosine residues -----Chargaff's rules

b. As studied by X-ray diffraction DNA has double helical str. With a uniform width.

c. According to structural study indication the nitrogenous base assumed as keto tautomeric form.

Cont………

d. According to chemical evidence indicated that DNA str. Was linked by phosphodiester bonds between the 3' and the 5' carbon of adjoining ribose units.

❑ Features of Watson-Crick model

❖ The two polynucleotide chains wind around the a central axis and forms a right-handed helix

❖ The polynucleotide chains are antiparallel to each other.

❖ The N-bases are occupied the core of the double helix, while the sugar- phosphate chains are the backbones of DNA, running along the outside of the helical structures.

Cont……..

❖ **The pairing bases are linked by H- bond.**

➢ The complementary strands of DNA immediately suggest that each strand of DNA can act as a template for the synthesis of its complementary strand and so transmit hereditary information across generations.

➢ The DNA of an organism (its genome) is unique to each organism and, in general, increases in amount in rough proportion to the complexity of the organism. In eukaryotes, genomic DNA occurs in discrete pieces called chromosomes.

Cont.....

➢ While single-stranded DNA is uncommon in cells, RNA (ribonucleic acid) is usually single-stranded. However, by complementary base pairing, RNA can form intrastrand double helical sections, which bend and fold these molecules into unique three-dimensional shapes.

❑ Functions of Nucleic Acid

➢ The link between DNA and proteins is RNA. DNA replication, its transcription into RNA, and the translation of messenger RNA into a protein constitute the central dogma of molecular biology: **DNA makes RNA makes protein**.

Carbohydrates

Focal points

☐ Major classes of COH

☐ Glycoconjugates

Carbohydrates

- **Most abundant class of biological molecules on Earth**

- **Originally produced through CO_2 fixation during photosynthesis**

❑**Roles of COH**

- **Energy storage (glycogen,starch)**

- **Structural components (cellulose,chitin)**

- **Cellular recognition**

- **Carbohydrate derivatives include DNA, RNA, co-factors, glycoproteins, glycolipids**

Major Classes of COH

❑ There are three major classes of COH:

A. Monosaccharides

B. Oligosaccharides

C. Polysaccharides

❑ Saccharide-meaning sugar in Greek

A. Monosaccharides

✓ **Simple sugars**

✓ **6-C sugar D-glucose (dextrose) its most abundant in nature**

✓ **Have cyclic str. If C no more than 4**

✓ **Has two families, aldehydes and Ketones with two or more hydroxyl groups**

✓ **Monosaccharides are chiral**

✓ **The simplest monosaccharidesare**

-Glyceraldehyde =aldotriose

-Dihydroxyacetone =Ketotriose

D-ribose

D-ribulose
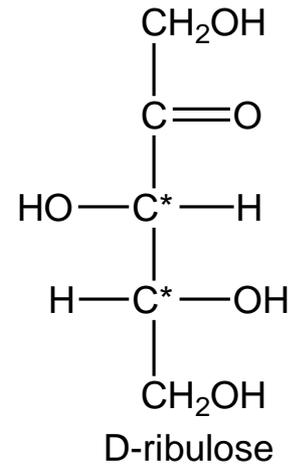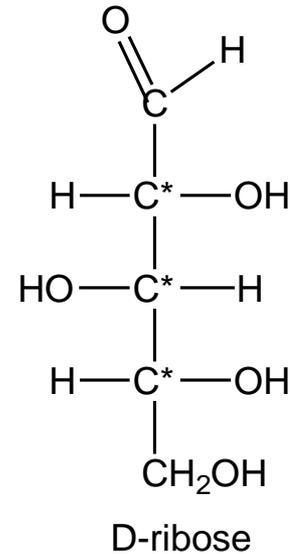
Cont………

- **Aldoses with 3C or more and ketoses with 4C or more are chiral**
- **The number of chiral carbons present in a ketose is always one less than the number found in the same length aldose**
- **Number of possible steroisomers = 2^n (n = the number of chiral carbons)**

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Figure 7.1

L-isomer   D-isomer

Glyceraldehyde

Dihydroxy-acetone

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D-glucose

D-fructose
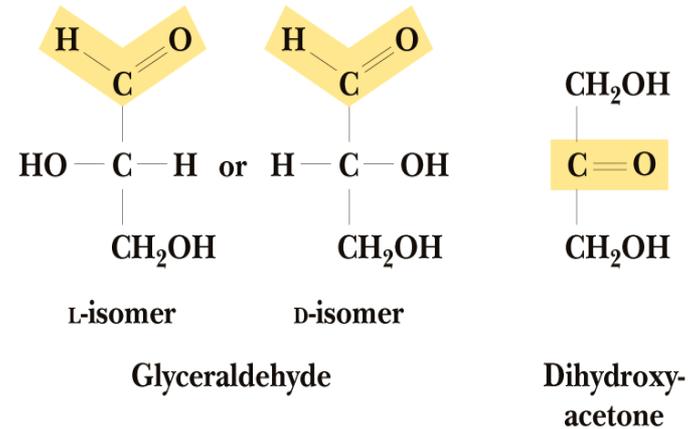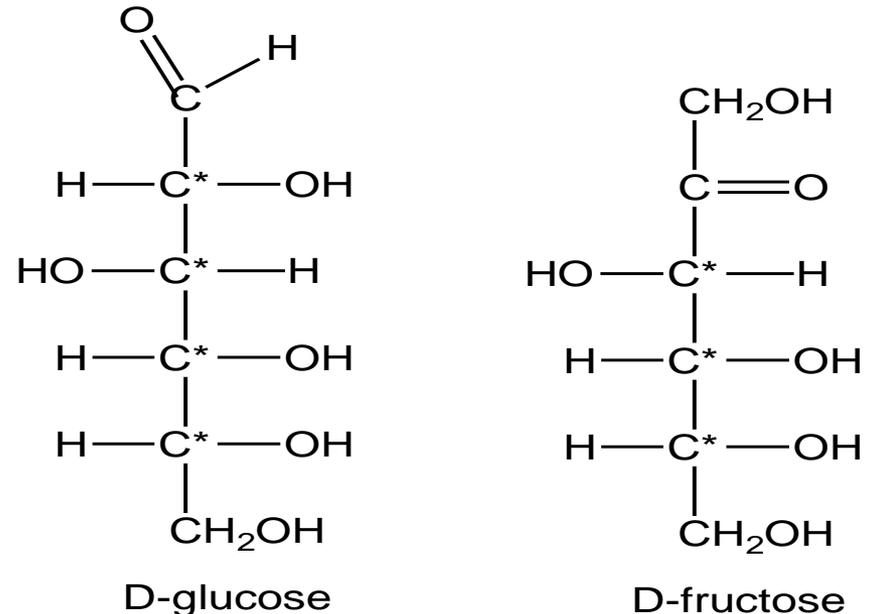
Stereochemistry

Enantiomers		Diastereomers		Epimers	
L-glucose	D-glucose	D-mannose	D-galactose	D-glucose	D-mannose
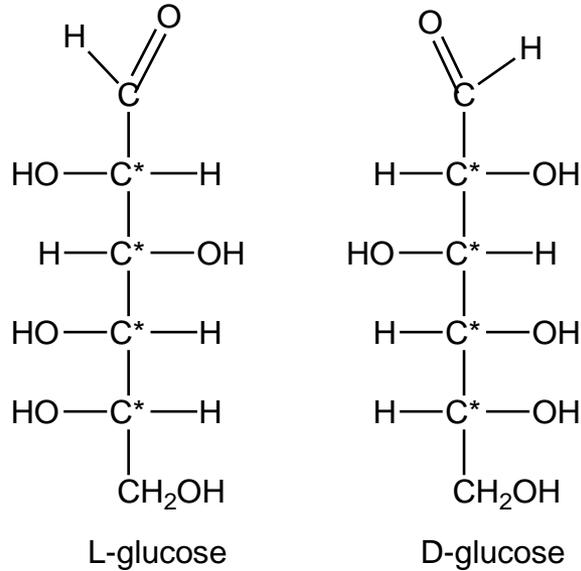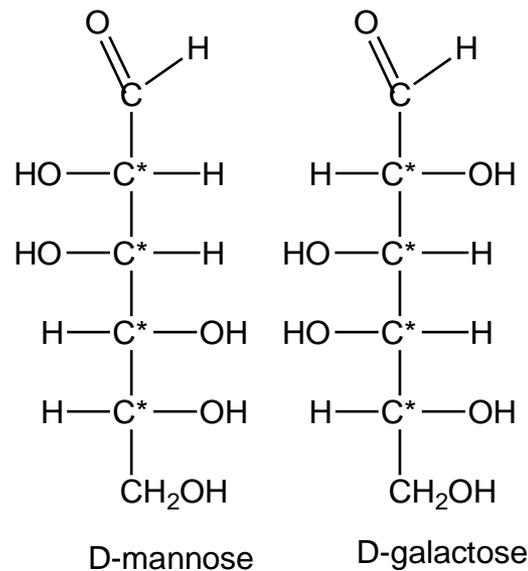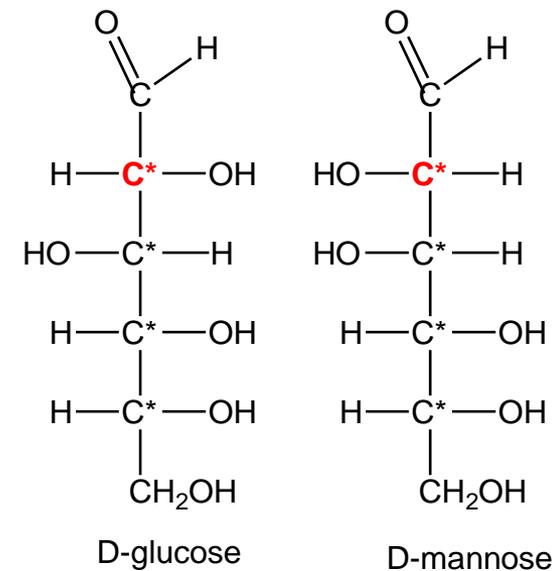
- **Enantiomers** = mirror images
- Pairs of isomers that have opposite configurations at one or more chiral centers but are NOT mirror images are **diastereomers**
- **Epimers** = Two sugars that differ in configuration at only one chiral center

Cyclization of aldose and ketoses introduces additional chiral center

- **Aldose sugars (glucose) can cyclize to form a cyclic hemiacetal**
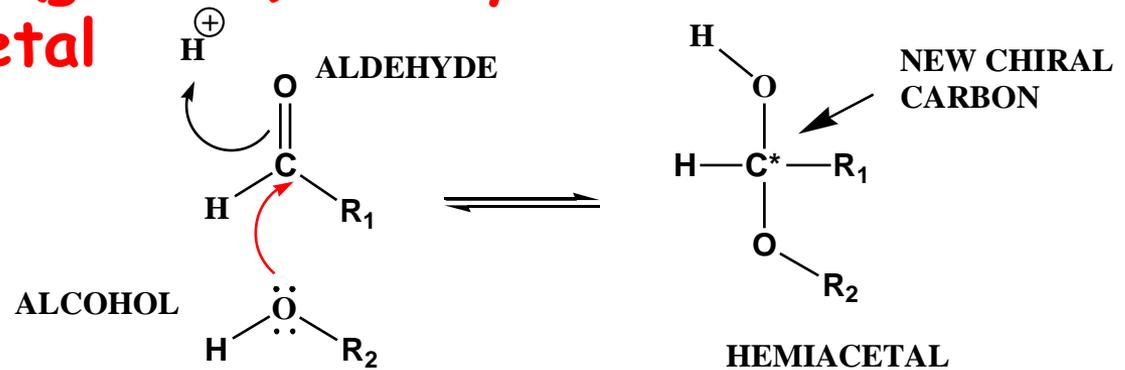
- **Ketose sugars (fructose) can cyclize to form a cyclic hemiketal**
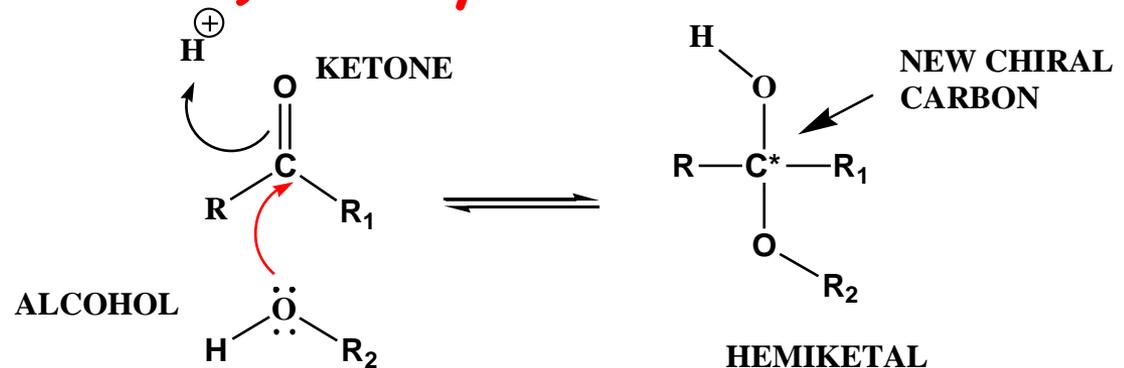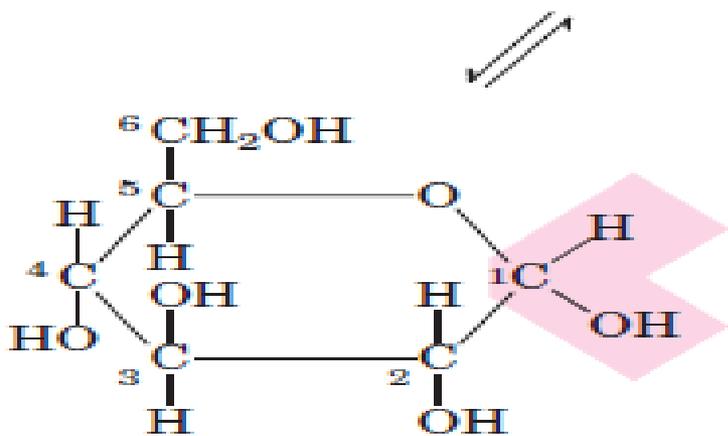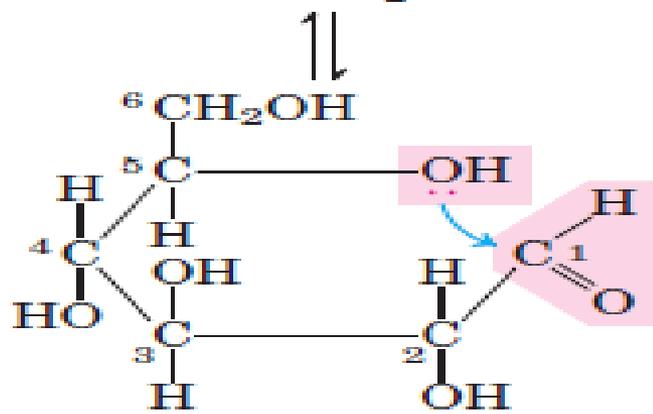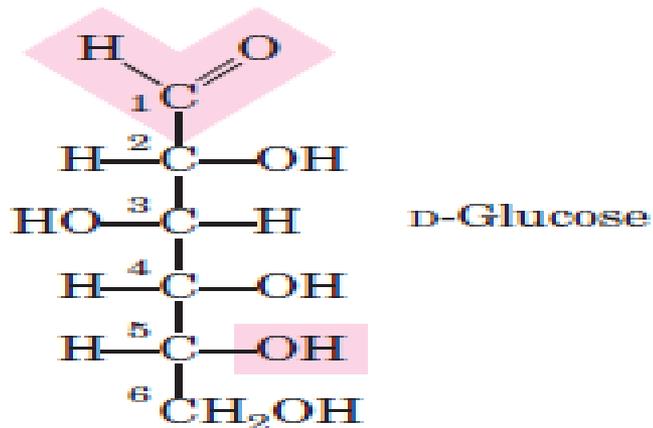
D-Glucose
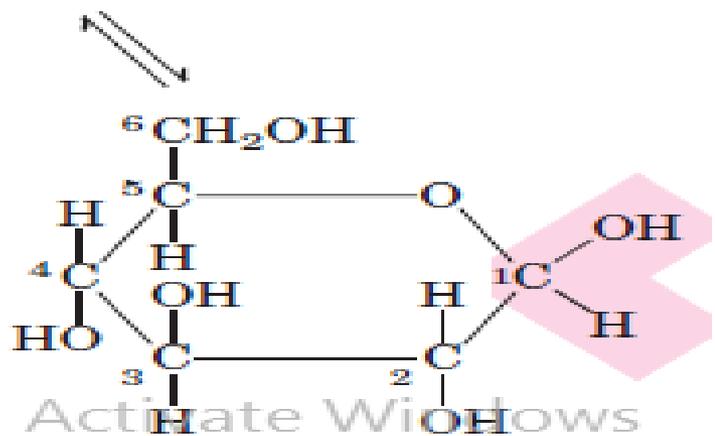
α-D-Glucopyranose
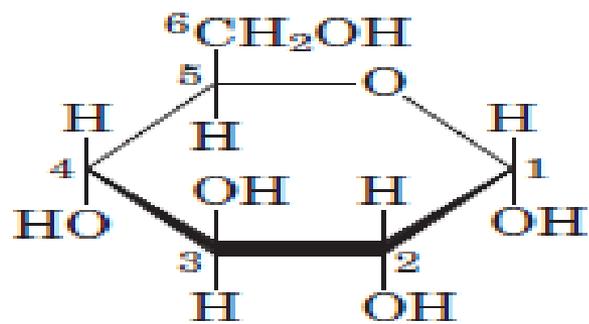
β-D-Glucopyranose

α-D-Glucopyranose
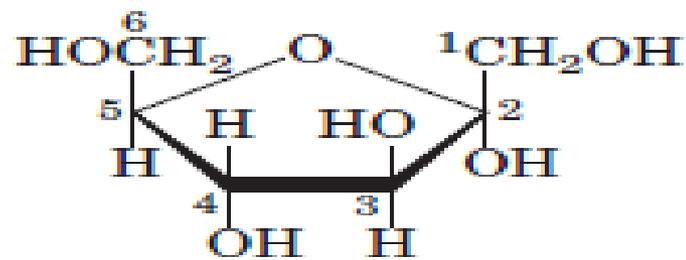
α-D-Fructofuranose
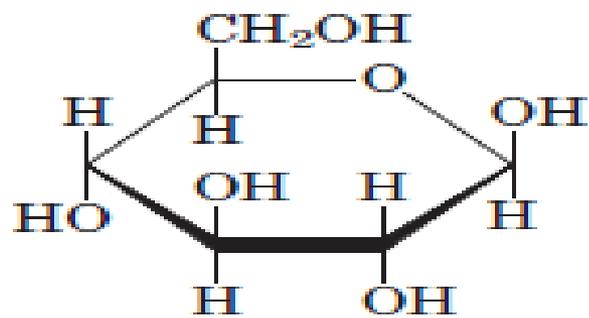
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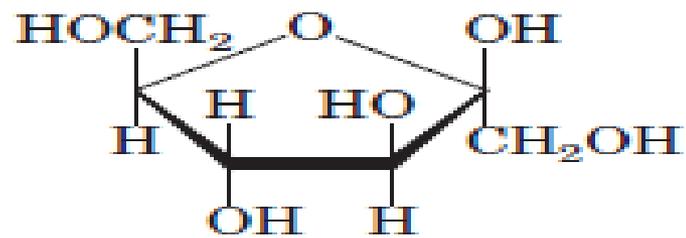
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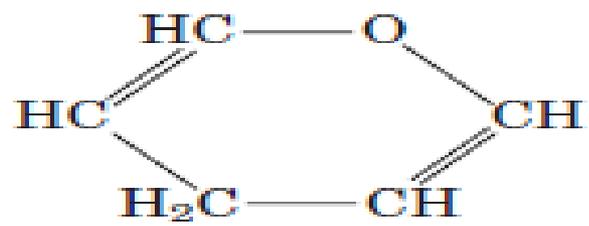
Pyran
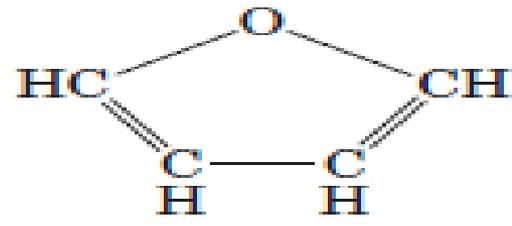
Furan

Haworth Projections
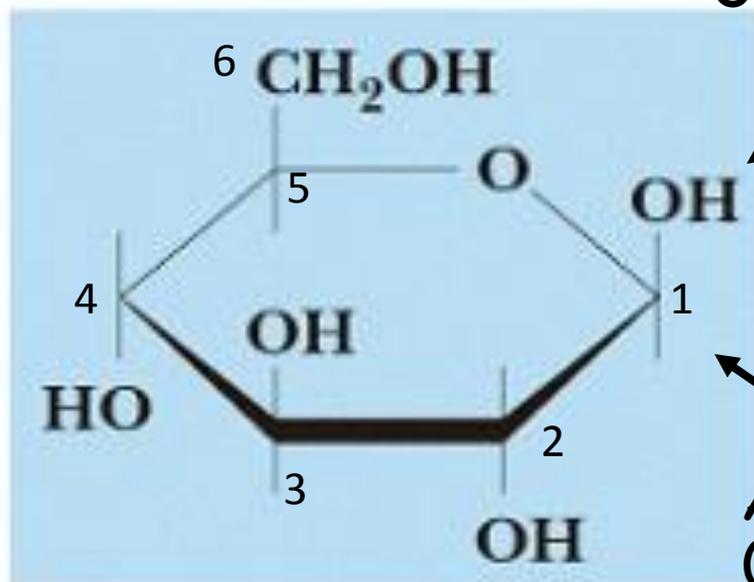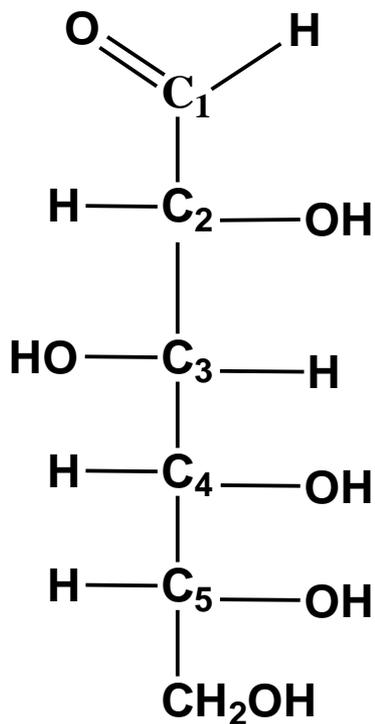
-OH up = beta
-OH down = alpha

Anomeric carbon (most oxidized)

For all non-anomeric carbons, -OH groups point down in Haworth projections if pointing right in Fischer projections

Reducing Sugars

- **When in the uncyclized form, monosaccharides act as reducing agents.**
- **Free carbonyl group from aldoses or ketoses can reduce Cu^{2+} and Ag^+ ions to insoluble products**

D-Glucose

Pyranose form

Furanose form

D-Glucose + O2   → glucose oxidase   D-Glucono -δ-lactone + H2O2
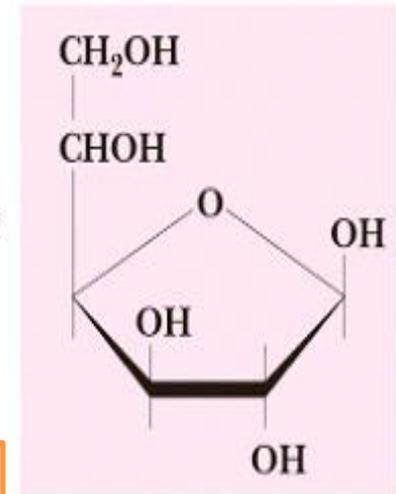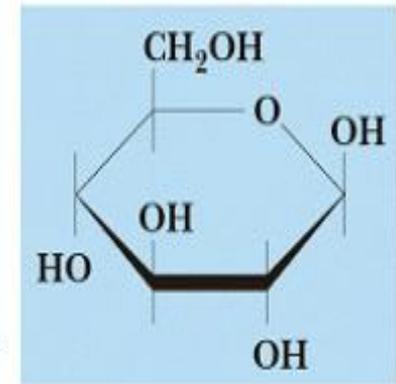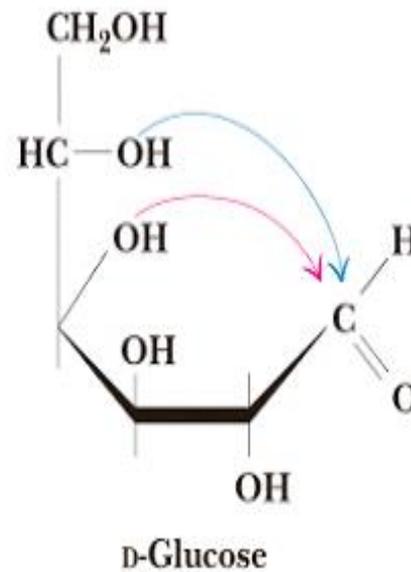
Derivatives of Monosaccharides

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Figure 7.13

α-D-Glucose-1-phosphate

α-D-Fructose-1,6-bisphosphate

Adenosine-5'-triphosphate
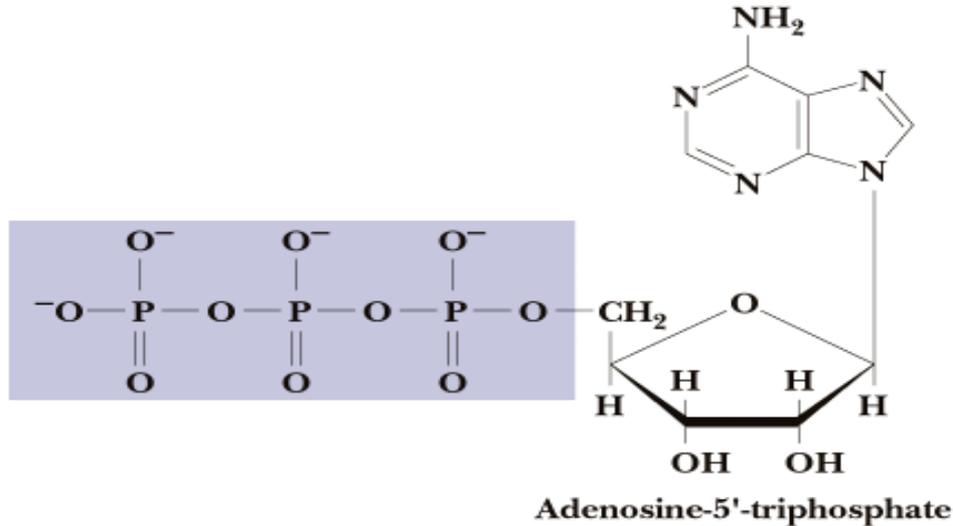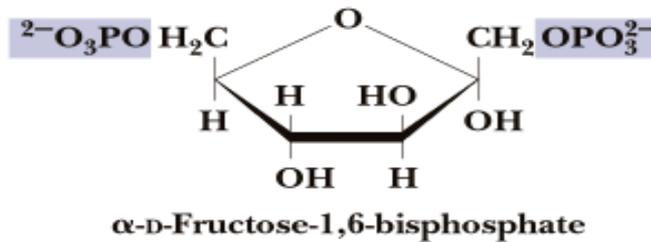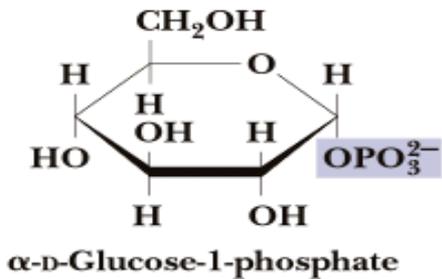
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Sugar Phosphate

Deoxy Acids

2-Deoxy-α-ᴅ-Ribose   α-ʟ-Rhamnose (Rha)   α-ʟ-Fucose (Fuc)
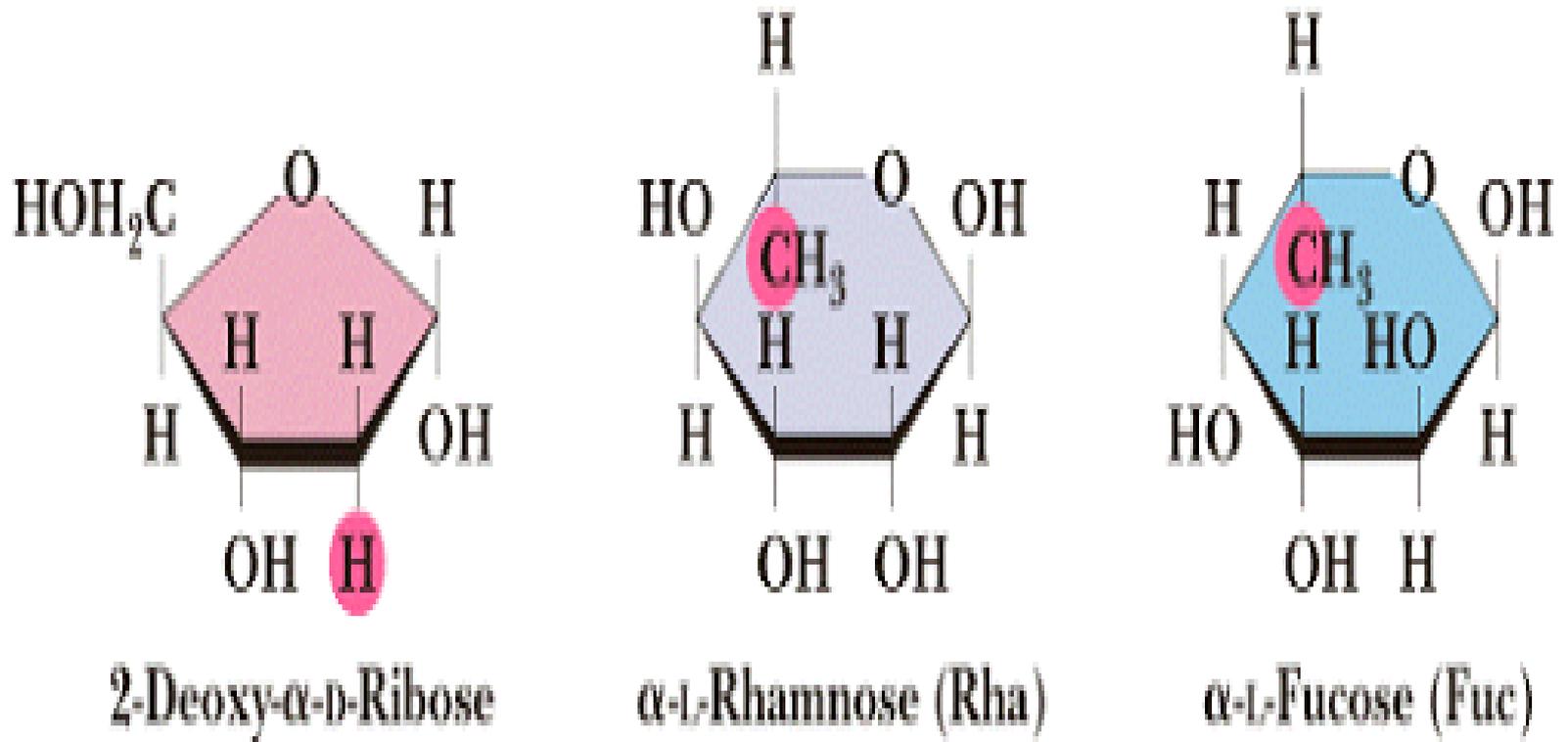
Amino Sugars
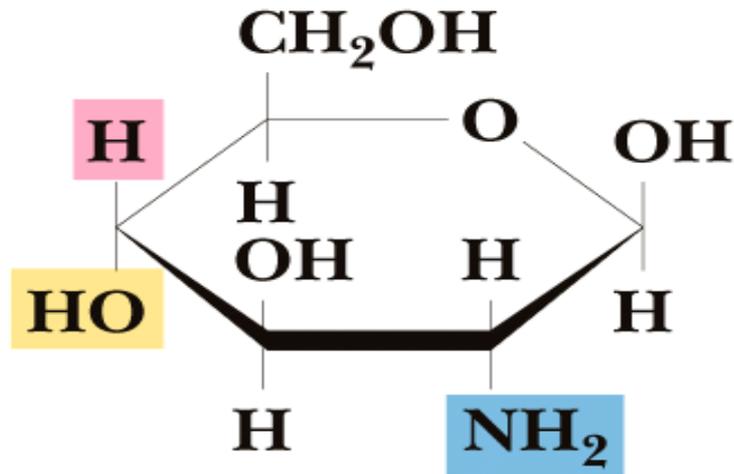
β-D-Glucosamine
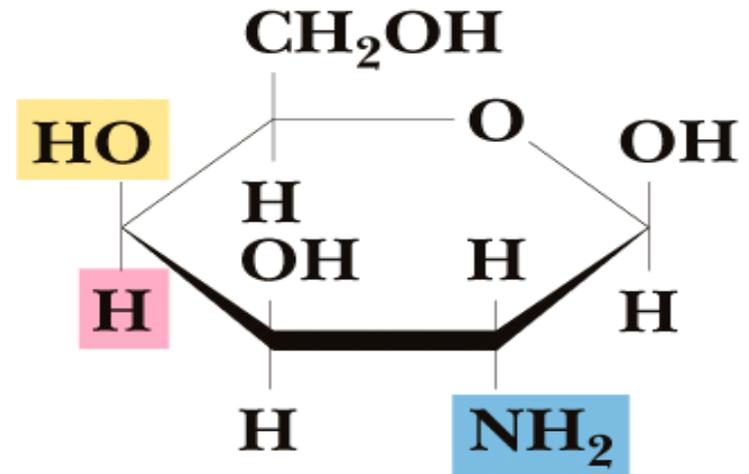
β-D-Galactosamine

Sugar alcohols

D-Glucitol (sorbitol)

D-Mannitol

D-Xylitol

D-Glycerol

myo-Inositol

D-Ribitol
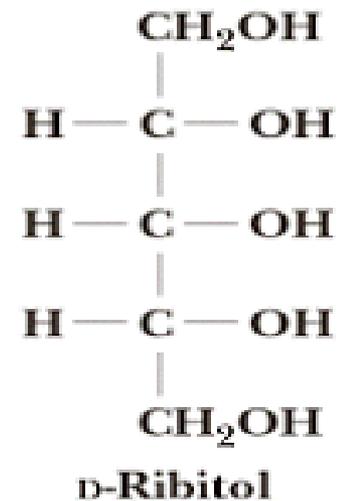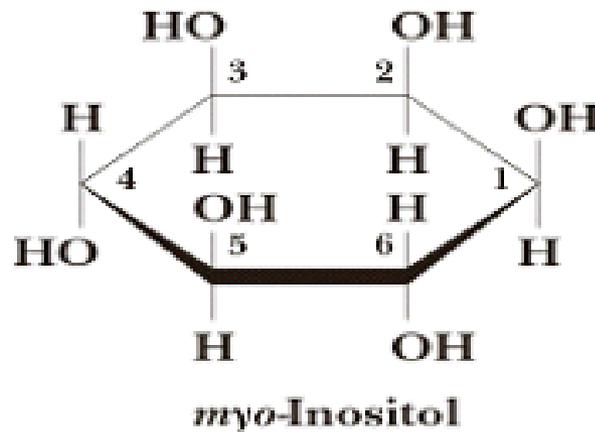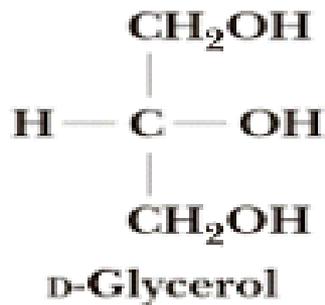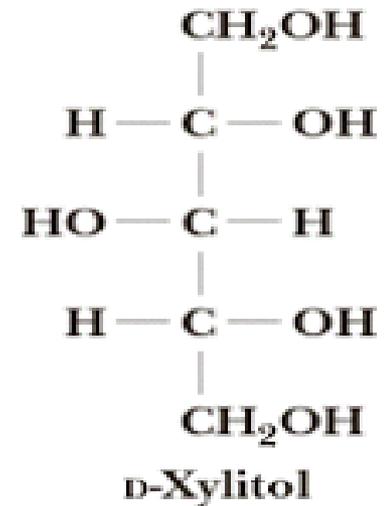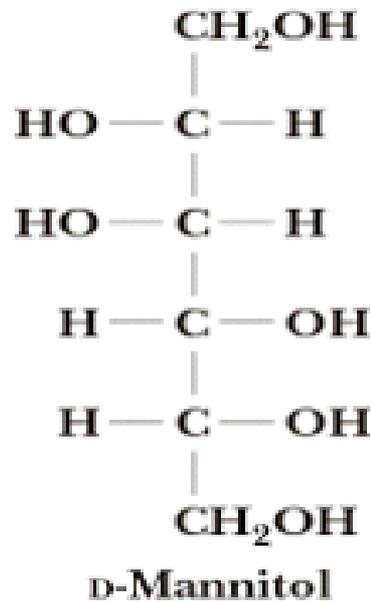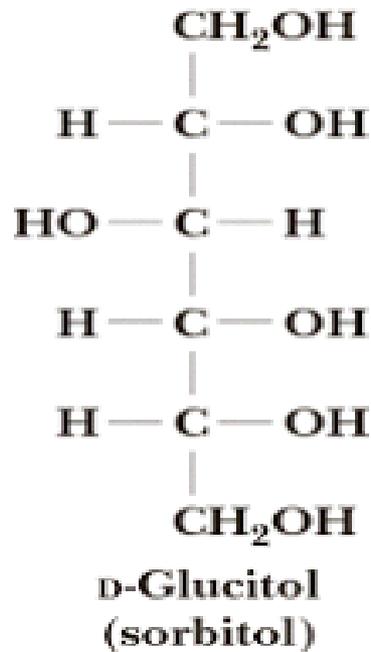
B. Oligosaccharides

❑ Consists of short chains of monosaccharide units or residues.

❑ The most abundant oligosaccharides are disaccharides-with two monosaccharide units.

❑ In cells, most oligosaccharides consisting of three or more units do no occur as free entities but are joined to nonsugar molecules such as lipids or proteins in glycoconjugates.

Cont……

α-D-Glucose + β-D-Glucose

hemiacetal

alcohol

hydrolysis   condensation

$H_2O \longrightarrow H_2O$

Maltose
α-D-glucopyranosyl-(1→4)-D-glucopyranose

acetal   hemiaceta
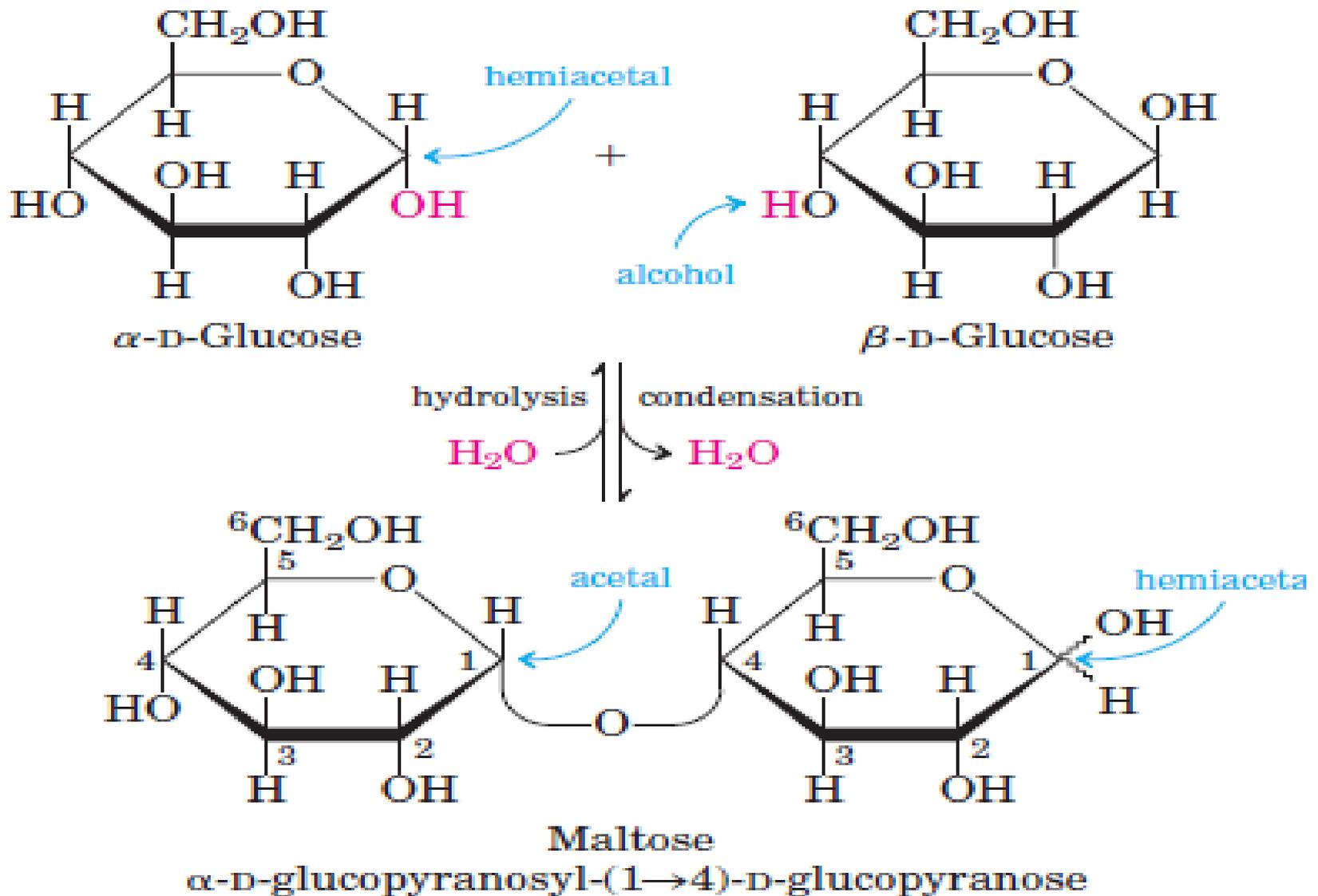
Cont……
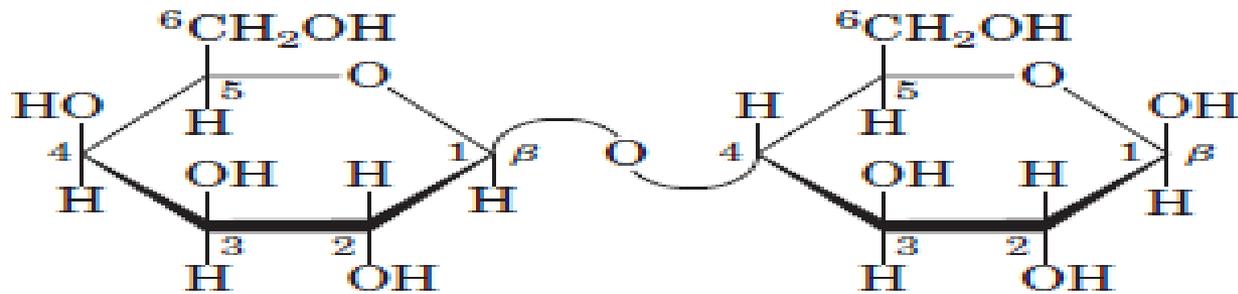
Lactose (β form)
β-D-galactopyranosyl-(1→4)-β-D-glucopyranose
Gal(β1→4)Glc
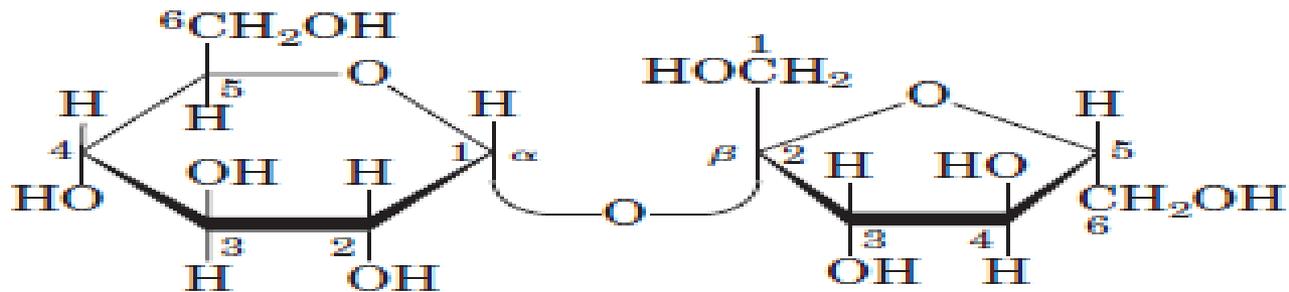
Sucrose
α-D-glucopyranosyl β-D-fructofuranoside
Glc(α1↔2β)Fru
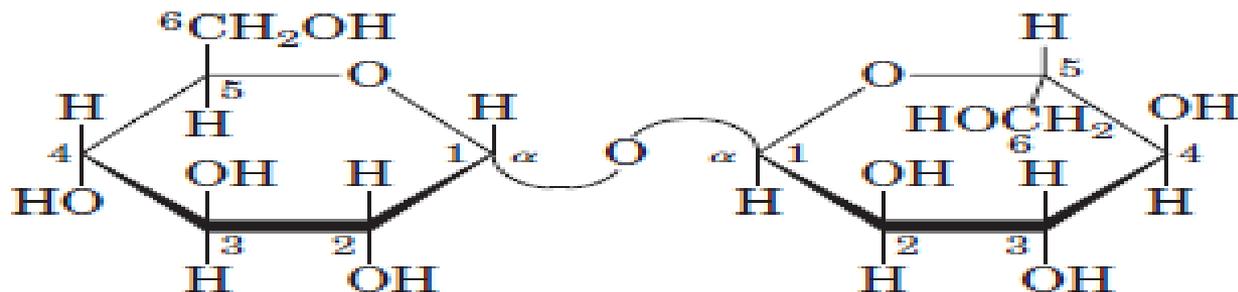
Trehalose
α-D-glucopyranosyl α-D-glucopyranoside
Glc(α1↔1α)Glc

C. Polysaccharides

❑Most COH found in nature occur as polysaccharides, polymers of medium to high molecular weight

❑Polysaccharides also known as glycans.

❑Polysaccharides could be homo polysaccharides and heteropolysaccharides

-Homopolysaccharides = contain only a single type of monomer

-Heteropolysaccharides= contain two or more different kinds

Cont………

Homopolysaccharides

Unbranched   Branched
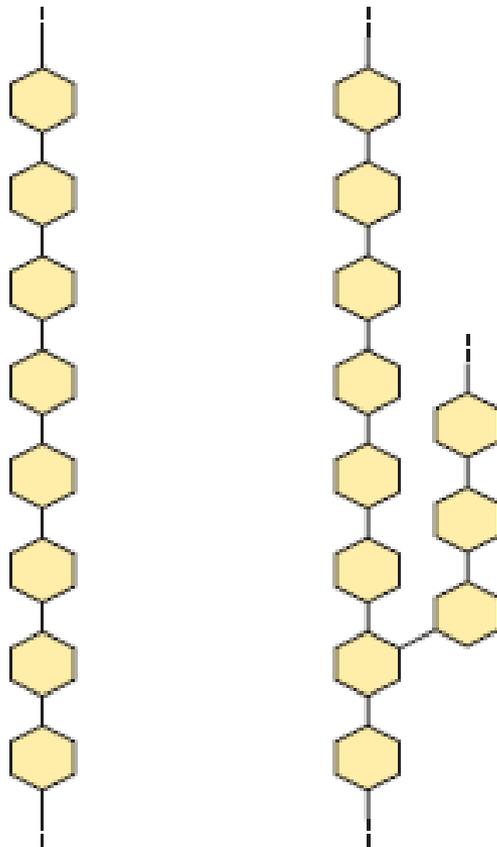
Heteropolysaccharides

Two monomer types, unbranched

Multiple monomer types, branched

Cont….
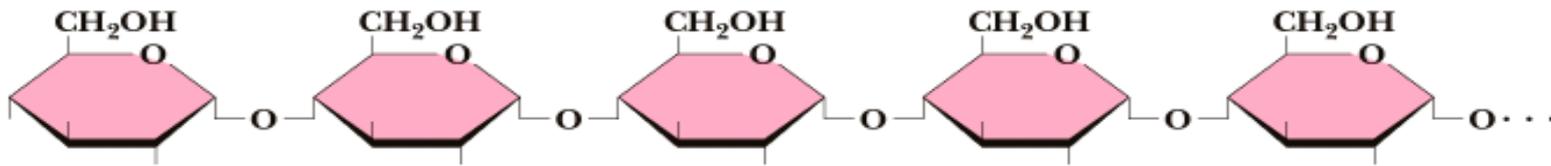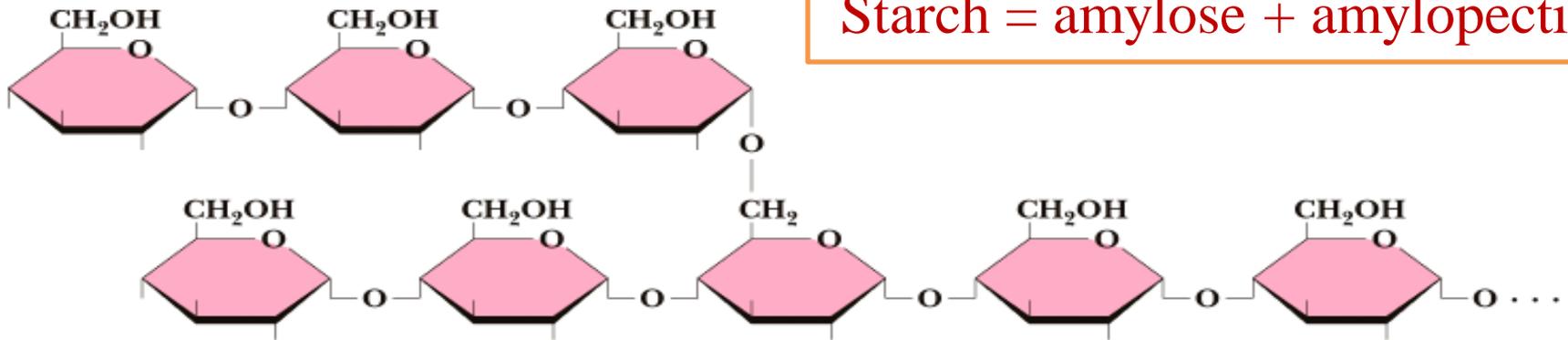
Amylose

Amylopectin

Starch = amylose + amylopectin

glycogen

a1,6-bond

a1,4-bond
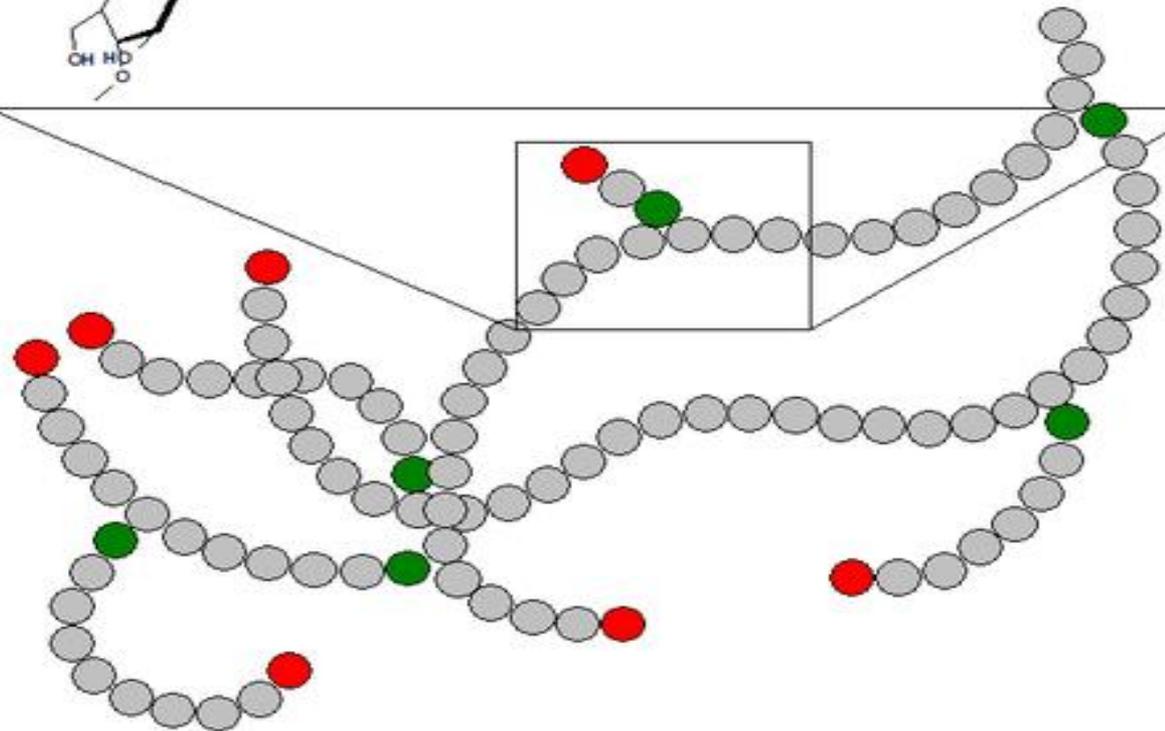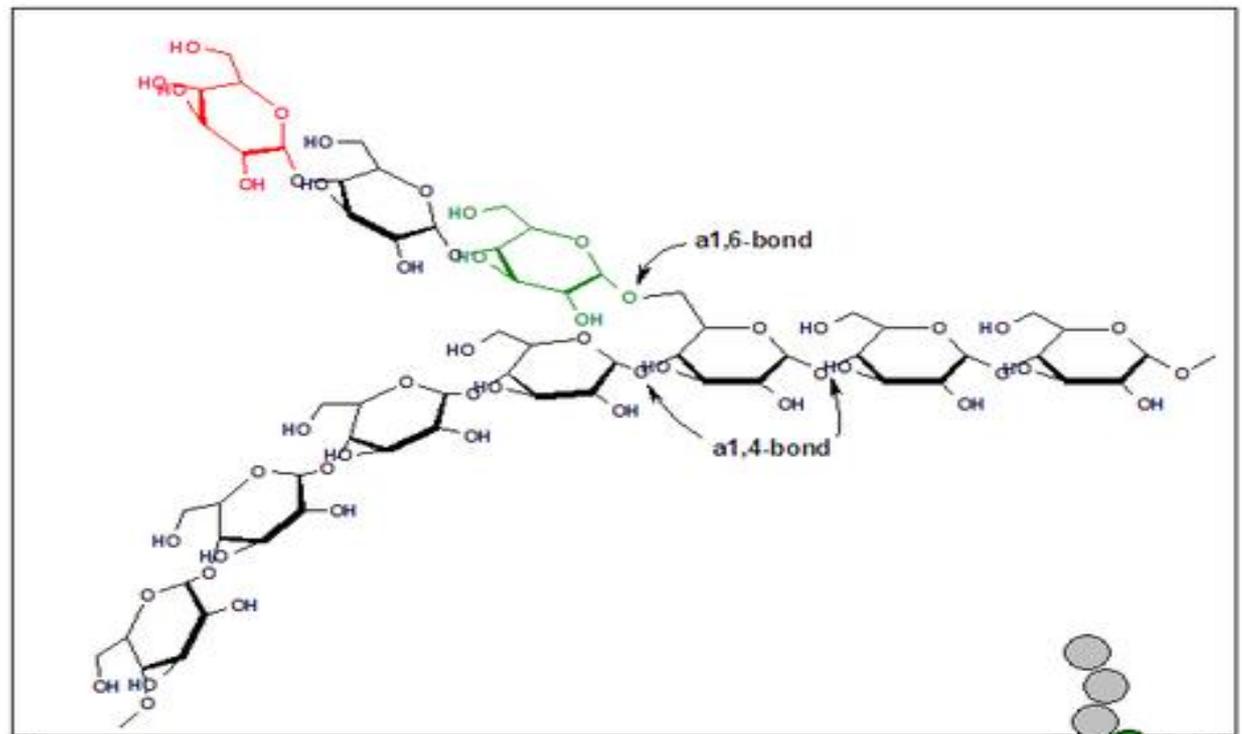
Dextrans

Dextran
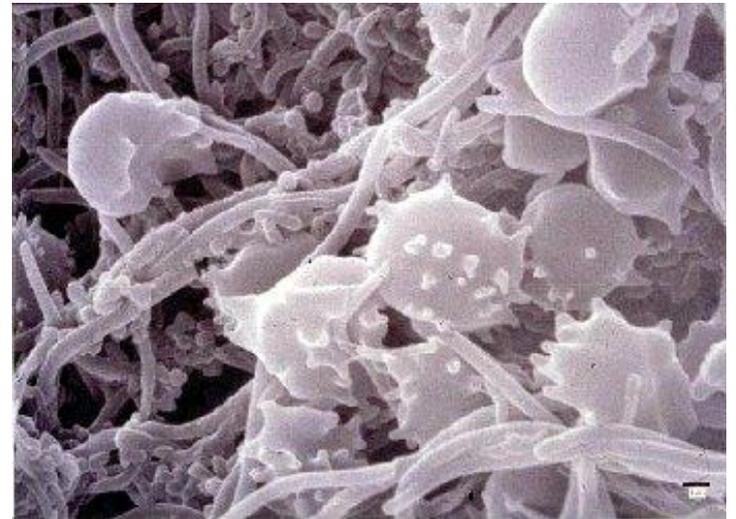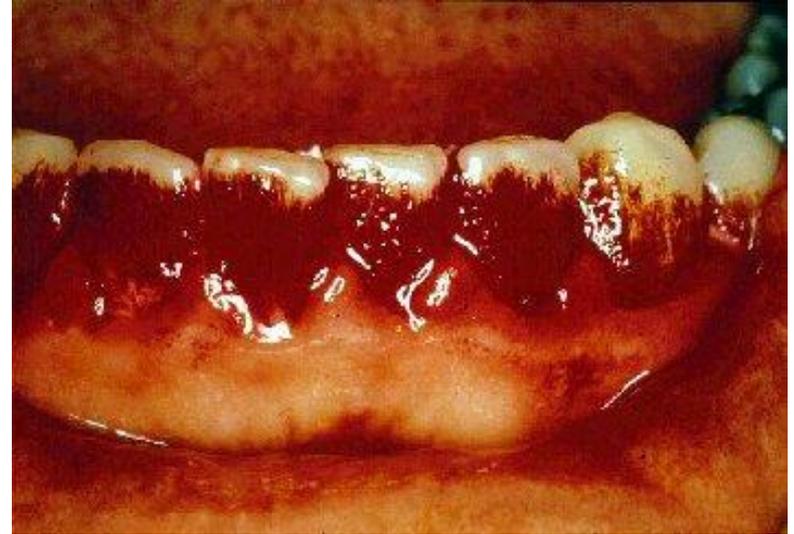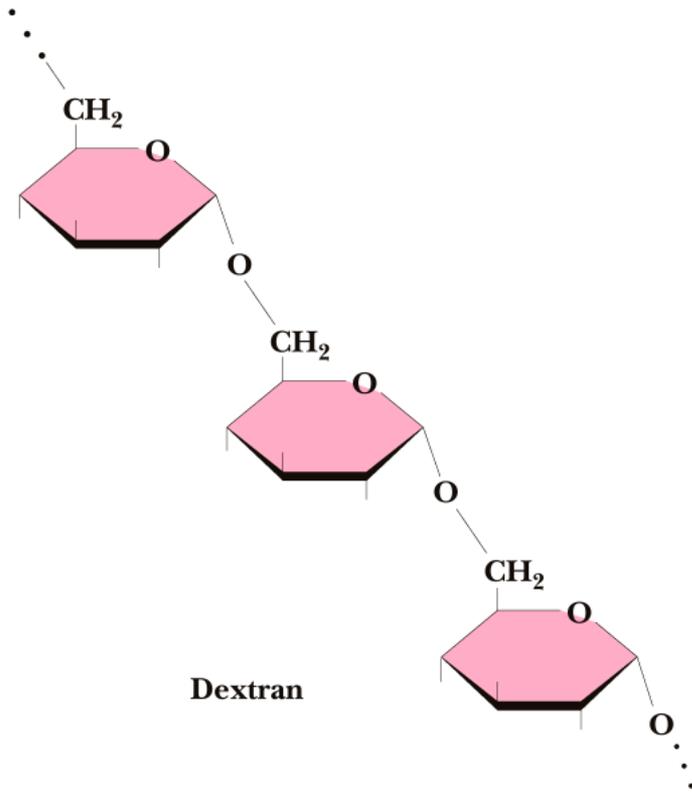
Cellulose vs Amylose

Garrett & Grisham: Biochemistry, 2/e
Figure 7.26

amylose

α-1,4-Linked D-glucose units

(a)

cellulose

β-1,4-Linked D-glucose units
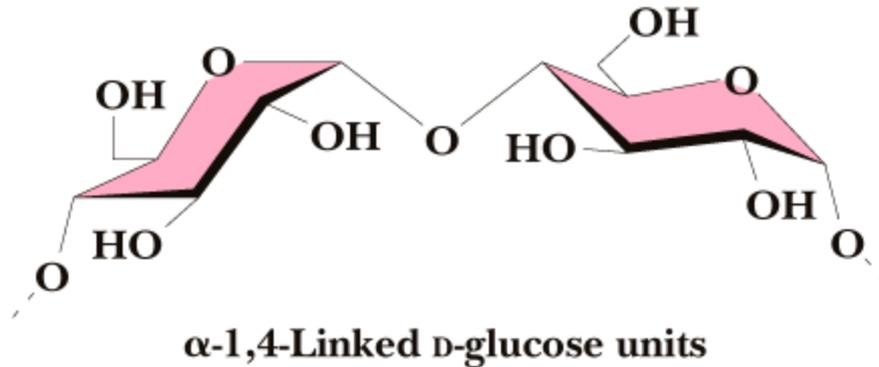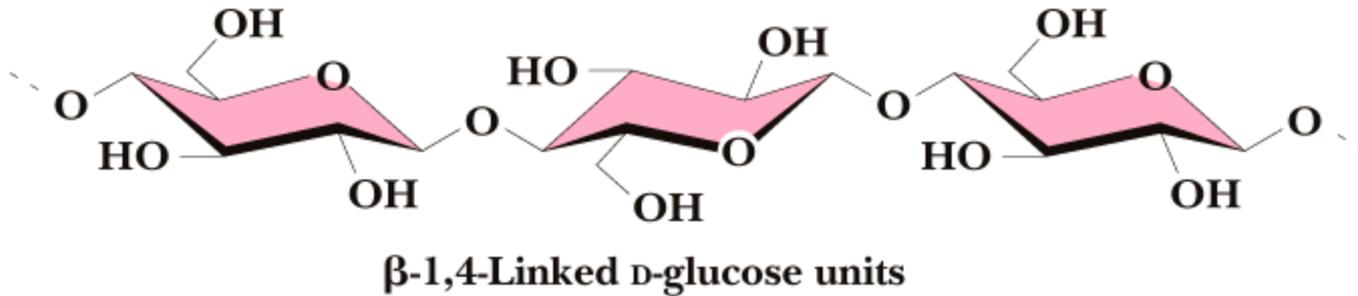
(b)

Saunders College Publishing

Glucose units rotated 180° relative to next residue

Chitin

(c) Chitin

Glucosamine

beta 1-4 glycosidic bond

N-acetyl group

Chitin

cuticle made of chitin
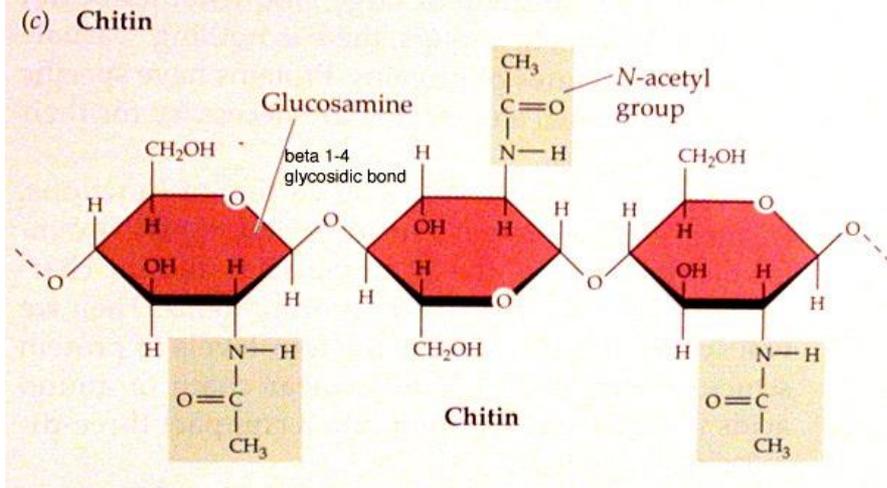
Chitin vs Cellulose
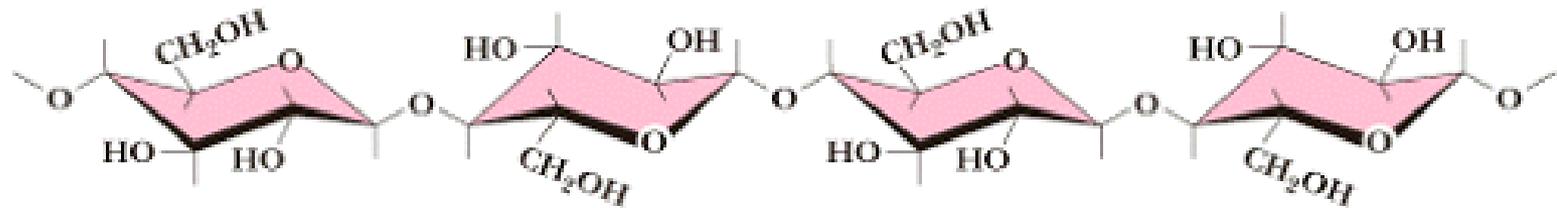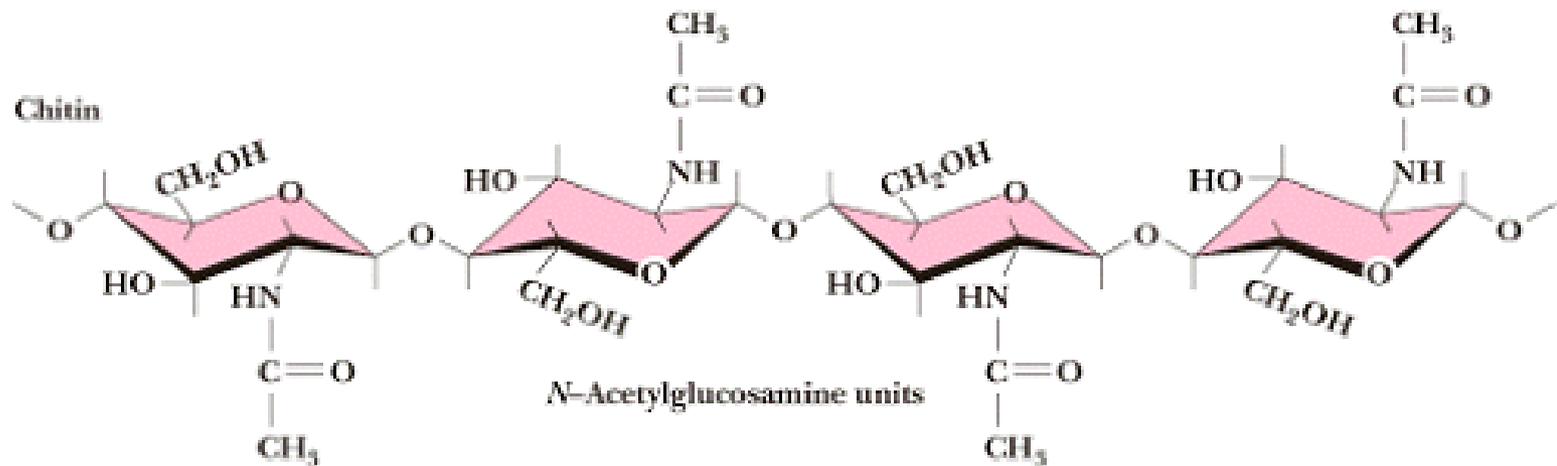
Glycoconjugates
❑Proteoglycan

✓ Are molecules of the cell surface or extracellular matrix in which one or more glycosaminoglycan chains are joined covalently to a membrane protein or a secreted protein.

✓ Proteoglycans are major components of connective tissue such as cartilage, in w/c their many non-covalent interactions with other proteoglycans, proteins and glycosaminoglycans provide stength and resilience.

Cont….
Glycoproteins

✓ Are found on the surface of the plasma membrane, in the extracellular matrix and in he blood.

✓ In side cells they are found in specific organelles such as Golgi complexes, secretory granules, and lysosoes.

✓ The oligosaccharide portions of glycoprotein are rich in information, forming highly specific sites for recognition and high-affinity binding by other proteins.

Cont...........
Glycolipids

✓ Are membrane lipids in which the hydrophilic head groups are oligosaccharides, which as in glycoproteins, act as specific sites for recognition by carbohydrate-binding proteins.

Excercise

1. Draw the following monosaccharides as Haworth projections:

A. α- anomer of D-ribose

B. β- anomer of D-glucose

C. Change these Haworth projections into Fischer projections

1.4.Vitamins and Co-factors

❑ vitamins

➤ Vitamins are organic molecules needed for variety of biological function within the body.

➤ The most important function of the vitamins is to serve as cofactors (co-enzymes) for enzymatic reactions.

Protein + coenzyme (vitamin)

↓ Enzyme ↑

Protein + cofactor (metal ion)

Cont……..

❑The vitamins comprise a diverse group of organic compounds that are nutritionally essential micronutrients. Vitamins function in vivo in several ways, including

(a) as coenzymes or their precursors (niacin, thiamin, riboflavin, biotin, pantothenic acid, vitamin B6, vitamin B12, and folate);

(b) as components of the antioxidative defense system (ascorbic acid, certain carotenoids, and vitamin E);

Cont……

(c) as factors involved in genetic regulation (vitamins A, D, and potentially several others); and

(d) in specialized functions such as vitamin A in vision, ascorbate in various hydroxylation reactions, and vitamin K in specific carboxylation reactions.

➢ The vitamins cannot be synthesized by mammalian cells and, therefore, must be supplied in the diet in small quantities (microgram or milligram per day).

Cont……

➢ Vitamins are two types:

Vitamins

Water soluble vitamins
- ✓ Vit. B1--Thymine
- ✓ Vit. B2--Riboflavin
- ✓ Vit.B3--Niacin
- ✓ Vit. B5—Pantothenic acid
- ✓ Vit. B6--Pyridoxine
- ✓ Vit. B12---cobalamine
- ✓ Pyridoxal, pyridoxamine
- ✓ Ascorbic acid—vit.C
- ✓ Folic acid
- ✓ Biotin

Fat soluble vitamins
- ✓ Vit. A
- ✓ Vit.D
- ✓ Vit. E
- ✓ Vit. K

111

Cont………

➢ Water soluble vitamins- soluble in water, consist of carbon, hydrogen, oxygen nitrogen, sulfur, cobalt.

➢ Deficiency: usually occur more than fat soluble vitamins.

➢ Water soluble vitamins are not stored in the body, body cannot store.

➢ Activate chemical reaction inside the body and act as coenzymes.

➢ Excretion in urine through urination.

➢ The most important water soluble vitamins are B complex and vitamin C.

Structures of water soluble vitamins

thiamine
vitamin B$_1$
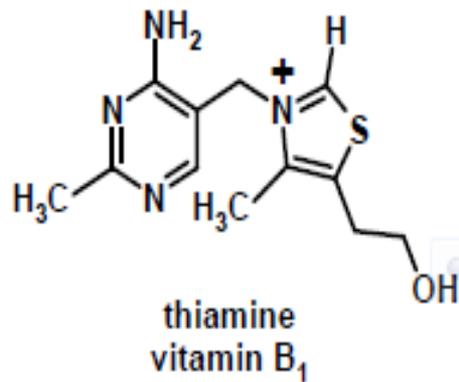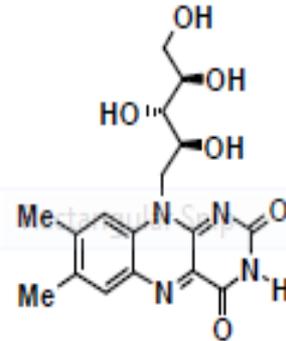
riboflavin
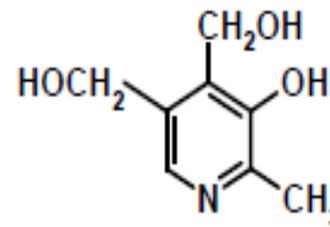
pyridoxine
vitamin B$_6$
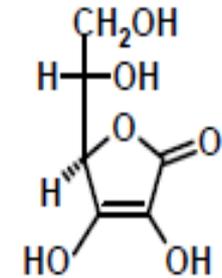
vitamin C
ascorbic acid
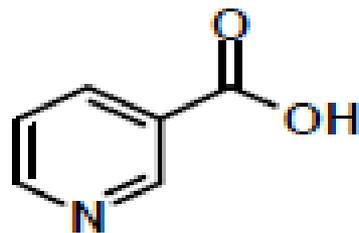
niacin
nicotinic acid
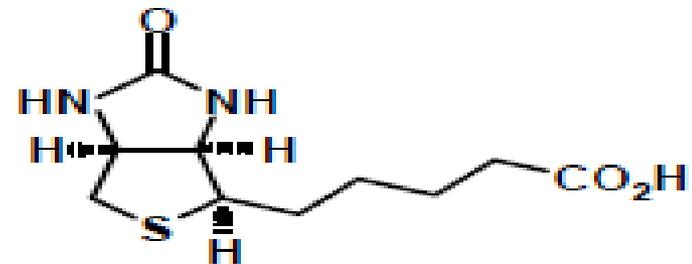
biotin
vitamin H

Cont………
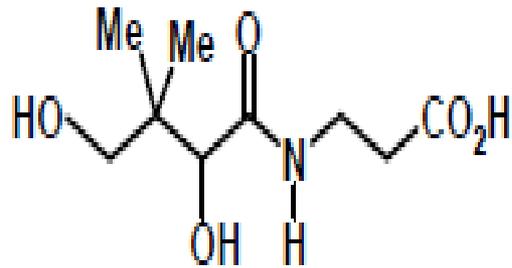
pantothenate

subunit of CoA-SH
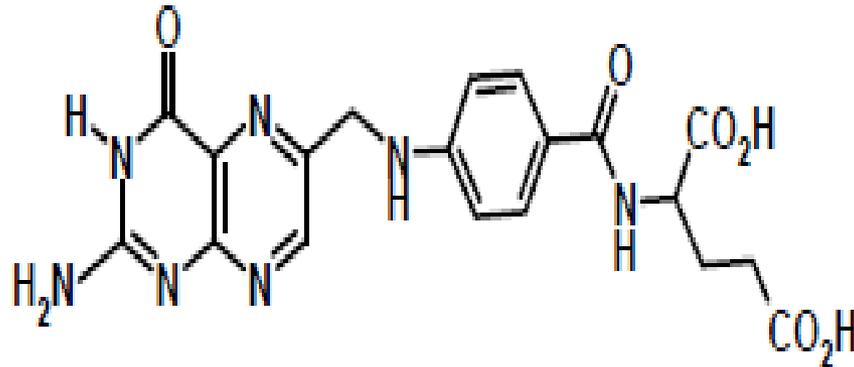
folate

vitamin B$_{12}$

Str. Of Fat soluble vitamins

vitamin A (all-trans)

CH$_2$OH

Me

Me

Me

Me

HO

vitamin D$_3$

HO

CH$_3$

H$_3$C

O

CH$_3$

vitamin E
α-tocopherol

O

CH$_3$

CH$_3$

O
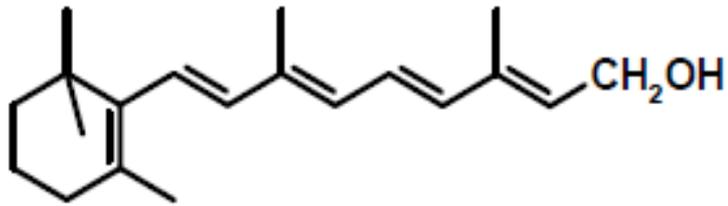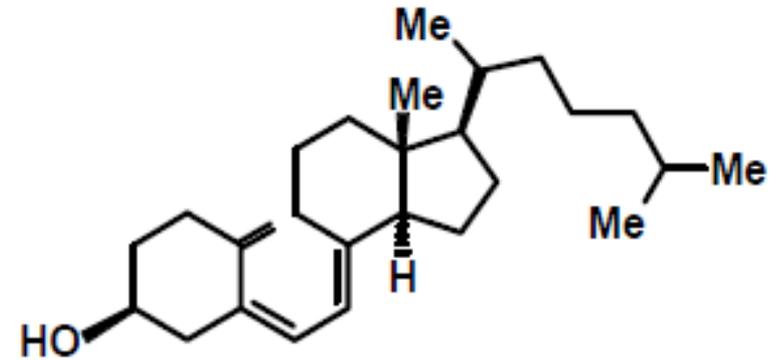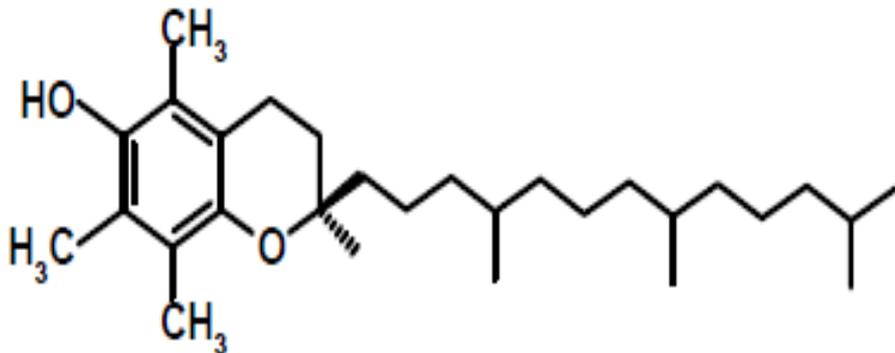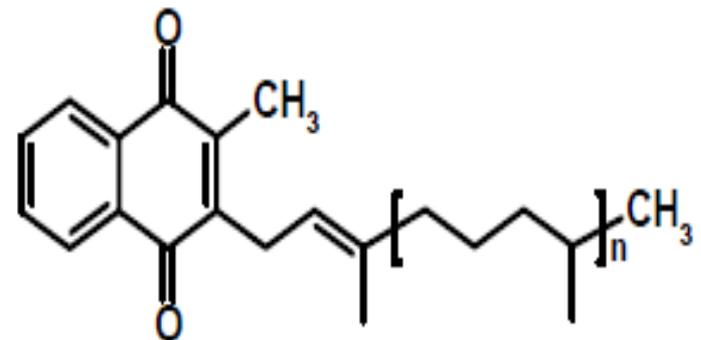
vitamin K$_1$

n = 3

Co-factors

❑Co-factors – are defined as small molecules that associate closely with enzymes during enzymatic reactions and are converted from one form to another in the reaction.

❑Two classes of co-factors:

1. Organic co-factors = known as coenzymes
2. Metallic co-factors = known as essential ions

❑ The enzyme involved in such a reaction is known as a holoenzyme, when cofactors presence while in the absence of the cofactor it is known as an apoenzyme.

❑Examples of co-enzymes are NAD (nicotinamide adenine dinucleotide) and FMN (flavin mononucleotide) both are involved in oxidation-reduction reactions.

❑Essential ions are required dietarily because they are used in small quantities in biochemical reactions. They participate in oxidation-reductions, stabilize charge complexes in enzymes, and serve other roles.

Cont…..

TABLE 6–1 Some Inorganic Elements That Serve as Cofactors for Enzymes

Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^+	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Se	Glutathione peroxidase
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

Cont………

TABLE 6-2 Some Coenzymes That Serve as Transient Carriers of Specific Atoms or Functional Groups

Coenzyme	Examples of chemical groups transferred	Dietary precursor in mammals
Biocytin	CO_2	Biotin
Coenzyme A	Acyl groups	Pantothenic acid and other compounds
5'-Deoxyadenosylcobalamin (coenzyme B$_{12}$)	H atoms and alkyl groups	Vitamin B$_{12}$
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B$_2$)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B$_6$)
Tetrahydrofolate	One-carbon groups	Folate
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B$_1$)