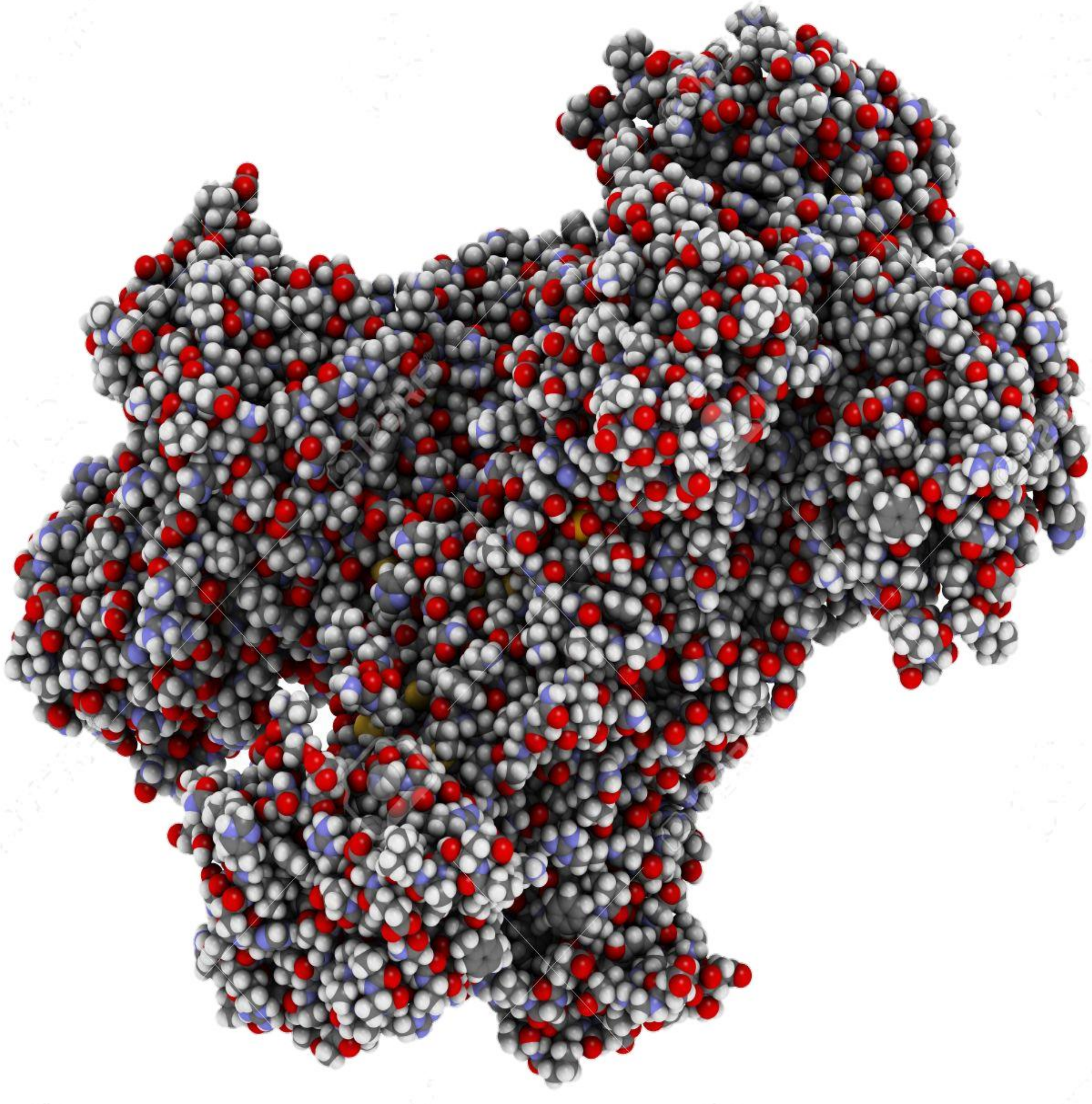
Biochemistry 01



MidTerm Handout

Written&Edited by : Enas Alamaireh

إنَّما نحنُ أيامٌ ماضية ، نسعَى لنصلَ إلى مبتغانا الَّذي لطالما كان حلُمًا لا يغيب ، حتى أخذَ بالتَّشكل شيئًا فشيئًا ليرسمَ بعضًا من معالمِه وملامحه التي أردناها له ، لا بدَّ له من اكتمال في يومٍ ما ، لحظةٍ ما ... تظنُّ أنَّكَ قد وصلتَ إلى نهاية هذا الطَّريق الذي أخذَ منكَ الكثير فتدرِكُ أنَّها البداية الموصِلة لاكتمالِه .

هذا بضعٌ من حصاد ، لا يتسمُّ بالكَمال فيحتملُ الخطأ والنَّقص، كتِبت حروفه بعناية ، بدقَّة ، ابتدأ باسم الله واختتِم بحمدِه ، كلِلَّت حروفه بالدُّعاء بأن يكونَ نافعًا غير ضار ، مرشدًا غير مضِل ، معينًا غير خاذِل . .

إيناس العَمايرة ..
2019/6/11-الثلاثاء

References ;

(1) Biochemistry 6th Edition by Mary K. Campbell .
(2) https://en.wikipedia.org/wiki/Biochemistry
(3) http://chemistry.elmhurst.edu/vchembook/560aminoacids.html
(4) https://opentextbc.ca/anatomyandphysiology/chapter/26-4-acid-base-balance/
(5) https://themedicalbiochemistrypage.org/
(6) http://www.namrata.co/
(7) https://en.wikibooks.org/wiki/Structural_Biochemistry/Protein_function/Heme_group/Myoglobin
(8) Slides uploaded on :
https://www.od.baumedicine.com/intro-to-biochemistry

Chemical bonds & compounds

•Before getting into biochemical aspects , you must be familiar with some basic themes related to chemistry and first of them is : chemical bonds & compounds.

•**Definition** of a bond ➡ a **force** that holds group of two or more atoms together and makes them function as a **unit** .

•The measure of bond strength in a chemical bond is referred to as :Bond Energy .

•**Definition** of bond energy(E) ➡the amount of **energy** required to break apart a mole of molecules into its component atoms(aka bond enthalpy).

•Types of bonds :

Generally, bonds are classified into 2 classes

(1) **Covalent** bonds .

(2) **Non-covalent** bonds , a chemical bond that involves the **sharing of electron pairs** between atoms that relatively have the same electronegativity .

•This table shows you types of bonds and their energies :

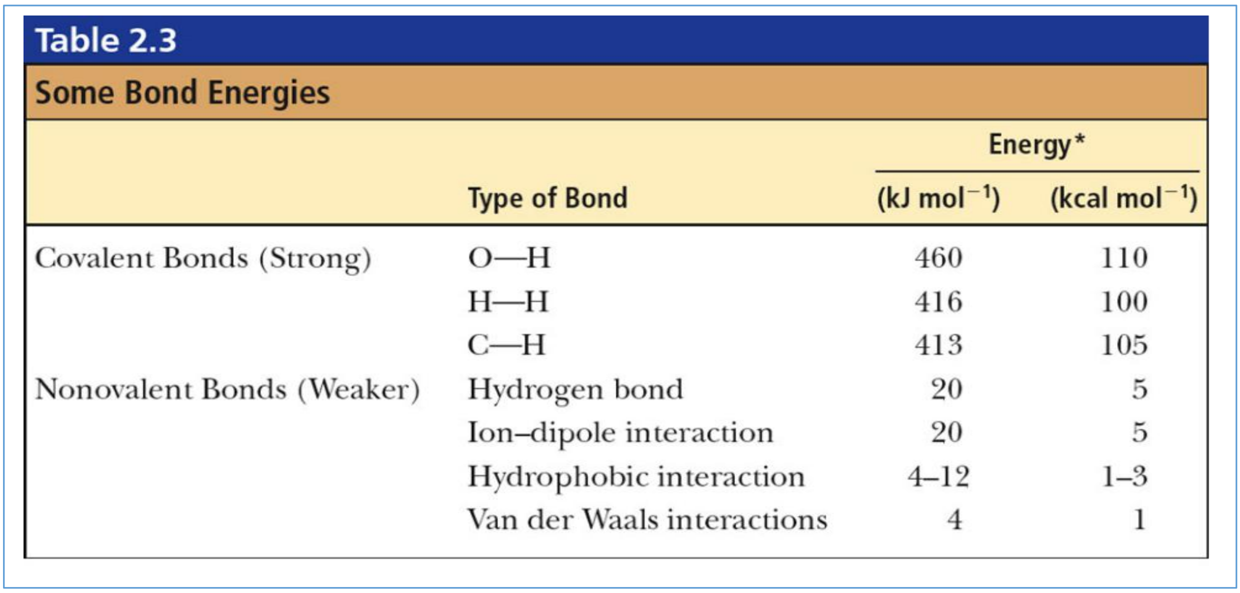


Table 2.3

Some Bond Energies

	Type of Bond	Energy* ($kJ\ mol^{-1}$)	Energy* ($kcal\ mol^{-1}$)
Covalent Bonds (Strong)	O—H	460	110
	H—H	416	100
	C—H	413	105
Nonovalent Bonds (Weaker)	Hydrogen bond	20	5
	Ion–dipole interaction	20	5
	Hydrophobic interaction	4–12	1–3
	Van der Waals interactions	4	1

- Notes :

•The strongest bonds that are oresentvin biochemicals are covalent bonds .

•The stronger the bond , the more energy required to break it .

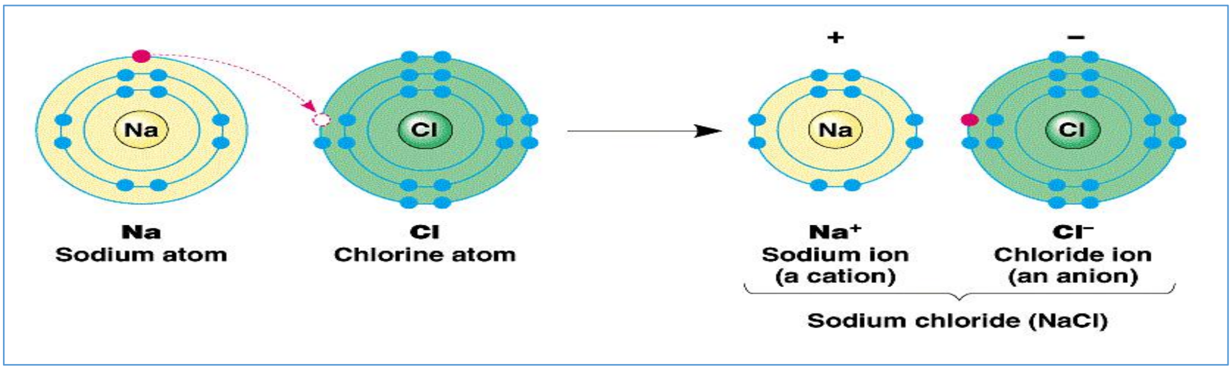
Ionic Bonds

•Definition of ionic bonds ➡type of chemical **bond** that generates *two oppositely charged* ***ions*** **.**

- In **ionic bonds**, the **metal** loses electrons to become a positively charged **cation**, whereas the **nonmetal** accepts those electrons to become a negatively charged **anion.**

•General properties :

1. Ionic bonds occur between atoms of different electronegativity .
2. Ionic bonds produce charged ions all states .
3. Ionic compounds are conductors having high melting and boiling points .
4. Ionic compounds are very stable and their crystals are very strong ,the shape of these crystals depends on the ratio of positive ions to negative ions and the size of the ions .



- Electron from Na is transferred to Cl, this causes a charge imbalance in each atom. The Na becomes (Na^+) and the Cl becomes (Cl^-), charged particles or ions.

Covalent Bonds

•It typically formed between **2 non-metals** having similar electronegativities ,meaning that neither atom is ‘strong’ enough to steal electrons from the other .Thus , atoms share electrons .

•The number of covalent bonds that an atom can form depends on the number of electrons that it has available for sharing.

- Atoms of (O,S···)can form two covalent bonds.
- Atoms of (N,P···) can form three covalent bonds.
- Atoms of (C, Si..)can form four covalent bonds .

- Bonds in all **polyatomic** ions and **diatomics** are all covalent bonds .

- Resonance structures :

In certain cases, molecules can be represent by more than one reasonable Lewis **structure** .

✔e.g.

benzene can be written in two equivalent ways called resonance structures. Benzene's **true structure** is a composite of its two resonance structures.

Benzene resonance structures

- **Lewis Structure & Molecular geometry**

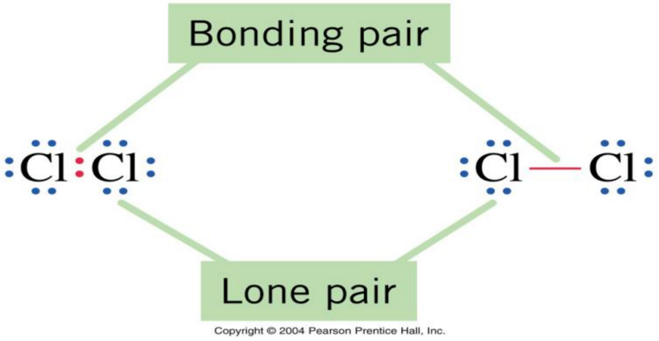
✔A Lewis structure is a **combination of Lewis symbols** that represents the formation of covalent bonds between atoms.

✔Lewis structure shows the bonded atoms with the electron configuration of a noble gas; that is, the atoms obey the **octet rule**.

The bonding in carbon dioxide (CO_2):
all atoms are surrounded by 8 electrons, fulfilling the **octet rule**.

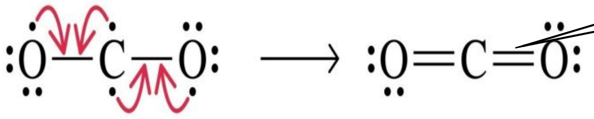
✔Basics of Lewis Structure :

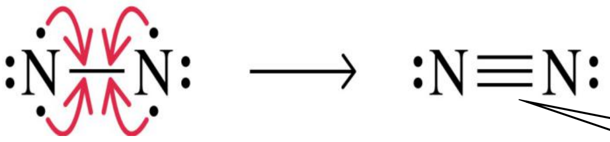
1. The shared pairs of electrons in a molecule are called **bonding pairs**.
2. In common practice, the bonding pair is represented by a dash (—).
3. The other electron pairs, which are **not shared**, are called **nonbonding pairs**, or **lone pairs**.



4. The covalent bond in which one pair of electrons is shared is called a single bond.
   - ➢ Multiple bonds can also form :

In a *double bond* two pairs of electrons are shared.

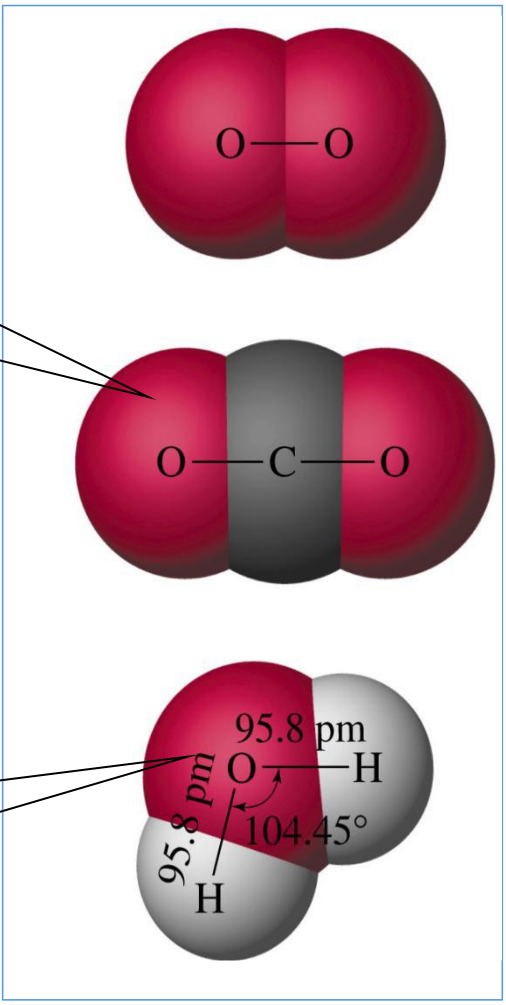




In a *triple bond* three pairs of electrons are shared.

✔Molecular geometry ➡ simply the shape of a molecule.

Molecular geometry is found using the Lewis structure, but the Lewis structure itself doesn’t necessarily represent the molecule’s shape.



A carbon dioxide molecule is *linear.*

A water molecule is *angular* or *bent.*

•Now , let’s make a comparison between both ionic and covalent compounds :

	Ionic compound	Covalent compounds
State	Crystalline solids	Gases/liquids/solids
Conduction	Conduct electricity when melted	Poor electrical conductors
Melting& boiling points	High melting&boiling points	Low melting&boiling points
Solubility	Many soluble in water but not in nonpolar liquids.	Many soluble in nonpolar liquids but not in water .

Reversible interactions of biomolecules are mediated by three kinds of noncovalent bonds

•Readily reversible, noncovalent molecular interactions are key steps in the dance of life. Such weak, noncovalent forces play essential roles in the following :

- Faithful **replication** of DNA .
- The **folding** of proteins into intricate 3D forms.
- The specific **recognition of substrates by enzymes**.
- The **detection of molecular signals**.

•The three fundamental noncovalent bonds are :

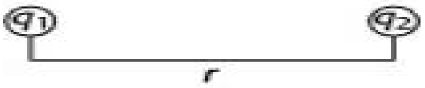
- **Electrostatic interactions.**
- **Hydrogen bonds.**
- **Van der Waals interactions.**

Electrostatic Interactions

•An electrostatic interaction depends on the electric charges on atoms.

•The energy of an electrostatic interaction is given by ***Coulomb's law***

$$E = kq_1q_2/Dr$$



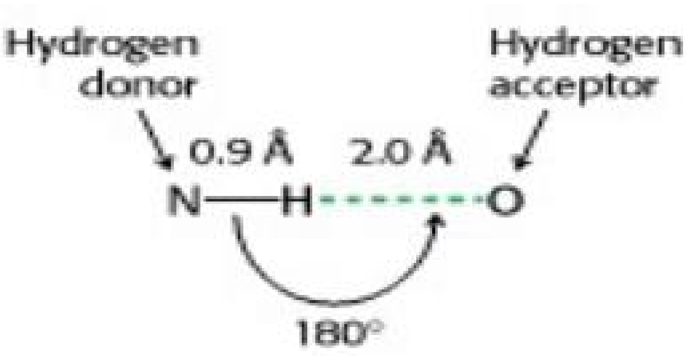
Hydrogen Bonds

•Hydrogen bonds are relatively weak interactions, which nonetheless are crucial for biological macromolecules such as **DNA** and **proteins**. These interactions are also responsible for many of the properties of water that make it such a special solvent.

•The hydrogen atom in a hydrogen bond is **partly shared** between two relatively electronegative atoms such as **nitrogen** or **oxygen**.

•The ***hydrogen-bond donor*** is the group that includes both the atom to which the hydrogen is more tightly linked and the hydrogen atom itself.

•Tthe ***hydrogen-bond acceptor*** is the atom less tightly linked to the hydrogen atom.



•The strongest hydrogen bonds have a tendency to be approximately **straight**, such that the hydrogen-bond donor, the hydrogen atom, and the hydrogen-bond acceptor lie along a straight line.

•Hydrogen bonds are fundamentally electrostatic interactions,explanation .

- ✓ The relatively electronegative atom to which the hydrogen atom is covalently bonded pulls electron density away from the hydrogen atom so that it develops a **partial positive charge** (**δ+**). It can interact with an atom having a **partial negative charge** (**δ-**) through an electrostatic interaction.

Van der waals interactions

•The basis of a van der Waals interaction is that **the distribution of electronic charge around an atom *changes with time***.

•At any instant, the charge distribution is not perfectly symmetric. This transient asymmetry in the electronic charge around an atom acts through electrostatic interactions to induce a complementary asymmetry in the electron distribution around its neighboring atoms.

•The resulting attraction between two atoms *increases as they come closer to each other*, until they are separated by the **van der Waals contact distance** . At a shorter distance, *very strong repulsive forces* become dominant because the outer electron clouds overlap.

Water:The solvent for biochemical reactions

•Water is the principal component of most cells. The geometry of the water molecule and its properties as a solvent play major roles in determining the properties of living systems.

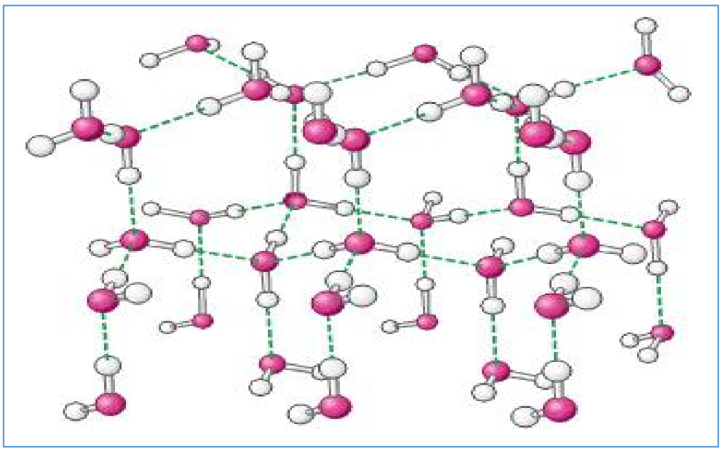
•The strength and specificity of weak interactions are highly dependent **on the medium in which they take place**, and the majority of biological interactions take place in water.

•Two properties of water are especially important biologically:

- Polarity .
- Cohesion ➡the highly cohesive nature of water dramatically affects the interactions between molecules in aqueous solution.

•Water molecules interact strongly with one another ***through hydrogen bonds***. These interactions are apparent in *the structure of ice* . Networks of hydrogen bonds hold the structure together; similar interactions **link molecules in liquid water** and account for the cohesion of liquid water, although, in the liquid state, some of the hydrogen bonds are broken.

•Structure of Ice.
Hydrogen bonds (shown as dashed lines) are formed between water molecules.



Q: Water is an excellent solvent for polar molecules ,explain ?
A:T he polarity and hydrogen-bonding capability of water make it a ***highly interacting molecule***, meaning that water greatly weakens electrostatic forces and hydrogen bonding between polar molecules by competing for their attractions.

•This table* shows electronegativities of different elements :

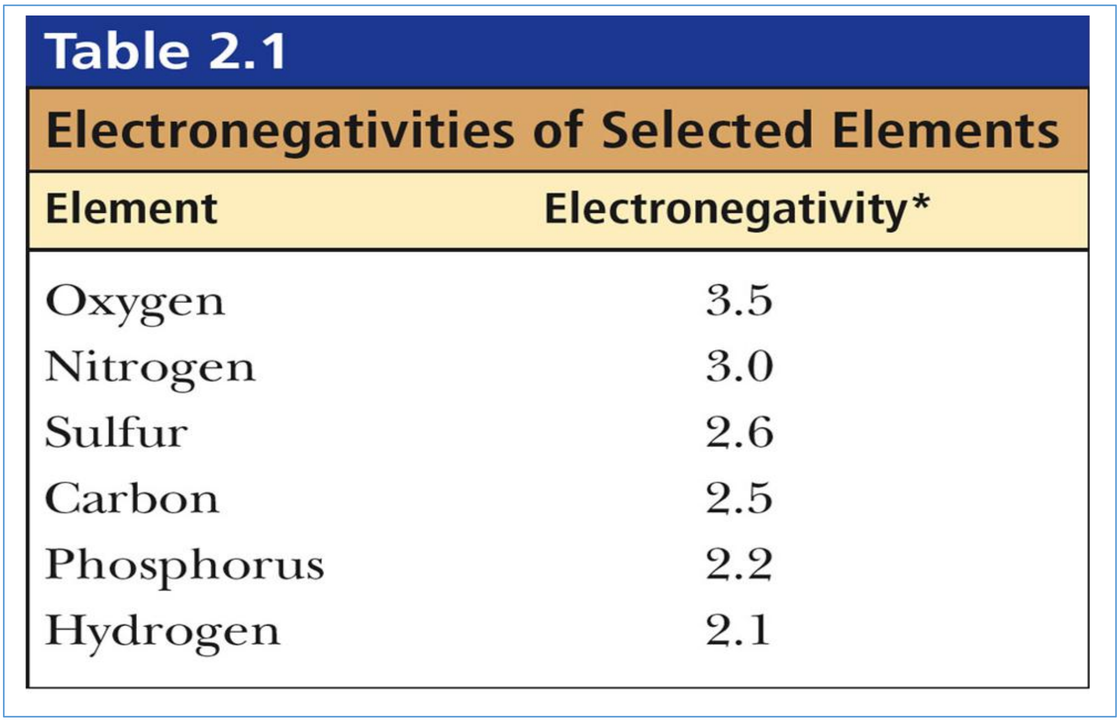
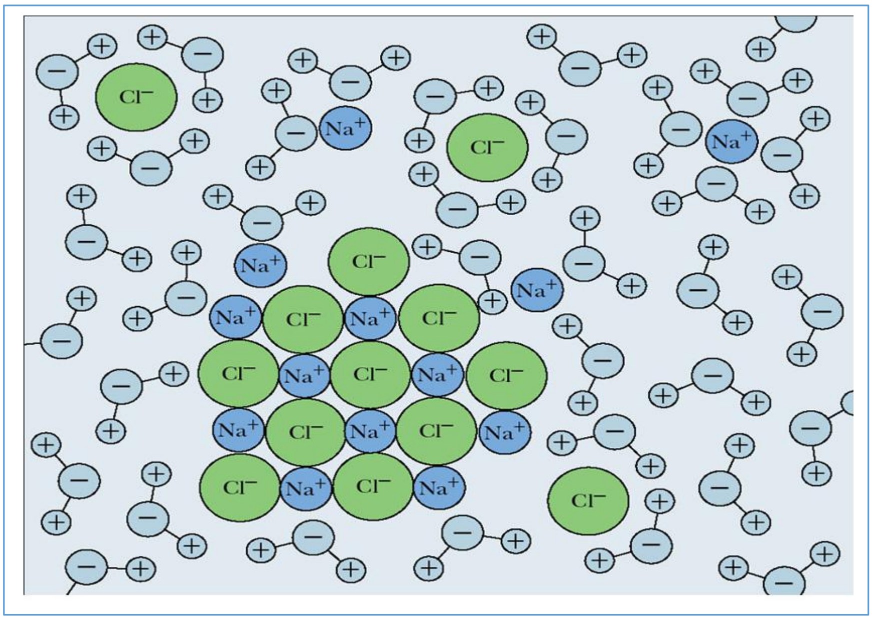


Table 2.1

Electronegativities of Selected Elements

Element	Electronegativity*
Oxygen	3.5
Nitrogen	3.0
Sulfur	2.6
Carbon	2.5
Phosphorus	2.2
Hydrogen	2.1

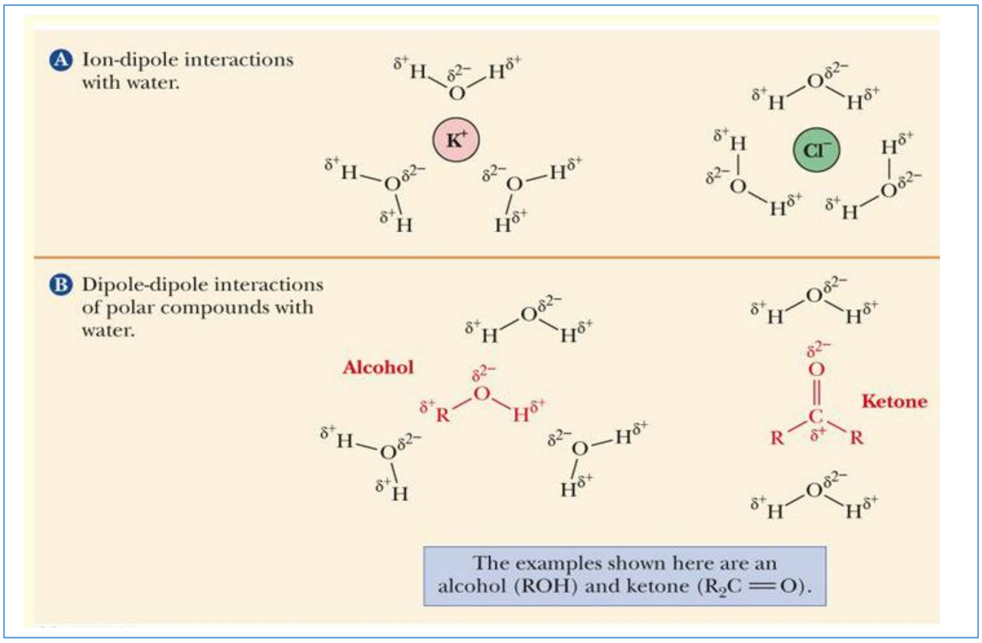
•Solvent properties of water
✔Water is capable of dissolving a variety of different substancs ,which why it is such a good solvent .Water is called to be ‘***Universal solvent***’ because of it dissolves more substances than any other liquid .



- Sodium chloride (NaCl) dissolves when water molecules continuously attack the NaCl crystal, pulling away the individual sodium (Na+) and chloride (Cl ⁻) ions.
- Hydration shells surrounding ions in solution. Unlike charges attract.
- The partial negative charge of water is attracted to positively charged ions. Likewise, the partial positive charge on the other end of the water molecule is attracted to negatively charged ions.

Collectively , The underlying physical principle is electrostatic attraction between unlike charges .

- Study this figure :



The examples shown here are an alcohol (ROH) and ketone ($R_2C{=}O$).

- Keep in mind that the favorable ***ion–dipole and dipole–dipole interactions*** responsible for the solubility of ionic and polar compounds don’t occur for ***nonpolar compounds***, so these compounds tend ***not to dissolve in water***.

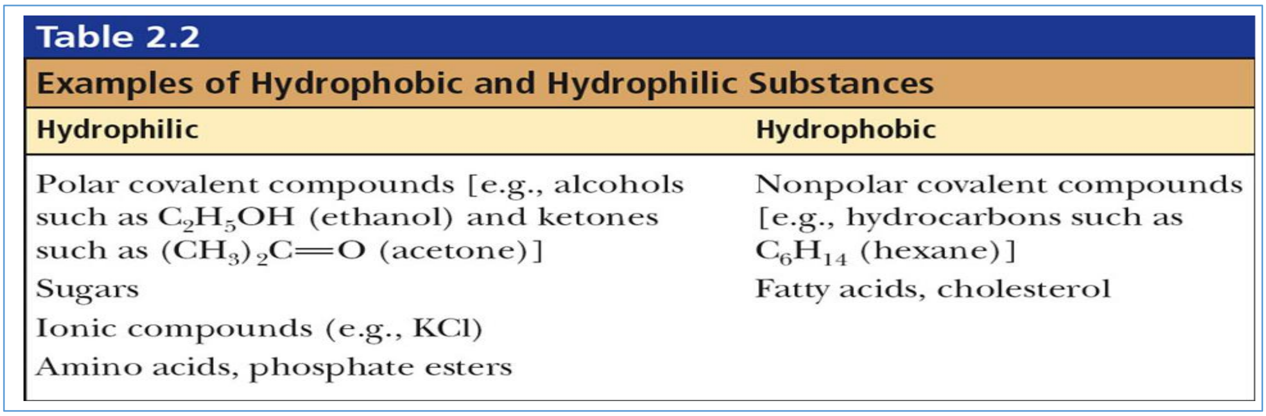


Table 2.2

Examples of Hydrophobic and Hydrophilic Substances

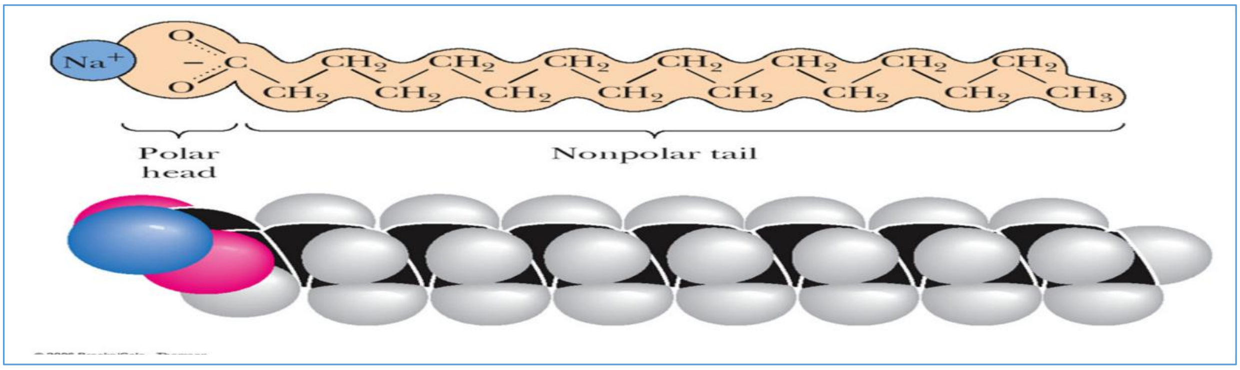
Hydrophilic	Hydrophobic
Polar covalent compounds [e.g., alcohols such as C_2H_5OH (ethanol) and ketones such as $(CH_3)_2C{=}O$ (acetone)]	Nonpolar covalent compounds [e.g., hydrocarbons such as C_6H_{14} (hexane)]
Sugars	Fatty acids, cholesterol
Ionic compounds (e.g., KCl)	
Amino acids, phosphate esters	

•*what happen when we put a drop of oil in the water*?

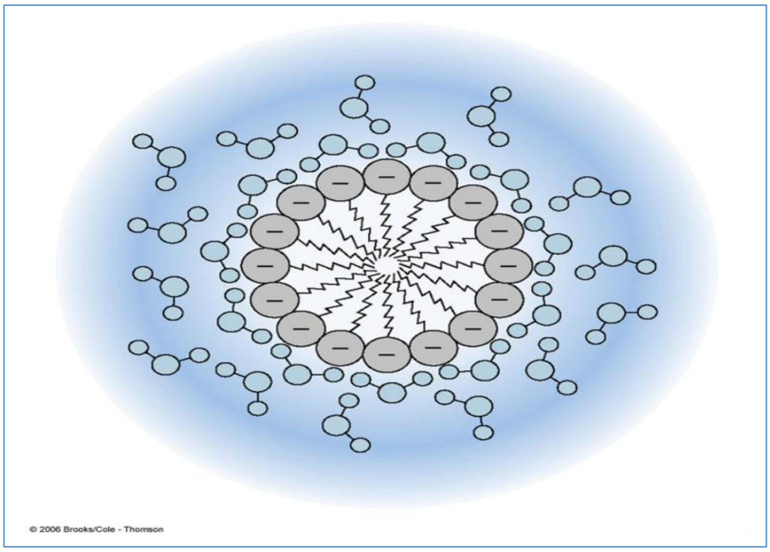
✔ **Amphipathic molecule** ➡A single molecule have both polar (hydrophilic) and nonpolar (hydrophobic) portions .

- ➢ long-chain fatty acid having a polar carboxylic acid group and a long nonpolar hydrocarbon portion is a prime example of an amphipathic substance.

•Amphiphilic molecules are frequently symbolized by a ball and zigzag line structure,•〰, where the **ball** represents the hydrophilic polar head and the **zigzag line** represents the nonpolar hydrophobic hydrocarbon tail.



•A typical micelle in aqueous solution forms an aggregate with **the hydrophilic head regions** in contact with surrounding solvent, sequestering the **hydrophobic single-tail regions** in the micelle centre.


© 2006 Brooks/Cole - Thomson

- ➢ Micelle formation by amphipathic molecules in aqueous solution. When micelles form, the ionized polar groups are in contact with the water, and the nonpolar parts of the molecule are protected from contact with the water.

•Both the melting point and the boiling point of water are significantly higher than would be predicted for a molecule of about the same molecular weight , study this table :

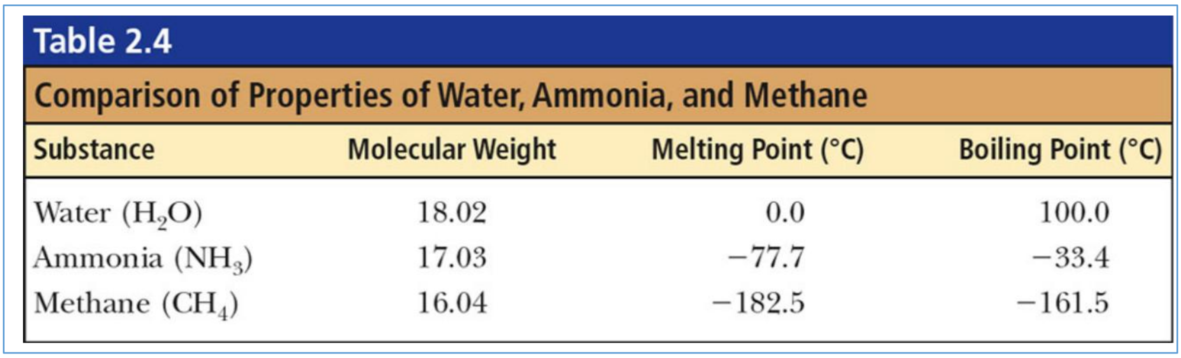


Table 2.4

Comparison of Properties of Water, Ammonia, and Methane

Substance	Molecular Weight	Melting Point (°C)	Boiling Point (°C)
Water (H_2O)	18.02	0.0	100.0
Ammonia (NH_3)	17.03	−77.7	−33.4
Methane (CH_4)	16.04	−182.5	−161.5

- ➢ Methane and ammonia(Gases), have **much lower melting and boiling points**,why?
  - ▪ The forces of attraction between the molecules of these substances are weaker than the attraction between water molecules, because of the number and strength of their hydrogen bonds .

Biologically Important Hydrogen Bonds

Hydrogen bonds have a vital involvement in stabilizing the 3D structures of biologically important molecules, including:

- ✓ **DNA** ➡The hydrogen bonds between complementary bases are one of the most striking characteristics of the double-helical structure of DNA
- ✓ **RNA** ➡Transfer RNA also has a complex 3D structure characterized by hydrogen-bonded regions
- ✓ **Proteins** ➡Hydrogen bonding in proteins gives rise to two important structures, the a-helix and b-pleated sheet conformations. Both types of conformation are widely encountered in proteins

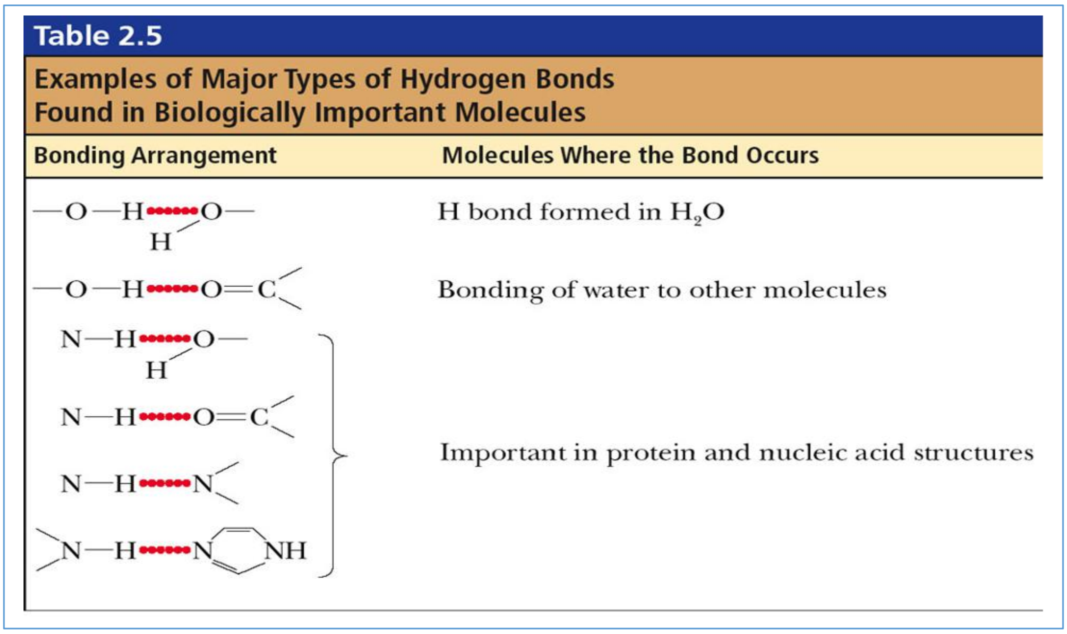
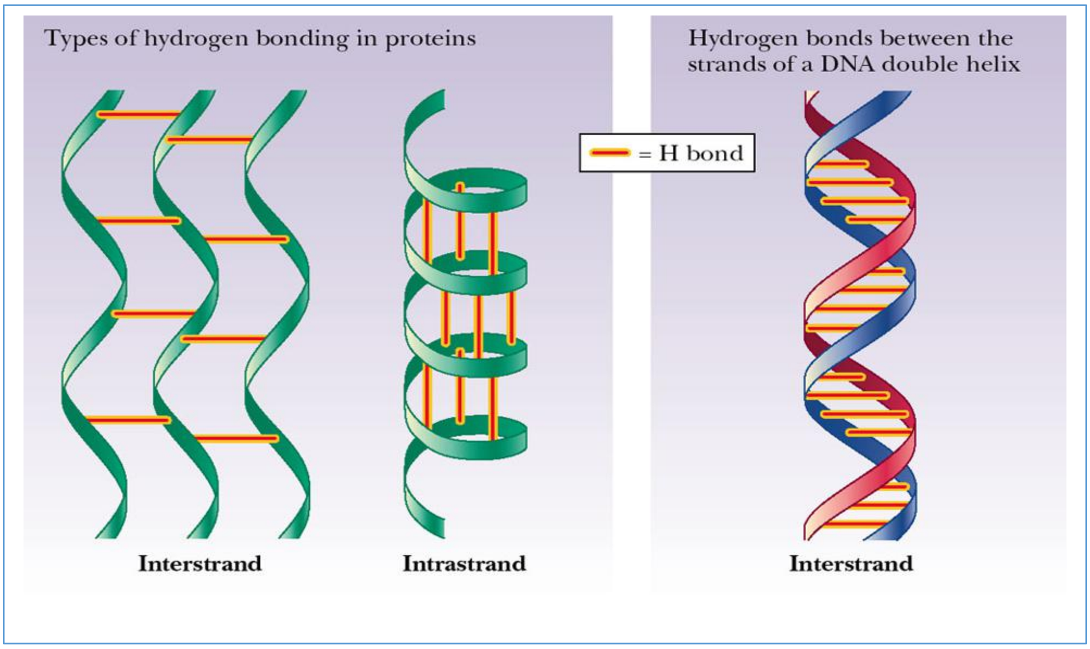


Table 2.5

Examples of Major Types of Hydrogen Bonds Found in Biologically Important Molecules

Bonding Arrangement	Molecules Where the Bond Occurs
—O—H••••••O— (H)	H bond formed in H_2O
—O—H••••••O=C<	Bonding of water to other molecules
N—H••••••O— (H)	Important in protein and nucleic acid structures
N—H••••••O=C<	
N—H••••••N<	
>N—H••••••N (ring) NH	



Acids,Bases and pH

•The biochemical behavior of many important compounds depends on their acid–base properties.

What are acids and bases?

A biologically useful definition of an acid & a base :

✔Acid ➡is a molecule that acts as a proton (hydro gen ion) donor.

✔Base➡is a molecule that acts as a proton acceptor.

Acids

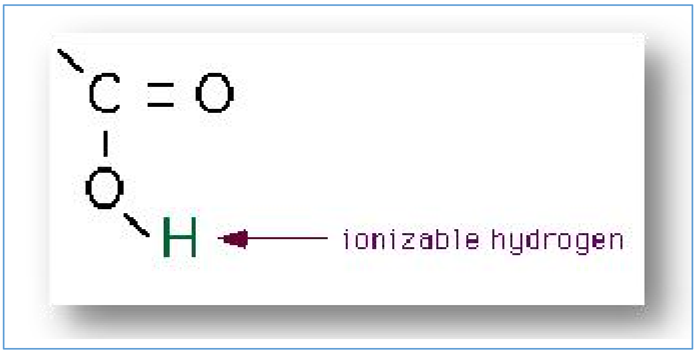
•***Properties*** :

1. Acids are **proton** (hydrogen ion, H^+) **donors** .
2. Acids have a pH **lower than 7**.
3. Acids taste **sour**.
4. Acids effect indicators:
   - Blue litmus turns red
   - Methyl orange turns red
5. Acids react with active metals, producing H_2.
6. Acids react with carbonates .
7. Acids neutralize bases.

•Strong and Weak acids

	Strong Acids	Weak Acids
Ionization	Assumed to be 100% ionized in solution (good H^+ donors).	less than 5% ionized in solution (poor H^+ donors).
Examples	HCL , H_2SO_4 , HNO_3	H_3PO_4 ,$HC_2H_3O_2$, Organic Acids

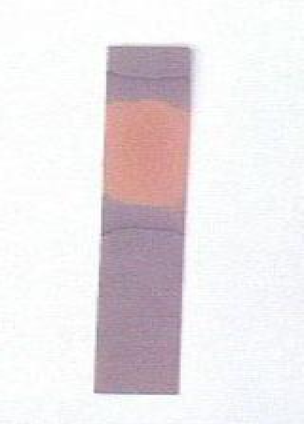
•Organic acids are weak acids. Some are used as flavoring agents in food. The carboxyl group is a poor proton donor, so all organic acids are weak acids.



✔e.g.(مهم)

- **Citric acid** in citrus fruit .
- **Malic acid** in sour apples .
- **Lactic acid** in sour milk and sore muscles .
- **Butyric acid** in rancid butter.
- **Acetic acid** is the acid ingredient in vinegar.

•Effect of acids on indicators (مهم):

Blue litmus paper	Methyl orange
Turns **red** in contact with an acid. 	Turns **red** with addition of an acid 

•*Reaction of acids with active metals*

Acids react with active metals to form salts and hydrogen gas.

- e.g.

$Mg + 2HCl \rightarrow MgCl_2 + H_{2(g)}$

$Zn + 2HCl \rightarrow ZnCl_2 + H_{2(g)}$

$Mg + H_2SO_4 \rightarrow MgSO_4 + H_{2(g)}$

•*Reaction of acids with carbonates*

$$2HC_2H_3O_2\text{(acetic acid)} + Na_2CO_3$$

$$\downarrow$$

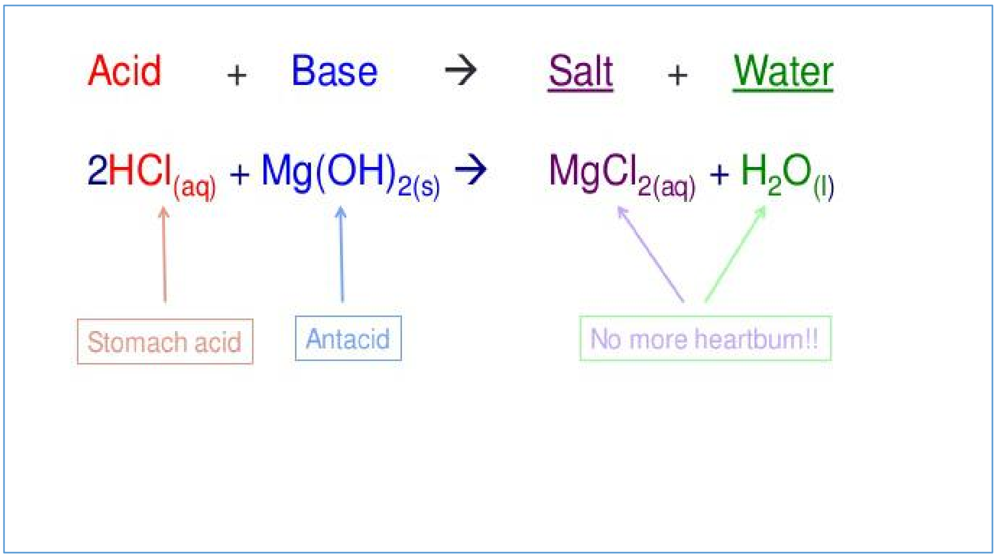
$$2NaC_2H_3O_2 + H_2O + CO_2$$

Best example on this ; effect of acid rain on marble .

•Reaction of acids& bases(Neutralization)

Neutralization reactions always produce ***Salt&Water*** .

- This is the basic principle by which antacids work in treatment of heartburn .



GOOD TO KNOW

•Urine pH

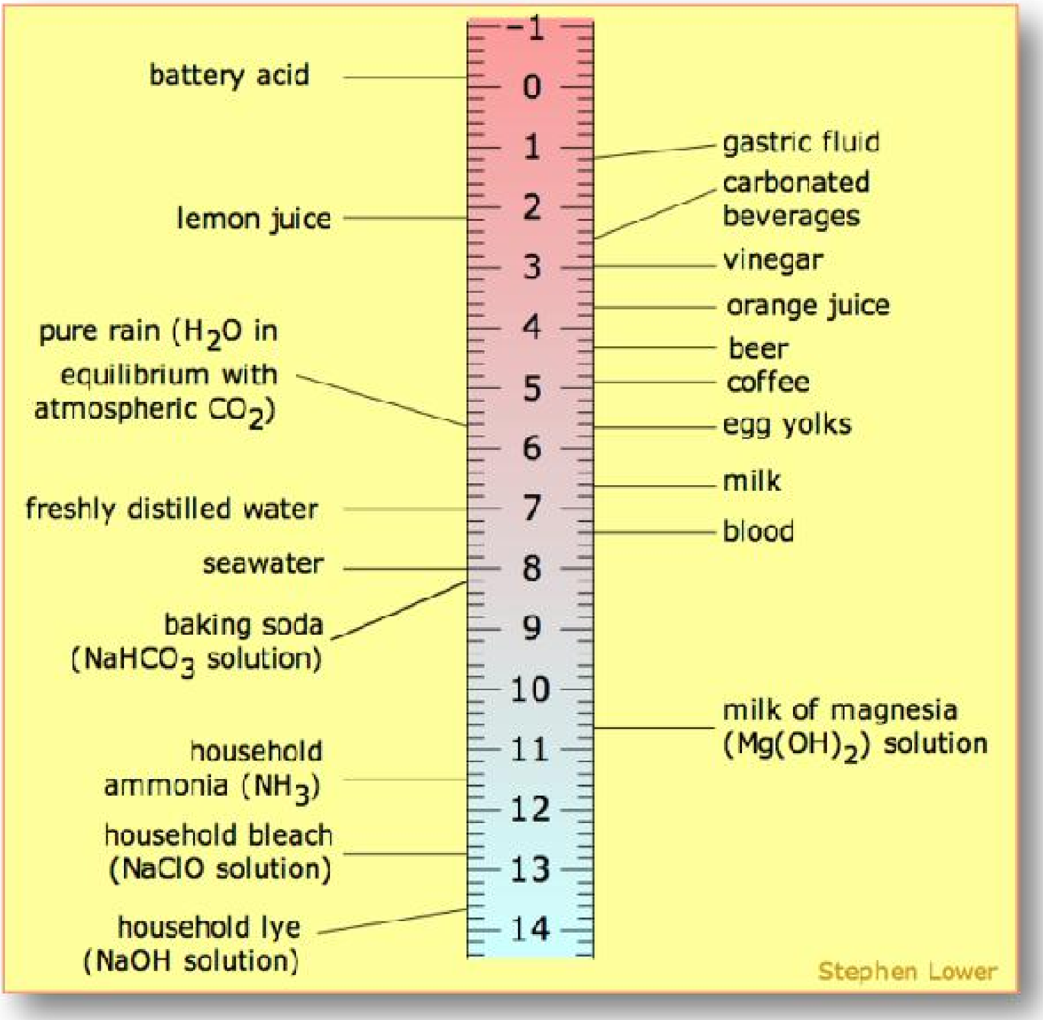
Depending on the person’s acid-base status , the pH of urine may range from 4.5-8 . Kidneys maintain normal acid-base balance primarily through the reabsorption of Na^+ and tubular secretion of hydrogen & amonium ions .

✔Acidic Urine ▸ As the amount of Na+ and excess acid retained by the body increases .

Occurs in ➡ Acidosis , Uncontrolled Diabetes , Respiratory diseases ,etc.

✔Alkaline Urine ▶ occurs in : Urinary tract obstruction ,Salicylate intoxication,Renal tubular acidosis , chronic renal failure , etc .

•Always remember ➡Blood pH : 7.35-7.45



Aqua Regia (King' water)

A mixture of Nitric Acid & Hydrochloric Acid , yellow-orange fuming liquid , so called by alchemists because of its ability to dissolve the nobel metals gold&platinum .

Bases

- ***Properties*** **:**

1. Bases are **proton** (hydrogen ion, H^+) **acceptors** .
2. Bases have a pH greater than 7 .
3. Bases taste bitter .
4. Bases effect indicators
   - Red litmus turns blue
   - Phenolphthalein turns purple
5. Solutions of bases feel slippery.
6. Bases neutralize acids.

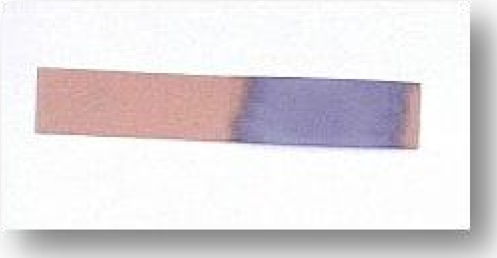
- **Ex:**

✓ Sodium hydroxide (**lye**), NaOH
✓ Potassium hydroxide, KOH
✓ Magnesium hydroxide, $Mg(OH)_2$
✓ Calcium hydroxide (**lime**), $Ca(OH)_2$

OH^- (hydroxide) in base combines with H^+ in acids to form **water**

$$H^+ + OH^- \rightarrow H2O$$

- *Effect of bases on indicators*

Red litmus paper	Phenolphthalein
Turns blue in contact with a base. 	**Turns bright pink in a base.** 

•*Bases Neutralize Acids*

Milk of Magnesia contains magnesium hydroxide, $Mg(OH)_2$, which neutralizes stomach acid, HCl.

$$2\ HCl + Mg(OH)_2 \longrightarrow MgCl_2 + 2\ H_2O$$

✔An acid and a base combine to make a salt and water. A salt is any ionic compound that could be made with the *anion of an acid* and the *cation of a base*. The hydrogen ion of the acid and the hydroxide ion of the base unite *to form water*.

•Strong bases that dissolve in water well, such as **sodium or potassium lye** are very dangerous because a great amount of the structural material of human beings is *made of protein*.

•Most of the alkaline organic compounds (and some inorganic materials) have an amino group **-(NH2)** rather than an ionizable hydroxyl group. By the Lowry- Bronsted definition, an amino group definitely acts as a base, and the effect of removing hydrogen ions from water molecules is the same as adding hydroxide ions to the solution.

Strong Acids & Strong Bases

Strong Acids	Strong Bases
✓ **HNO3** - nitric acid	✓ **LiOH**- lithium hydroxide
✓ **HCl** - hydrochloric acid	✓ **NaOH** - sodium hydroxide
✓ **H_2SO_4** - sulfuric acid ,**the only one that is diprotic.**	✓ **KOH** - potassium hydroxide
✓ **$HClO_4$** - perchloric acid	✓ **RbOH** - rubidium hydroxide
✓ **HBr** - hydrobromic acid	✓ **CsOH** - cesium hydroxide
✓ **HI** - hydroiodic acid	✓ **$Ba(OH)_2$**-bariumhydroxide, **the only dibasic strong base**

Weak Acids & Weak Bases

	Weak Acid	Weak Base
Chemical Equation	$HA \rightarrow A^- + H^+$	$XOH \rightarrow (OH)^- + X^+$
equilibrium expression	$k_A = \frac{[H^+][A^-]}{[HA]}$	$k_B = \frac{[X^+][OH^-]}{[XOH]}$

- There are four perfectly correct ways to write the H-OH equation :

$$pH = pk_A - \log \frac{[HA]}{[A^-]}$$

or

$$pH = pk_A + \log \frac{[A^-]}{[HA]}$$

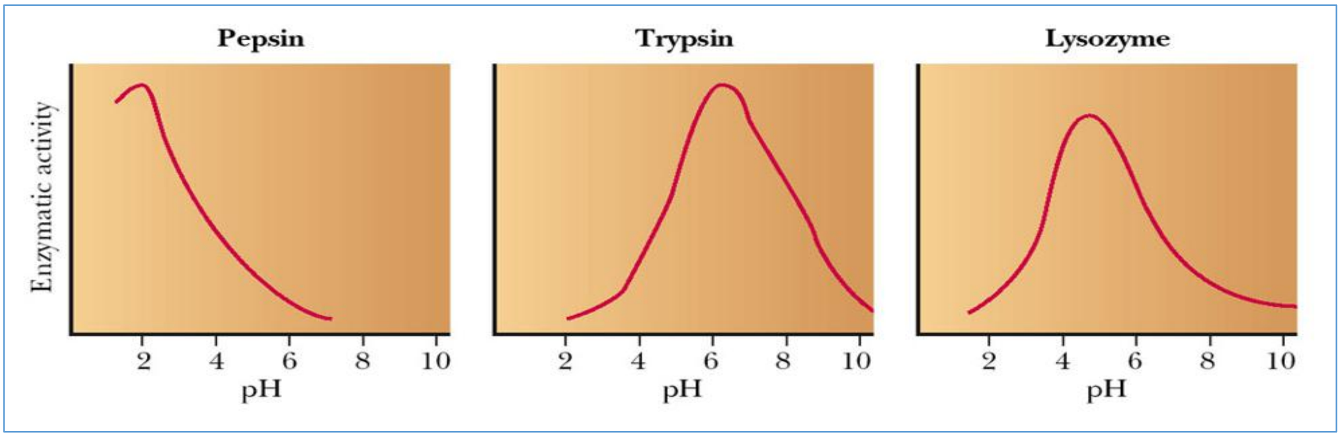
or

$$pOH = pk_B + \log \frac{[\text{conjugate cation}]}{[\text{base}]}$$

or

$$pOH = pk_B - \log \frac{[\text{base}]}{[\text{conjugate cation}]}$$

- *<u>Effect of pH on enzymatic activity</u>* (مهم)

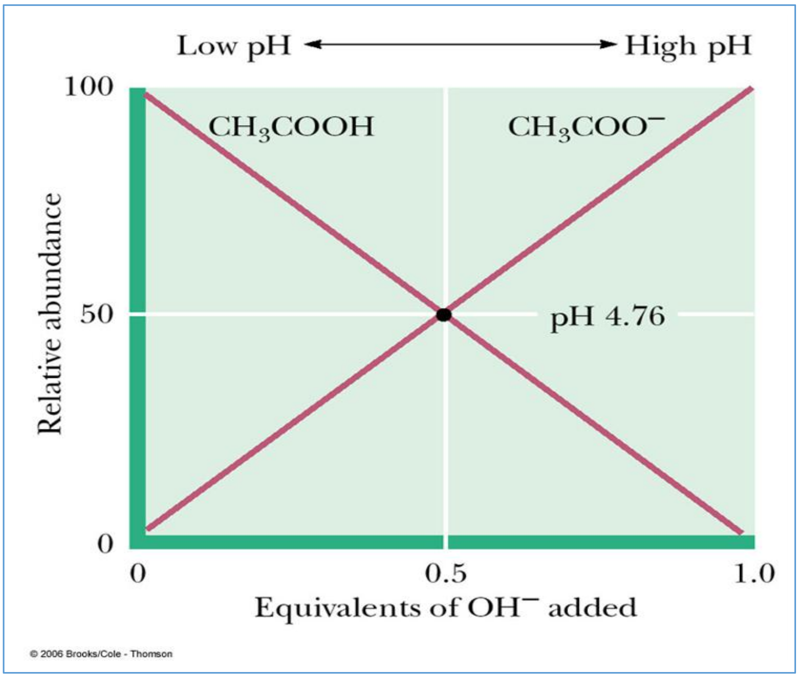


- Pepsin, trypsin, and lysozyme all have steep pH optimum curves.
  - ✓ Pepsin ➡ **2-2.5**
  - ✓ Lysozyme ➡ **6-7** (avg:5)
  - ✓ Trypsin ➡**7.8-8.7**

Titration Curves

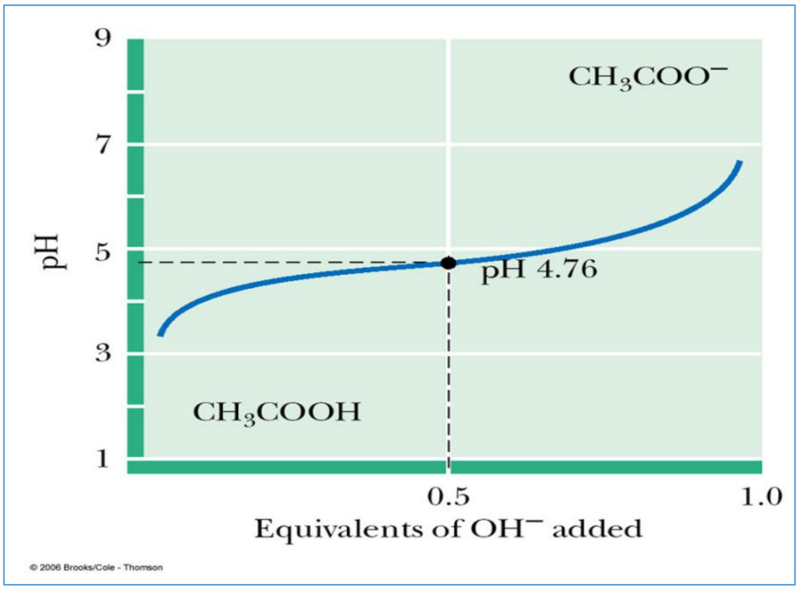
- Titration is an experiment in which measured amount of ***base*** is added to measure amount of ***acid*** .
- In aqueous solution, the relative concentrations of a weak acid and its conjugate base can be related to the titration curve of the acid.

- This figure shows titration curve for acetic acid :



© 2006 Brooks/Cole - Thomson

- If the pH is monitored as base is added to a sample of acetic acid in the course of a titration, an inflection point in the titration curve is reached when the pH equals the pKa of acetic acid



© 2006 Brooks/Cole - Thomson

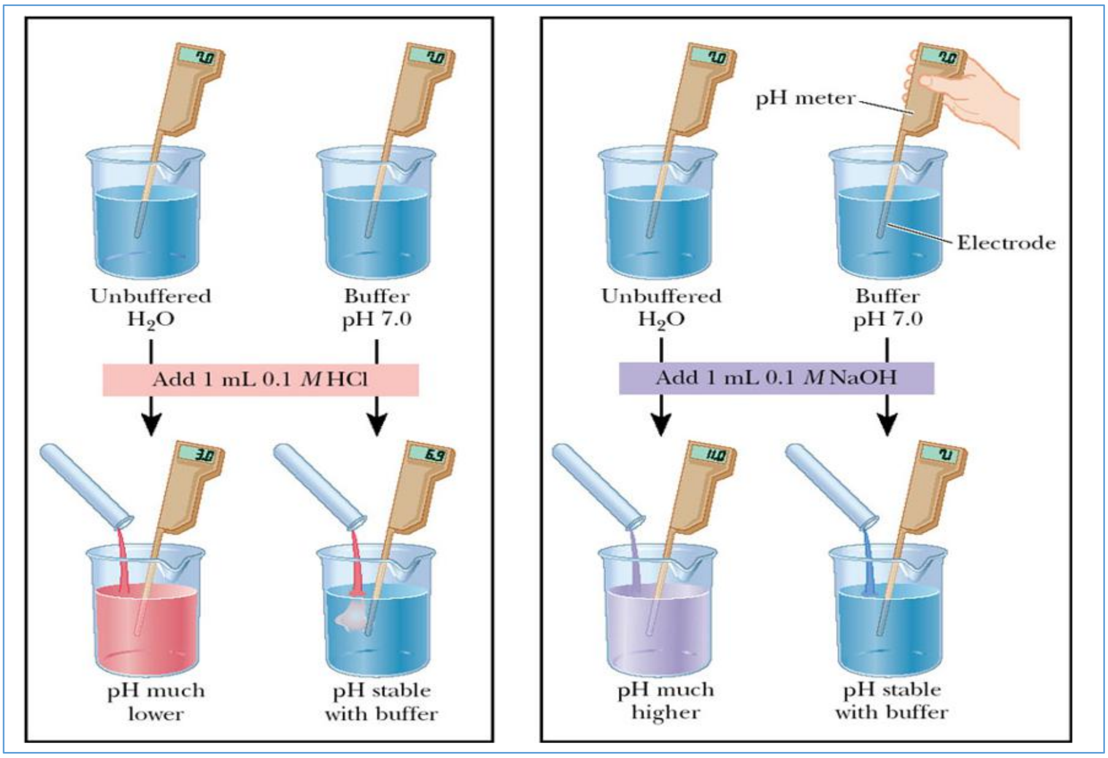
- The pH at the inflection point is 4.76, which is the pKa of acetic acid. The inflection point occurs when 0.5 mol of base has been added for each mole of acid present.
- Near the inflection point, the pH changes very little as more base is added.
- The point in the titration at which the acid is exactly neutralized is called the equivalence point.
- (In our example)When 1 mol of base has been added for each mole of acid, the equivalence point is reached, and essentially all the acetic acid has been converted to acetate ion(Its conjugate base).
- The acid (acetic acid) is progressively converted to its conjugate base (acetate ion) as more NaOH is added and the titration proceeds.

Buffers

•In terms of acid and base chemistry, a buffer solution tends to resist change in pH when small to moderate amounts of a strong acid or strong base are added.

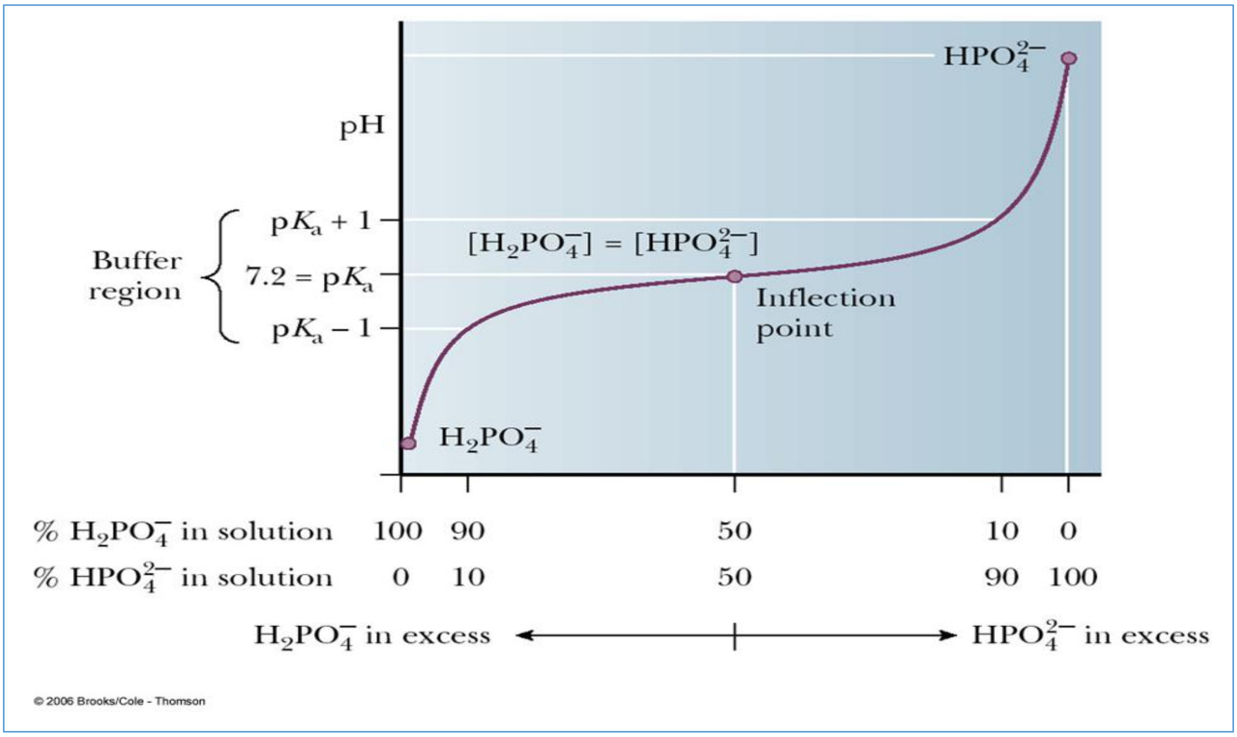
•A buffer solution consists of a mixture of a weak acid and its conjugate base.The control of pH by buffers depends on the fact that their compositions reflect the acid/base concentration ratio in the region of the titration curve in which there is little change in pH.

•How do buffers work ?



- In the previous figure ➞Acid is added to the two beakers on the left. The pH of unbuffered H2O drops dramatically while that of the buffer remains stable. Base is added to the two beakers on the right. The pH of the unbuffered water rises drastically while that of the buffer remains stable.

•*The relationship between the titration curve and buffering action in* ***$H_2PO_4^-$***



© 2006 Brooks/Cole - Thomson

- The titration curve of **$H_2PO_4^-$**, showing the buffer region for the **$H_2PO_4^-/HPO_4^{2-}$** pair.

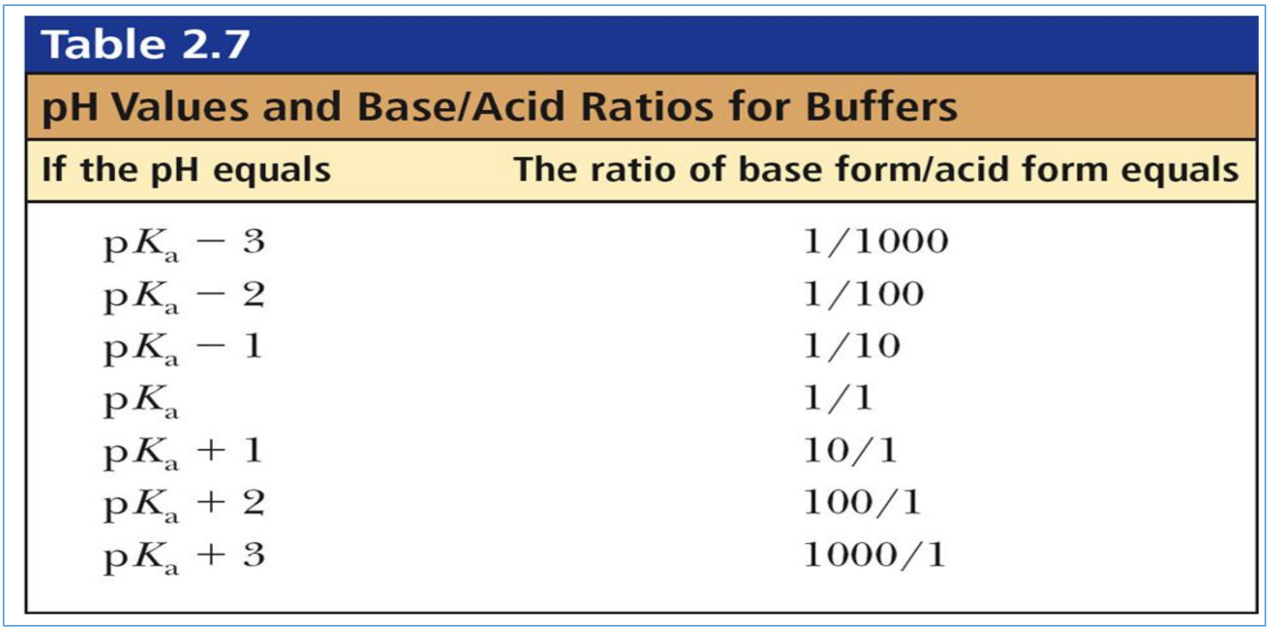


Table 2.7

pH Values and Base/Acid Ratios for Buffers

If the pH equals	The ratio of base form/acid form equals
$pK_a - 3$	1/1000
$pK_a - 2$	1/100
$pK_a - 1$	1/10
pK_a	1/1
$pK_a + 1$	10/1
$pK_a + 2$	100/1
$pK_a + 3$	1000/1

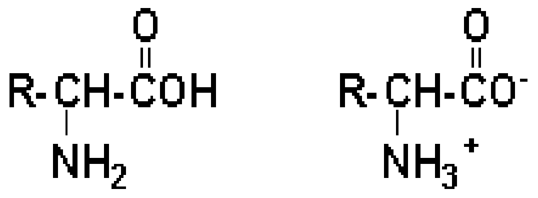
- Imp : Maximum buffering capacity for buffers is when the ratio of base for/acid form ➞ 1/1

Amino Acids & Peptides

- ✓ Amino acids are molecules containing an **amine group**(NH_2), a **carboxylic acid group**(R-C=O-OH) and a **side-chain**(usually denoted as R) that varies between different amino acids.
- ✓ Composed from **α-carbon** to which Hydrogen atom ,R-group, **α-amino group** and **α-carboxyl group** are attached.
- ✓ The key elements of an amino acid are :
  - Carbon.
  - Hydrogen.
  - Oxygen .
  - Nitrogen.
- ✓ **α-Amino acid**: A molecule containing an **amino** group and a carboxylic **acid** group that are separated by one carbon, called the **α**-carbon.

> - ➢ In biochemistry there are **twenty standard amino acids** which differ in the makeup of the side chain (R group) **attached to the α-carbon.**
> - ➢ **Only α-amino acids are physiologically important ,used by cells to synthesize proteins.**

- ✓ Although amino acids are commonly written in the unionized form, they are more properly written in **the zwitterion form**:



$$R\text{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{-}\overset{\displaystyle O}{\overset{\|}{C}}OH \qquad R\text{-}\underset{\displaystyle NH_3^+}{\underset{|}{CH}}\text{-}\overset{\displaystyle O}{\overset{\|}{C}}O^-$$

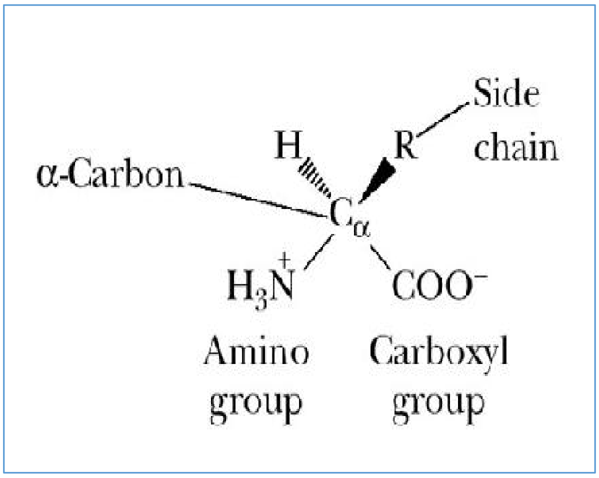
unionized form

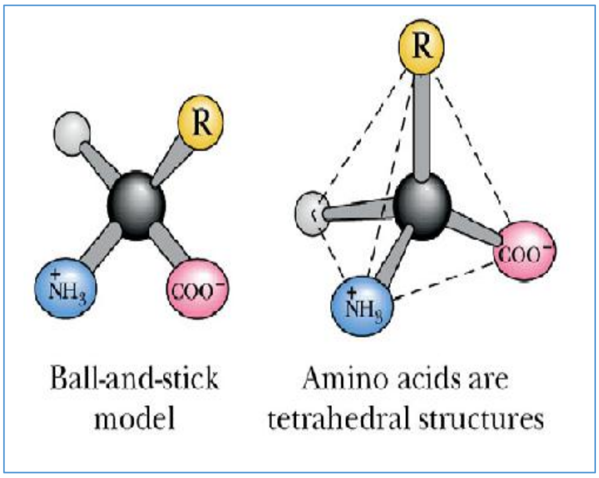
zwitterion

A molecule with both –ve (COO^-) and +ve($NH3^+$) charges .

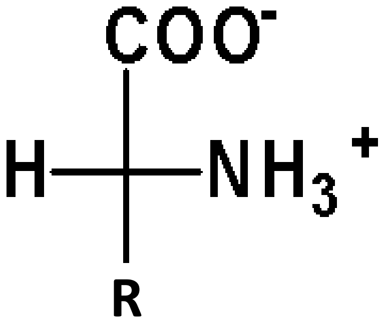
- ✓ With the exception of **glycine**, all protein-derived amino acids **are chiral** and have at least one asymmetric (chiral) carbon (normally the α-carbon).

✓ Representation of 3D shape of an aa :





•By Fischer projections (imp):

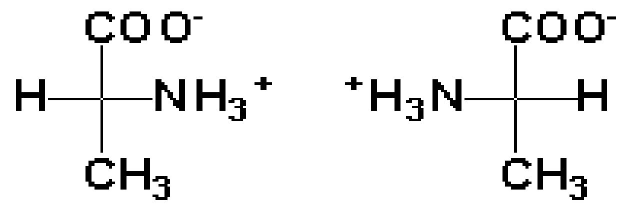


✓ ***Chirality of Amino Acids***

Chiral amino acids have two *stereoisomers* (=optical isomers=**different spatial arrangement of atoms around chiral carbon**)

The mirror-image stereoisomers are called enantiomers.Two forms of enantiomers : L- form & D- form .

e.g.➡



D-Alanine L-Alanine

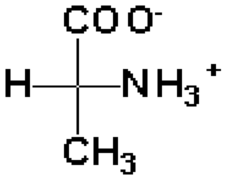
(Fischer projections)

<**The majority of amino acids have the L-configuration at the α-carbon**>

•**Naturally occuring amino acids are those of L-configuration ,whereas naturally occuring sugars are those of D-configuration .**

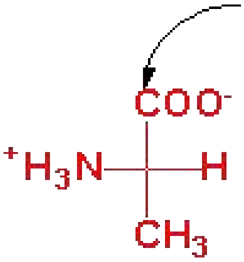
•**Imp** : In most cases, ***D-amino acids*** are toxic .They occur in nature in **antibiotics** and **bacterial cell walls**.

•Comparison of the stereochemistry (3D structure) of **alanine** and **glyceraldehyde** :



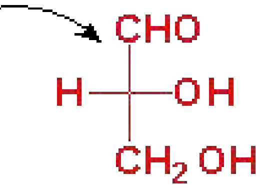
D-Alanine

the naturally occurring form

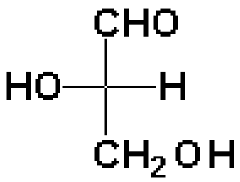


L-Alanine

the naturally occurring form



D-Glyceraldehyde



L-Glyceraldehyde

Amino Acids & Classification

Note :You must be familiar with the 20 amino acids , and all of their classifications (You won't be asked to memorize the structure .However,you will memorize the classifications based on the structure)

The 20 standard amino acids found in protein structure are divided into seven distinct groups.

1.Aliphatic amino acids:

2.Hydroxyl group containing amino acids:

3.Sulfur containing amino acids:

4.Acidic amino acids and their amides:

5.Basic amino acids:

6.Aromatic amino acids:

7.Imino acids:

- **Aliphatic aa** have R-group with hydrocarbon chain.
- **Aromatic aa** have benzene ring in the R-group.

- aa can be abbreviated in two ways : 3-letter(which is you must memorize) & 1-letter ➡See the table below :

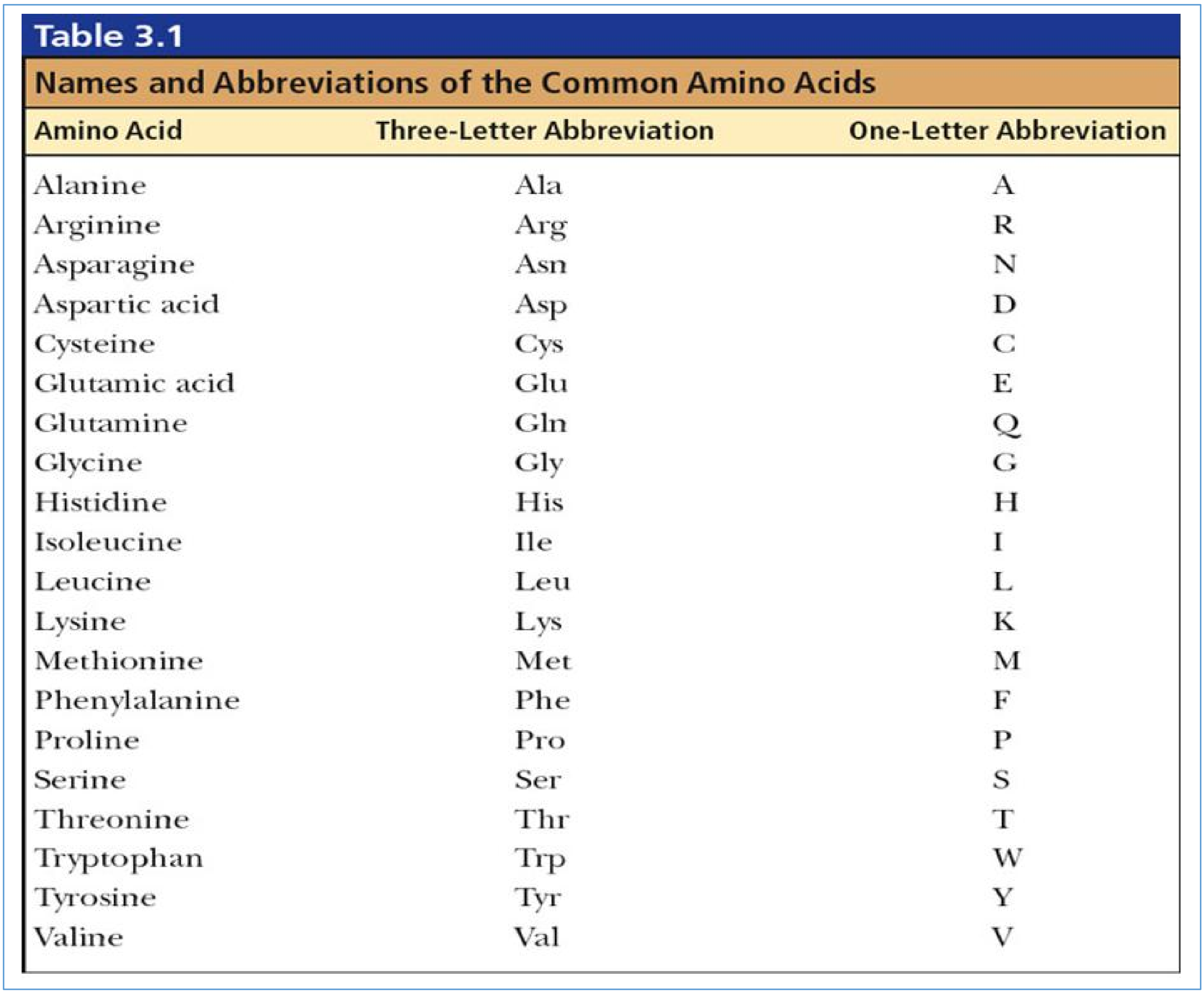


Table 3.1

Names and Abbreviations of the Common Amino Acids

Amino Acid	Three-Letter Abbreviation	One-Letter Abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

•**Aliphatic** aa (**GAVLI**) :

Glycine , **A**lanine , **V**aline , **L**eucine , **I**soleucine .

•**Hydroxyl-containing** aa :

Serine , **Thr**eonine , **Tyr**osine

•**Sulfur-containing** aa :

Cysteine , **Met**hionine

•**Acidic** aa (**A**CID kills **Ag**riculture) :

Aspartate , **Glu**tamate

•**Basic** aa :

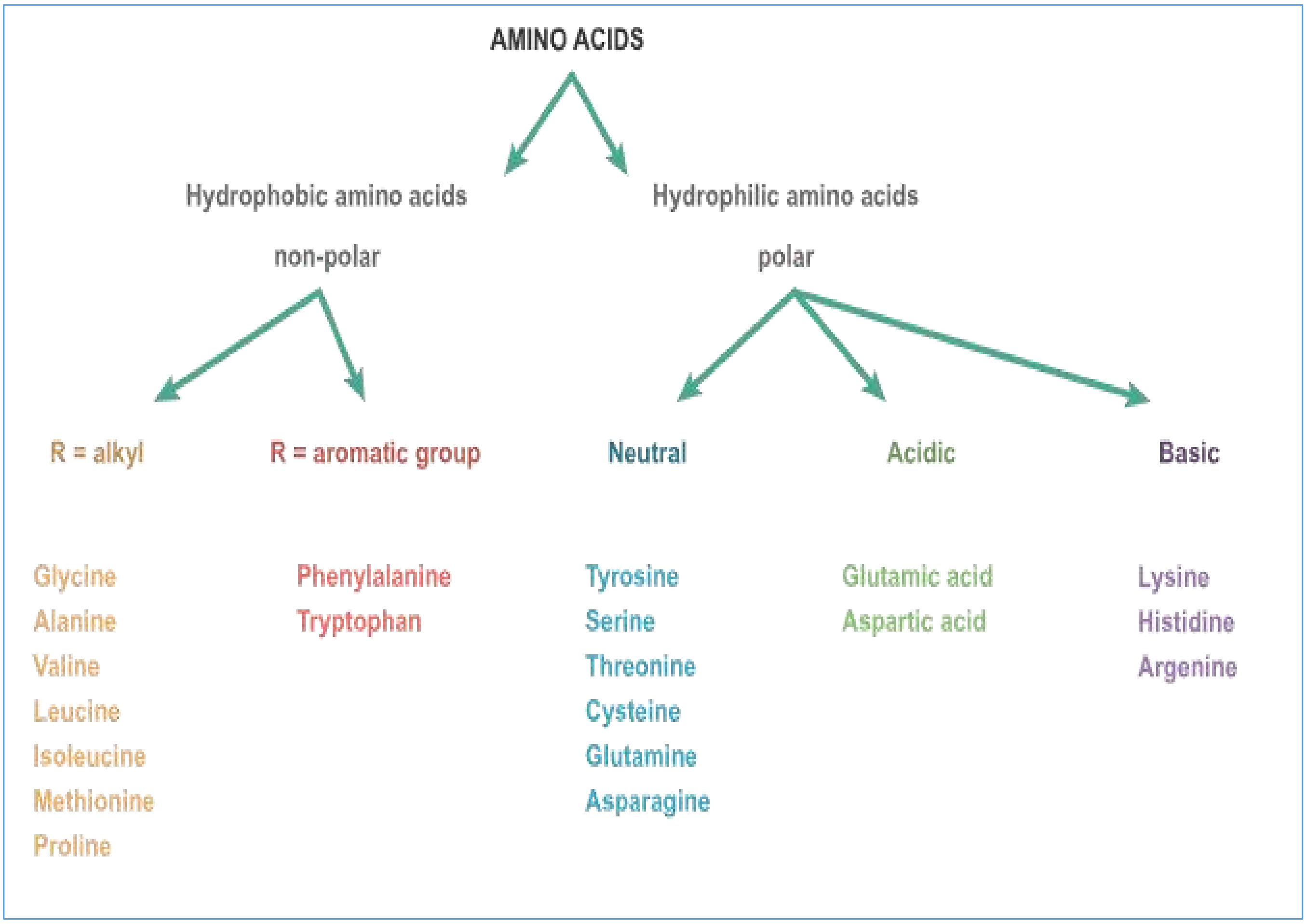
Histidine , **Arg**inine , **Lys**ine

•**Aromatic** aa :

Phenylalanine , **Tryp**tophan , **Tyr**osine

•**Imino** aa :

Proline



Source :*www.khanacademy.org*

•Notice these structural features :(imp)

- ✓ For 19 of the 20, the -amino group is primary; for proline, it has secondary amine (called imino acid).
- ✓ With the exception of **glycine**, amino acids are chiral.
- ✓ Glycine is the **smallest** amino acid has no D-/L- isomers .
- ✓ Isoleucine and threonine contain a **second chiral carbon** .
- ✓ Chiral amino acids are optically active ; meaning that they have L-&D-configurations .

•Essential & Non-essential amino acids

ESSENTIAL AMINO ACIDS

VERSUS

NONESSENTIAL AMINO ACIDS

ESSENTIAL AMINO ACIDS	NONESSENTIAL AMINO ACIDS
Essential amino acids cannot be synthesised by the human body	Nonessential amino acids can be synthesised by the human body
Must be obtained from the daily diet	Can be synthesized by the human body
Known as indispensable amino acids	Known as dispensable amino acids
Adults cannot synthesise 9 amino acids	Adults can synthesise 11 amino acids

Essential aa mnemonic :

PVT Tim Hall

Phenylalanine
Valine
Threonine
Tryptophan
Isoleucine
Methionine
Histidine
Arginine
Leucine
Lysine

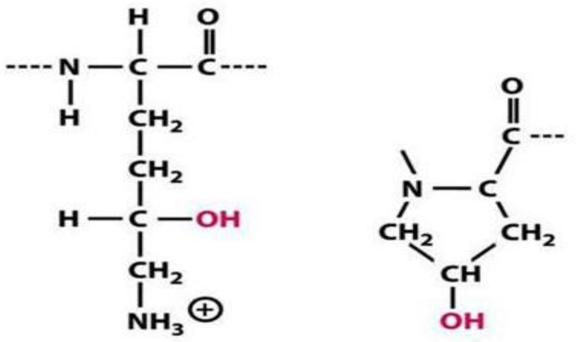
- ➢ **His & Arg are essential only in children .**

•***Modified Amino Acids***

Formed by modification of one of the 20 amino acids *after synthesis of proteins* (**Post-Translational Modification**)

(1) **Hydroxyproline** & **Hydroxylysine**

Proline and Lysine are hydroxylated enzymatically after translation to form hydroxyproline&hydroxylysine which are important in collagen synthesis

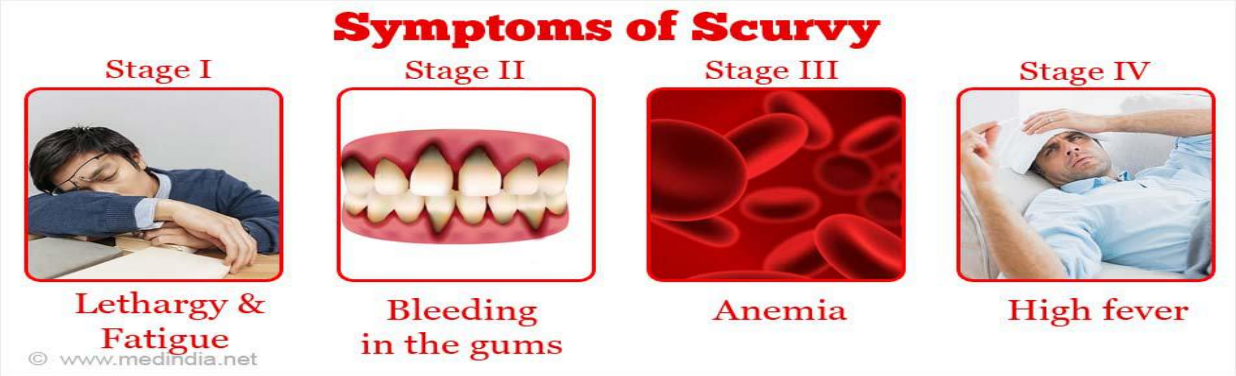


hydroxylysine in protein **hydroxyproline in protein**

✓ Both hydroxyproline and hydroxylysine are formed via the ***enzyme-catalyzed oxidations*** of the proline and lysine amino acid side chains, which occur after the collagen polypeptide has been synthesized. These enzymatic reactions *require* **ascorbic acid** (**vitamin** C) as a cofactor **.**

Scurvy is a disease caused by severe and chronic vitamin C (ascorbic acid) deficiency. Most people think of scurvy as a disease of the past, when sailors had to spend months at sea without access to fresh fruit and vegetables. While scurvy may be uncommon in modern society, it does still exist. Anyone whose diet is inadequate in vitamin C is at risk.

The onset of symptoms of scurvy depends on how long it takes for the person to use up their limited stores of vitamin C. The human body is unable to make vitamin C. For example, if the diet includes no vitamin C at all, the average onset of symptoms is about four weeks.

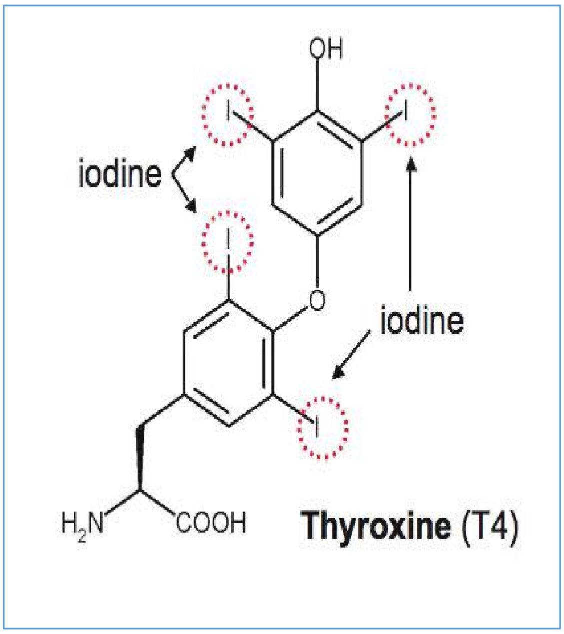


.

(2) **Thyroxine** -T4-

✓ Only in the Thyroid Gland

✓ The main hormone secreted into the bloodstream by the thyroid gland. It plays vital roles in *digestion*, *heart* and *muscle* function, *brain development* and *maintenance of bones*.



Thyrotoxicosis is the condition that occurs due to excessive production of thyroid hormone by the thyroid gland.
Signs and symptoms vary between people and may include :
irritability, **muscle weakness**, **sleeping problems**, a **fast heartbeat**, **heat intolerance**, **diarrhea**, **enlargement of the thyroid**, **hand tremor**, and **weight loss**.

- ✓ ***Ornithine*** is one of the three amino acids involved in the **Urea cycle**(Will be discussed in details in Biochemistry 102), alongside L-**Arginine** and L-**Citrulline**; this amino acids appears to reduce elevated amonia levels when supplemented.
- ✓ ***Ornithine*** is a **nonprotein** amino acid (not used to create proteins) .

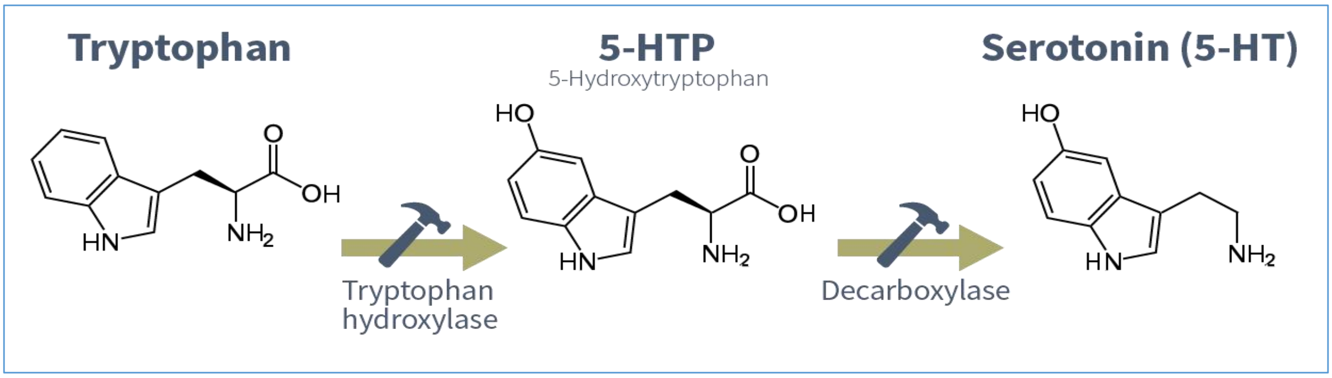
Ammonia Test

It's a simple blood test that lets your doctor measure how much ammonia is in your blood. Bacteria in your gut and in your cells create ammonia when your body breaks down protein.

Ammonia is a waste product. Your liver turns ammonia into a chemical called urea. This chemical is water-soluble . It leaves your body in your urine. But if you have certain health conditions, like kidney or liver failure, your body can't make or get rid of urea. In either case, ammonia builds up. This can cause a number of problems, like confusion, extreme tiredness, and in some cases, coma or even death.

Amino Acids & Neurotransmitters

(1) Trp & Serotonin



- ✓ The high concentration of tryptophan **in milk** protein may mildly elevate the levels of serotonin, which relaxes the brain.
- ✓ Serotonin is regarded as a chemical that is responsible for maintaining mood balance, and that a deficit of serotonin leads to depression.
- ✓ The **tryptophan** in milk might make you sleepy, whereas the **tyramine** in cheese should wake you up.

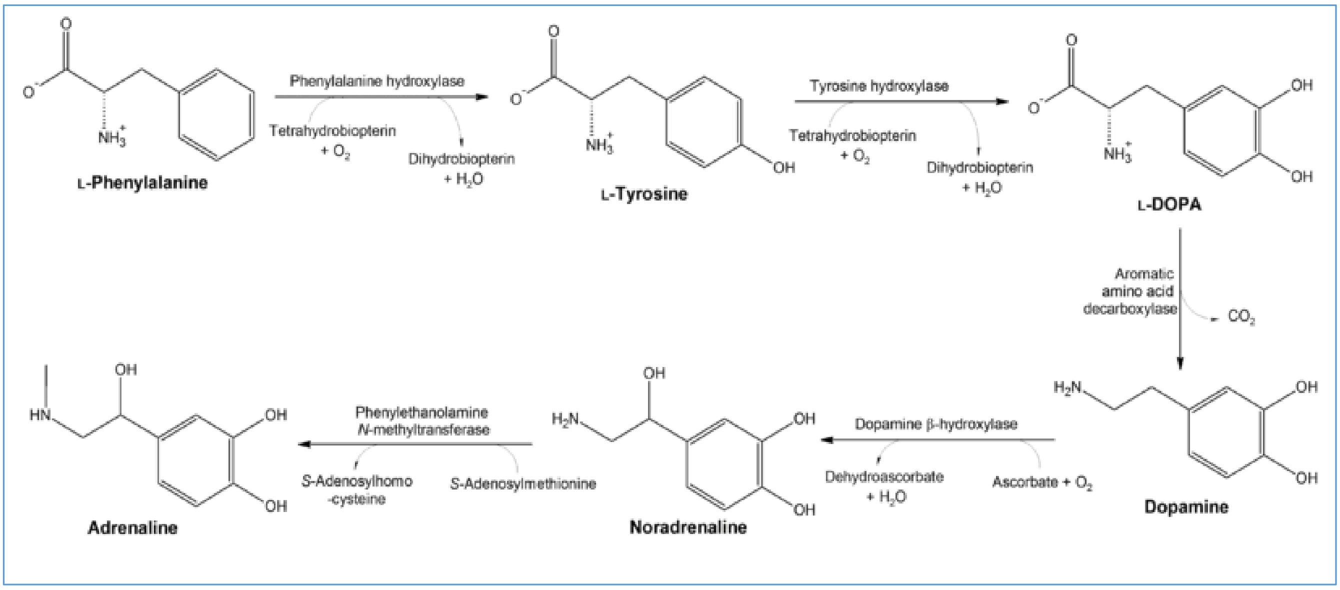
(2) Phe

Phenylalanine is a precursor for :

a) **Tyrosine**.

b) The **monoamine neurotransmitters** :
Dopamine, **Norepinephrine** (noradrenaline), and **Epinephrine** (adrenaline).

c) The skin pigment **melanin**.



Phenylketonuria (**PKU**) is an inborn error of metabolism that results in decreased metabolism of the amino acid phenylalanine.
The enzyme **phenylalanine hydroxylase** normally converts the amino acid phenylalanine into the amino acid tyrosine. If this reaction does not take place(In case of PKU), phenylalanine accumulates and tyrosine is deficient. Excessive phenylalanine can be metabolized into phenylketones.
Metabolites include **phenylacetate, phenylpyruvate and phenethylamine**. Elevated levels of phenylalanine in the blood and detection of phenylketones in the urine is diagnostic, however most patients are diagnosed via newborn screening.

Tyrosine (which is normally a non-essential amino acid) , in patients of PKU ,Tyrosine becomes essential meaning it must be provided by diet .

(PKU will be discussed later in details)

✓ Parkinson's disease

PD is a neurodegenerative disorder that affects predominately dopamine-producing ("dopaminergic") neurons in a specific area of the brain called substantia nigra.

Levodopa, the most effective Parkinson's disease medication, is a natural chemical that passes into your brain and is converted to dopamine.

As we previously mentioned , in a free amino acid, the carboxyl group and amino group of the general structure are charged at neutral pH—the carboxylate portion negatively and the amino group positively. Amino acids without charged groups on their side chains exist in neutral solution as zwitterions with no net charge. A zwitterion has equal positive and negative charges; in solution, it is electrically neutral.

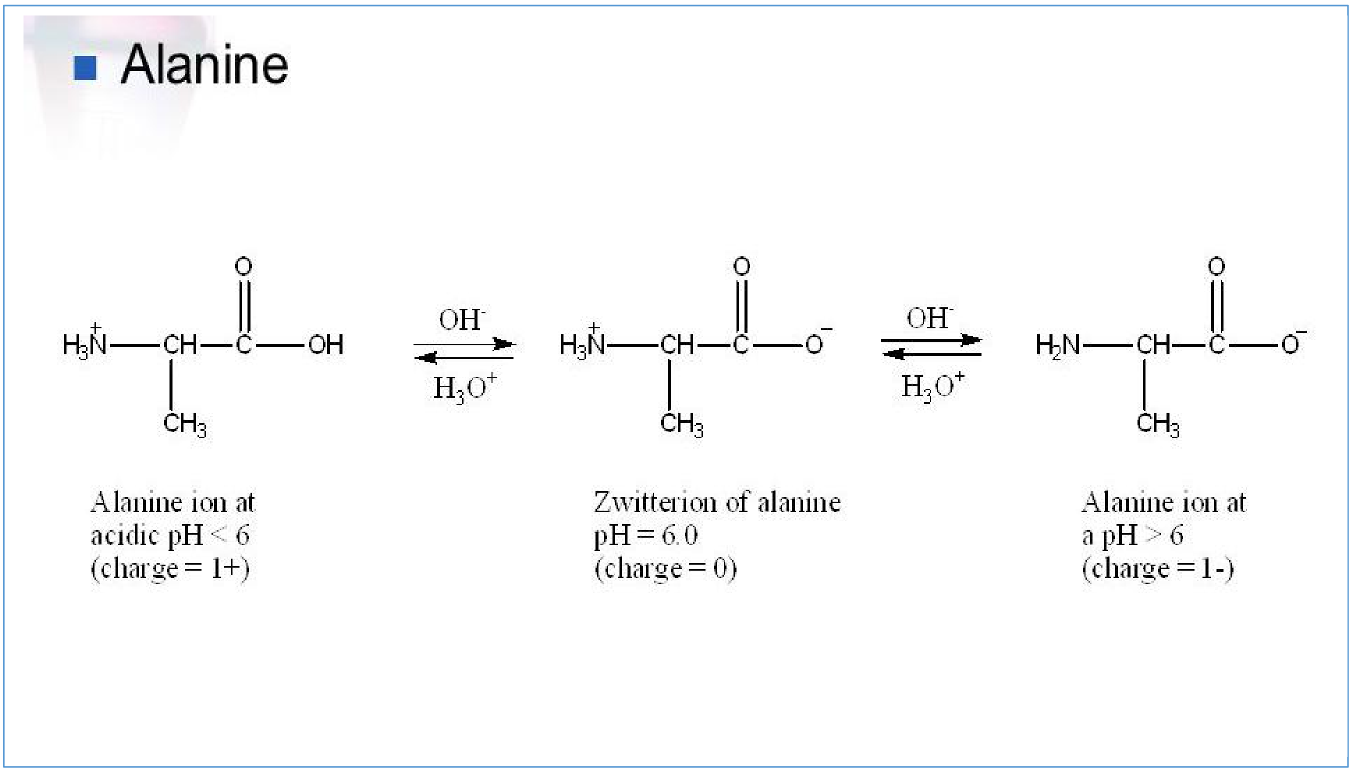
Titration of amino acids

What happens when we titrate an amino acid?

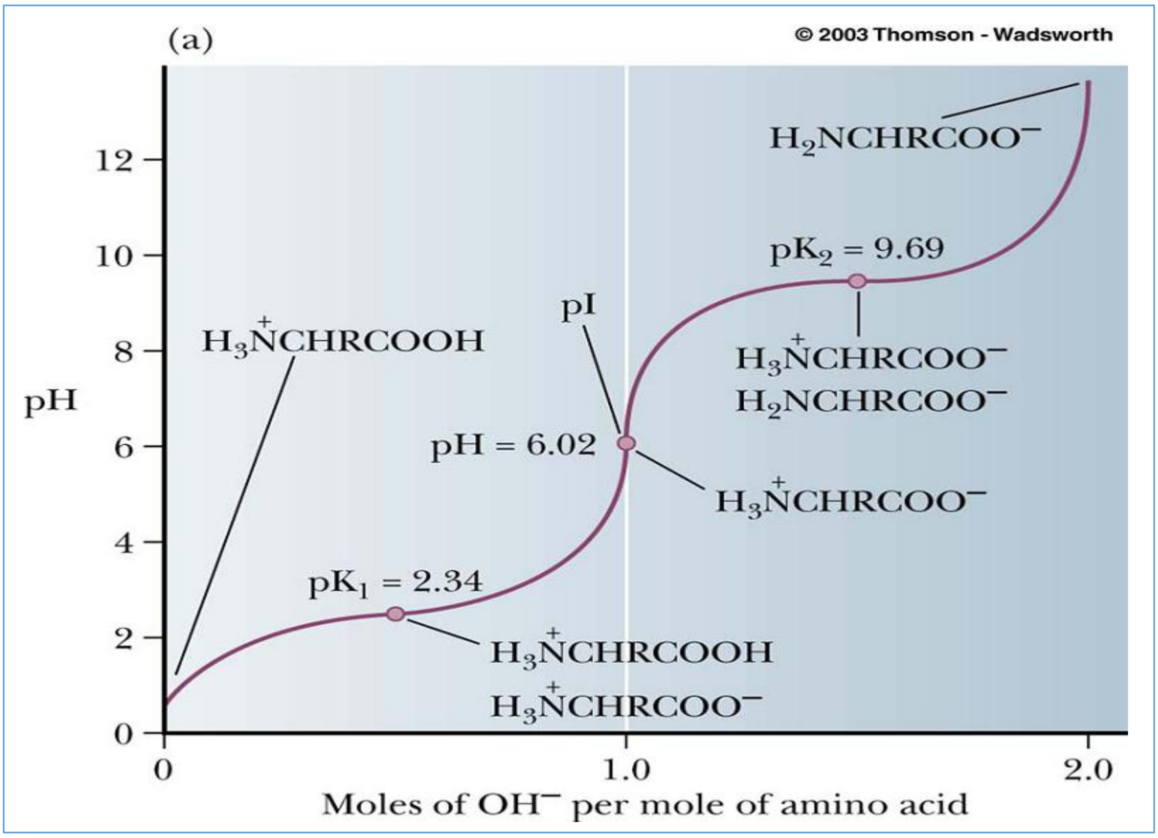
When an amino acid is titrated, its titration curve indicates the reaction of each functional group with hydrogen ion.

We'll study Ala as an ex :

- ✓ In alanine, the carboxyl and amino groups are **the two titratable groups**. At very low pH, alanine has a protonated (and thus uncharged) carboxyl group and a positively charged amino group that is also protonated. Under these conditions, the alanine ***has a net positive charge of 1***.
- ✓ As base is added, the carboxyl group **loses its proton to become a negatively charged carboxylate group** (as shown in the figure below), and the pH of the solution **increases**. Alanine now ***has no net charge***.
- ✓ As the pH increases still further *with addition of more base*, the protonated amino group (a weak acid) loses its proton, and the alanine molecule now ***has a negative charge of 1.***

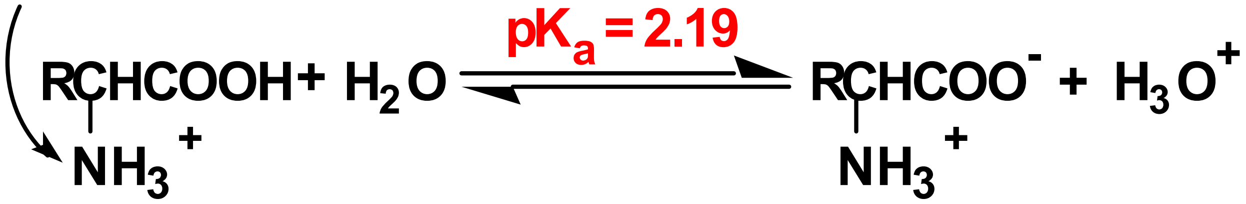


•*This figure shows titration curve of Alanine is that of a diprotic acid :*



- ✓ The average **pKa** of an **α-carboxyl group** is 2.19,which makes them considerably ***stronger acids than acetic acid*** (pKa 4.76) ,why ?
  - ➢ the greater acidity of the amino acid carboxyl group is **due to the electron-withdrawing inductive effect of the -NH3+ group**

The ammonium ion has an electron-withdrawing inductive effect



$$\underset{\displaystyle NH_3^+}{RCHCOOH} + H_2O \underset{}{\overset{pK_a = 2.19}{\rightleftharpoons}} \underset{\displaystyle NH_3^+}{RCHCOO^-} + H_3O^+$$

- ✓ As we already know , given the value of pKa of each functional group, we can calculate the ratio of each acid to its conjugate base as a function of pH :
  - ➢ Consider the ionization of an α-COOH
  - ➢ writing the acid ionization constant and rearranging terms

$$\alpha\text{-COOH} + H_2O \underset{}{\overset{pK_a = 2.00}{\rightleftharpoons}} \alpha\text{-COO}^- + H_3O^+$$

$$K_a = \frac{[H_3O^+][\alpha\text{-COO}^-]}{[\alpha\text{-COOH}]} \quad \text{or} \quad \frac{[\alpha\text{-COO}^-]}{[\alpha\text{-COOH}]} = \frac{K_a}{[H_3O^+]}$$

✓ We can also calculate the ratio of acid to conjugate base for an α-NH_3^+ group; for this calculation, assume a value **10.0** for pKa

$$\alpha\text{-NH}_3^+ + H_2O \overset{pK_a = 10.00}{\rightleftharpoons} \alpha\text{-NH}_2 + H_3O^+$$

➢ writing the acid ionization constant and rearranging gives

$$\frac{[\alpha\text{-NH}\ _O]}{[\alpha\text{-NH}\ _P{}^H]} = \frac{K_\sim}{[H\ _P O^H]}$$

Isoelectric pH

•**Isoelectric pH** (point), **pI** ➡ the pH at which the majority of molecules of compound in solution ***have no net charge.***

➢ pI for glycine, for example, falls midway between the pKa values for the carboxyl and amino groups,as follow :

$$pI = \frac{1}{2}(pK_a\ \alpha\text{-COOH} + pK_a\ \alpha\text{-NH}_3^+)$$
$$= \frac{1}{2}(2.35 + 9.78) = 6.06$$

•every amino acids have its PI . Check the table in the next page (Only for knowledge)

Amino acid: pK and pI

$$pI = \frac{pK_1 + pK_2}{2}$$

Amino Acid	Abbreviation		pK_1	pK_2	pK_R	pI
	3-Letters	1-Letter	-COOH	$-NH_3^+$	R group	
Alanine	Ala	A	2.34	9.69	-	6.00
Arginine	Arg	R	2.17	9.04	12.48	10.76
Asparagine	Asn	N	2.02	8.80	-	5.41
Aspartic Acid	Asp	D	1.88	9.60	3.65	2.77
Cysteine	Cys	C	1.96	10.128	8.18	5.07
Glutamic Acid	Glu	E	2.19	9.67	4.25	3.22
Glutamine	Gln	Q	2.17	9.13	-	5.65
Glycine	Gly	G	2.34	9.60	-	5.97
Histidine	His	H	1.82	9.17	6.00	7.59
Isoleucine	Ile	I	2.36	9.60	-	6.02
Leucine	Leu	L	2.36	9.60	-	5.98
Lysine	Lys	K	2.18	8.95	10.53	9.74
Methionine	Met	M	2.28	9.21	-	5.74
Phenylalanine	Phe	F	1.83	9.13	-	5.48
Proline	Pro	P	1.99	10.60	-	6.30
Serine	Ser	S	2.21	9.15	-	5.58
Threonine	Thr	T	2.09	9.10	-	5.60
Tryptophan	Trp	W	2.83	9.39	-	5.89
Tyrosine	Tyr	Y	2.20	9.11	10.07	5.66
Valine	Val	V	2.32	9.62	-	5.96

`From Lehninger Principle of Biochemistry.`

✓ ***Buffering systems in Human body***

Blood buffers	Kidney buffers
✧ Hemoglobin . ✧ Phosphate . ✧ Proteins . ✧ HCO_3^-/H_2CO_3 . ✧ NH_3/NH_4^+ .	✧ HCO_3^-/H_2CO_3 . ✧ NH_3/NH_4^+ . ✧ Phosphate .

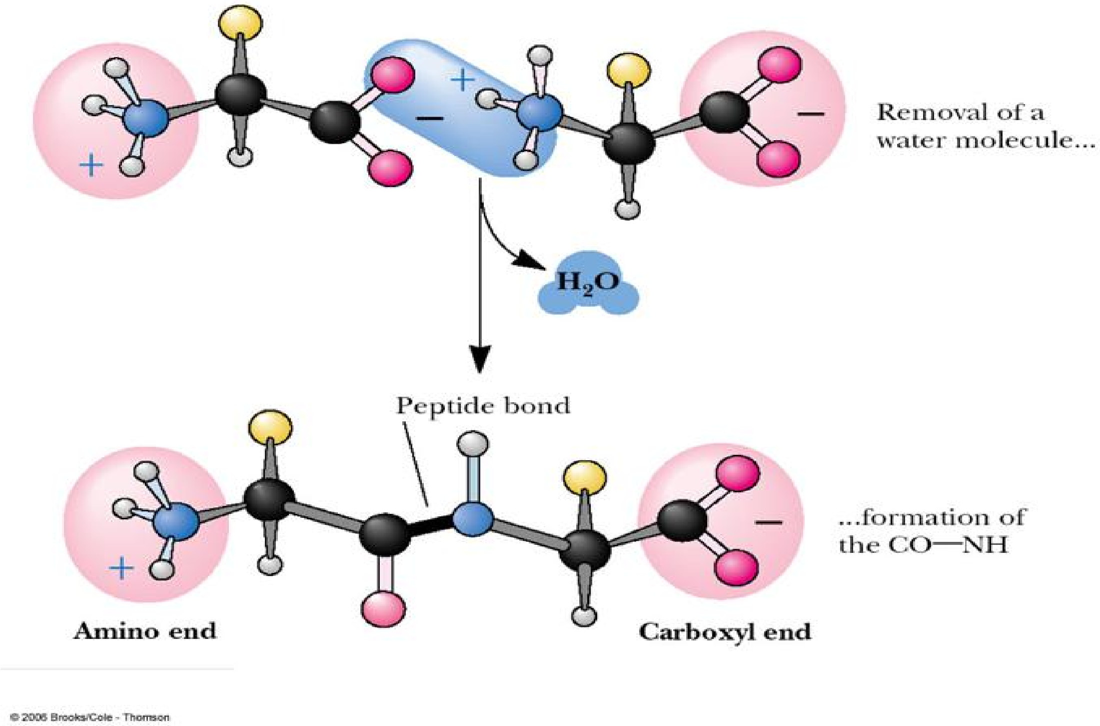
Peptides & Peptide bond

✓ In 1902, Emil Fischer proposed that proteins are long chains of amino acids joined by amide bonds to which he gave the name peptide bonds

✓ **Peptide bond**: the special name given to the amide linkage between the α-carboxyl group of one amino acid and the α-amino group of another.

✓

Formation of a peptide bonds involves removal of a water molecule ; see the figure :



© 2006 Brooks/Cole - Thomson

✓ peptides: the name given to a polymer of amino acids joined by peptide bonds; **they are classified by the number of amino acid residues in the chain as follow :**

➢ Dipeptide: a molecule containing ***two*** amino acid residues joined by a peptide bond

➢ Tripeptide: a molecule containing ***three*** amino acids joined by peptide bonds

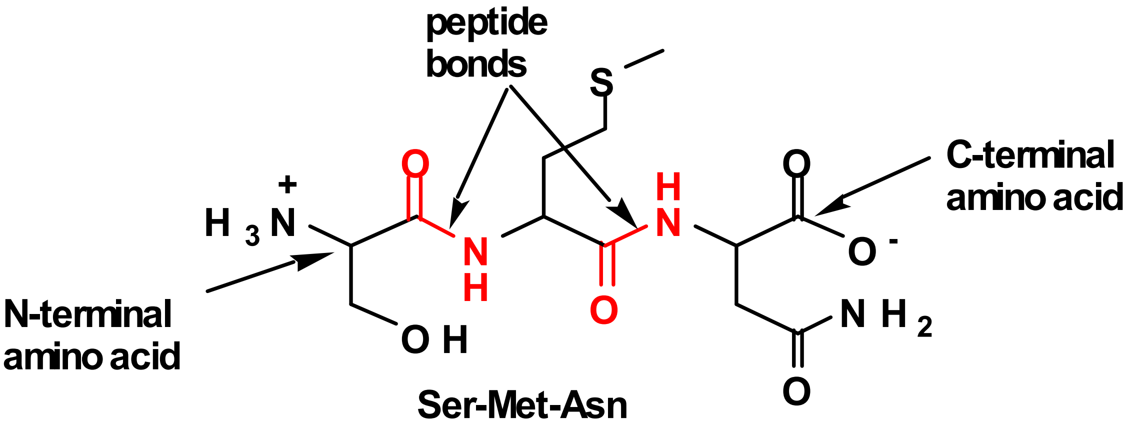
➢ Oligopeptide: few amino acids (***10 or less***)

➢ Polypeptide: a macromolecule containing many amino acids (normally ***>100***) joined by peptide bonds

➢ Protein: a biological macromolecule of molecular weight 5000 g/mol or greater, consisting of ***one or more polypeptide chains .***

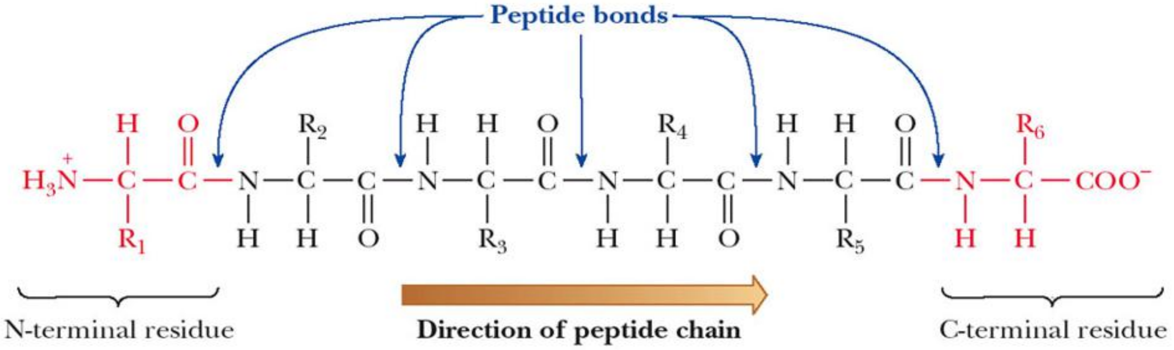
How to write peptides ?

By convention, peptides are written from the left, beginning with **the free -NH_3^+** group and ending with the **free -COO^- group.**



Ser-Met-Asn

•Notice the direction of peptide chain :

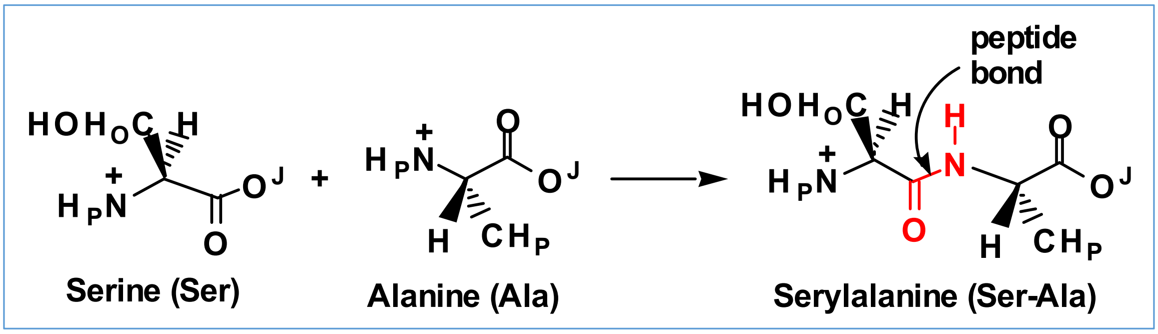


© 2006 Brooks/Cole - Thomson

<u>*Examples on peptides*</u> :

- **Dipeptides**

(1) Serylalanine

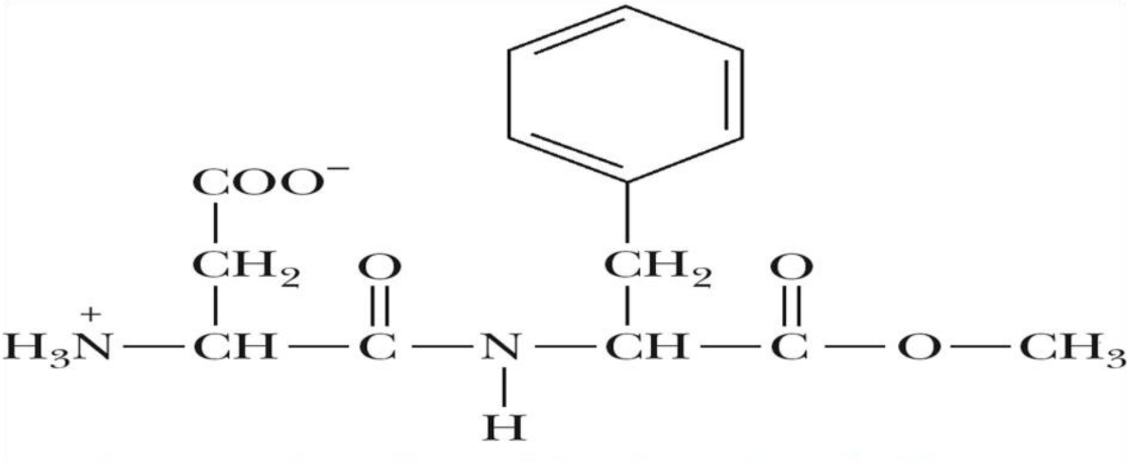


(2) Aspartame

✓ An artificial non-saccharide sweetener used as a sugar substitute in some foods and beverages.

✓ Sold under brand names such as **NutraSweet®** and **Equal®**

✓ Aspartame is around ***180-200*** times sweeter than sucrose (table sugar).



L-Aspartyl-L-phenylalanine (methyl ester)

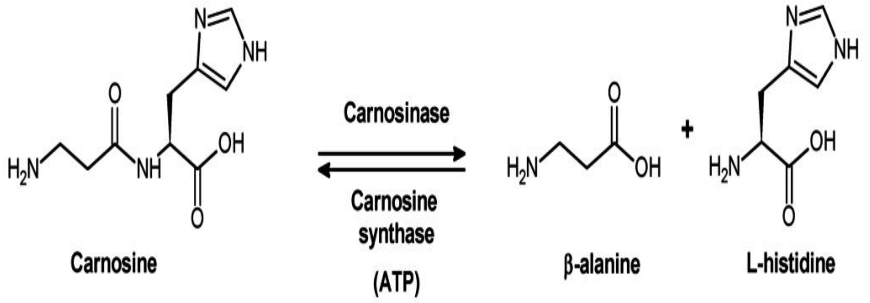
(3) Carnosine (beta-alanyl-L-histidine)

✓ A dipeptide molecule, made up of the amino acids ***beta-alanine and histidine***

✓ It is highly concentrated in ***muscle and brain tissues***.

✓ It has a number of ***antioxidant properties*** that may be beneficial.

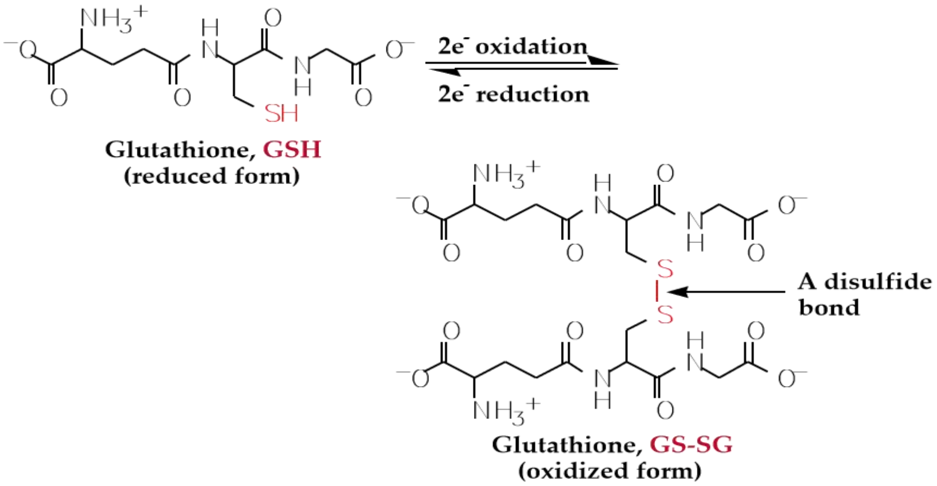
✓ Carnosine has been proven to scavenge reactive oxygen species (ROS) as well as alpha-beta unsaturated aldehydes formed from peroxidation of cell membrane fatty acids during oxidative stress.



- **Tripeptides**

Glutathione

- ✓ It is ***γ–glutamyl-L-cysteinylglycine***, a tripeptide scavenger for oxidizing agents (antioxidant).
- ✓ Glutathione exists in both **reduced** (**GSH**) and **oxidized** (**GSSG**) states.



- ✓ In the reduced state, the **thiol group** of cysteine is able to donate a reducing equivalent (H^{+}+ e^{-}) to other molecules, such as ROS to neutralize them, or to protein cysteines to maintain their reduced forms.
- ✓ With donating an electron, glutathione itself becomes reactive and readily reacts with another reactive glutathione to form glutathione disulfide (GSSG).

- **Pentapeptides**

Enkephalins

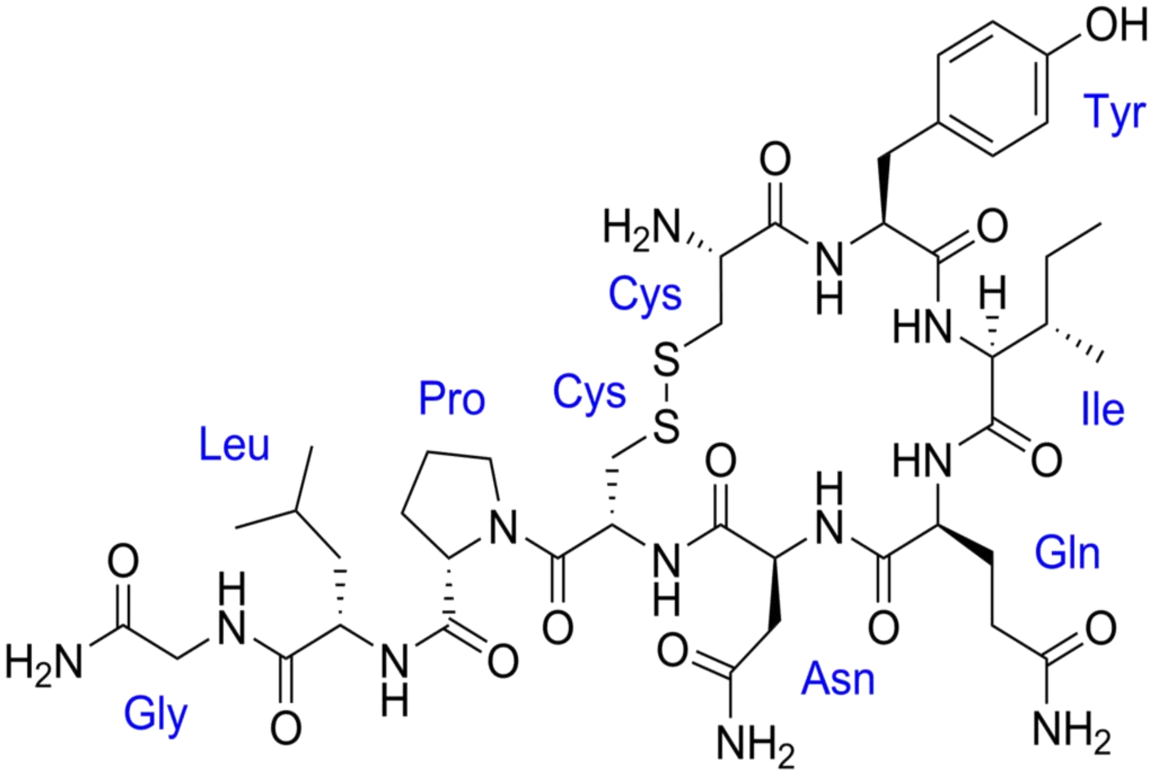
- ✓ Natural pain killers found in the brain .
- ✓ Involved in regulating nociception in the body .
- ✓ 2 forms of enkephalin have been found :
  - ■ Met-enkephalin .
    Gly-Gly-Phe-**Met**.
  - ■ Leu-enkephalin .
    Tyr-Gly-Gly-Phe-**Leu** **.**

- **Nanopeptides**

Oxytocin

- ✓ Peptide hormone and neuropeptide.
- ✓ Oxytocin is normally produced in the *hypothalamus* and released by the *posterior pituitary*.

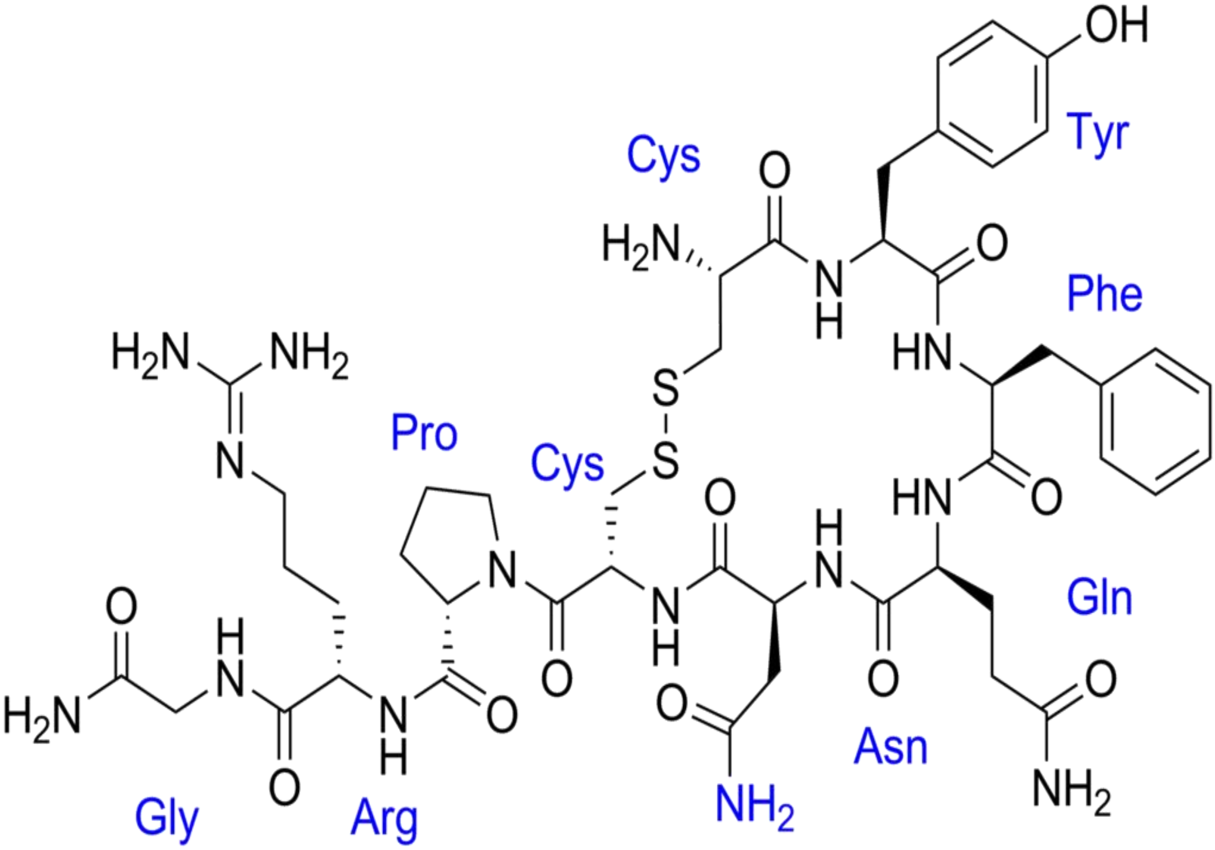
- ✓ Oxytocin is released into the bloodstream as a hormone in response to stretching of the cervix and uterus during labor and with stimulation of the nipples from breastfeeding.



- ✓ Oxytocin has an ***isoleucine at position 3 and a leucine at position 8***; it stimulates smooth muscle contraction in the uterus during labor and in the mammary glands during lactation.

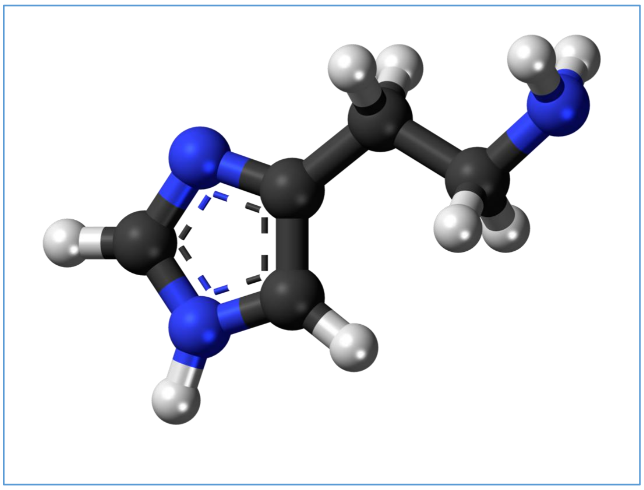
Vasopressin

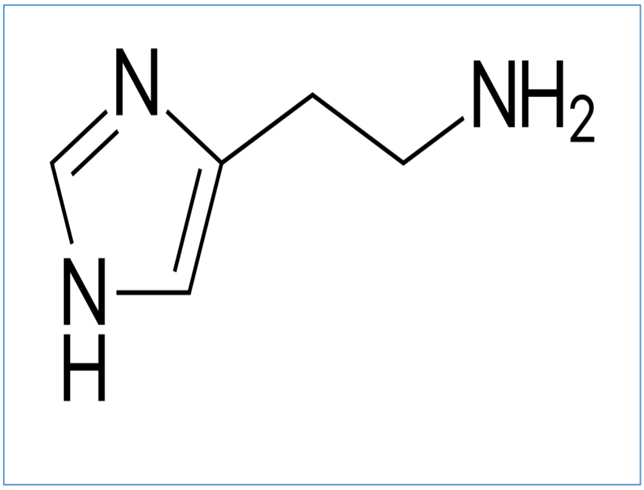
- ✓ Other names of it you must be familiar with :
  Antidiuretic hormone (ADH).
  Arginine vasopressin (AVP).
  Argipressin .
- ✓ Synthesized as a peptide prohormone in neurons in the *hypothalamus*, and is converted to AVP. It then travels down the axon of that cell, which terminates in the *posterior pituitary*, and is released from vesicles into the circulation ***in response to extracellular fluid hypertonicity*** (hyperosmolality).
- ✓ Vasopressin (ADH) has a ***phenylalanine at position 3 and an arginine at position 8***; it stimulates resorption of water by the kidneys, thus raising blood pressure.



The last to talk about in this aspect is **Histamine**

- ✓ It is an organic nitrogenous compound involved in local immune responses as well as regulating physiological function in the gut and acting as a neurotransmitter.
- ✓ Histamine is involved in the inflammatory response .
- ✓ Histamine is derived from the decarboxylation of the amino acid histidine, a reaction catalyzed by the enzyme **L-histidine decarboxylase**.





The Three-Dimensional Structure of Proteins

Biologically active proteins are polymers consisting of amino acids linked by covalent peptide bonds. Many different conformations (three-dimensional structures) are possible for a molecule as large as a protein.

What are the levels of protein structure?

- ✓ **Primary structure** which is the order in which the amino acids are covalently linked together.
- ✓ Two three-dimensional aspects of a single polypeptide chain, called the **secondary** and **tertiary** structures :
  - ➢ **Secondary structure** is the arrangement in space of the atoms in the peptide backbone.
  - ➢ **Tertiary structure** includes the three-dimensional arrangement of all the atoms in the protein, including those in the side chains and in any prosthetic groups (i.e. , groups of atoms other than amino acids).
- ✓ **Quaternary structure** describes a protein that has multiple polypeptide chains.

✌ lets get into them in details ✌

Primary structure

•The amino acid sequence (the primary structure) of a protein determines its 3D structure, which, in turn, determines its properties.

•In every protein, the correct three-dimensional structure is needed for correct functioning.

•One of the most striking demonstrations of the importance of primary structure is found in the hemoglobin associated with *sickle-cell anemia*.

*Normally, your red blood cells are flexible and round, moving easily through your blood vessels. In sickle cell anemia, the red blood cells become rigid and sticky and are shaped like sickles or crescent moons. These irregularly shaped cells can get stuck in small blood vessels, which can slow or block blood flow and oxygen to parts of the body .

*Caused by a change in one amino acid in each of two of the four chains of hemoglobin(HbS the β-chain of sickle cell Hb a valine residue has replaced a glutamic acid residue at position 6).

•Always keep in mind :

▶The primary structure of a protein determines the other levels of structure.

▶A single amino acid substitution can give rise to a malfunctioning protein, as is the case with sickle-cell anemia.

Secondary Structure

•The secondary structure of proteins is the hydrogen-bonded arrangement of the backbone of the protein, the polypeptide chain. The nature of the bonds in the peptide backbone plays an important role here.

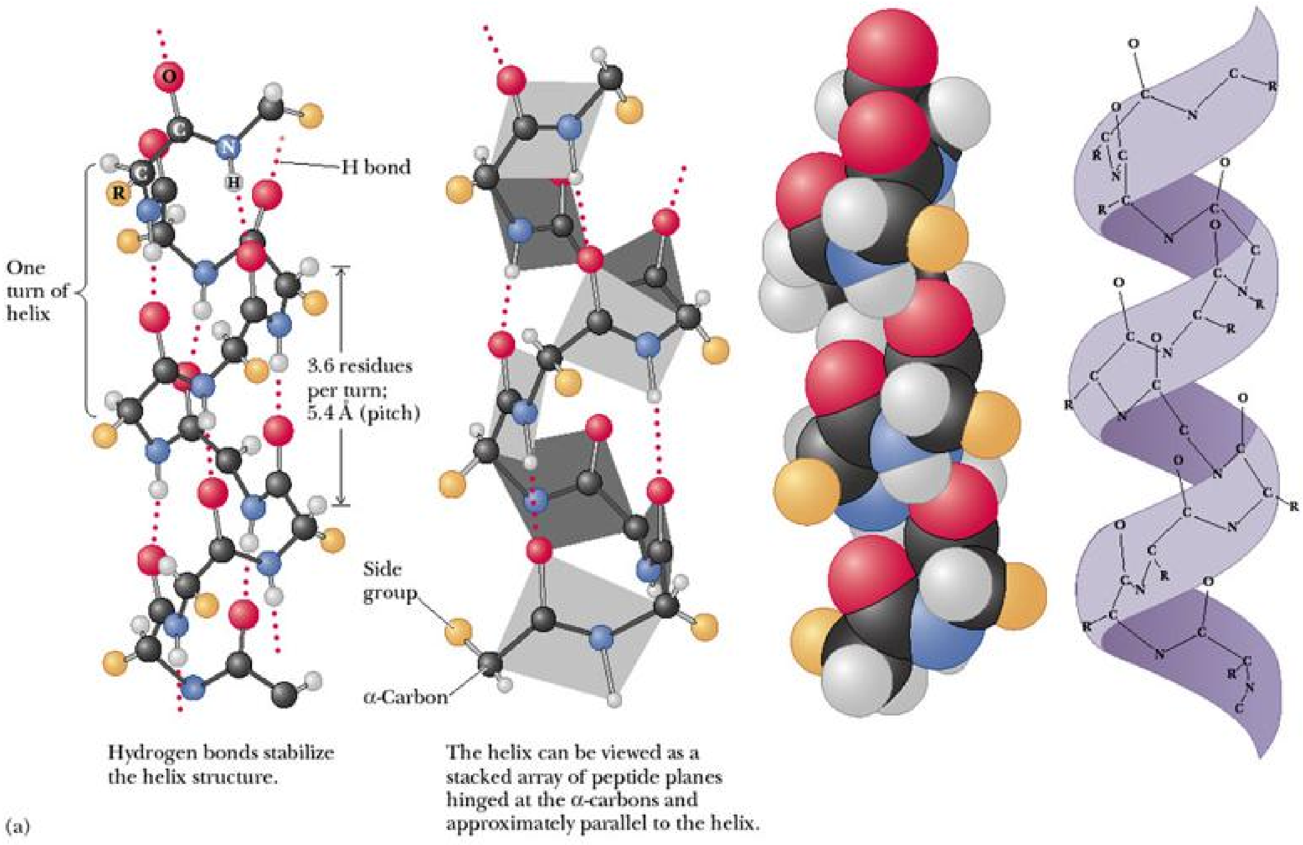
• Two kinds of secondary structures that occur frequently in proteins are the repeating **α**-helix and **β**-pleated sheet hydrogen-bonded structures. The **α**-helix and **β**-pleated sheet are not the only possible secondary structures, but they are by far the most important .

✓ α-Helix

•The a-helix is stabilized by hydrogen bonds parallel to the helix axis within the backbone of a single polypeptide chain.

•**Char**acteristics :

- Coil of the helix is clockwise or right-handed .
- There are 3.6 amino acids per turn .
- Full turn distance is 5.4 Å (pitch) .
- Each peptide bond is trans and planar .
- C=O of each peptide bond is hydrogen bonded to the N-H of the 4th amino acid away .
- C=O······H-N hydrogen bonds are parallel to helical axis.
- All R groups point outward from helix.
- R groups are not involved in the H-bonds.



Hydrogen bonds stabilize the helix structure.

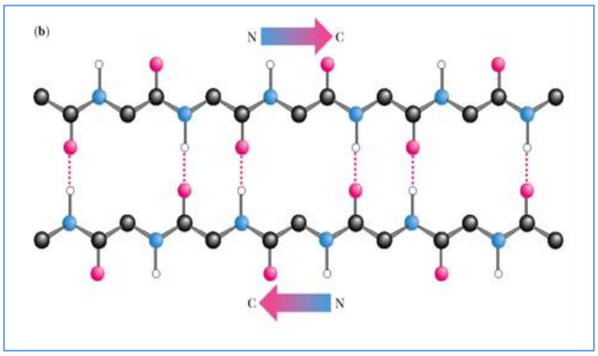
The helix can be viewed as a stacked array of peptide planes hinged at the α-carbons and approximately parallel to the helix.

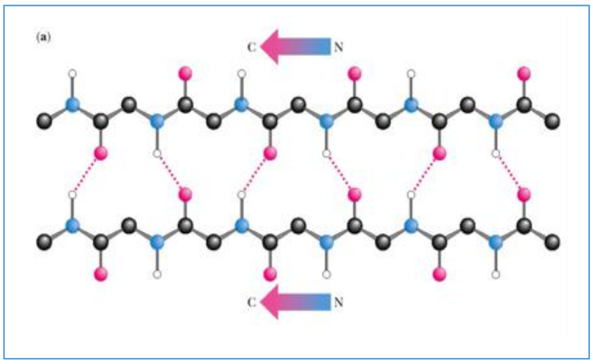
✓ **β-**pleated sheet

•Beta sheets consist of **β-strands** connected laterally by at least two or three backbone hydrogen bonds, forming a generally twisted, pleated sheet.

•**Characteristics** :

- Polypeptide chains are folded back on itself.
- Polypeptide chains lie adjacent to one another; may be parallel or antiparallel(As shown below).



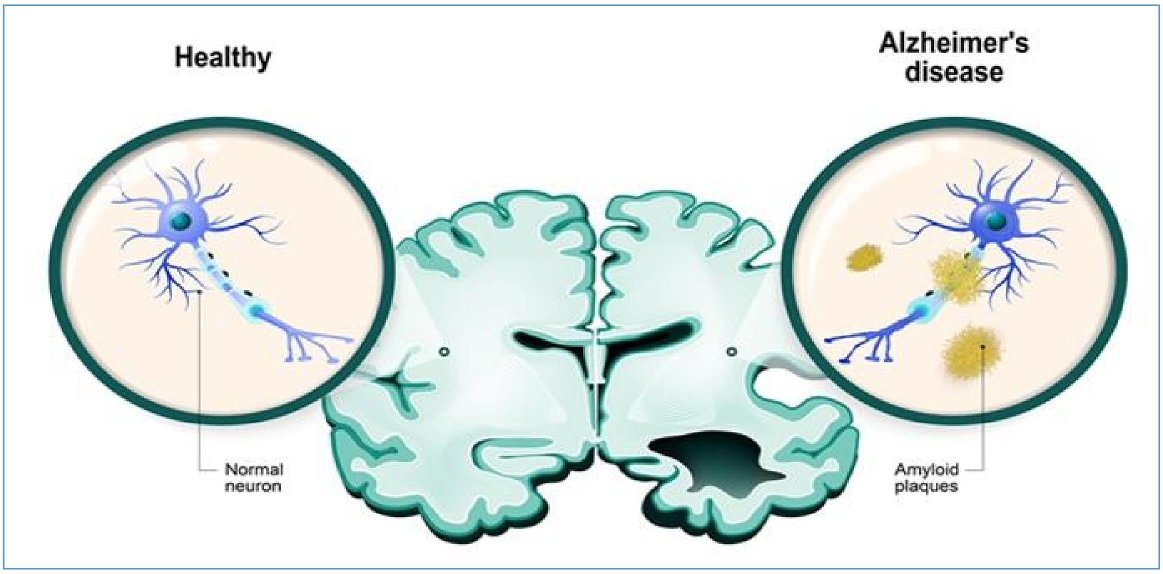


<Anti-parallel beta-sheets are more stable>

- R-groups alternate, first above and then below plane.
- Each peptide bond is trans and planar.
- C=O and N-H groups of each peptide bond are perpendicular to axis of the sheet.
- C=O……H-N hydrogen bonds are between adjacent sheets and perpendicular to the direction of the sheet.

β turns (aka tight turns, reverse turns) are a type of non-regular secondary structure in proteins that cause a change in direction of the polypeptide chain.
Often a reverse turn marks a transition between one secondary structure and another.
For steric (spatial) reasons, glycine&proline are frequently encountered in reverse turns, at which the polypeptide chain changes direction.

✓ In Alzheimer's disease patients, levels of β-amyloid become elevated, and this protein undergoes a conformational transformation from a soluble α helix–rich state to a state rich in β-sheet and prone to self-aggregation.



➢ Alzheimer's disease has been identified as a protein misfolding disease (proteopathy), caused by plaque accumulation of abnormally folded amyloid beta protein and tau protein in the brain.

Tertiary Structure

•The 3D arrangement in space of all atoms in a polypeptide chain

•Bonds stabilize the 3° structure:

- Metal ion coordination.
- Side chain H-bond.
- Electrostatic attraction.
- Disulfide bond.
- Hydrophobic interaction.

Quaternary Structure

•Quaternary structure is the final level of protein structure and pertains to proteins that consist of more than one polypeptide chain. Each chain is called a subunit.

There are two types of protein conformations :

1) ***Fibrous*** proteins .
2) ***Globular*** proteins .

Start talking about Fibrous conformation of proteins ➡

- ***Fibrous proteins***: contain polypeptide chains organized approximately parallel along a single axis.
- They consist of long fibers or large sheets tend to be mechanically strong are insoluble in water and dilute salt solutions .
- Fibrous proteins are adapted for a structural function .
- α-Keratin, collagen, and elastin provide clear examples of the relationship between protein structure and biological function (As table in the next page shows).
- These proteins share properties that give strength and/or elasticity to structures in which they occur.

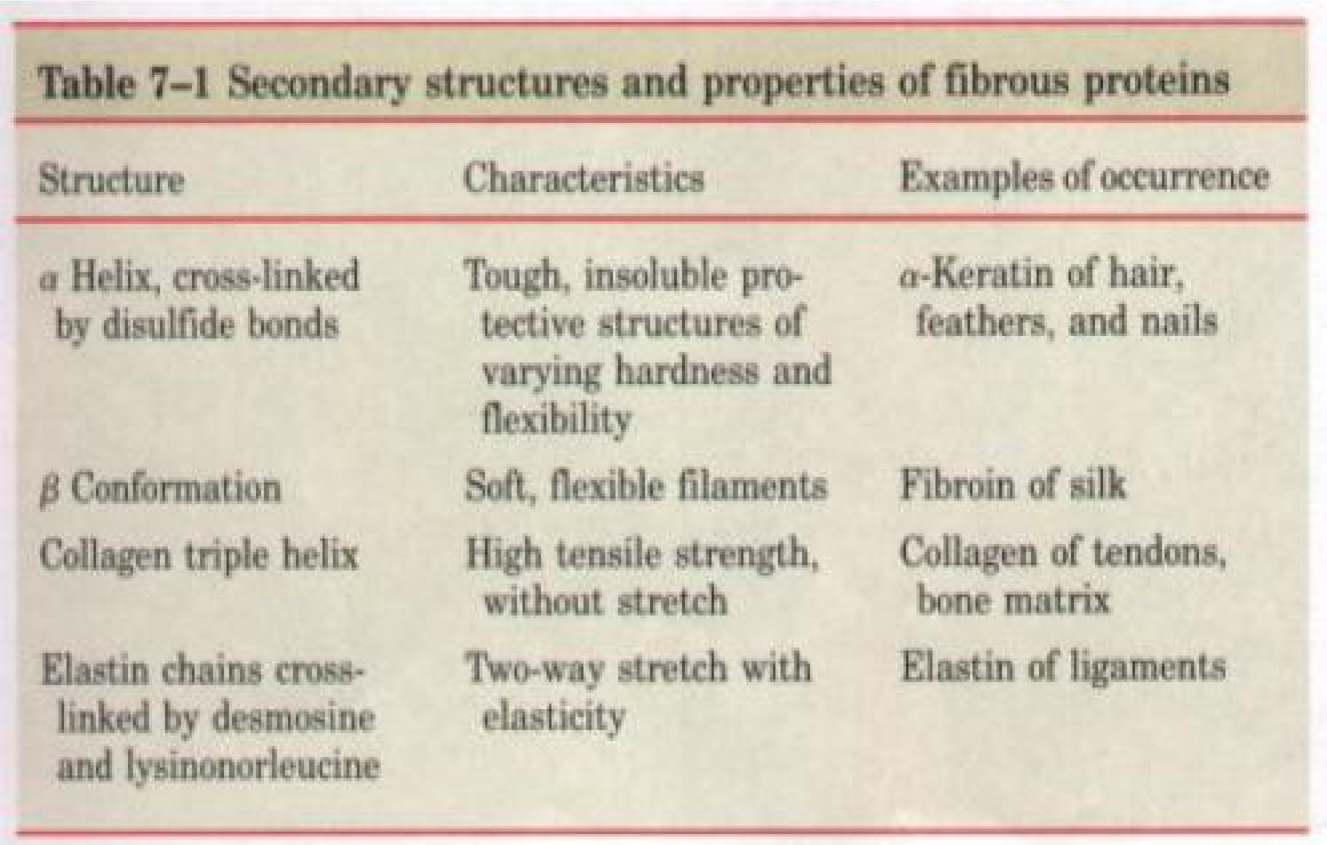
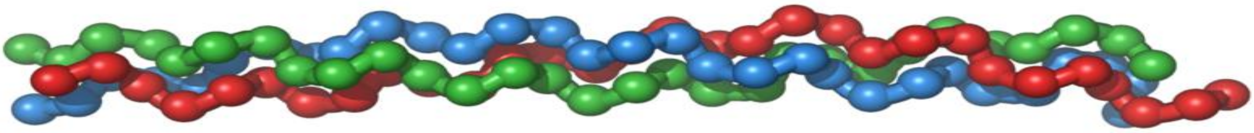


Table 7–1 Secondary structures and properties of fibrous proteins

Structure	Characteristics	Examples of occurrence
α Helix, cross-linked by disulfide bonds	Tough, insoluble protective structures of varying hardness and flexibility	α-Keratin of hair, feathers, and nails
β Conformation	Soft, flexible filaments	Fibroin of silk
Collagen triple helix	High tensile strength, without stretch	Collagen of tendons, bone matrix
Elastin chains cross-linked by desmosine and lysinonorleucine	Two-way stretch with elasticity	Elastin of ligaments

Collagen triple helix



Collagen has an unusual amino acid composition and sequence:

- ✓ Glycine is found at almost every third residue .
- ✓ Proline (Pro) makes up about 17% of collagen .
- ✓ Collagen contains two uncommon derivative amino acids not directly inserted during translation. These amino acids are found at specific locations relative to glycine and are modified post-translationally by different enzymes, both of which require vitamin C as a cofactor.
  - ➢ ***Hydroxyproline*** (Hyp), derived from proline.
  - ➢ ***Hydroxylysine*** (Hyl), derived from lysine.

Cortisol

- ✓ Cortisol is a steroid hormone that regulates a wide range of processes throughout the body, including metabolism and the immune response. It also has a very important role in helping the body respond to stress.
- ✓ Almost every cell contains receptors for cortisol and so cortisol can have lots of different actions depending on which sort of cells it is acting upon.
- ✓ Cortisol stimulates degradation of (skin) collagen into amino acids.

The best-known defect in collagen biosynthesis is ***Scurvy***, a result of a dietary deficiency of vitamin C required by prolyl and lysyl hydroxylases.
The resulting *deficit in the number of hydroxyproline and hydroxylysine residues* undermines the conformational stability of collagen fibers, leading to bleeding gums, swelling joints, poor wound healing, and ultimately to death.

Menkes syndrome

- ✓ A disorder that reflects a dietary deficiency of the copper required by lysyl oxidase, which catalyzes a key step in formation of the covalent cross-links that strengthen collagen fibers.
- ✓ It is characterized by sparse, kinky hair; failure to gain weight and grow at the expected rate (failure to thrive); and deterioration of the nervous system.
- ✓ Additional signs and symptoms include weak muscle tone (hypotonia), sagging facial features, seizures, developmental delay, and intellectual disability. Children with Menkes syndrome typically begin to develop symptoms during infancy and often do not live past age 3.
- ✓ Early treatment with copper may improve the prognosis in some affected individuals. In rare cases, symptoms begin later in childhood.

Genetic disorders of collagen biosynthesis include :

- ✓ Several forms of *osteogenesis imperfecta*, characterized by fragile bones.
- ✓ In *Ehlers-Dahlos syndrome*, a group of connective tissue disorders that involve impaired integrity of supporting structures, defects in the genes that encode α collagen-1, procollagen N-peptidase, or lysyl hydroxylase result in mobile joints and skin abnormalities.

Moving to discuss ***Globular Proteins*** ➡

- ✧ Globular proteins: proteins which are folded to a more or less spherical shape .
- ✧ They tend to be ***soluble*** in water and salt solutions .
- ✧ Most of their *polar side chains are on the outside* and interact with the aqueous environment by hydrogen bonding and ion-dipole interactions .
- ✧ Most of their *nonpolar side chains are buried inside* .
- ✧ Nearly all have substantial sections of α-helix and **β**-sheet .

A good example on globular proteins : **Myoglobin.**

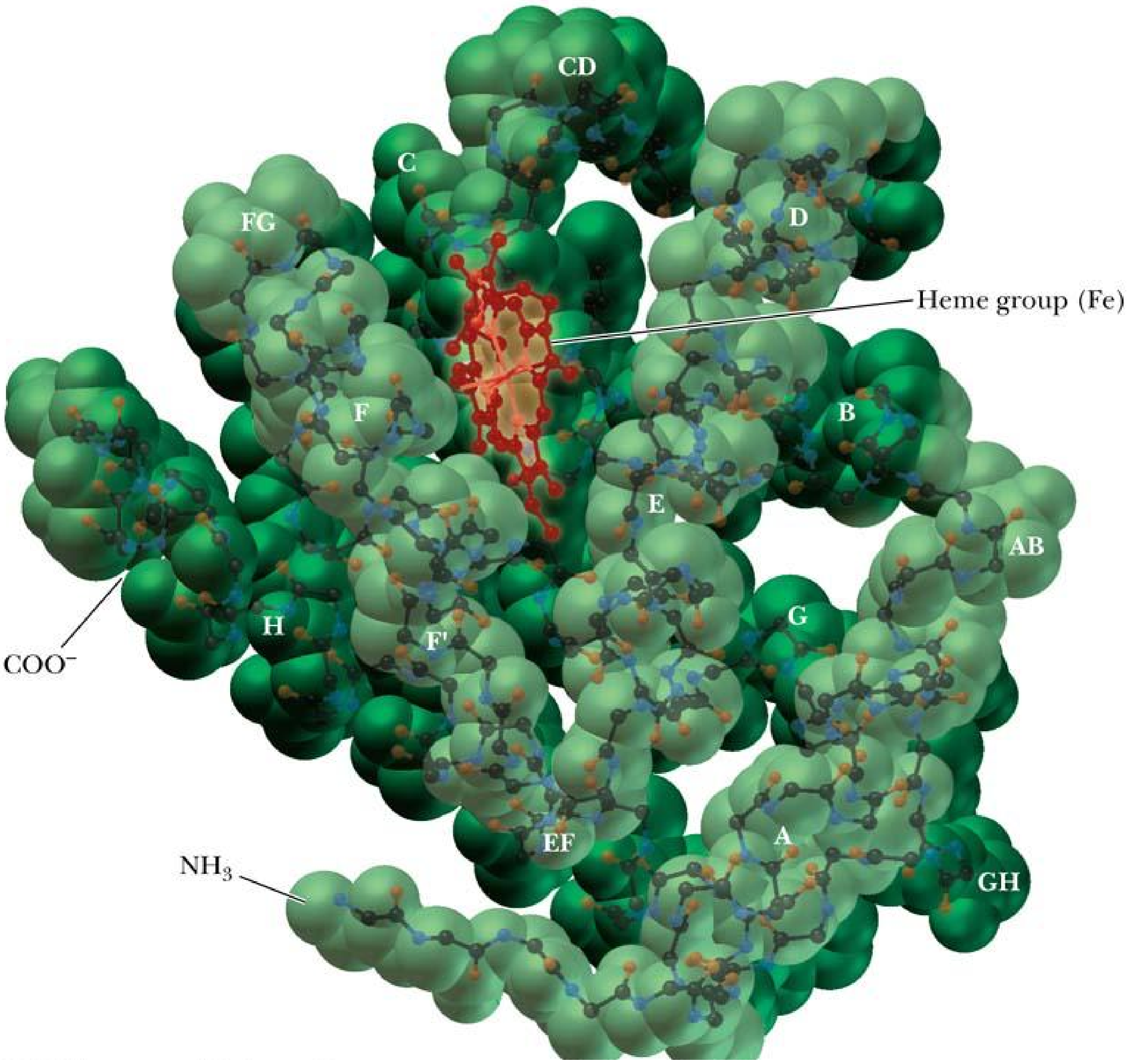
- ✓ Function in O_2 storage in muscles.
- ✓ The complete myoglobin molecule consists of a single polypeptide chain of 153 amino acid residues and includes a prosthetic group, the heme group, which also occurs in hemoglobin.
- ✓ Compact with a single heme group in a hydrophobic pocket .(See the figure in the next page).

- ✓ Most polar side chains are on the surface ,nonpolar side chains are folded to the interior.

- ✓ Myoglobin has ***eight α-helical regions*** and ***no β -pleated sheet regions***.

- ✓ Myoglobin was the first protein for which the complete tertiary structure was determined by X-ray crystallography (see the figure below).

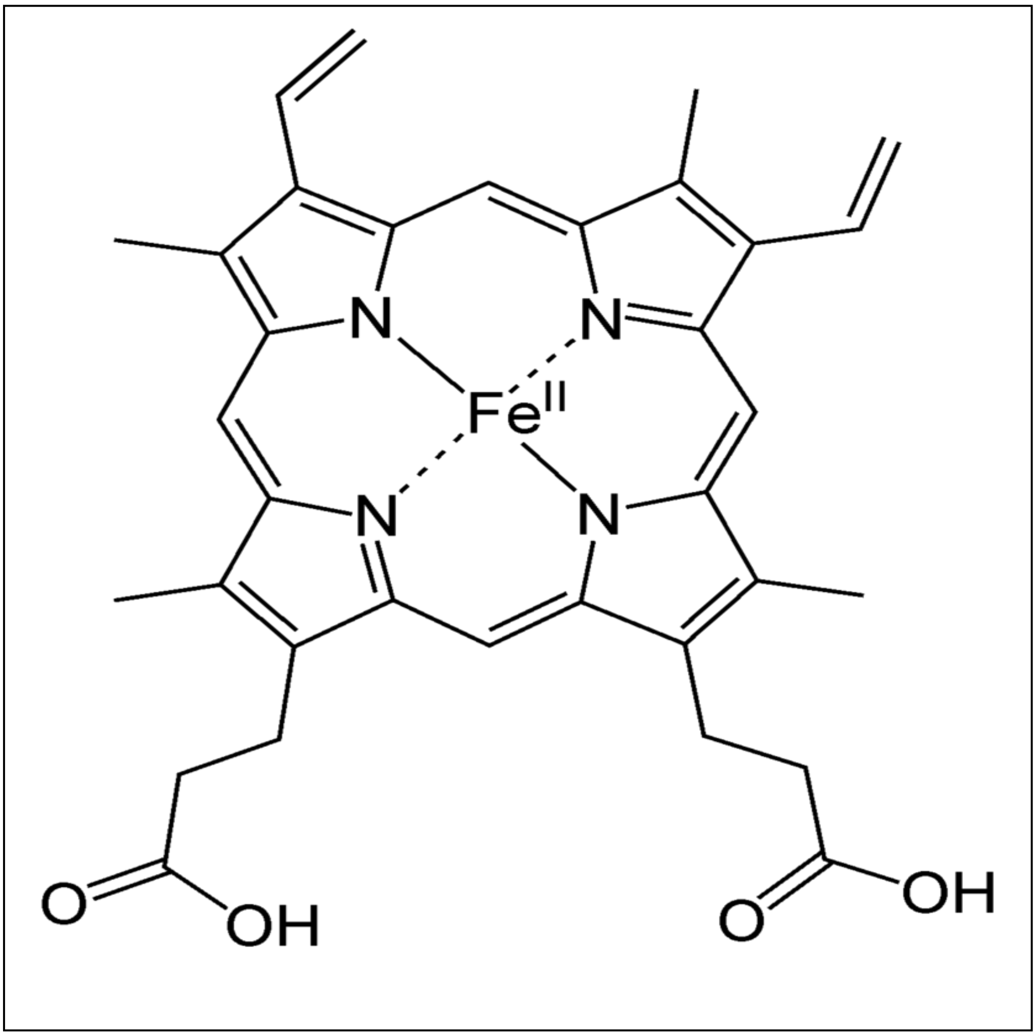
- ✓ ***Two polar histidine residues*** are found in the interior; they are *involved in interactions with the heme group and bound oxygen*, and thus play an important role in the function of the molecule.
  - ➢ A *proximal histidine group* (proximal F8 his) is attached directly to iron, and a *distal histidine group* (distal E7 his) hovers near the opposite face.



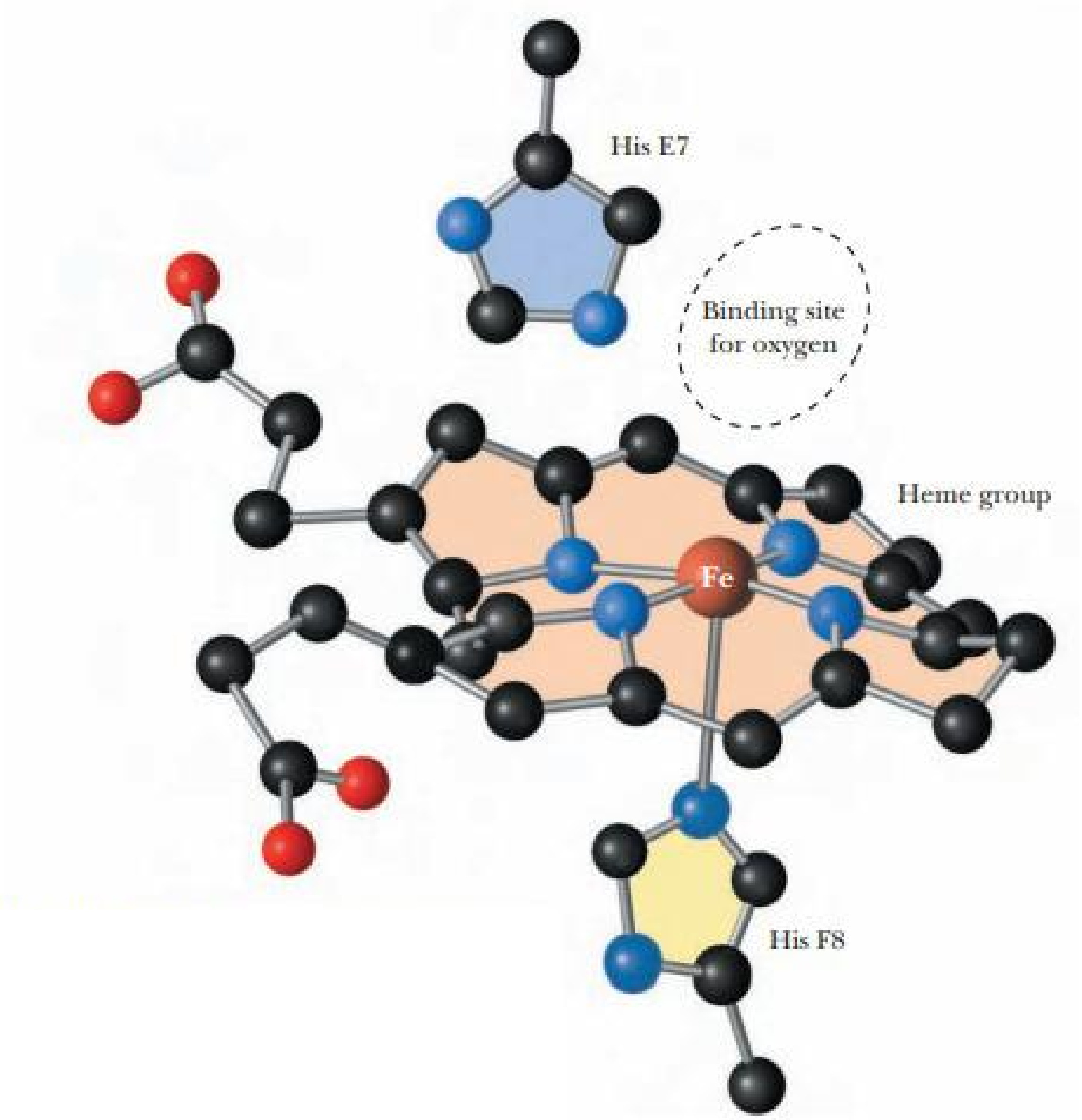
Heme Structure

(هاي أساسيات رح تمرّ لقدام معكم ورح تنسألوا عنها كتير ومهم كذلك تكونوا عارفينها ، ما في اشي بكير تعرفوه هلأ كونوا أكيدين إنه يلي بتتعلمه اليوم رح يفرق معك بكره كتير)

- ✓ Myoglobin contains a heme (prosthetic) group which is responsible for its main function (carrying of oxygen molecules to muscle tissues).
- ✓ Heme group consists of protoporphyrin organic component consists of four pyrrole rings that are linked by methine bridges and an iron atom located in its center.
- ✓ At the center of protporphyrin, the iron atom is bonded to nitrogen atoms from four pyrrole rings. The iron atom can form ***two additional bonds***, one on each side of the heme plane. These binding sites are called the ***fifth and sixth coordination sites,****the fifth and sixth coordination sites lie perpendicular to, and on opposite sides of, the plane of the porphyrin ring.*
- ✓ In myoglobin, ***the fifth coordination site is occupied by the imidazole ring from a histidine residue on the protein***. This hisitidine is referred to as the *proximal histidine*. The sixth coordination site is available ***to bind oxygen or an N of the second His side chain.***



- ✓ ***The heme group gives muscle and blood their distinctive red color ;****oxidation of the iron atom (Fe^{2+} -> Fe^{3+}) is mainly responsible for the color of muscle and blood.*
- ✓ *From the figure below ,* you notice that His F8 occupies the 5th coordination site of the iron , oxygen is bound at the 6th coordination site of the iron and his E7 lies close to the oxygen. Actually ,*the E7 histidine sterically inhibits oxygen from binding perpendicularly to the heme plane, with biologically important ramifications.*

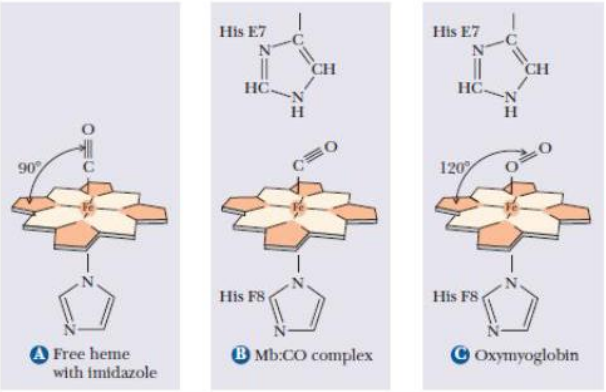


Why does oxygen have imperfect binding to the heme group?

At first, it would seem counterintuitive that oxygen would bind imperfectly to the heme group. After all, the job of both myoglobin and hemoglobin is to bind to oxygen. Wouldn't it make sense that oxygen should bind strongly?

- Besides oxygen, carbon monoxide also binds to heme. *The affinity of free heme*(in the absence of globin) ***for carbon monoxide (CO)*** is 25,000 times greater than its affinity for oxygen.
- In the presence of globin, Fe(II) affinity to O_2 ***increases*** and its affinity to CO ***decreases*** ;however it remains *higher than that of O2* , it's ***210x*** its affinity to O_2 .
- In the absence of the protein, the iron of the heme group can be oxidized to Fe(III); the oxidized heme will not bind oxygen. Thus, the combination of both heme and protein is needed to bind O_2 for oxygen storage.
- CO is a potent poison in larger quantities because of its effect both on *oxygen binding to hemoglobin* and on *the final step of the electron transport chain*.
- It is also important to remember that although our metabolism requires that hemoglobin and myoglobin bind oxygen, it would be equally disastrous if the heme never let the oxygen go(If heme tighlty binds to O_2). Thus having binding be too perfect would defeat the purpose of having the oxygen-carrying proteins.

- The second histidine His E7 – not bound to the heme, but acts a gate to open and closes as oxygen enter the hydrophobic pocket
- E7 inhibit O2 to bind to perpendicularly to heme
- The presence of His E7 – will force CO to bind at the 120 angle – make it lose it affinity to heme



Ⓐ Free heme with imidazole

Ⓑ Mb:CO complex

Ⓒ Oxymyoglobin

Quaternary Structure of proteins

- ✓ Quaternary structure is the final level of protein structure and pertains to proteins that consist of more than one polypeptide chain. Each chain is called a subunit.
- ✓ Commonly occurring examples are *dimers*, *trimers*, and *tetramers*, consisting of two, three, and four polypeptide chains, respectively.
- ✓ *You have to memorize number o subunits in each protein written in the table below .*

Globular protein	# of subunits
Alcohol Dehydrgenase	**2**
Triosephosphate isomerase	**2**
Aldolase	**3**
Hemoglobin	**2+2**
Lactate Dehydrogenase	**4**
Pyruvate Kinase	**4**
Insulin	**2**

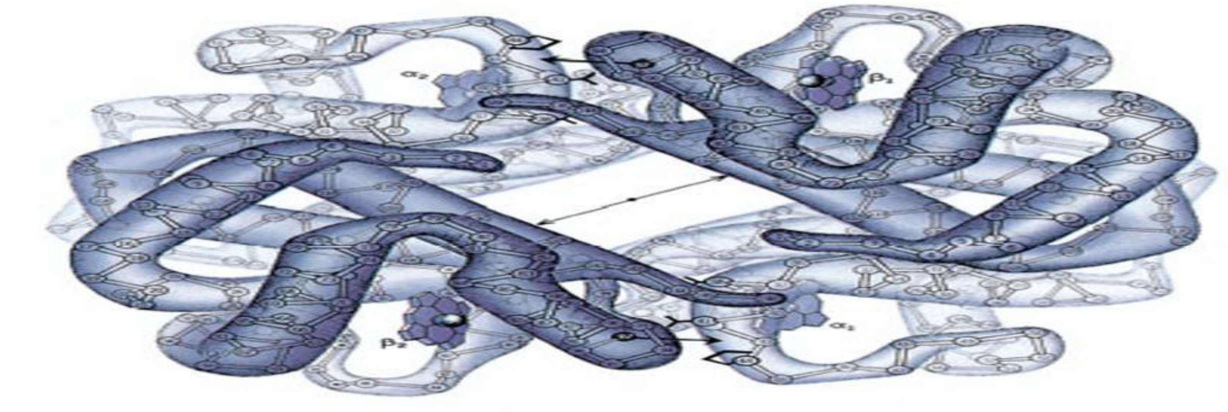
•We'll discuss only hemoglobin ; the rest will be mentioned in Biochem(102) .

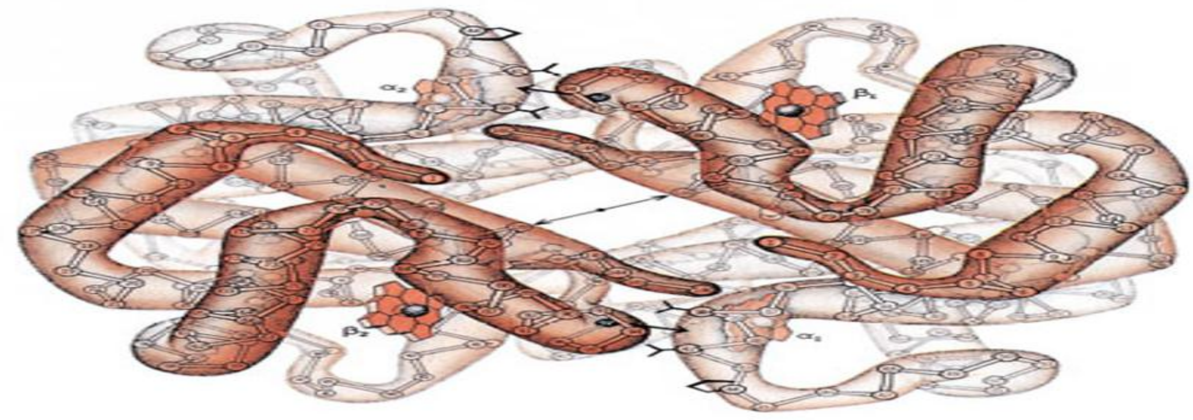
Hemoglobin is the oxygen-transporting protein of red blood cells and is a globular protein with a quaternary structure.

- Hemoglobin consists of ***four polypeptide subunits***;of two α-chains (**141** amino acids each) and two **β**-chains (**153** amino acids each),each chain has 1 heme group.
- Hemoglobin can bind up to 4 molecules of O_2 ,binding is *cooperative*; when one O_2 is bound, it becomes easier for the next O_2 to bind (positive cooperativity)
- Hemoglobin transports oxygen in the blood from the lungs to the rest of the body.

- There are two states in the hemoglobin, the ***T state*** (the tense state) and the ***R state*** (the relaxed state). The T state has *less of an affinity for oxygen than the R state*.

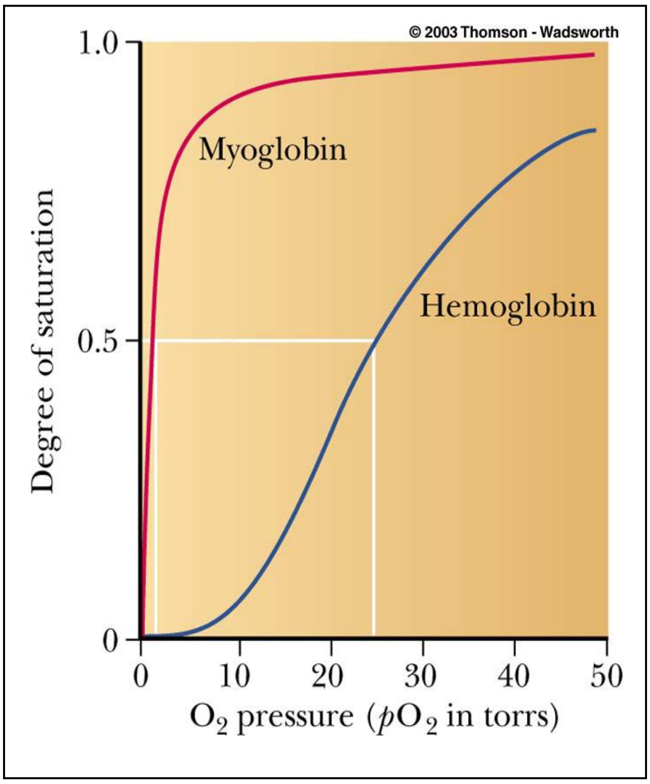
- Hemoglobin has different quaternary structures in the bound (oxygenated) and unbound (deoxygenated) forms. The two β-chains are much closer to each other in oxygenated hemoglobin than in deoxygenated hemoglobin.<See the next page>





O2 binding of hemoglobin and myoglobin

- ✓ The following graphshows the oxygen-binding properties of hemoglobin and myoglobin .



© 2003 Thomson - Wadsworth

- ✓ The oxygen-binding curve of myoglobin is thus said to be ***hyperbolic,***[can bind with O2 at any pressure]. In contrast, the shape of the oxygen-binding curve for hemoglobin is ***sigmoidal***. This shape indicates that the binding of the first oxygen molecule facilitates the binding of the second oxygen, which facilitates the binding of the third, which in turn facilitates the binding of the fourth. This is precisely what is meant by the term cooperative binding.
- ✓ (imp):Note that even though cooperative binding means that binding of each subsequent oxygen is easier than the previous one, the binding curve is *still lower than that of myoglobin at any oxygen pressure*. In other words, at any oxygen pressure, myoglobin will have a higher percentage of saturation than hemoglobin .

•There are several important factors that affect the affinity of hemoglobin to oxygen as therefore affect the oxygen-hemoglobin dissociation curve. These factors include :

(1) pH / [H^+] .
(2) Temperature .
(3) carbon dioxide .
(4) 2,3-BPG .
(5) Carbon monoxide.

Hemoglobin and Bohr Effect

✓ The effect of pH on the oxygen-binding ability of Hb is called the ***Bohr effect.***

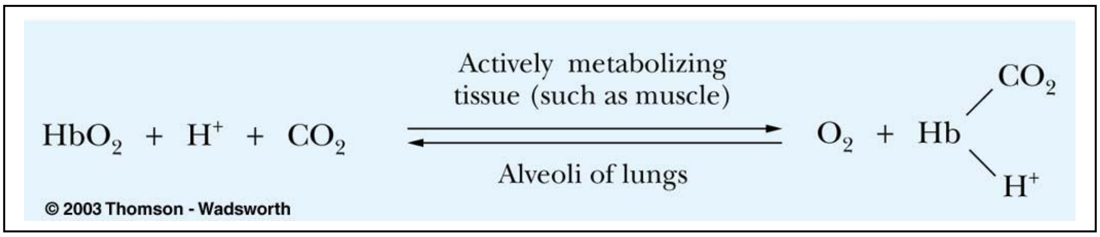
Explanation :(It's only to get the idea fully , focus on the highlighted sentences)

As cells carry our their metabolic processes as a higher rate, they will produce more waste by-products. One of the major waste by-products is carbon dioxide. Carbon dioxide is a non-polar molecule and that means it cannot easily dissolve inside the blood (a polar substance).

The way that our cells solve this problem is by first transferring the carbon dioxide to the red blood cells located in nearby capillaries. Once inside the red blood cells, an enzyme called carbonic anhydrase combines gaseous carbon dioxide with liquid water to produce aqueous carbonic acid. Carbonic acid, which is a weak acid, readily dissociates into a hydrogen ion and bicarbonate ion. Since these two ions are polar, they readily and easily dissolve inside the polar blood plasma. These two molecules can bind to hemoglobin at special allosteric sites and change the conformation of the protein in such as way as to decrease its affinity for oxygen.

Therefore, by increasing the concentration of carbon dioxide, we increase the concentration of hydrogen and therefore decrease the pH . This decreases hemoglobins affinity for oxygen and shifts the oxygen-hemoglobin dissociation curve to the right.

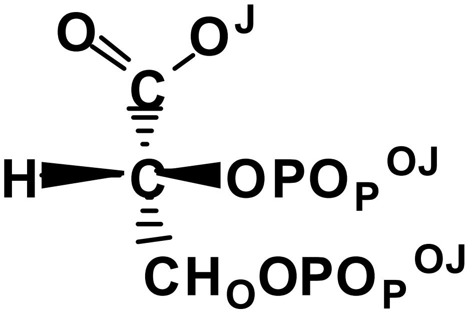
On the other hand, if we increase our pH, we shall shift the curve to the left and increase hemoglobin's affinity for oxygen.



$$HbO_2 + H^+ + CO_2 \underset{\text{Alveoli of lungs}}{\overset{\text{Actively metabolizing tissue (such as muscle)}}{\rightleftharpoons}} O_2 + Hb\begin{matrix} CO_2 \\ H^+ \end{matrix}$$

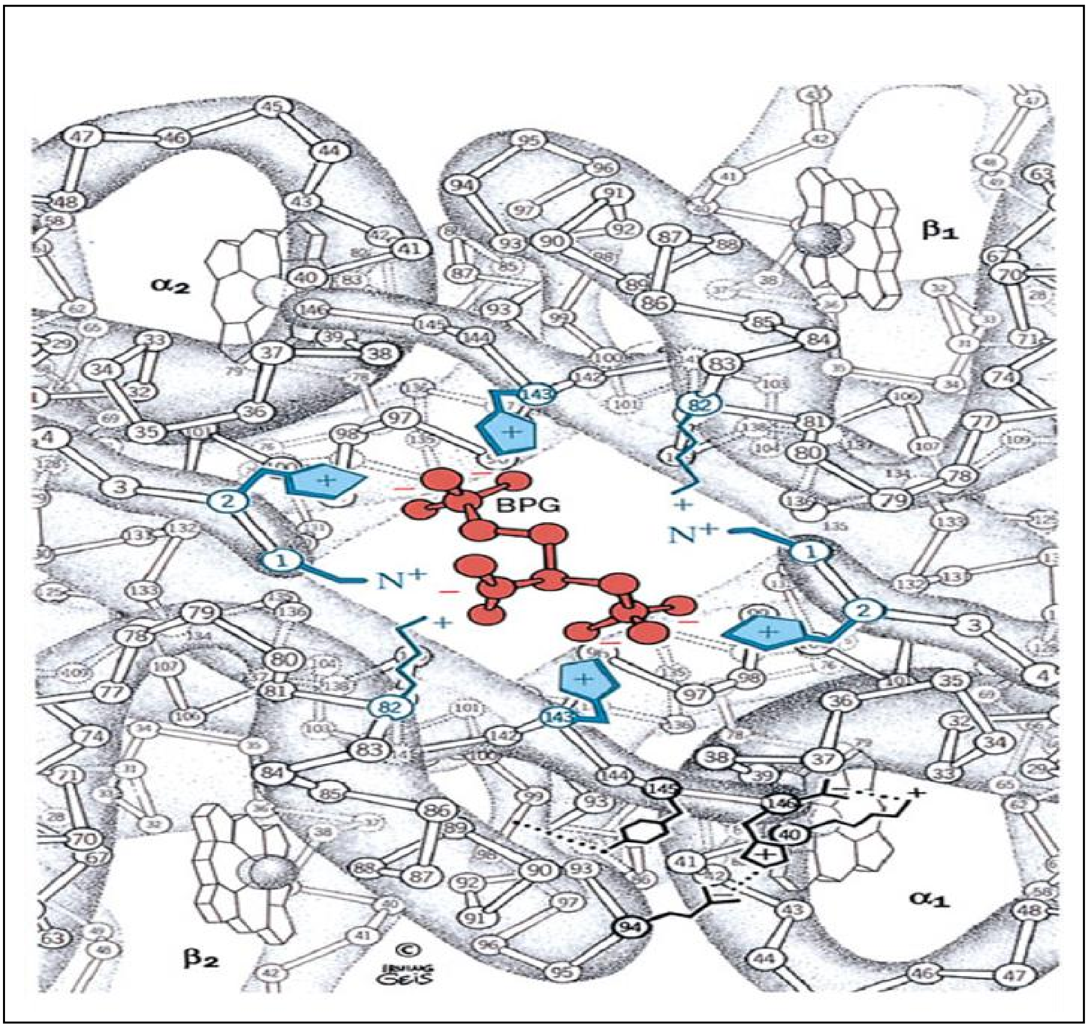
2,3 BPG and Hemoglobin

- 2,3-biphosphoglycerate or simply 2,3-BPG is a biological molecule that is produced as an intermediate ***during the process of glycolysis***. When a cell is exercising and has a high metabolic rate, it will produce excess 2,3-BPG molecules. Some of these 2,3-BPG molecules will exit the cell and enter the blood plasma of nearby capillaries.
- Once inside the blood plasma, the biphosphoglycerate can then enter the red blood cells ***and bind to deoxyhemoglobin***.
- *Only deoxyhemoglobin* contains a cavity (space) between the two beta subunits that is large enough for 2,3-BPG to actually bind to via electrostatic forces between negative charges on BPG and positive side chains
- (e.g., Lys, Arg) of hemoglobin.
- ***Oxyhemoglobin does not contain this space*** and therefore 2,3-BPG does not readily bind to it.
- Once bound, the 2,3-BPG *changes the shape of the deoxyhemoglobin and makes it much less likely to actually bind to oxygen*. Therefore it shifts the entire oxygen-hemoglobin dissociation curve to the right. This ultimately bring more oxygen molecules to the exercising cells of our tissue.

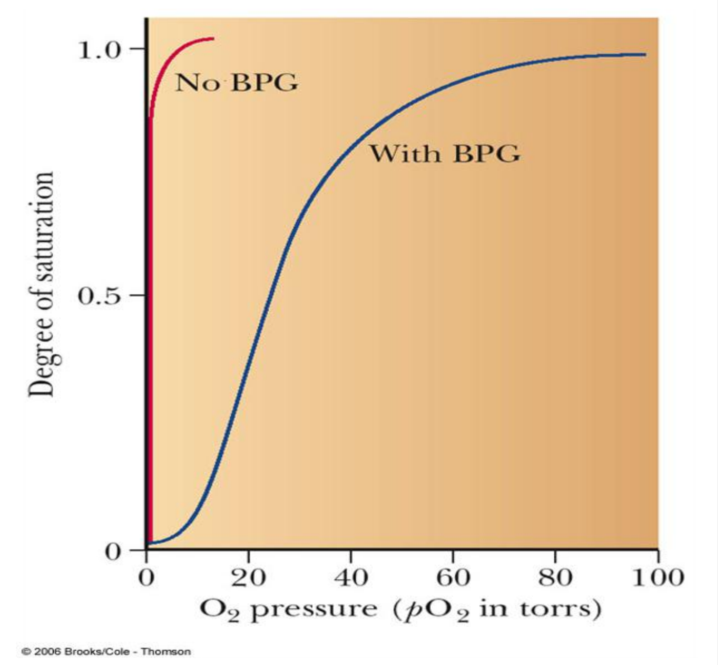


2,3-Bisphosphoglycerate (BPG)

- ✓ The binding of BPG to deoxyhemoglobin. Note the electrostatic interactions between the BPG and the protein.



- ✓ A comparison of the oxygen-binding properties of hemoglobin in the presence and absence of BPG. Note that the presence of the BPG markedly decreases the affinity of hemoglobin for oxygen.



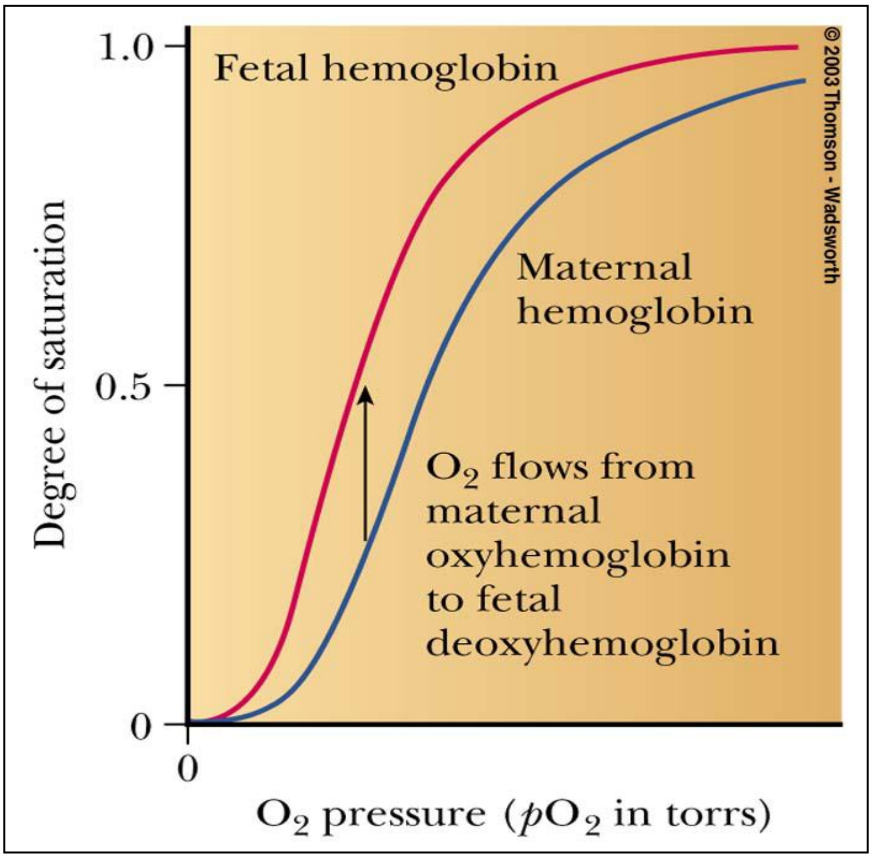
© 2006 Brooks/Cole - Thomson

Some Hemoglobin Variants

(1) Hb S : The primary hemoglobin in people with sickle cell anemia , *The presence of hemoglobin S causes the red blood cell to deform and assume a sickle shape when exposed to decreased amounts of oxygen.*

*(2) Hb F :*The primary hemoglobin produced by the fetus, and its role is to transport oxygen efficiently in a low oxygen environment. Production of Hb F decreases sharply after birth and reaches adult levels by 1-2 years of age.

- *Has a higher affinity for O_2 than maternal Hb A*
- *Consists of 2α2γ*
- *Binds less strongly to BPG than does Hb A*
- *This curve shows Oxygen binding capacity of Hb F :*



(3) Hb A :**Adult hemoglobin**, $\alpha_2\beta_2$, is the most common human **hemoglobin** tetramer, accounting for over 97% of the total red blood cell **hemoglobin**.

Glycated hemoglobin(HbA1c)

- ✓ Is a form of hemoglobin that is covalently bound to glucose ,hemoglobin carries oxygen in the blood. When hemoglobin is exposed to glucose in the blood, they are bound together through the glycation process.
- ✓ The fraction of hemoglobin glycosylated, normally about **5%**, is proportionate to blood glucose concentration.
- ✓ Since the half-life of an erythrocyte is typically 60 days, the level of glycosylated hemoglobin (HbA1c) reflects the mean blood glucose concentration ***over the preceding 6–8 weeks***.Measurement of HbA1c therefore provides valuable information for *management of diabetes mellitus*.

Adaptation to High Altitude

Physiological changes that accompany prolonged exposure to high altitude include an *increase in the number of erythrocytes* and *increase in their concentrations of hemoglobin and of BPG*.

Elevated BPG lowers the affinity of HbA for O2 (decreases P_{50}), which enhances release of O2 at the tissues.

BIOMEDICAL IMPLICATIONS

(1) Myoglobinuria

The presence of myoglobin in the urine, usually associated with rhabdomyolysis(i.e. ,*a condition in which damaged skeletal muscle breaks down rapidly*) or muscle destruction.

Urine from a person with rhabdomyolysis showing the characteristic brown discoloration as a result of myoglobinuria .

(2) Anemias

Reductions in the number of red blood cells or of hemoglobin in the blood, can reflect impaired synthesis of hemoglobin (eg, in iron deficiency; or impaired production of erythrocytes (eg, in folic acid or vit.B12 def.)

Factors Directing Folding

- ✓ Protein folding ➡the physical process by which a protein chain acquires its native 3D structure, a conformation that is usually biologically functional.
- ✓ Noncovalent interactions, such as :
  - ✧ Hydrogen bonding between polar side chains, e.g., Ser and Thr
  - ✧ Hydrophobic interaction between nonpolar side chains, e.g., Val and Ile
  - ✧ Electrostatic attraction between side chains of opposite charge, e.g., Lys and Glu
  - ✧ Electrostatic repulsion between side chains of like charge, e.g., Lys and Arg, Glu and Asp
- ✓ Formation of disulfide (-S-S-) covalent bonds between side chains of cysteines

---

Denaturation

- ✓ *Denaturation of proteins* involves the disruption and possible destruction of both the *secondary and tertiary structures*.
- ✓ Since denaturation reactions are not strong enough to break the peptide bonds, the primary structure (sequence of amino acids) remains the same after a denaturation process.
- ✓ Denaturation disrupts the normal α-helix and β-sheets in a protein and uncoils it into a random shape.
- ✓ Denaturation can be brought about by :
  1. Heat
  2. Large changes in pH, which alter charges on side chains, e.g., $-COO^-$ to $-COOH$ or $-NH^+$ to $-NH$
  3. Detergents such as sodium dodecyl sulfate (SDS) which disrupt hydrophobic interactions
  4. Urea or guanidine HCL, which form H-bonds with protein that are stronger than those within the protein disrupt H-bonding
  5. Mercaptoethanol, which reduces disulfide bonds.

Protin Purificartion & Characterization Techniques

✓ Extracting Pure Proteins from Cells

- Many different proteins exist in a single cell. A detailed study of the properties of any one protein requires a homogeneous sample *consisting of only one kind of molecule*.
- The separation and isolation, or purification, of proteins constitutes an essential *first step* to further experimentation.
- In general, separation techniques focus on *size*, *charge*, and *polarity*—the sources of differences between molecules.
- Many techniques are performed to eliminate contaminants and to arrive at a pure sample of the protein of interest.
- As the purification steps are followed, we make a *table*(see the table below) of the recovery and purity of the protein to gauge our success.

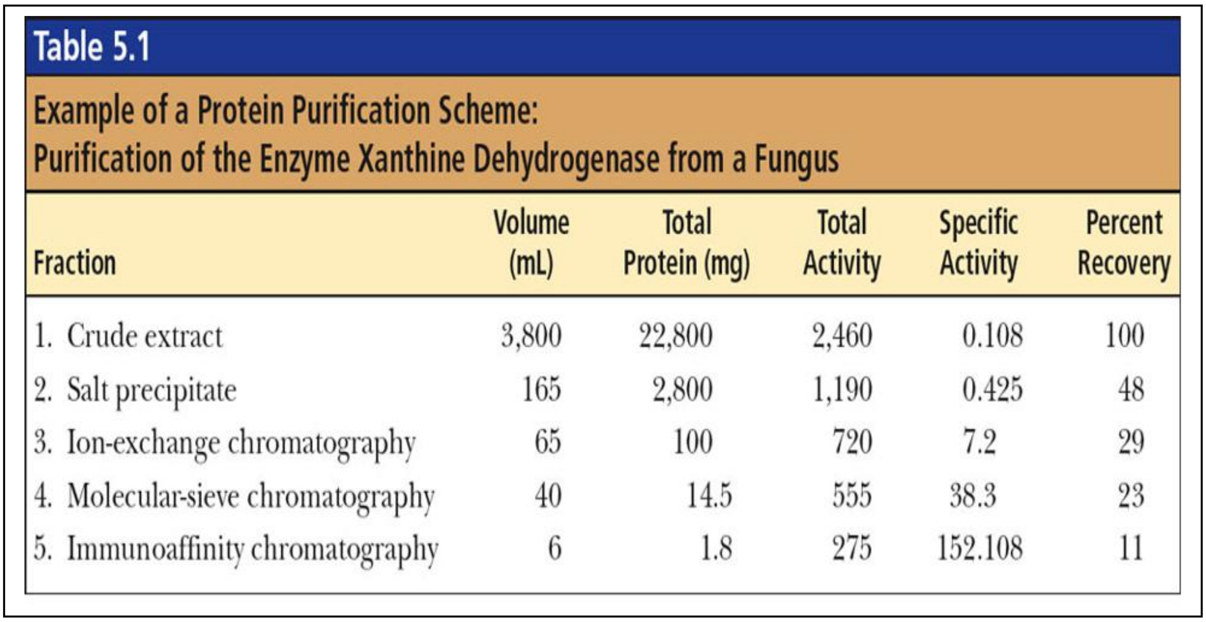


Table 5.1

Example of a Protein Purification Scheme: Purification of the Enzyme Xanthine Dehydrogenase from a Fungus

Fraction	Volume (mL)	Total Protein (mg)	Total Activity	Specific Activity	Percent Recovery
1. Crude extract	3,800	22,800	2,460	0.108	100
2. Salt precipitate	165	2,800	1,190	0.425	48
3. Ion-exchange chromatography	65	100	720	7.2	29
4. Molecular-sieve chromatography	40	14.5	555	38.3	23
5. Immunoaffinity chromatography	6	1.8	275	152.108	11

✓ Notes :

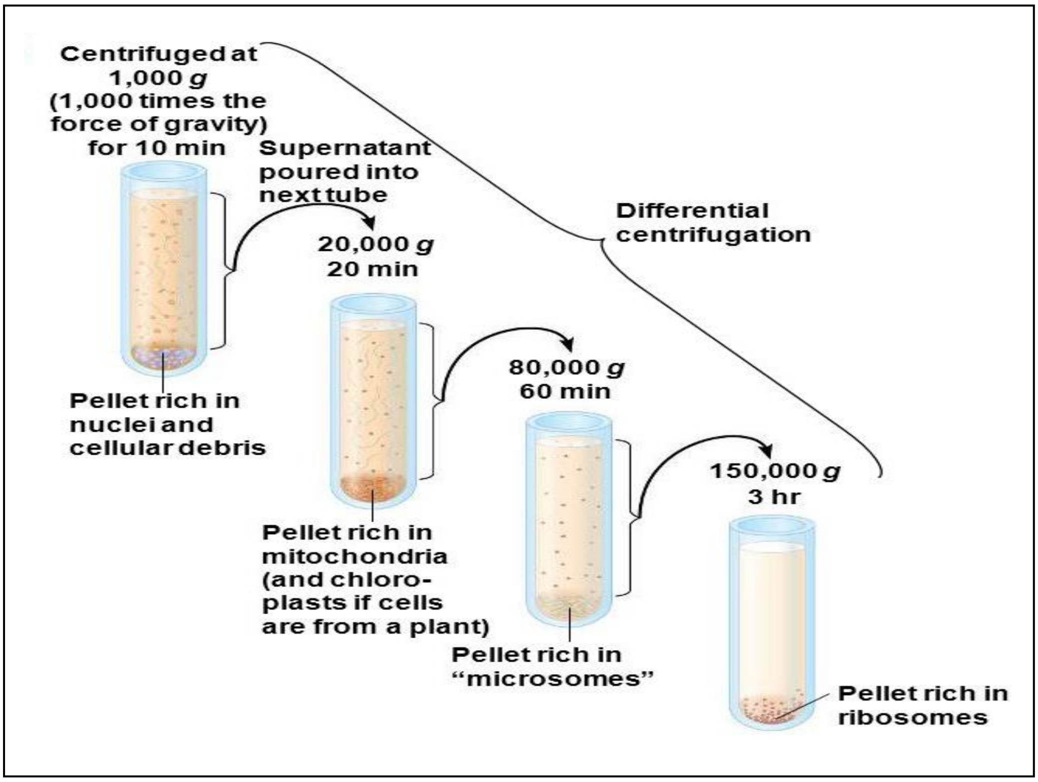
- ➢ The ***percent recovery*** column tracks how much of the protein of interest has been retained at each step. This number usually ***drops steadily during the purification***, and we hope that by the time the protein is pure, sufficient product will be left for study and characterization.
- ➢ The ***specific activity*** column compares the purity of the protein at each step, and this value ***should go up if the purification is successful***.
- ➢ Volume & Total protein values ***decrease*** during purification .

How do we get the proteins out of the cells?

- Disruption of cells(i.e. ,homogenization, involves breaking open the cells) is the first step in protein purification. The various parts of cells can be separated by centrifugation. This is a useful step because proteins tend to occur in given organelles.
- After the proteins are solubilized, they are often subjected to a crude purification based on solubility. ***Ammonium sulfate*** is the most common reagent to use at this step, and this procedure is referred to as salting out.
- So ,Initial purification steps are accomplished using differential centrifugation and salting out with ammonium sulfate. Then , further separation is done by *chromatography* and *electrophoresis.*

Differential Centrifugation

- It is a common procedure in biochemistry and cell biology used to separate organelles and other sub-cellular particles on the basis of sedimentation rate.
- Procedure (explained briefly) :
  - ✓ Differential centrifugation can be used with intact particles (e.g. biological cells, microparticles, nanoparticles), or used to separate the component parts of a given particle.
  - ✓ Using the example of a separation of eukaryotic organelles from intact cells, the cell must first be lysed and homogenized . Once the crude organelle extract is obtained, it may be subjected to a varying centrifugation speeds to separate the organelles .



- ✓ Two of the most important methods for separating amino acids, peptides, and proteins are *chromatography* and *electrophoresis*.

Column Chromatography

- Chromatography is based on the fact that different compounds can distribute themselves to varying extents between different phases, or separable portions of matter. One phase is the stationary phase, and the other is the mobile phase.
- The mobile phase flows over the stationary material and carries the sample to be separated along with it.
- The components of the sample interact with the stationary phase to different extents. Some components interact relatively strongly with the stationary phase and are therefore carried along more slowly by the mobile phase than are those that interact less strongly. The differing mobilities of the components are the basis of the separation.
- Many chromatographic techniques used for research on proteins are forms of column chromatography, in which the material that makes up the stationary phase is packed in a column. The sample is a small volume of concentrated solution that is applied to the top of the column; the mobile phase, called the eluent, is passed through the column.
- The sample is diluted by the eluent, and the separation process also increases the volume occupied by the sample.
- The various forms of chromatography rely on differences in *charge*, *polarity*, or *size* of the molecules to be separated, depending on the application.

Now,What are the different types of chromatography?

(1) Size-exclusion chromatography<aka, gel-filtration chromatography>
(2) Affinity chromatography .
(3) Ion-exchange chromatography .

Let's explain them and general principle of each ;

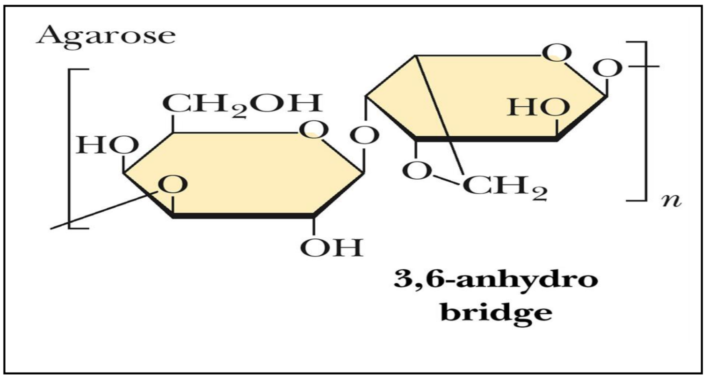
- Size-exclusion chromatography:

separates molecules on the basis of size, making it a useful way to sort proteins of varied molecular weights.

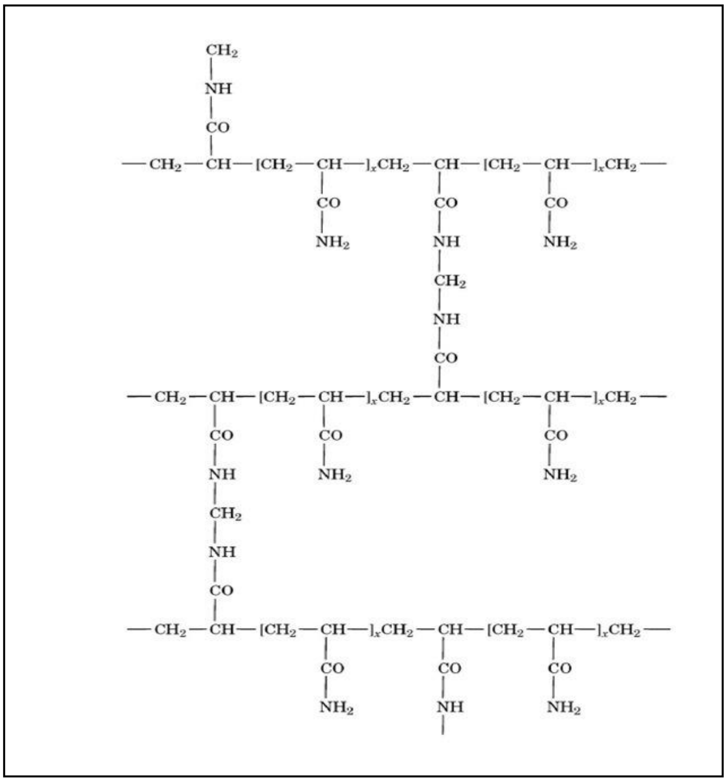
It is a form of column chromatography in which the stationary phase consists of cross-linked gel particles.

The gel particles are usually in bead form and consist of one of two kinds of polymers.

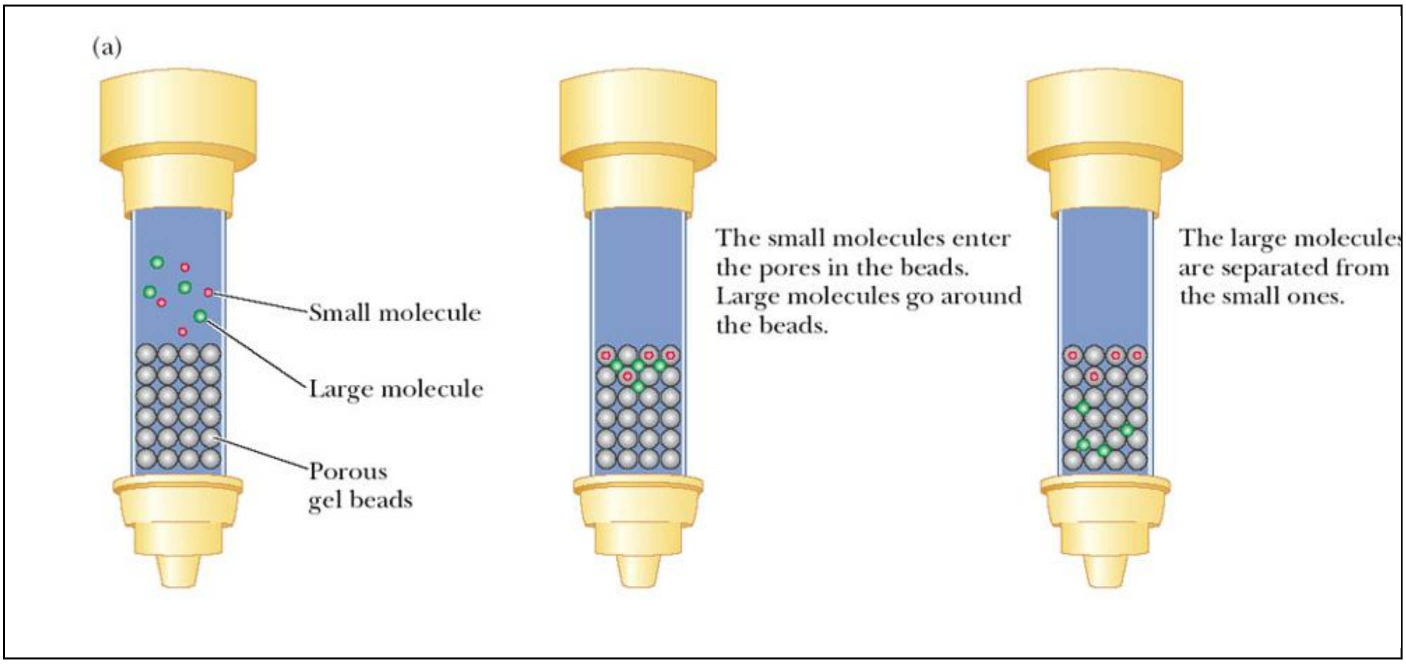
- ➢ The 1st is a carbohydrate polymer, such as dextran or agarose.



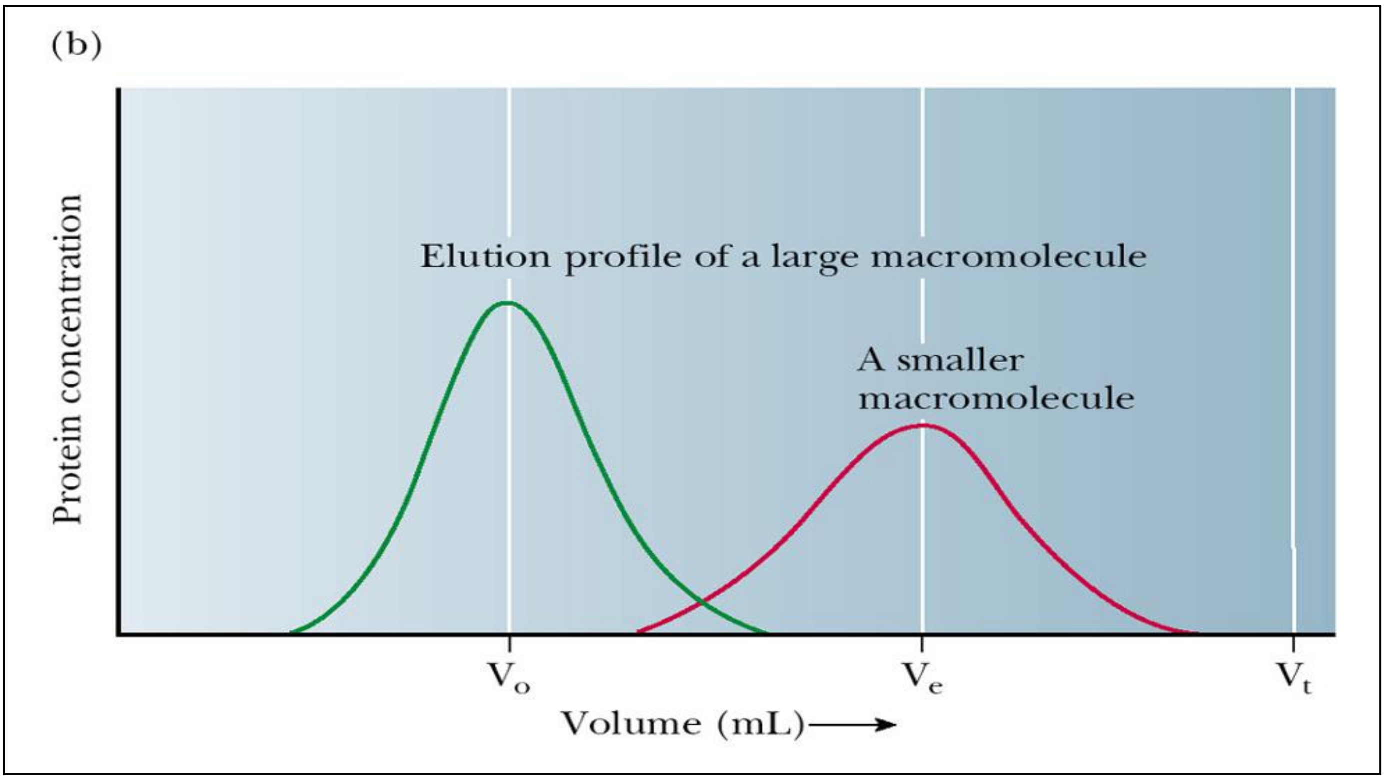
- ➢ The 2nd is based on polyacrylamide .



- ✓ The cross-linked structure of these polymers produces pores in the material ,the extent of cross-linking can be controlled to select a desired pore size.



- When a sample is applied to the column, smaller molecules, which are able to enter the pores, tend to be delayed in their progress down the column, unlike the larger molecules. As a result, the larger molecules are eluted first, followed later by the smaller ones, after escaping from the pores.



- The advantages of this type of chromatography are :

(1) It's convenience as a way to separate molecules on the basis of size.
(2) The fact that it can be used to estimate molecular weight by comparing the sample with a set of standards.

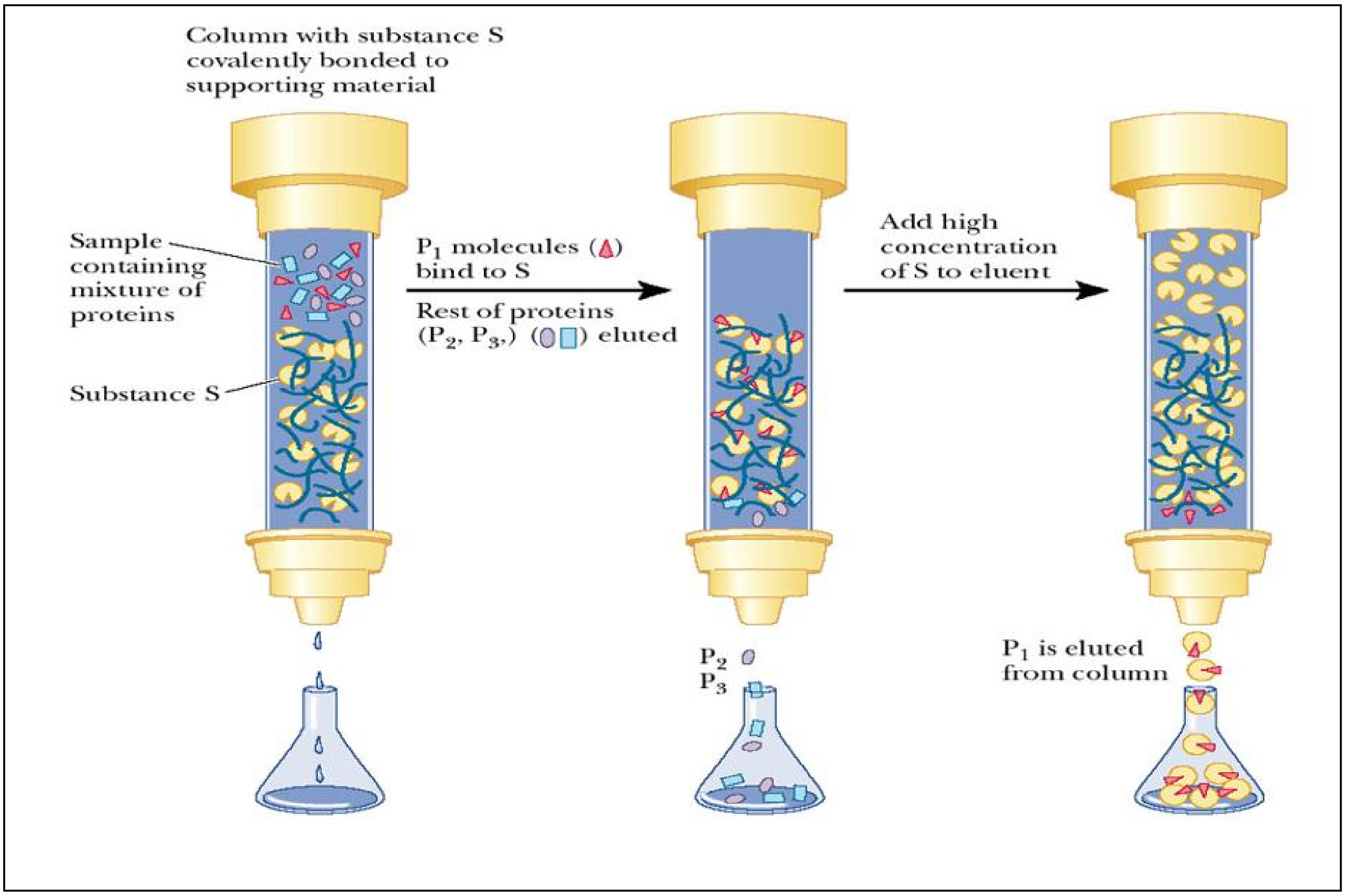
Affinity chromatography :

This type uses the specific binding properties of many proteins. It is another form of column chromatography with a polymeric material used as the stationary phase.

The distinguishing feature of affinity chromatography is that the polymer is covalently linked to some compound, called a ligand, that binds specifically to the desired protein , the other proteins in the sample do not bind to the column and can easily be eluted with buffer, while the bound protein remains on the column.

The bound protein can then be eluted from the column by adding high concentrations of the ligand in soluble form, thus competing for the binding of the protein with the stationary phase.

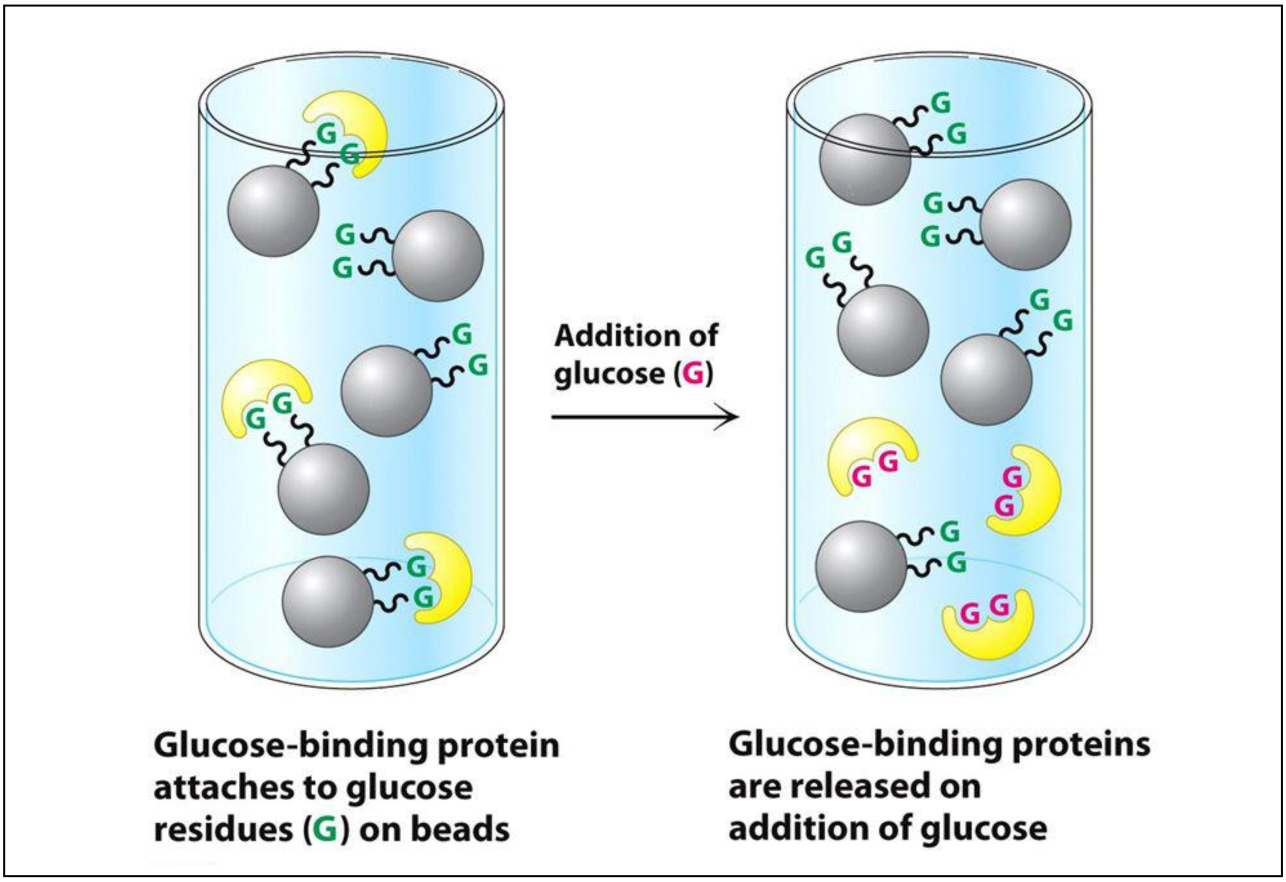
Affinity chromatography is a convenient separation method and has the advantage of producing very pure proteins.



- In a mixture of proteins, only one (designated P1) will bind to a substance (S) called the substrate. The substrate is attached to the column matrix. Once the other proteins (P2 and P3) have been washed out, P1 can be eluted, either by adding a solution of high salt concentration or by adding free S.

➡Imp example :

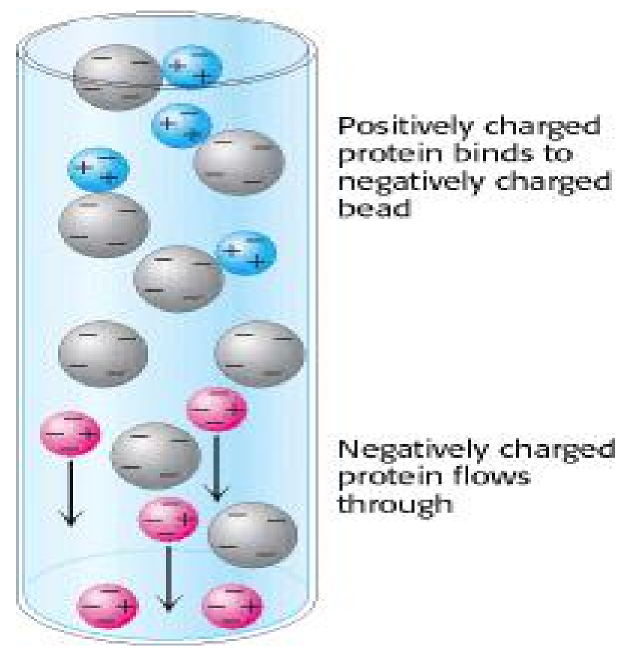
Affinity chromatography of concanavalin A (shown in yellow) on a solid support containing covalently attached glucose residues (G).



- The plant protein concanavalin A can be purified by passing a crude extract through a column of beads containing covalently attached glucose residues.
- Concanavalin A binds to such a column because it has affinity for glucose, whereas most other proteins do not. The bound concanavalin A can then be released from the column by adding a concentrated solution of glucose.

- Ion-exchange chromatography :

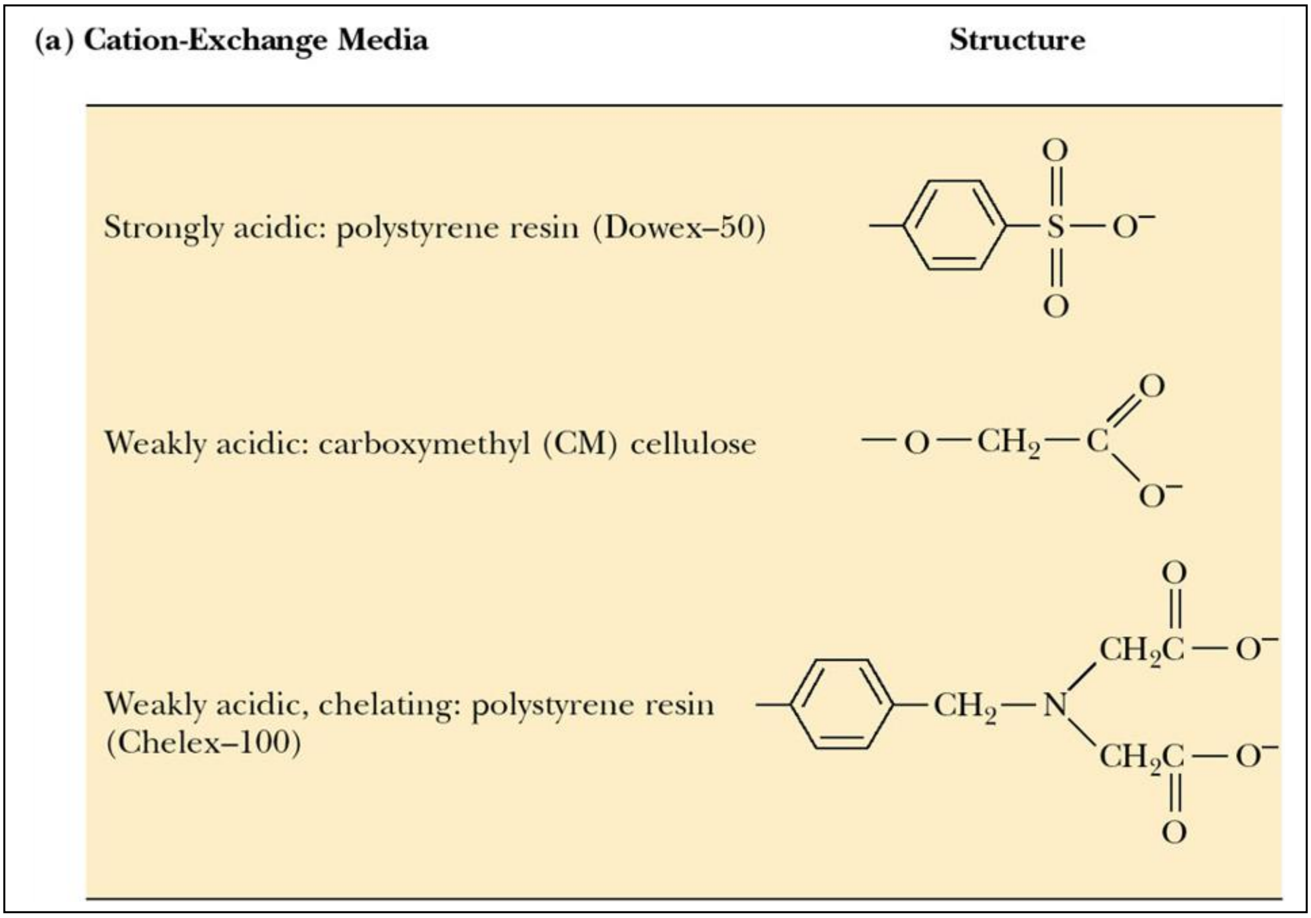
Similar to affinity chromatography. Both use a column resin that binds the protein of interest. With ion-exchange chromatography, however, the interaction is less specific and is based on net charge.



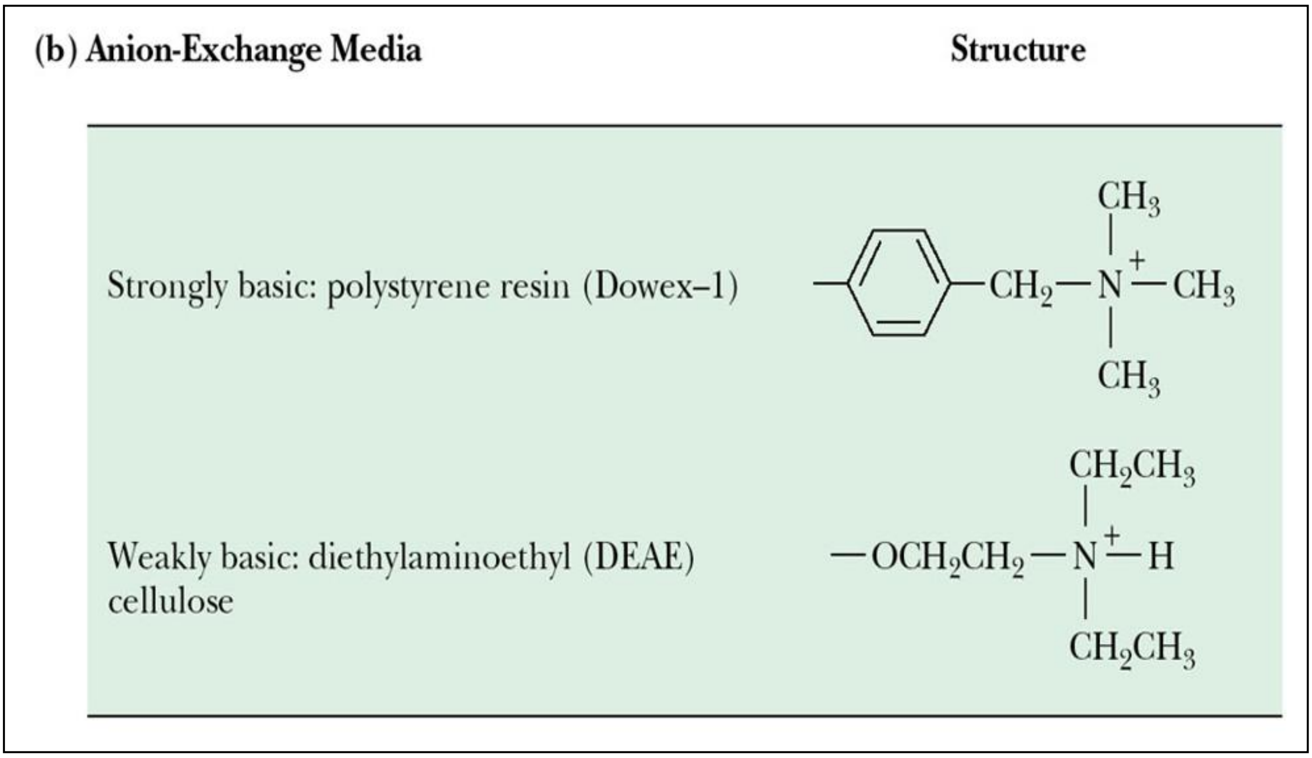
Positively charged protein binds to negatively charged bead

Negatively charged protein flows through

An ion-exchange resin has a ligand with a positive charge or a negative charge. A ***negatively charged resin is a cation exchanger***, and a ***positively charged one is an anion exchanger***.



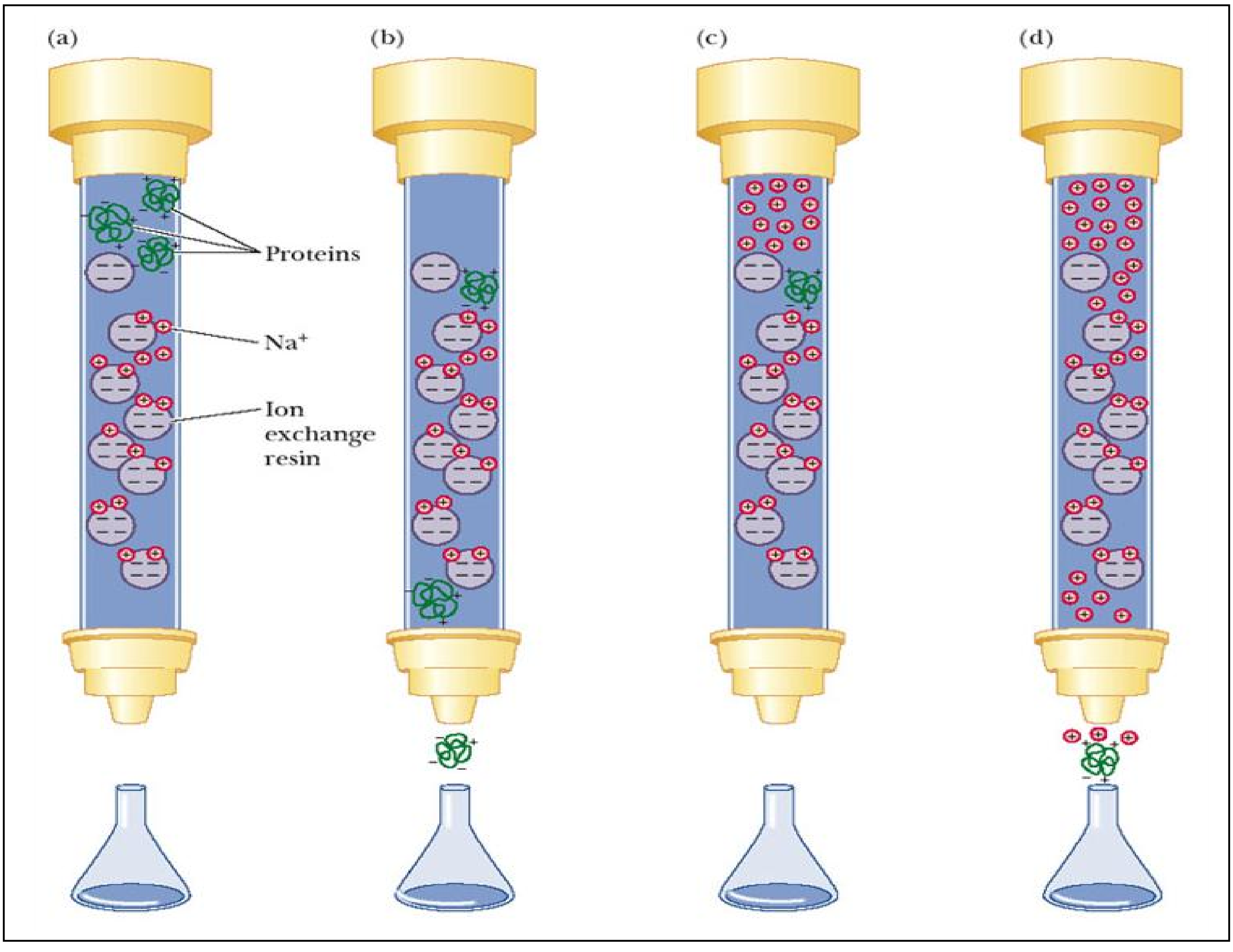
(a) Cation-Exchange Media	Structure
Strongly acidic: polystyrene resin (Dowex–50)	
Weakly acidic: carboxymethyl (CM) cellulose	
Weakly acidic, chelating: polystyrene resin (Chelex–100)	



(b) Anion-Exchange Media	Structure
Strongly basic: polystyrene resin (Dowex–1)	$-C_6H_4-CH_2-\overset{+}{N}(CH_3)_3$
Weakly basic: diethylaminoethyl (DEAE) cellulose	$-OCH_2CH_2-\overset{+}{N}H(CH_2CH_3)_2$

*You aren’t asked to memorize the structures , you only have to know the name and its classification .

(This figure shows Ion-exchange chromatography using a cation exchanger)



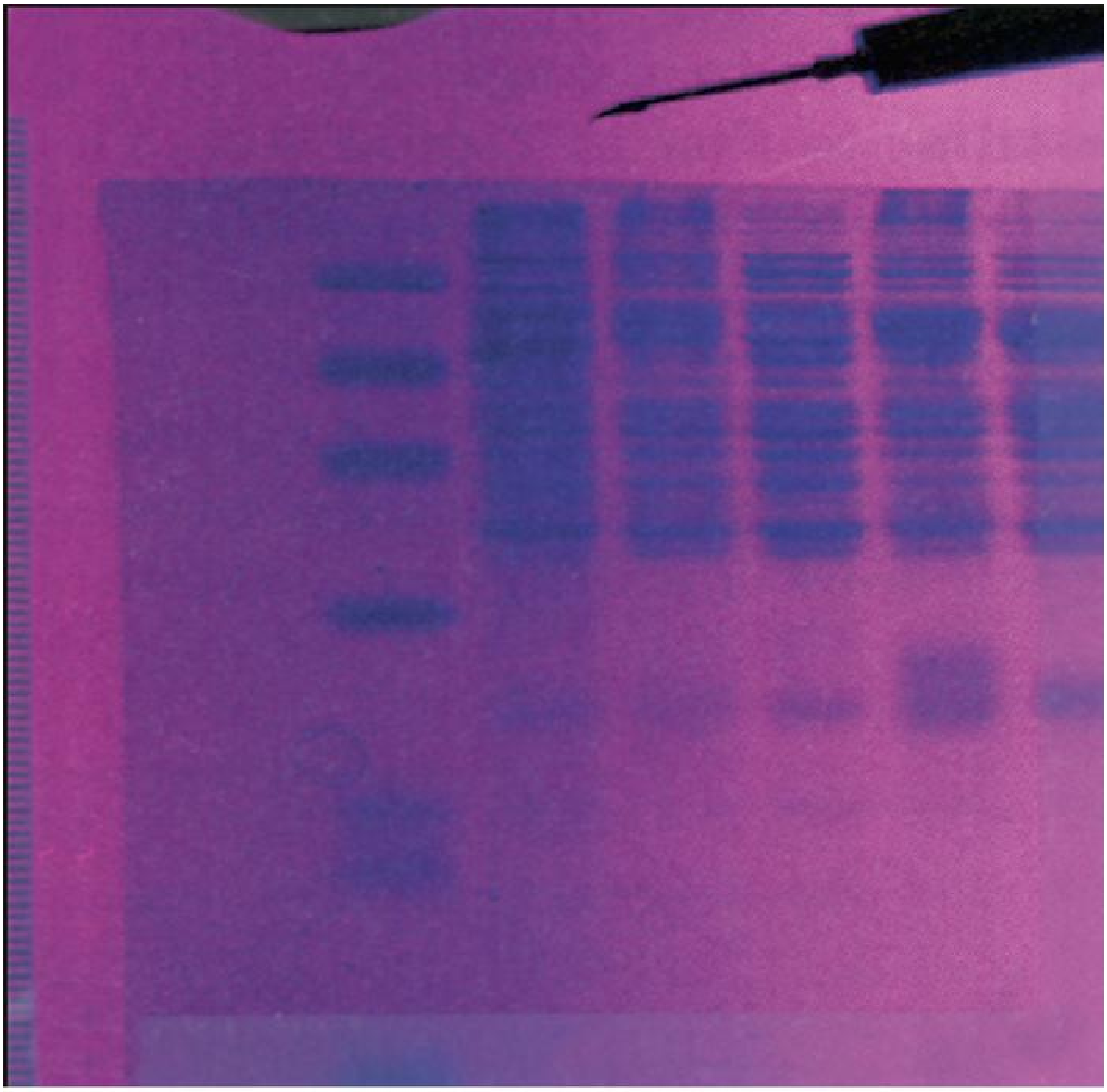
(a) At the beginning of the separation, various proteins are applied to the column. The column resin is bound to Na+ counterions (small red spheres).
(b) Proteins that have no net charge or a net negative charge pass through the column. Proteins that have a net positive charge stick to the column, displacing the Na+.
(c) An excess of Na+ ion is then added to the column.
(d) The Na+ ions outcompete the bound proteins for the binding sites on the resin, and the proteins elute.

In a nutshell :

- ✓ Column chromatography refers to several common techniques for purification of proteins.
- ✓ In gel-filtration chromatography, proteins are separated by size .
- ✓ In ion-exchange chromatography, molecules with a specific charge are selectively bound to a column, separated from proteins that don't bind, and then eluted.
- ✓ In affinity chromatography, molecules are bound to the column via specific interactions for a bound ligand. Once nonbinding proteins are removed, the protein of interest can be eluted.

Electrophoresis

- Electrophoresis is the process of separating compounds on the basis of their electric charge & size
- In electrophoresis, differences in *charge and in size* are the criteria for separation.
- Although many supporting media have been used for electrophoresis, including paper and liquid, the most common support is a *polymer of agarose or acrylamide* that is similar to those used for column chromatography.
- A sample is applied to wells that are formed in the supporting medium. An electric current is passed through the medium at a controlled voltage to achieve the desired separation.
- After the proteins are separated on the gel, the gel is stained to reveal the protein locations (As seen in the figure in the next page) .



- Electrophoresis of amino acids can be carried out using paper, starch, agar, certain plastics, and cellulose acetate as solid supports .

  ✓ In ***paper electrophoresis*** :

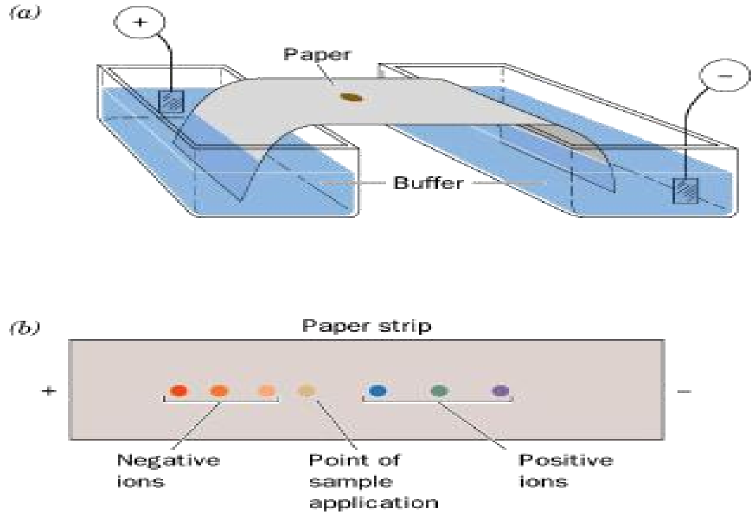
  ➞A paper strip saturated with an aqueous buffer of predetermined pH serves as a bridge between two electrode vessels.

  ➞A sample of amino acids is applied as a spot (the origin) on the solid support strip

  ➞An electric potential is applied to the electrode vessels and amino acids migrate toward the electrode with charge opposite their own .

  ➞Molecules with a high charge density move faster than those with a low charge density ,molecules at their isoelectric point remain at the origin .

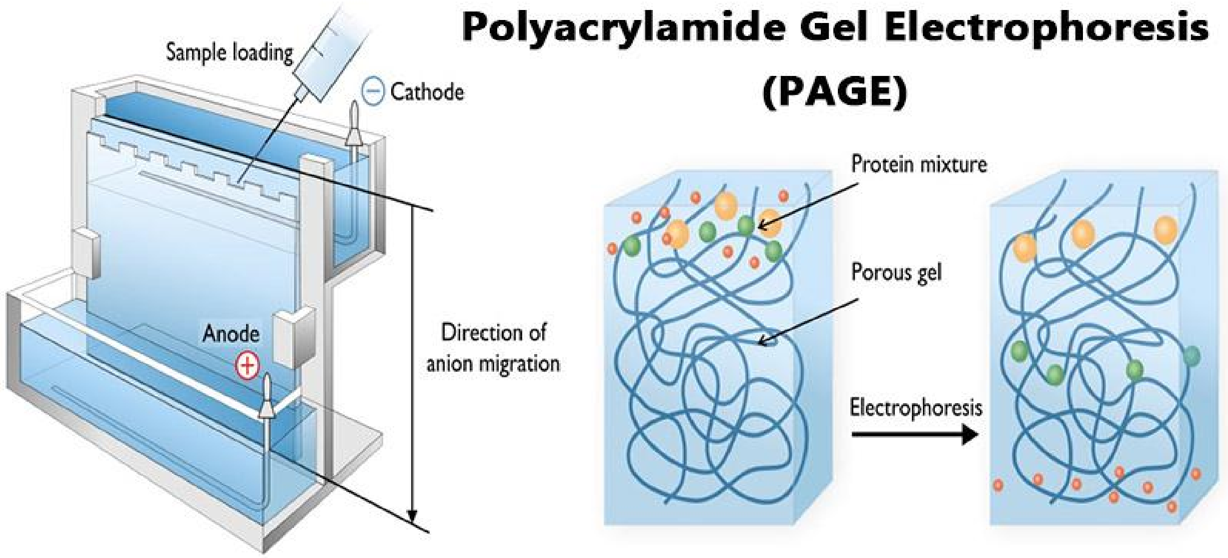
  ➞After separation is complete, the strip is dried and developed to make the separated amino acids visible .



Paper electrophoresis

SDS – polyacrylamide-gel electrophoresis (SDS – PAGE)

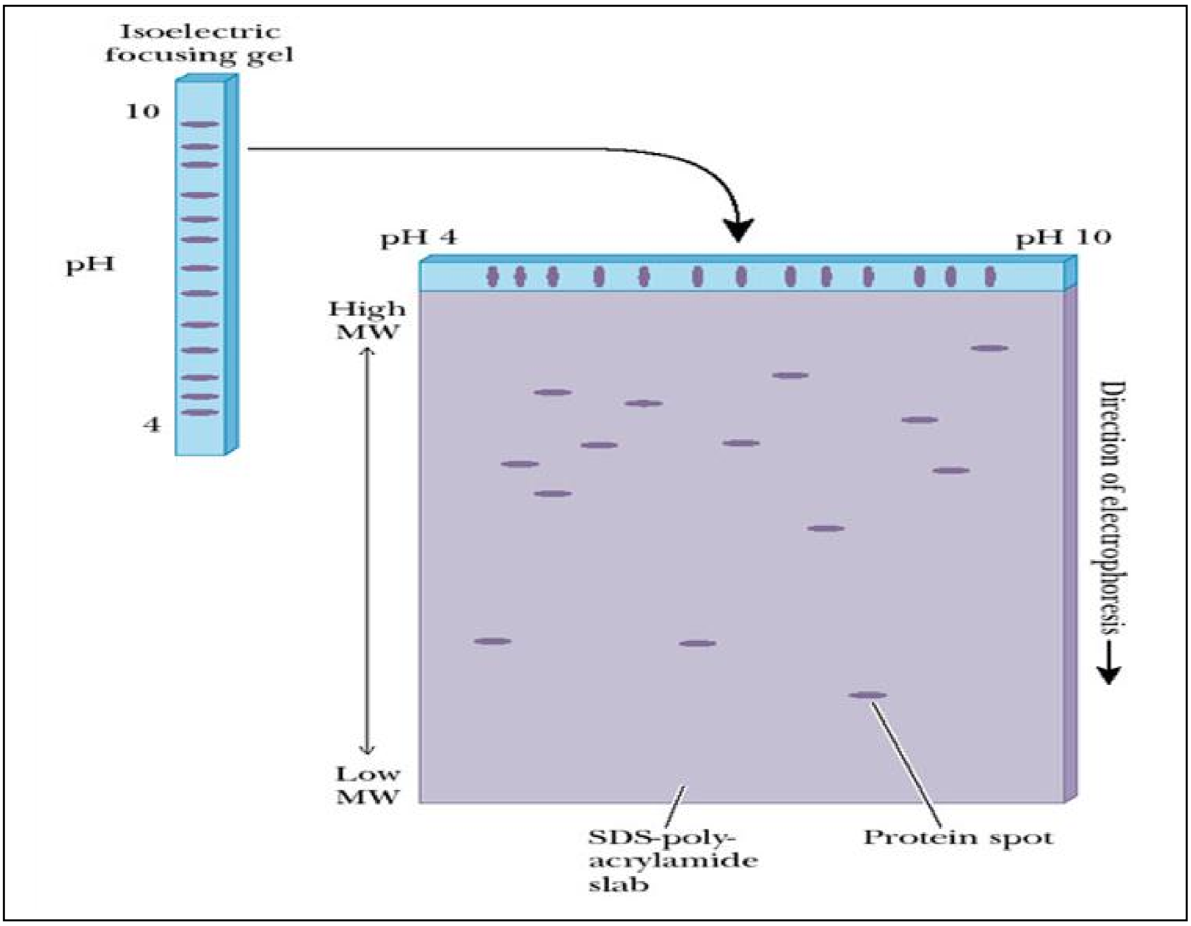
- The acrylamide offers more resistance to large molecules than to small molecules. Because the shape and charge are approximately the same for all the proteins in the sample, *the size of the protein becomes the determining factor in the separation* ; small proteins move faster than large ones. Like molecular-sieve chromatography, SDS – PAGE can be used *to estimate the molecular weights of proteins by comparing the sample with standard samples*.


Polyacrylamide Gel Electrophoresis (PAGE)

- ✓ An ingenious combination, known as *two-dimensional gel electrophoresis* (2-D gels), allows for enhanced separation by using isoelectric focusing in one dimension and SDS – PAGE run at 90° to the first .

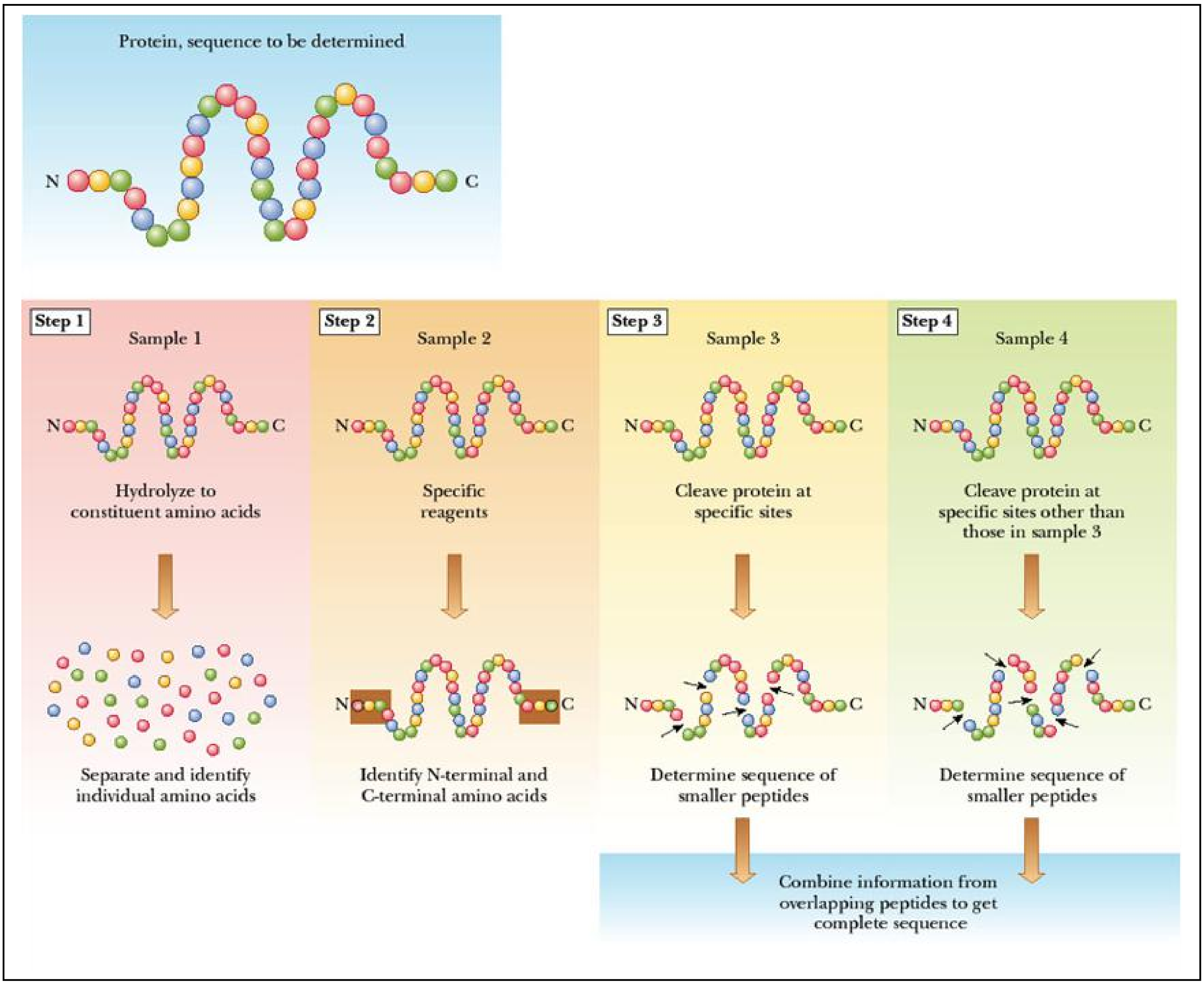
- ✓ Isoelectric focusing is another variation of gel electrophoresis. Because different proteins have different titratable groups, they also have different isoelectric points. Recall that the isoelectric pH (pI) is the pH at which a protein (or amino acid or peptide) has no net charge.
- ✓ At the pI, the number of positive charges exactly balances the number of negative charges.

- ✓ In an isoelectric focusing experiment, the gel is prepared ***with a pH gradient that parallels the electric-field gradient***. As proteins migrate through the gel under the influence of the electric field, they encounter *regions of different pH*, so the charge on the protein changes. Eventually each protein reaches the point at which it has no net charge—its isoelectric point—and no longer migrates. Each protein remains at the position on the gel corresponding to its pI, allowing for an effective method of separation.



How Do We Determine the Primary Structure of a Protein?

Determination of the N-terminal and C-terminal amino acids of proteins depends on the use of these separation methods after the ends of the molecule have been chemically labeled. Selective cleavage of the protein into peptides by enzymatic or chemical hydrolysis produces fragments of manageable size for sequencing.

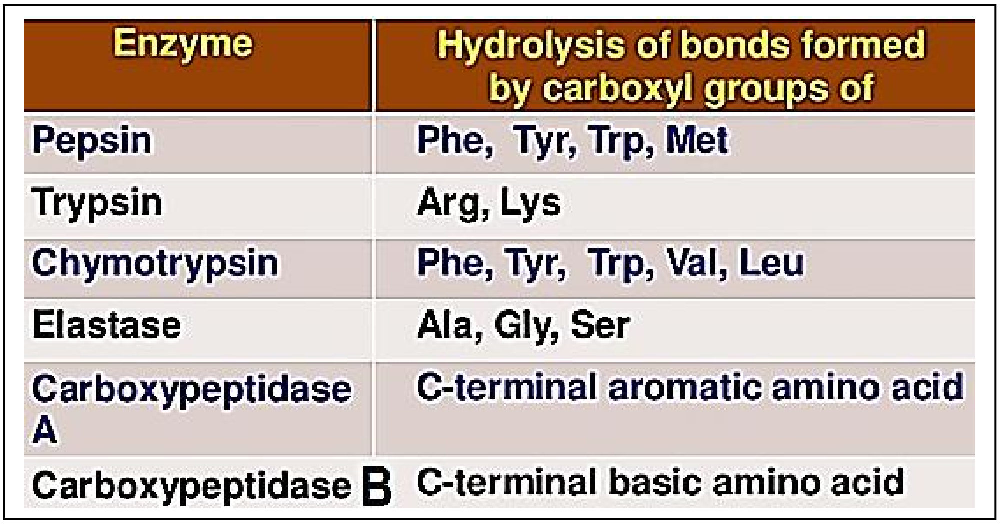


➡*Highly purified protein is essential for determination of its amino acid sequence*. Cells contain thousands of different proteins, each in widely varying amounts. The isolation of a specific protein in quantities sufficient for analysis presents a challenge that may require multiple successive purification techniques.

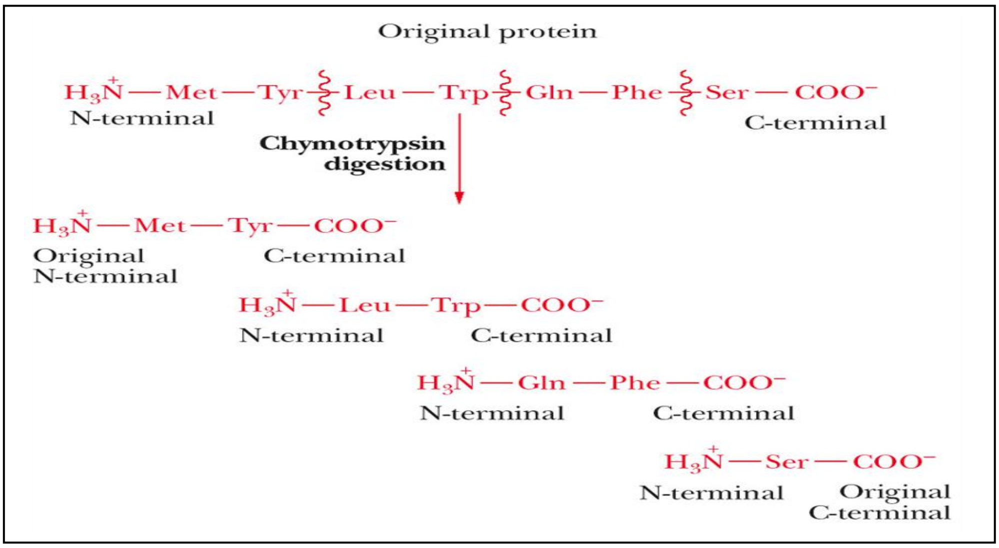
Cleavage of proteins into peptides :

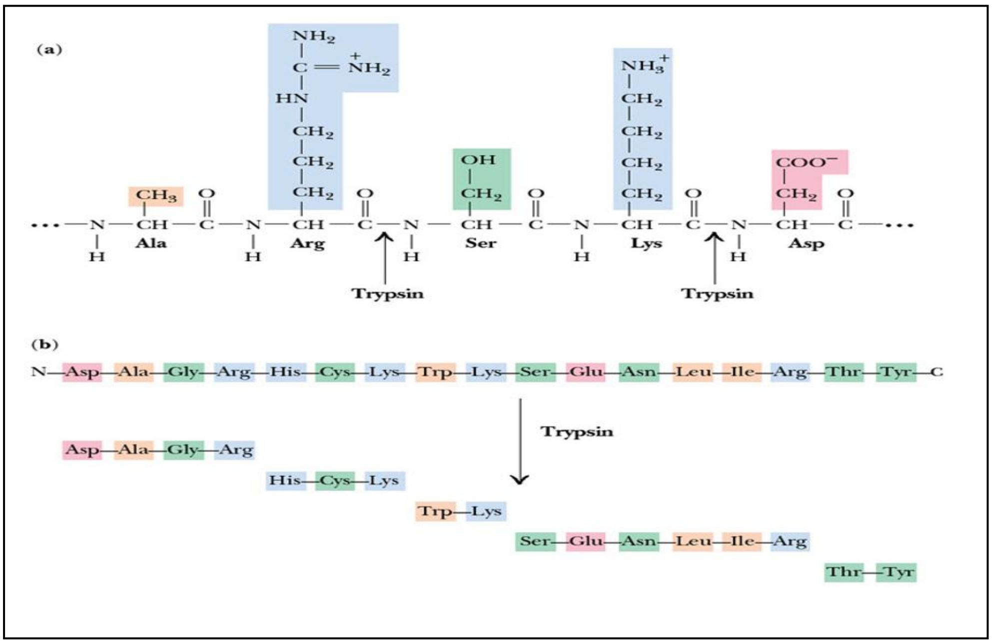
- Proteins can be cleaved at specific sites by enzymes or by chemical reagents.

➞Enzymatic Cleavage :



Enzyme	Hydrolysis of bonds formed by carboxyl groups of
Pepsin	Phe, Tyr, Trp, Met
Trypsin	Arg, Lys
Chymotrypsin	Phe, Tyr, Trp, Val, Leu
Elastase	Ala, Gly, Ser
Carboxypeptidase A	C-terminal aromatic amino acid
Carboxypeptidase B	C-terminal basic amino acid

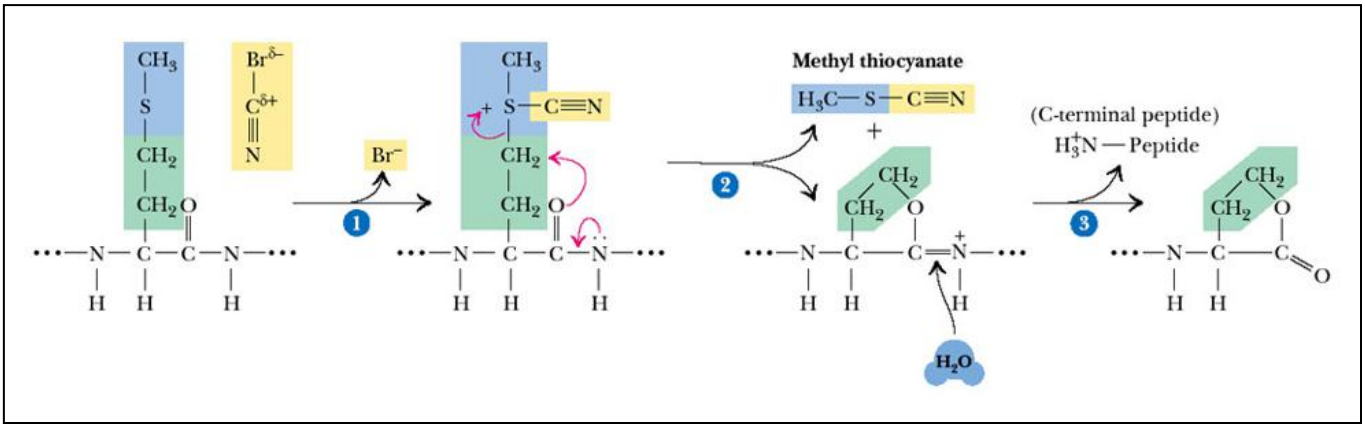


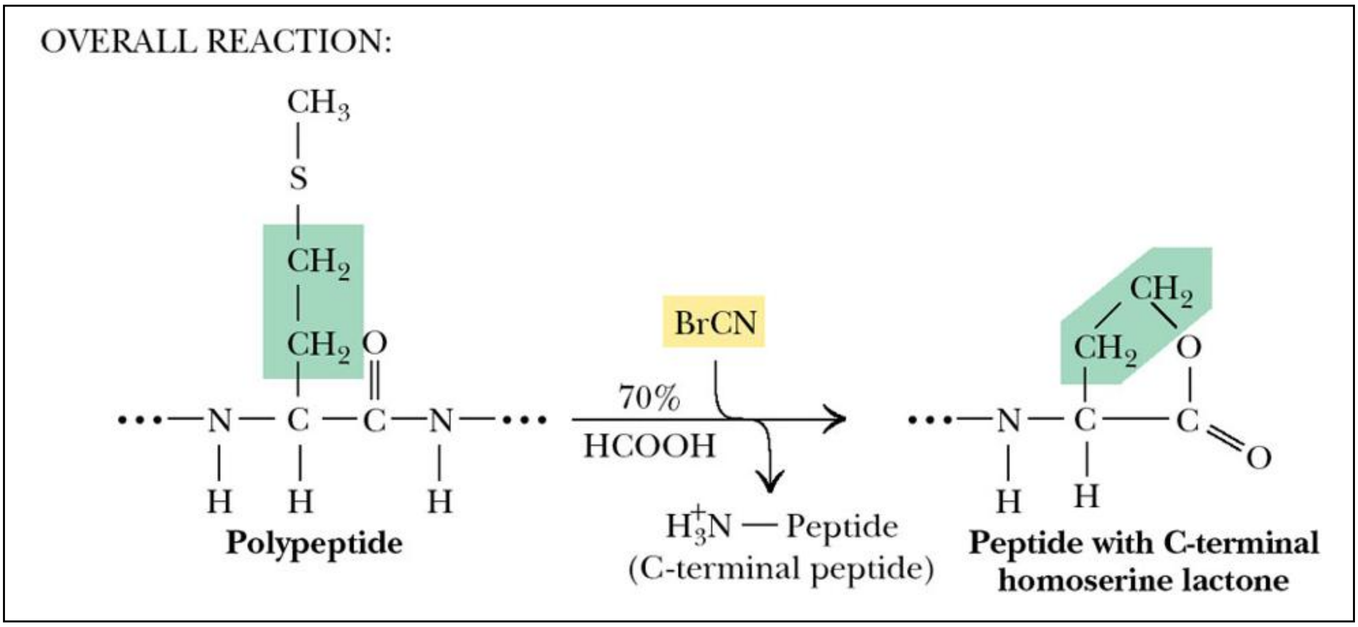


➞Chemical Cleavage :

(1) Cyanogen Bromide

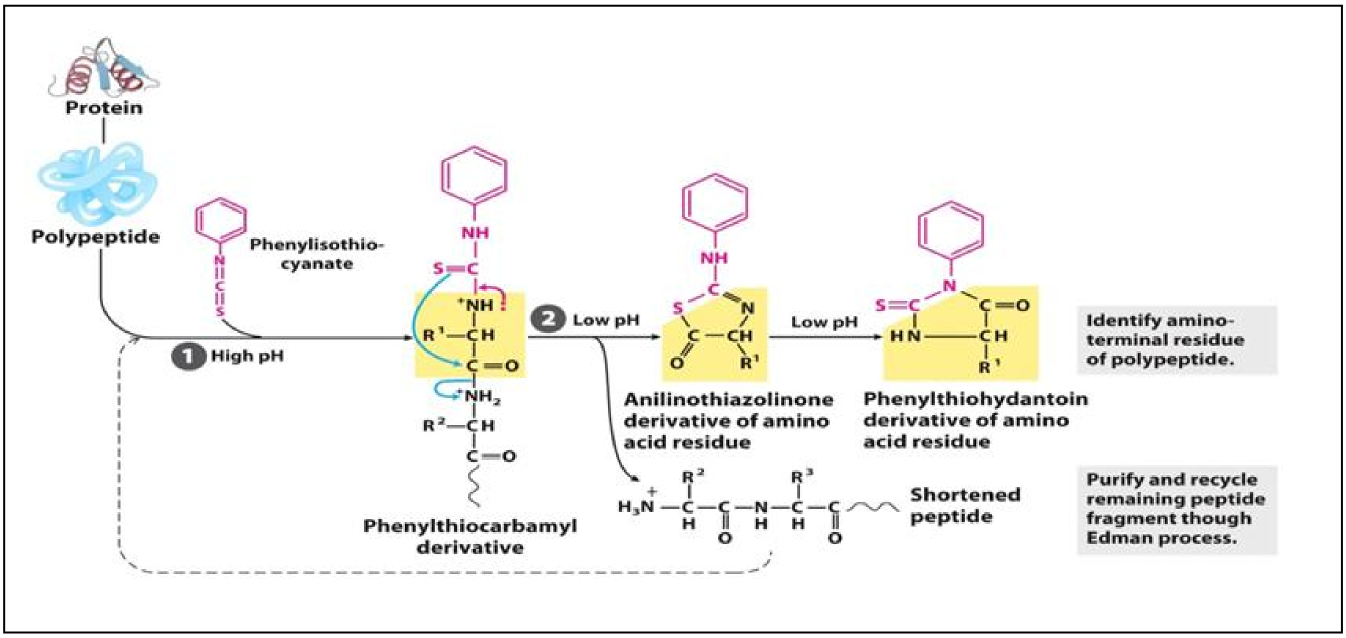
- In the case of the chemical reagent cyanogen bromide (CNBr), the sites of cleavage are at internal methionine residues. The sulfur of the methionine reacts with the carbon of the cyanogen bromide to produce a homoserine lactone at the C-terminal end of the fragment .





(2) Edman Degradation

- Is a method of sequencing amino acids in a peptide. In this method, the amino-terminal residue is labeled and cleaved from the peptide without disrupting the peptide bonds between other amino acid residues.
- The Edman reagent ➡ phenylisothiocyanate (PITC) .



Carbohydrates

هاي المحاضرة كتير بيسك ومهمة ، مش صعبة بس طويلة وهي الأخيرة بهاد الملخص :/

When the word carbohydrate was coined, it originally referred to compounds of the general formula **$Cn(H_2O)n$**. However, only the simple sugars, or monosaccharides,fit this formula exactly.

The other types of carbohydrates, oligosaccharides and polysaccharides, are based on the monosaccharide units and have slightly different general formulas.

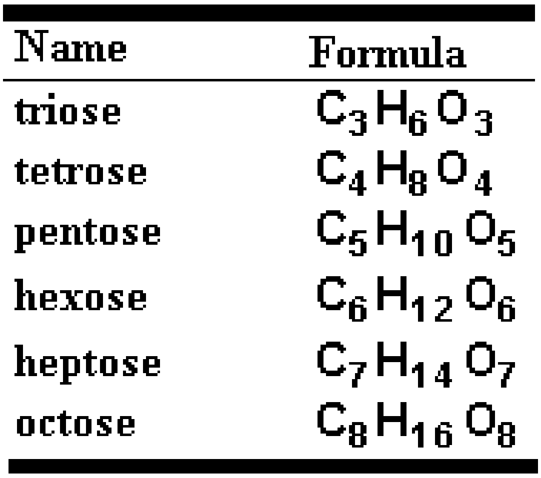
Carbohydrates are carbon compounds that contain large quantities of hydroxyl groups. The simplest carbohydrates also contain either an ***aldehyde moiety*** (these are termed polyhydroxyaldehydes) or a ***ketone moiety*** (polyhydroxyketones).

✓ All carbohydrates can be classified as:

- Monosaccharides.
- Oligosaccharides.
- Polysaccharides.

Monosaccharides

•The monosaccharides commonly found in humans are classified according to the number of carbons they contain in their backbone structures. The major monosaccharides contain four to six carbon atoms.

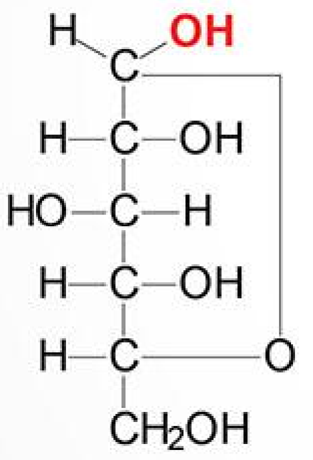


Name	Formula
triose	$C_3H_6O_3$
tetrose	$C_4H_8O_4$
pentose	$C_5H_{10}O_5$
hexose	$C_6H_{12}O_6$
heptose	$C_7H_{14}O_7$
octose	$C_8H_{16}O_8$

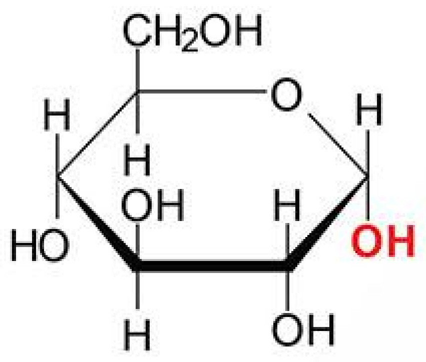
•The aldehyde and ketone moieties of the carbohydrates with five and six carbons will spontaneously react ***with alcohol groups present in neighboring carbons*** to produce intramolecular hemiacetals or hemiketals, respectively. This results in the formation of five- or six-membered rings. Because the five-membered ring structure resembles the organic molecule ***furan***, derivatives with this structure are termed ***furanoses***. Those with six-membered rings resemble the organic molecule ***pyran*** and are termed ***pyranoses.***

- Such structures can be depicted by either ***Fischer*** or ***Haworth*** style diagrams.
- The numbering of the carbons in carbohydrates proceeds from the carbonyl carbon, for aldoses, or the carbon nearest the carbonyl, for ketoses.

•Ex:



α-D-Glucose
cyclic Fischer projection



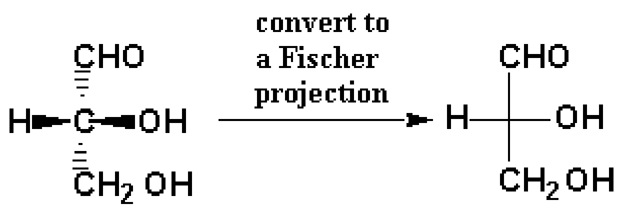
α-D-Glucose
Haworth projection

- ▪ The rings can open and re-close, allowing rotation to occur about the carbon bearing the reactive carbonyl yielding two distinct configurations (**α** and **β**) of the hemiacetals and hemiketals.
- ▪ The carbon about which this rotation occurs is the ***anomeric carbon*** and the two forms are termed anomers. Carbohydrates can change spontaneously between the α and β configurations .

Fisher Projection :

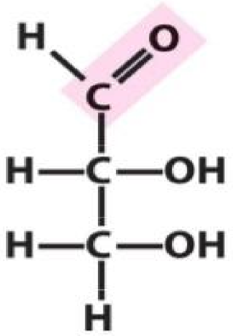
- 2D representation for showing the configuration of tetrahedral .
  - Horizontal lines represent bonds projecting forward .
  - Vertical lines represent bonds projecting to the rear .

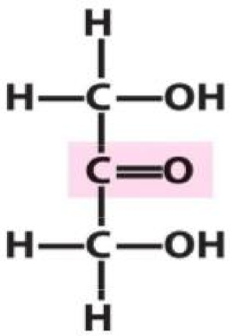
•Ex:



D-Glyceraldehyde D-Glyceraldehyde

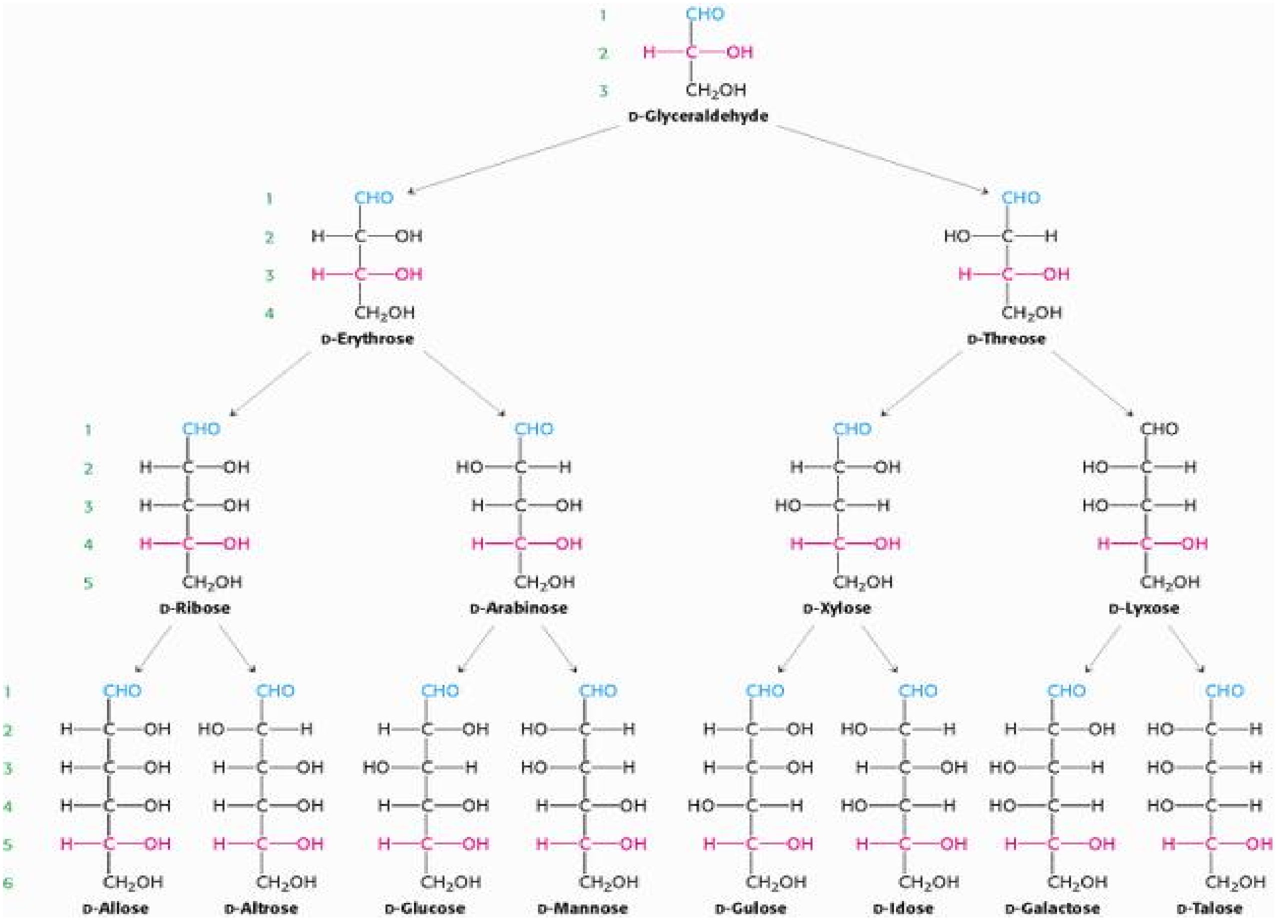
The only known trioses :


Glyceraldehyde,
an aldotriose

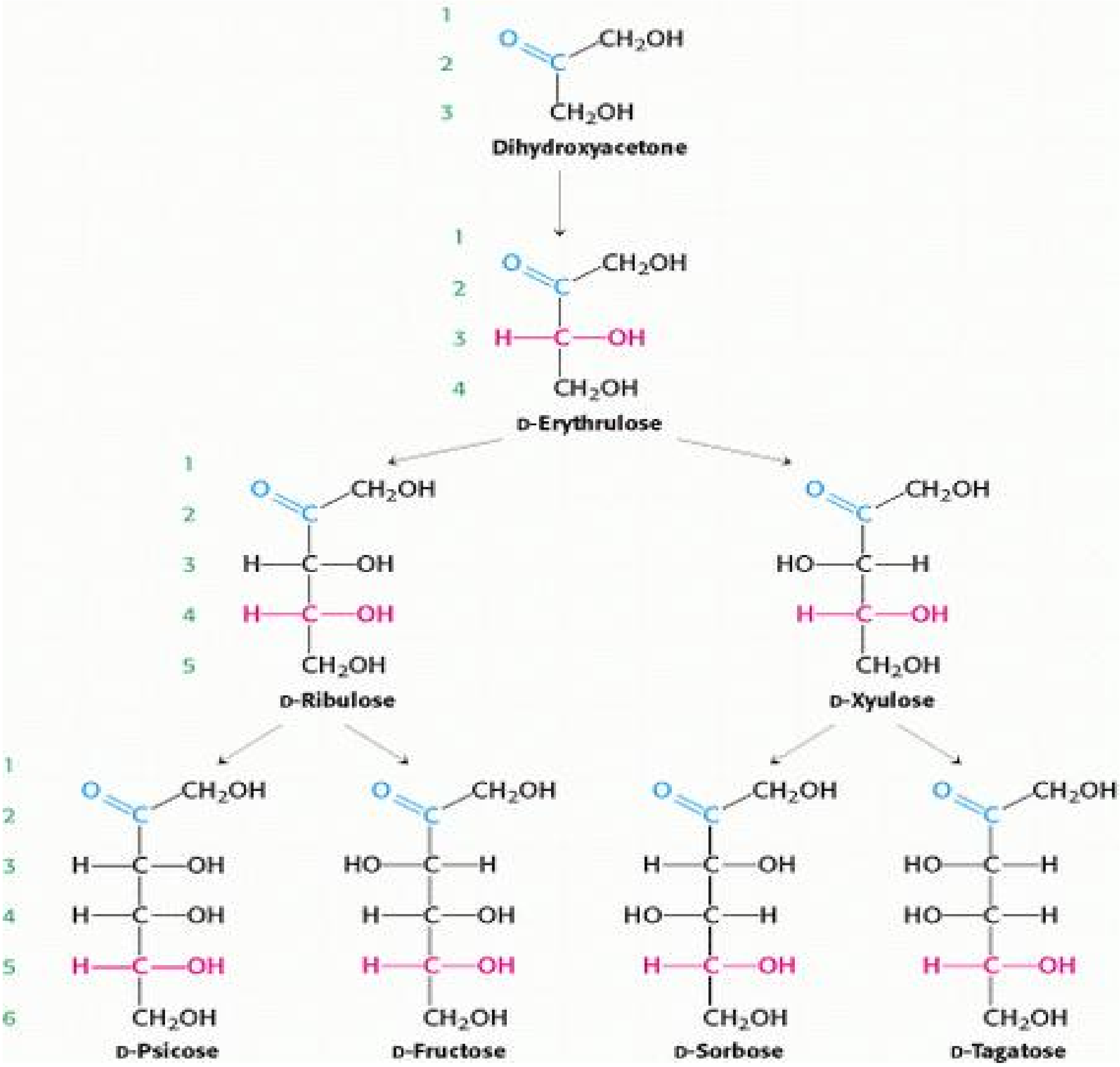

Dihydroxyacetone,
a ketotriose

- ✓ ***Aldo-*** and ***keto-*** are often omitted and these compounds are referred to simply as trioses; although this designation does not tell the nature of the carbonyl group, it at least tells the number of carbons .

D-Aldoses



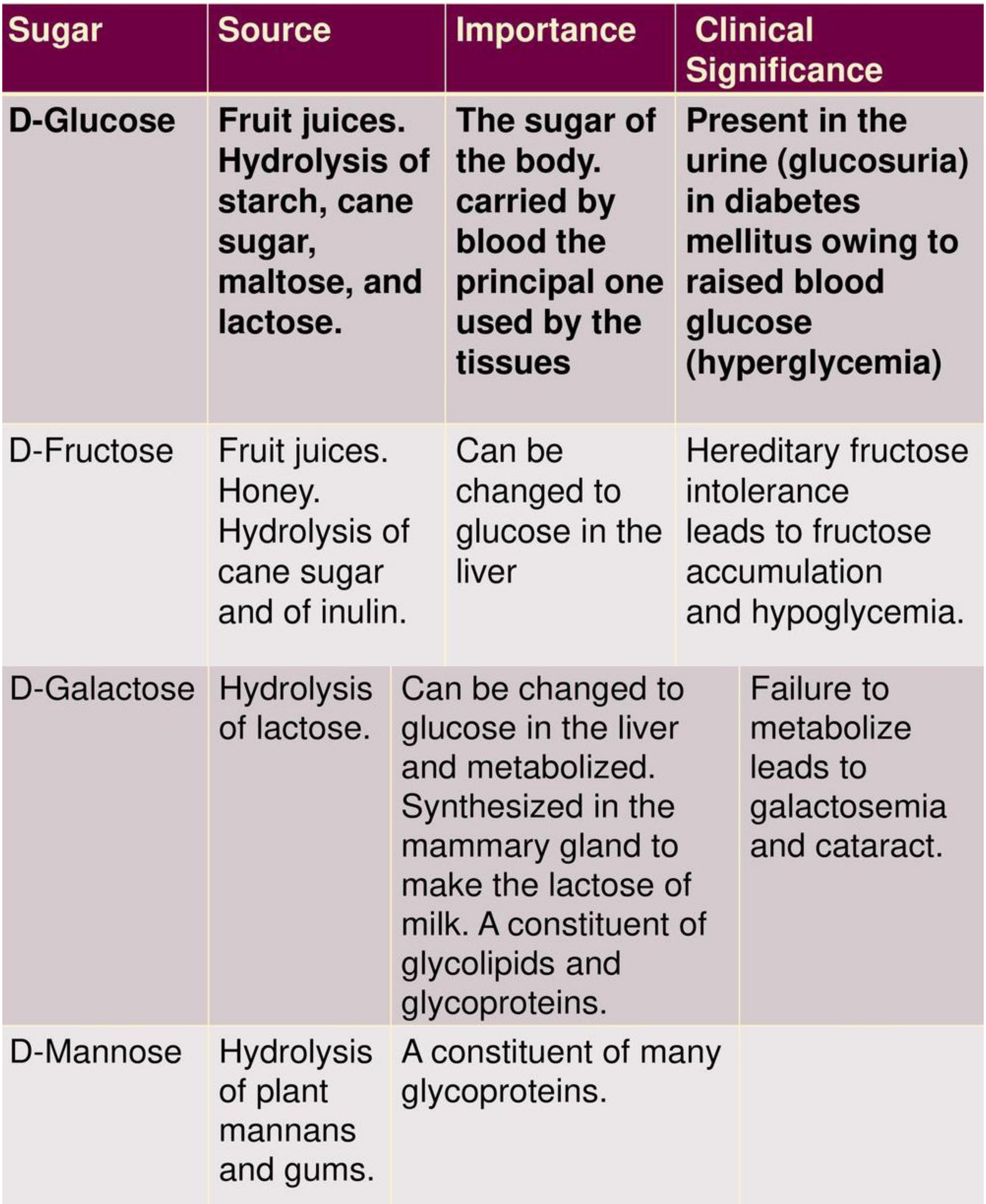
D-Ketoses



Sweetness Values for Sugars : مهم

- ✓ Sucrose(Table Sugar) ➡ 100% sweetness (Reference Sugar) .
- ✓ Fructose (Fruit Sugar)➡ 170% sweetness .
- ✓ Lactose (Milk Sugar) ➡ 15%-17% sweetness (Least sweet sugar) . For infants , it's the main source of carbs during their early stages of life .
- ✓ Maltose (Malt Sugar) ➡ 39% sweetness .
- ✓ Glucose ➡70%-74% sweetness .

Hexoses of physiologic importance(imp):



Sugar	Source	Importance	Clinical Significance
D-Glucose	**Fruit juices. Hydrolysis of starch, cane sugar, maltose, and lactose.**	**The sugar of the body. carried by blood the principal one used by the tissues**	**Present in the urine (glucosuria) in diabetes mellitus owing to raised blood glucose (hyperglycemia)**
D-Fructose	Fruit juices. Honey. Hydrolysis of cane sugar and of inulin.	Can be changed to glucose in the liver	Hereditary fructose intolerance leads to fructose accumulation and hypoglycemia.
D-Galactose	Hydrolysis of lactose.	Can be changed to glucose in the liver and metabolized. Synthesized in the mammary gland to make the lactose of milk. A constituent of glycolipids and glycoproteins.	Failure to metabolize leads to galactosemia and cataract.
D-Mannose	Hydrolysis of plant mannans and gums.	A constituent of many glycoproteins.	

Sugars that aren't found in our diets and their importance

- ✓ Fucose :
  - ▸ Found in breast milk.
  - ▸ Present in several medicinal mushrooms.
  - ▸ Benefits the immune system.

- ✓ Xylose :
  - ▸ Present in some sugarless gums and candies.
  - ▸ Added to some nasal sprays to discourage the binding of allergens and pathogens to mucuos membranes.
  - ▸ Anti-bacterial and anti-fungal properties.
  - ▸ May help prevent certain cancers.

- ✓ N-acetyl-neuraminic acid (NANA) :
  - ▸ Present in breast milk.
  - ▸ Assists in brain development.
  - ▸ Boosts immune function.
  - ▸ Anti-viral properties.

- ✓ N-acetyl-glucosamine :
  - ▸ Beneficial for cartilage regeneration and joint inflammation.
  - ▸ Glucosamine comes from this compound.
  - ▸ Deficiencies have been linked to diseases of the bowel.

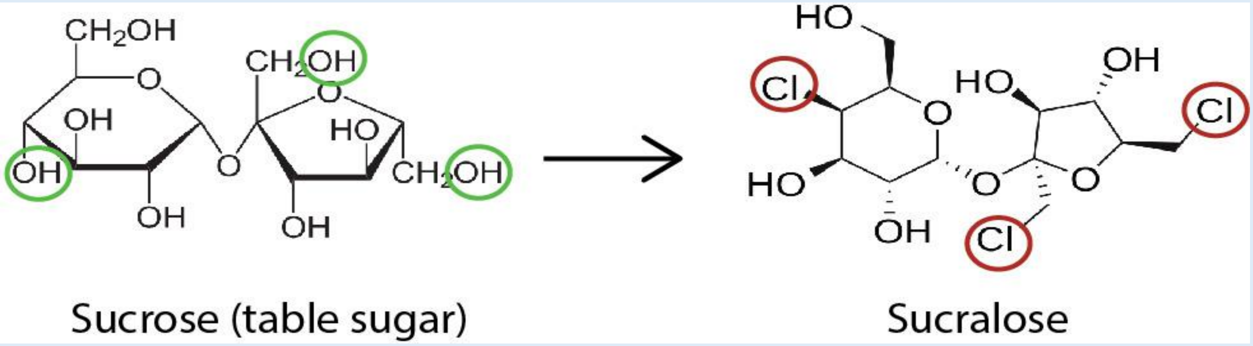
- ✓ N-acetyl-galactosamine :
  - ▸ May inhibit the growth of some tumors.
  - ▸ Assists in cell to cell communication.

Breast milk contains five of the essential sugars :

- ✔Fucose .
- ✔Galactose .
- ✔N-acetylneuraminic acid .
- ✔N-acetylglucosamine .
- ✔Glucose .

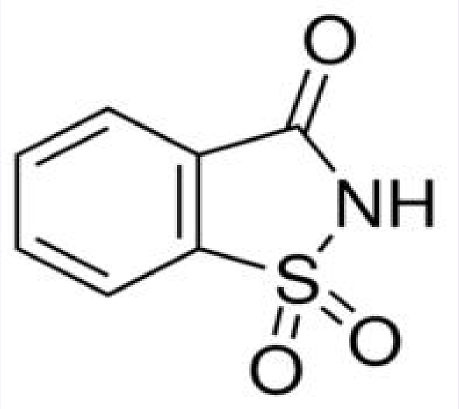
Sucralose (Splenda)

Discovered in 1976, sucralose is 600 times sweeter than sugar and does not metabolize to produce energy, thus it does not contain calories. It is the only low calorie sweetener that is made from sugar, which has been changed so passes through the body unchanged and unmetabolized. Substituting for three alcohol groups on the sugar molecule with 3 chlorine atoms.



Saccharine

Sodium saccharin (benzoic sulfimide) is an artificial sweetener with effectively no food energy. It is about ***300–400 times as sweet as sucrose*** but has a bitter or metallic aftertaste, especially at high concentrations. Saccharin is used to sweeten products such as drinks, candies, cookies, and medicines

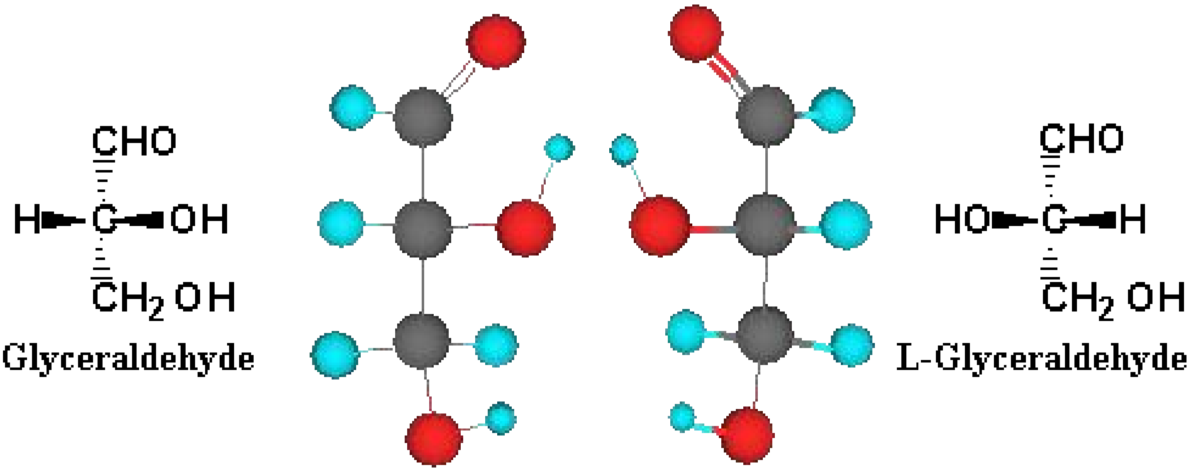


Stevia

Stevia is a sweetener and sugar substitute extracted from the leaves of the plant species Stevia rebaudiana which is grown in Brazil and Paraguay.

Stevia has no calories, and it is 200 times sweeter than sugar in the same concentration.

- ✓ Glyceraldehyde (Aldotriose) contains a stereocenter (chiral C) and exists as a pair of stereoisomers (enantiomers) .



D-Glyceraldehyde

L-Glyceraldehyde

Note: Number of stereoisomers= **2^n**

n is the number of stereocenters (chiral carbon)

✍Recall:

A chiral atom has 4 different groups attached, and is optically active (impair return of polarised light, either to the right <+ve> , or to the left <-ve>)

- Dextro ➡ to the right .
- Levo ➡to the left .

Now ,What are **D-**&**L-**Monosaccharides ?

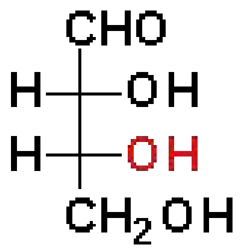
- According to the conventions proposed by Fischer projection (previously mentioned):

✓ **D**-monosaccharide: a monosaccharide that, when written as a Fischer projection, has the -OH on its penultimate carbon (the one before the last= the highest numbered chiral carbon) ***on the right***

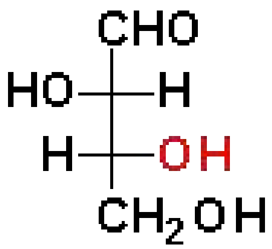
✓ **L**-monosaccharide: a monosaccharide that, when written as a Fischer projection, has the -OH on its penultimate carbon (the one before the last= the highest numbered chiral carbon) ***on the left***

We finshed talking about aldotrioses , now let's start talking about the rest ➡

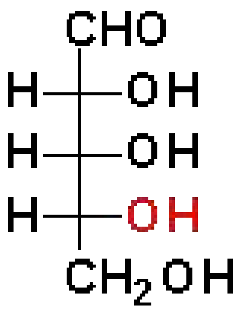
The following sugars are the two most common ***D-aldotetroses*** and the two most common ***D-aldopentoses*** :



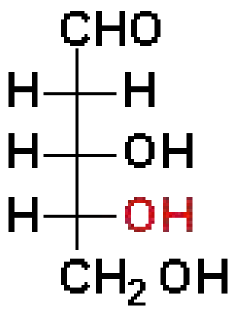
D-Erythrose



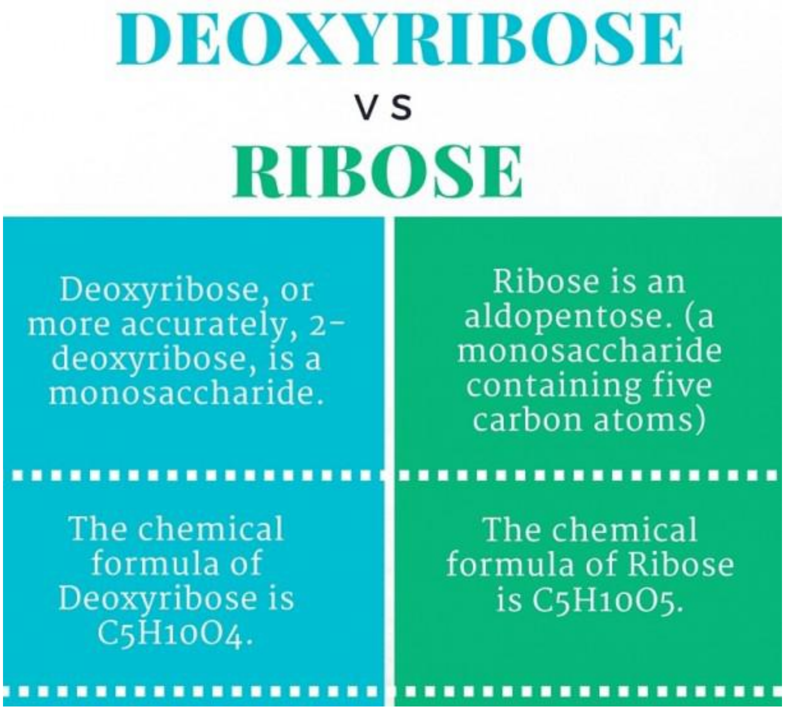
D-Threose



D-Ribose



2-Deoxy-D-ribose



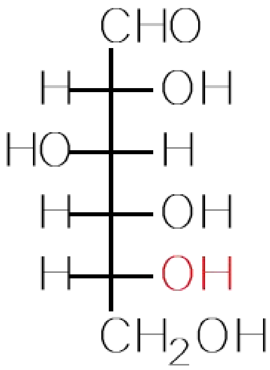
DEOXYRIBOSE

VS

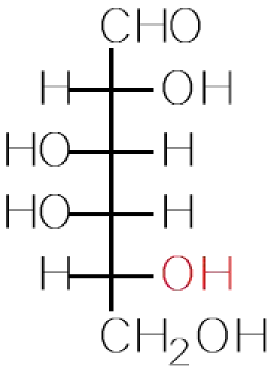
RIBOSE

Deoxyribose, or more accurately, 2-deoxyribose, is a monosaccharide.	Ribose is an aldopentose. (a monosaccharide containing five carbon atoms)
The chemical formula of Deoxyribose is C5H10O4.	The chemical formula of Ribose is C5H10O5.

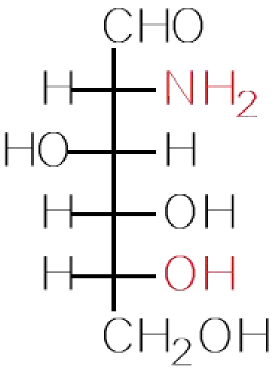
- The three most common D-aldohexoses(from lt-->Rt).Note that the third of these is an amino sugar.
- The 4th is the most common 2-keto-D-hexose (D-Fructose).



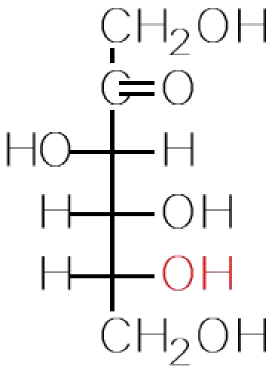
D-Glucose



D-Galactose



D-Glucosamine



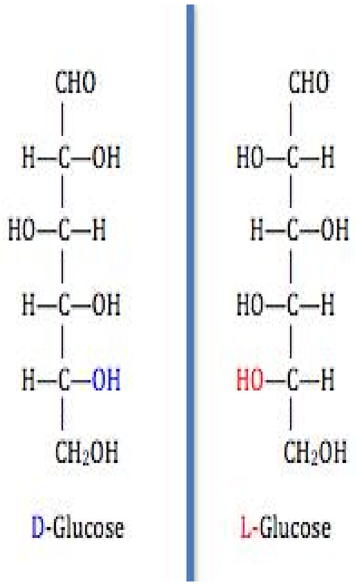
D-Fructose

Stereochemistry of Carbs

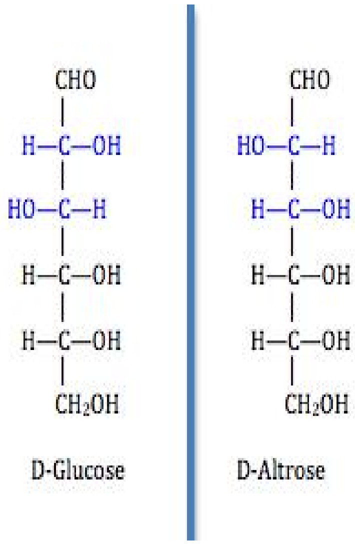
- Ww will discuss the following class. of steroisomers :

✓ Enantiomers .
✓ Diasteromers .
✓ Epimers .
✓ Anomers .

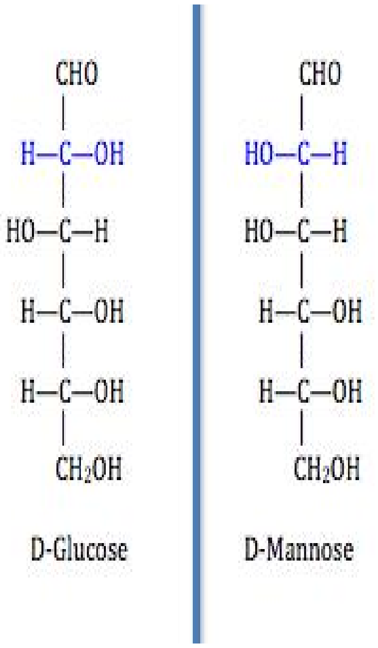
▶Two carbohydrates are said to be enantiomers if they are nonsuperimposable mirror images of one another. An example of an enantiomer is the D- and L-isomers of glucose, as shown by the figure below .



▶Carbohydrates are classified as diastereomers if their chiral carbons are connected to the exactly the same substrates but connected at differing configurations (R or S). Unlike an enantiomer, diastereomers are NOT object and mirror image. An example of two carbohydrates that are diastereoisomers are D-Glucose and D-Altrose as seen in the figure below .

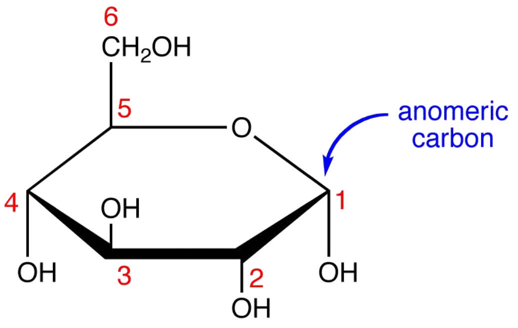


▶Another type of isomer that carhbohydrates that can take on are epimers. Epimers are two diastereomers that differ only at one stereocenter.As shown in the figure below, D-Glucose and D-Mannose are an example of an epimer.

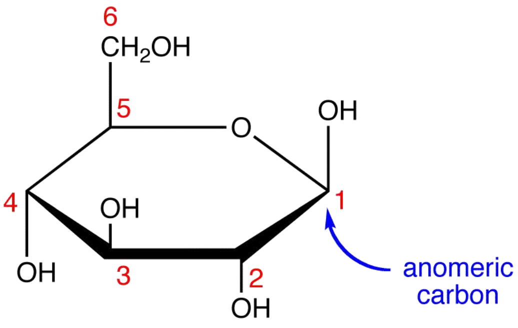


▶Anomers are cyclic monosaccharides or glycosides that are epimers, differing from each other in the configuration of C-1 if they are aldoses or in the configuration at C-2 if they are ketoses.

Ex: ***α-D-Glucopyranose*** and ***β-D-glucopyranose*** are anomers.



α-D-glucopyranose



β-D-glucopyranose

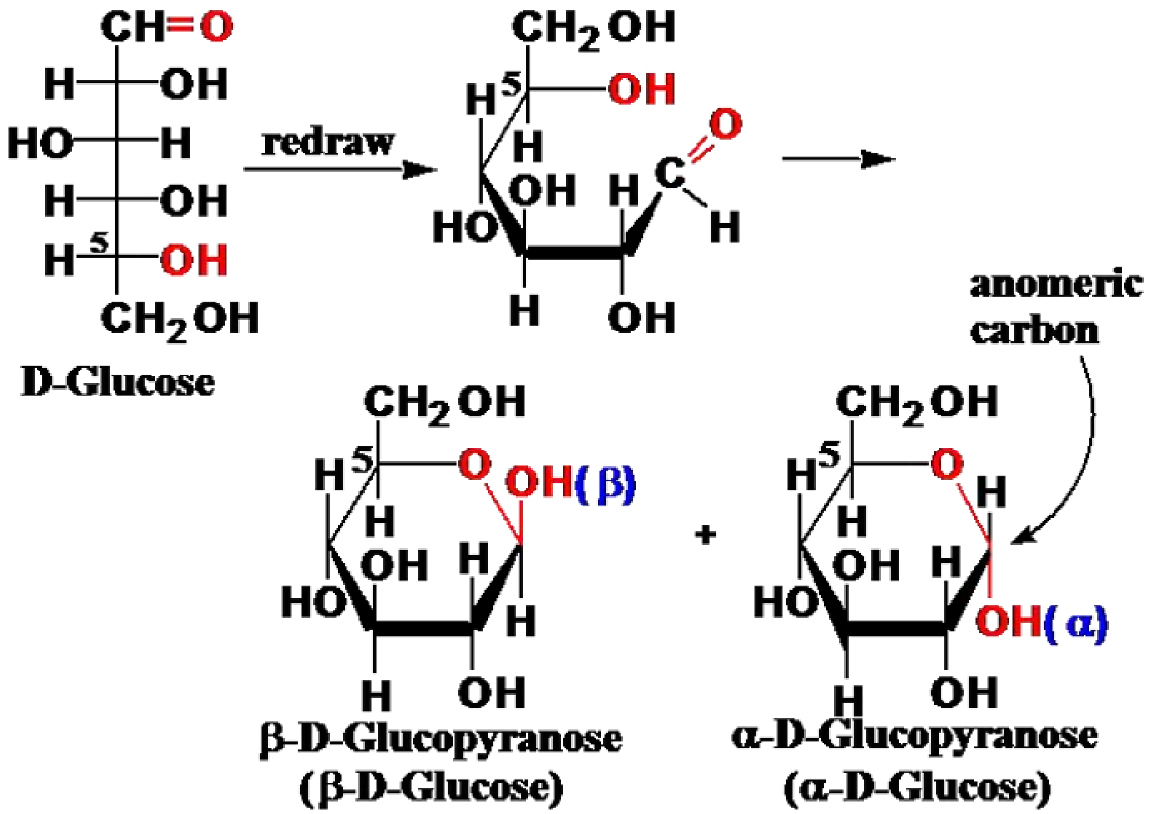
➡Anomeric carbon: the new stereocenter resulting from cyclic hemiacetal or hemiketal formation .

Haworth Projections :

- A Haworth projection is a common way of writing a structural formula to represent the cyclic structure of monosaccharides with a simple 3D perspective.

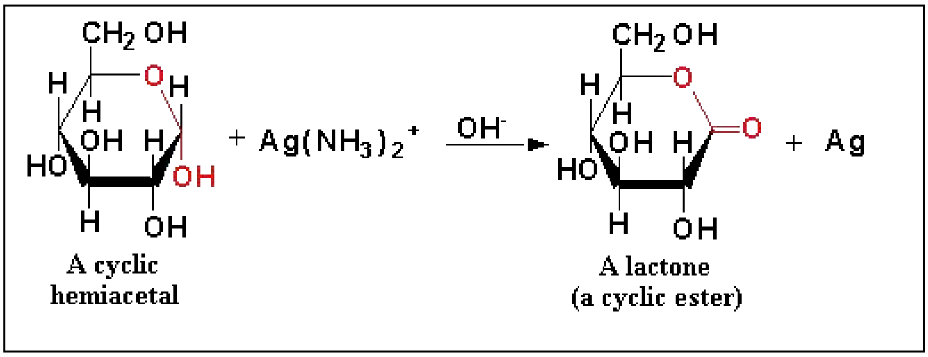
▶ Basics of haworth projection :

- ✓ Five- and six-membered hemiacetals are represented as planar pentagons or hexagons .
- ✓ Most commonly written with the anomeric carbon on the right and the hemiacetal oxygen to the back right .
- ✓ The designation β-means that -OH on the anomeric carbon is cis to the terminal $-CH_2OH$ (above the plane); α- means that it is trans to the terminal -CH2OH (below the plane)
- ✓ The OH groups on the right in Fischer below in Haworth. Those to the left in Fischer upward in Haworth.
- ✓ Counting is clockwise starting from anomeric carbon .

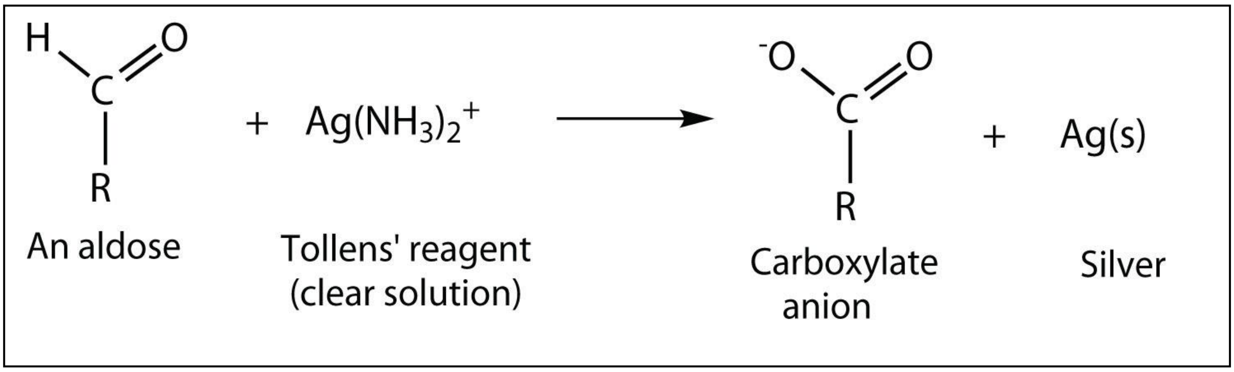


Oxidation&Reduction

- Oxidation and reduction reactions of sugars play key roles in biochemistry.
- Oxidation of sugars provides energy for organisms to carry out their life processes; the highest yield of energy from carbohydrates occurs when sugars are completely oxidized to CO_2 and H_2O in aerobic processes. The reverse of complete oxidation of sugars is the reduction of CO_2 and H_2O to form sugars, a process that takes place in photosynthesis.
- Reducing sugar: one that reduces an oxidizing agent, has free aldehyde or ketone group.
  - In the cyclic form, the compound produced by oxidation of an aldose is a lactone (a cyclic ester linking the carboxyl group and one of the sugar alcohols)



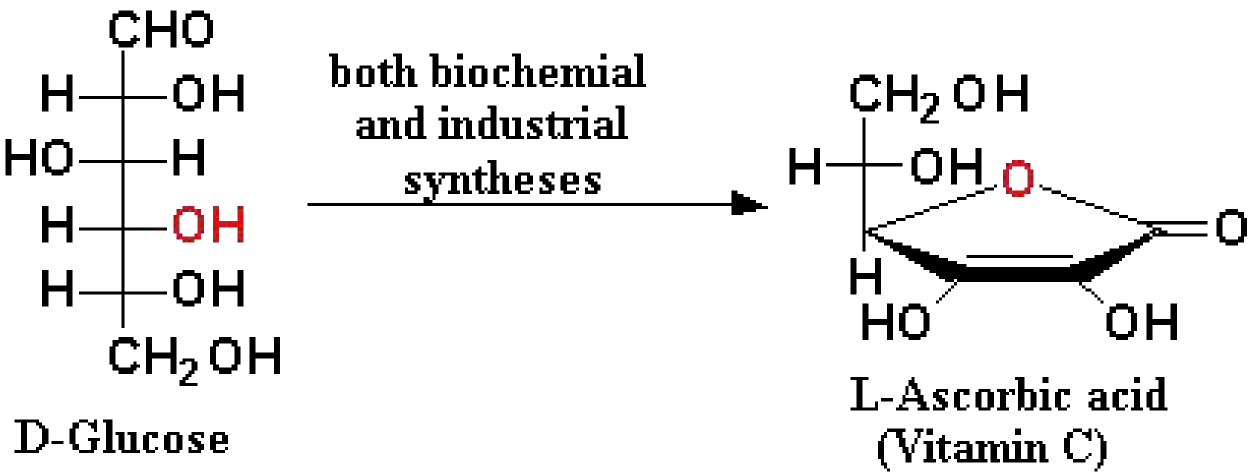
- Two types of reagent are used in the laboratory to detect the presence of reducing sugars.
  - The first of these is Tollens reagent, which uses the silver ammonia complex ion, $Ag(NH_3)^{2+}$, as the oxidizing agent. A silver mirror is deposited on the wall of the test tube if a reducing sugar is present, as a result of the Ag+ in the complex ion being reduced to free silver metal .



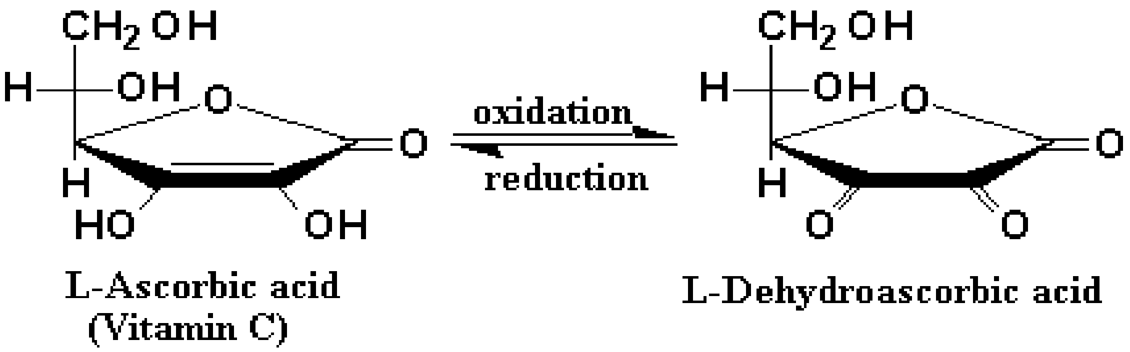
  - A more recent method for detection of glucose, but not other reducing sugars, is based on the use of the enzyme glucose oxidase, which is specific for glucose.

Vitamin C is related to sugars !

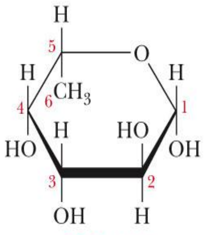
- L-Ascorbic acid (vitamin C) is synthesized both biochemically and industrially from oxidation of D-glucose



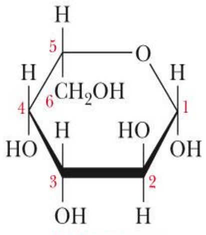
- L-Ascorbic acid is very easily oxidized to L-dehydroascorbic acid ,both are physiologically active and are found in most body fluids.



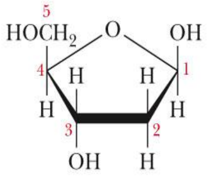
In addition to oxidized sugars, there are some important reduced sugars. In *deoxy sugars*, a hydrogen atom is substituted for one of the hydroxyl groups of the sugar. One of these deoxy sugars is L-fucose (L-6-deoxygalactose), which is found in the carbohydrate portions of some glycoproteins ,including the ABO blood-group antigens. An even more important example of a deoxy sugar is D-2-deoxyribose, the sugar found in DNA .



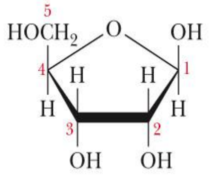
β-L-Fucose
(6-Deoxy-β-L-galactose)



β-L-Galactose



β-D-Deoxyribose
(2-Deoxy-β-D-ribose)



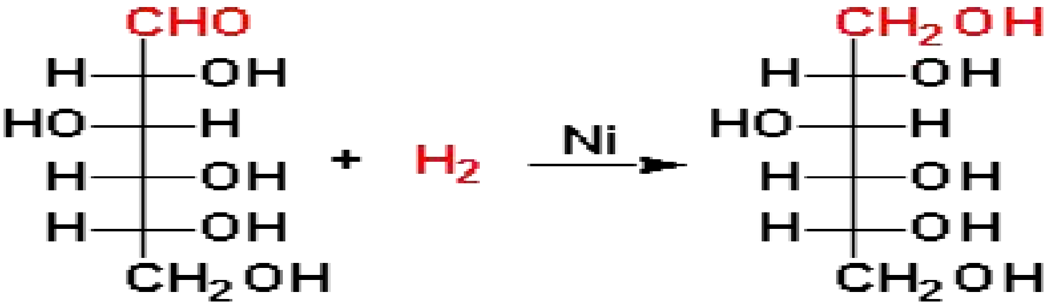
β-D-Ribose

Reduction

- The carbonyl group of a monosaccharide can be reduced to a hydroxyl group by a variety of reducing agents.

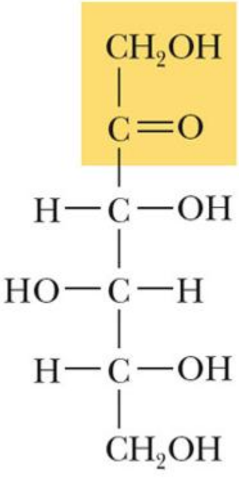
Reduction of the CHO group of a monosaccharide gives a polyhydroxy compound called an alditol .

- Two compounds of this kind, xylitol and sorbitol, derivatives of the sugars xylulose and sorbose, respectively, have commercial importance as sweeteners in sugarless chewing gum and candy.

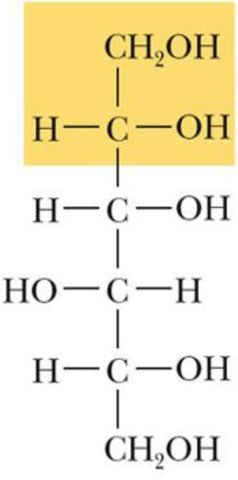


D-Glucose

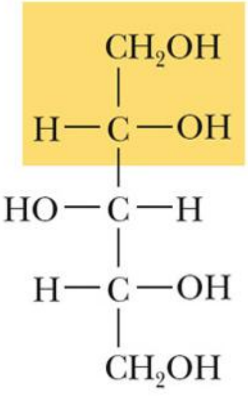
D-Glucitol
(D-Sorbitol)



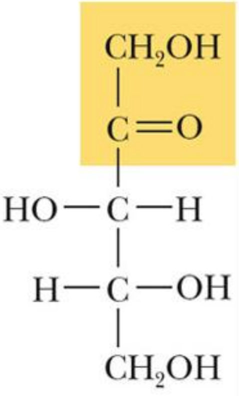
D-Sorbose



D-Sorbitol



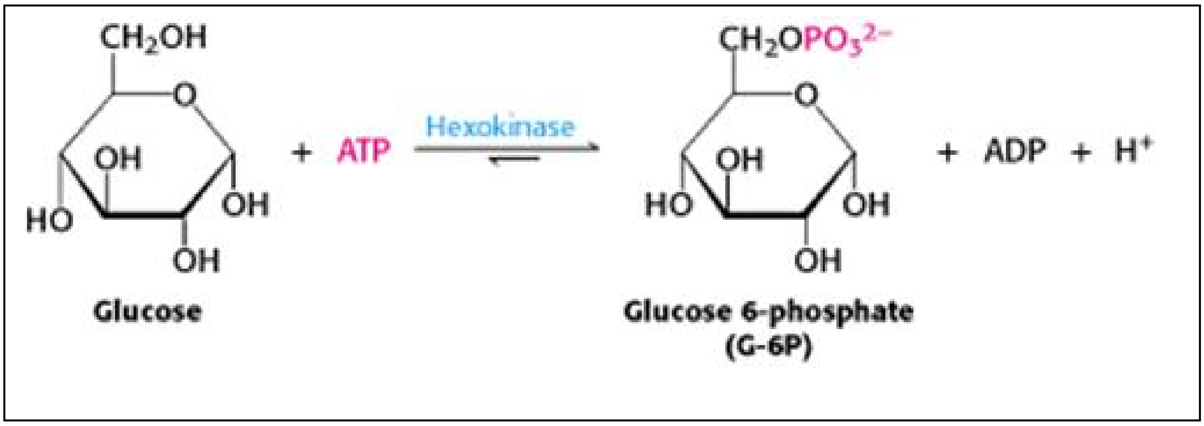
D-Xylitol



D-Xylulose

Phosphoric Esters

The hydroxyl groups of sugars behave exactly like all other alcohols in the sense that they can react with acids and derivatives of acids to form esters. The phosphate esters are particularly important because they are the usual intermediates in the breakdown of carbohydrates to provide energy. Phosphate esters are frequently formed by transfer of a phosphate group from ATP to give the phosphorylated sugar and ADP. Such reactions play an important role in the metabolism of sugars .



- Phosphorylation allows cells to accumulate sugars because the phosphate group prevents the molecules from diffusing back across their transporter. Phosphorylation of glucose is a key reaction in sugar metabolism because many sugars are first converted to glucose before they are metabolized further.

What are glycosides, and how do they form?

- It is possible for a sugar hydroxyl group (ROH) bonded to the anomeric carbon to react with another hydroxyl (R'—OH) to form a glycosidic linkage (R'—O—R).
- This type of reaction involves the anomeric carbon of the sugar in its cyclic form.

<Recall that the anomeric carbon is the carbonyl carbon of the open-chain form of the sugar and is the one that becomes a chiral center in the cyclic form.>

So ,

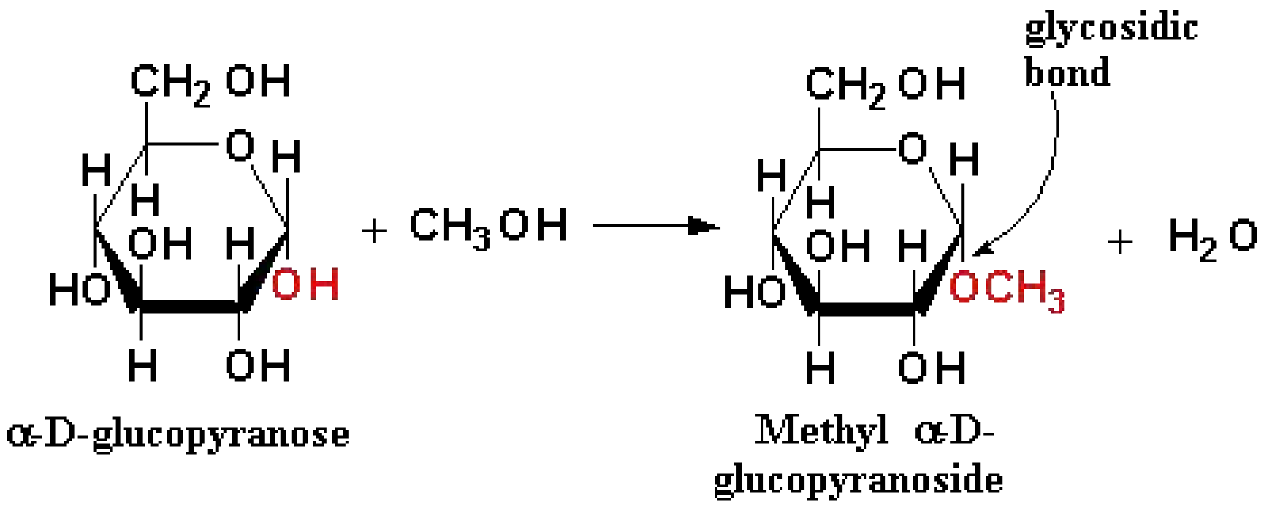
- Glycoside: a carbohydrate in which the -OH of the anomeric carbon is replaced by -OR

➡Those derived from furanoses are furanosides .

➡Those derived from pyranoses are pyranosides .

- Glycosidic bonds between monosaccharide units are the basis for the formation of oligosaccharides and polysaccharides.

- Glycosidic linkages can take various forms; the anomeric carbon of one sugar can be bonded to any one of the —OH groups on a second sugar to form an ***α- or β-glycosidic linkage***.

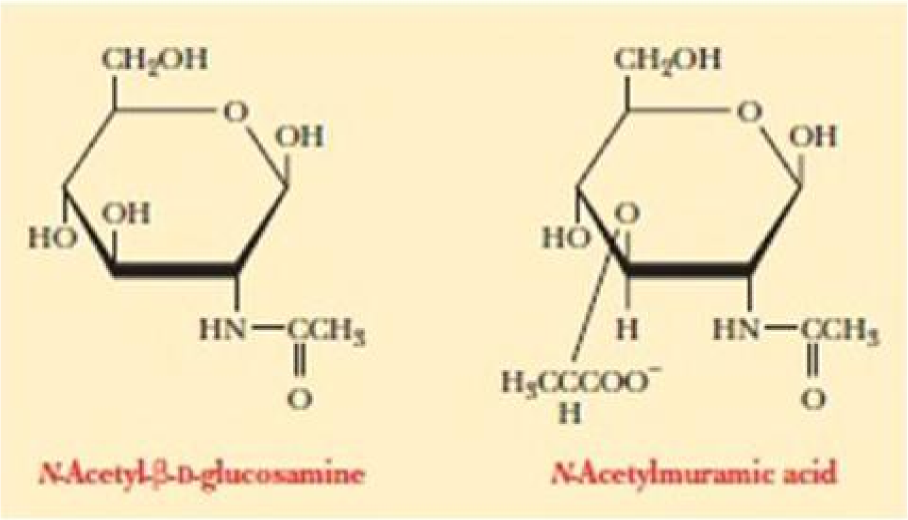


Before getting into di-&poly-saccharides , let's discuss important derivatives of sugars which are Amino Sugars ;

✓ Amino sugars are an interesting class of compounds related to the monosaccharides.

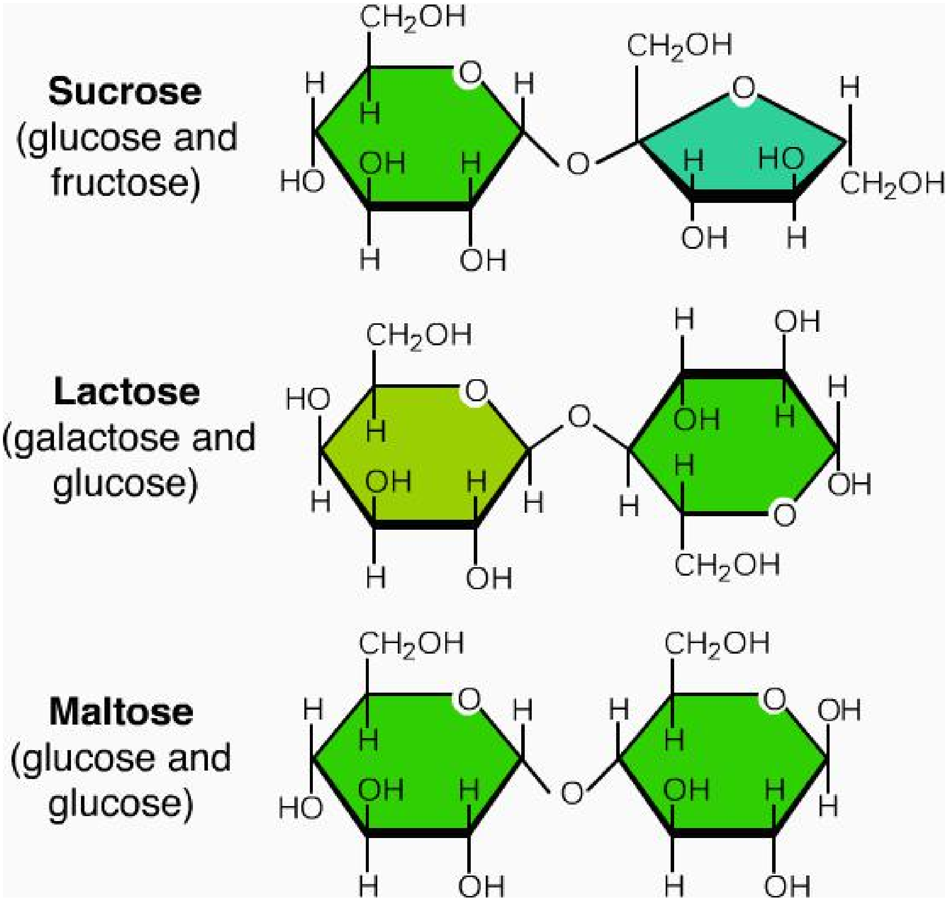
✓ In sugars of this type, an amino group (—NH_2) or one of its derivatives is substituted for the hydroxyl group of the parent sugar. In N-acetyl amino sugars, the amino group itself carries an acetyl group (CH_3—CO—) as a substituent.

Two particularly important examples are ***N-acetyl- β -D-glucosamine*** and its derivative ***N-acetyl- β -muramic acid***, which has an added carboxylic acid side chain (Shown below) .



Some Important Oligosaccharides

Oligomers of sugars frequently occur as disaccharides, formed by linking two monosaccharide units by glycosidic bonds. Three of the most important examples of oligosaccharides are disaccharides. They are sucrose, lactose, and maltose . Two other disaccharides, isomaltose and cellobiose.

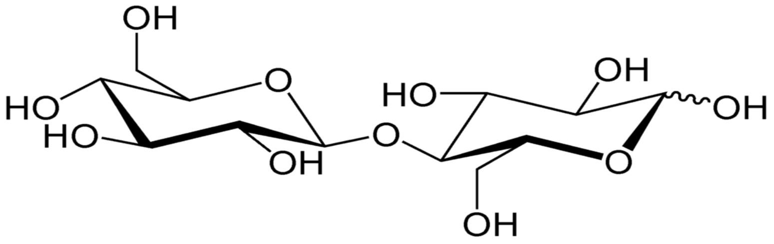


Sugar	Composition	Source
Isomaltose	*O*-α-D-glucopyranosyl-(1->6)-α-D-glucopyranose	Enzymic hydrolysis of starch (the branch points in amylopectin)
Maltose	*O*-α-D-glucopyranosyl-(1->4)-α-D-glucopyranose	Enzymic hydrolysis of starch (amylase); germinating cereals and malt
Lactose	*O*-α-D-galactopyranosyl-(1->4)-β-D-glucopyranose	Milk (and many pharmaceutical preparations as a filler)
Sucrose	*O*-α-D-glucopyranosyl-(1->2)-β-D-fructofuranoside	Cane and beet sugar, sorghum and some fruits and vegetables

Celloboise!

Cellobiose is a disaccharide with the formula $C_{12}H_{22}O_{11}$. Cellobiose, a reducing sugar, consists of two β-glucose molecules linked by a β(1→4) bond. It can be hydrolyzed to glucose enzymatically or with acid.Cellobiose has eight free alcohol (OH) groups, one acetal linkage and one hemiacetal linkage, which give rise to strong inter- and intramolecular hydrogen bonds.
It can be obtained by enzymatic or acidic hydrolysis of cellulose and cellulose rich materials .

Imp info about it !
Cellobiose can be used as an indicator carbohydrate for Crohn's disease and malabsorption syndrome.(will be discussed later)

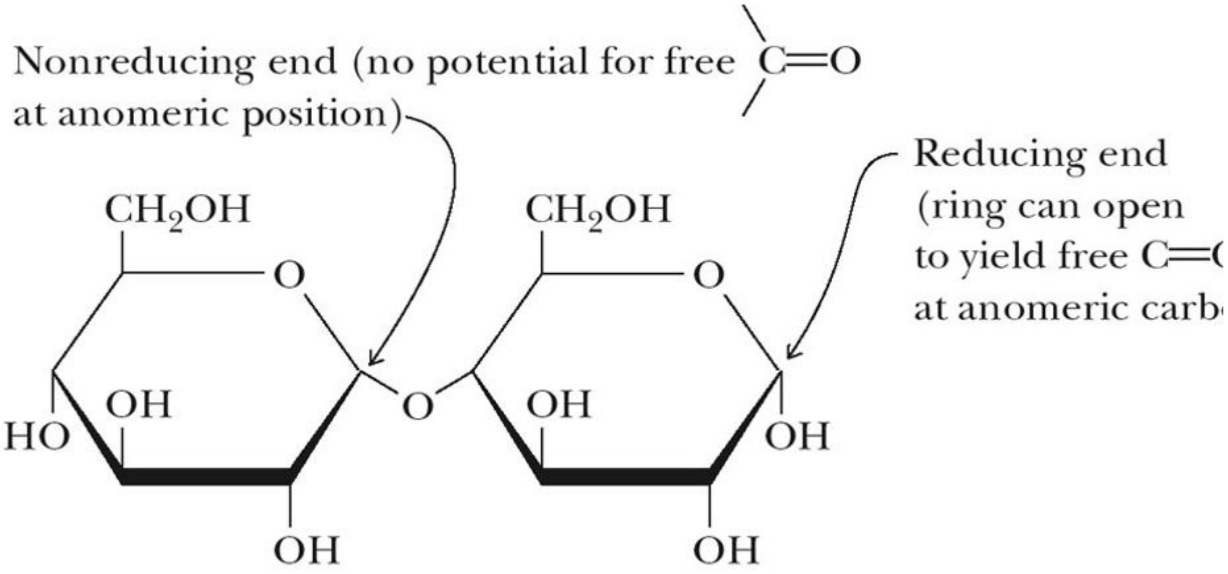


Reducing Disaccharides :

- Reducing disaccharidases, e.g. lactose, maltose, etc. are reducing in nature due to presence of a free carbonyl group in one of the two constituent monsaccharides.

Recall++Like monosaccharides, reducing disaccharidases reduce Fehling and Benedict reagents to cuprous oxide (Cu2O), a yellow to brick-red precipitate. The cupric ions (Cu++) of Fehling and Benedict reagents are reduced to cuprous ions (Cu+).

- However, non-reducing disaccharidase ,sucrose (Known to be the only non-reducing disaccharide),is non-reducing in nature since it possess no free carbonyl group.
  - In non-reducing disaccharidases, the carbonyl groups of both constituent monsaccharides are involved in formation of O-glycosidic linkage, therefore, no free carbonyl group is found in non-reducing disaccharidases.



Dimer of α-D-glucose with α(1→ 4) linkage

Lactose Intolerance :

Why do so many people not want to drink milk ?!

Lactose intolerance is a digestive disorder caused by the inability to digest lactose, the main carbohydrate in dairy products.

It can cause various symptoms, including bloating, diarrhea and abdominal cramps,People with lactose intolerance experience these symptoms when they eat dairy,they can have a negative effect on quality of life.

People with lactose intolerance don't make enough of the enzyme lactase, which is needed to digest lactose.

The lactase enzyme is needed to break lactose down into glucose and galactose, which can then be absorbed into the bloodstream and used for energy.

Without sufficient lactase, lactose moves through your gut undigested and causes digestive symptoms .

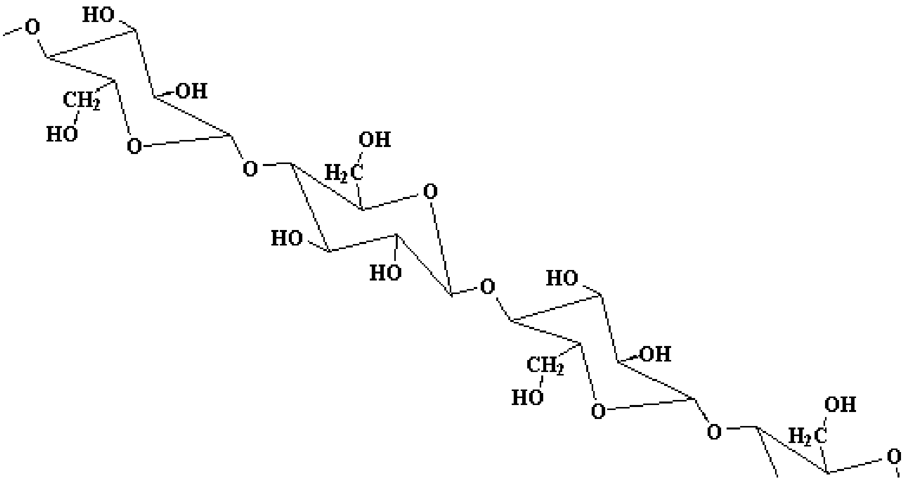
<Furthur details will be mentioned for you next year >

Polysaccharides

✓ When many monosaccharides are linked together, the result is a polysaccharide. Polysaccharides that occur in organisms are usually composed of a very few types of monosaccharide components. A polymer that consists of only one type of monosaccharide is a homopolysaccharide; a polymer that consists of more than one type of monosaccharide is a heteropolysaccharide.

We'll start with Cellulose ▶ ▶

- Cellulose is the major structural component of plants, especially of wood and plant fibers. It is a linear homopolysaccharide of β -D-glucose, and all residues are linked in β (1➡4) glycosidic bonds .
- Fully extended conformation with alternating 180° flips of glucose units(approximately **2800** D-glucose units per molecule).



- Individual polysaccharide chains are hydrogen-bonded together, giving plant fibers their mechanical strength. Animals lack the enzymes, called cellulases, that hydrolyze cellulose to glucose. Such enzymes attack the β -linkages between glucoses, which is common to structural polymers.
- Cellulases are found in certain bacteria, including the bacteria that inhabit the digestive tracts of insects, such as termites, and grazing animals, such as cattle and horses. The presence of these bacteria explains why cows and horses can live on grass and hay but humans cannot.

Why Do You Need Cellulose in Your Diet?

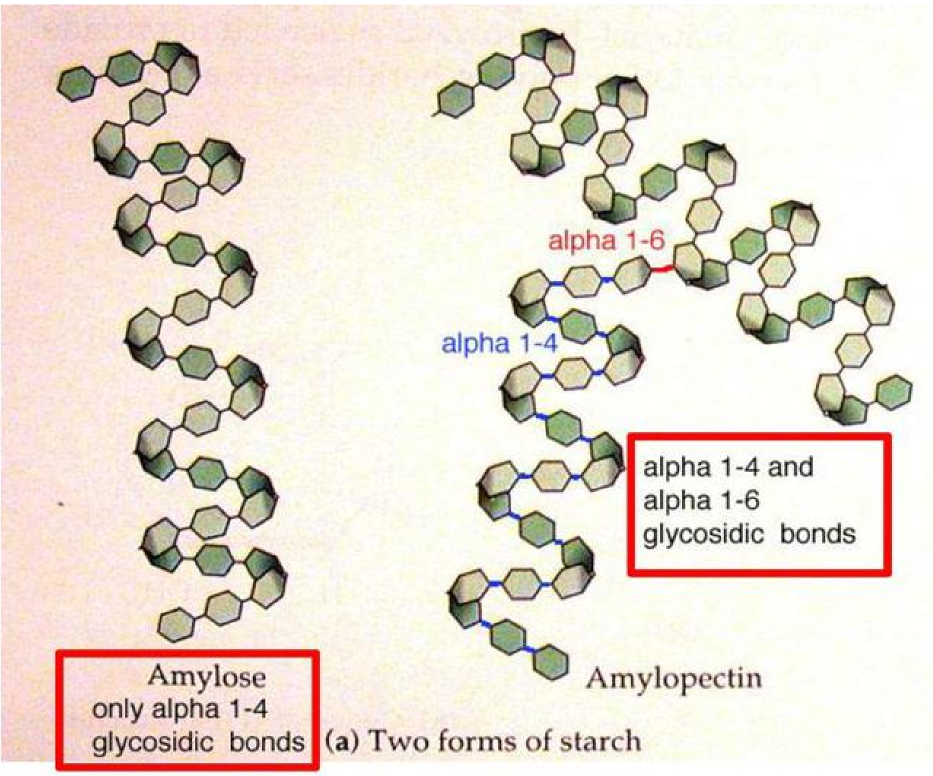
Cellulose, a component of plant foods that is indigestible by the human body. It might not seem intuitive that something your body can't digest can benefit your health, but it can. Cellulose is a type of fiber called insoluble fiber, and its benefits include helping food move through your digestive system more quickly, thus preventing constipation, and reducing the risk of developing a condition called diverticular disease<Discussed later >.

Starch

- Starch is the major form of stored carbohydrate in plant cells. Its structure is identical to glycogen, except for a much lower degree of branching (about every 20–30 residues). Unbranched starch is called ***amylose***; branched starch is called ***amylopectin***.
- Starches are polymers of α-D-glucose that occur in plant cells, usually as starch granules in the cytosol. Note that there is an α-linkage in starch, in contrast with the β-linkage in cellulose. The types of starches can be distinguished from one another by their degrees of chain branching.
  - Amylose is a linear polymer of glucose, with all the residues linked together by α(1 -->4) bonds.
  - Amylopectin is a branched chain polymer, with the branches starting at α(1 -->6) linkages along the chain of α(1 -->4) linkages .
- Because starches are storage molecules, there must be a mechanism for releasing glucose from starch when the organism needs energy. *Both plants and animals contain enzymes that hydrolyze starches*.
- Two of these enzymes, known as α- and β-amylase (***the α and β do not signify anomeric forms in this case***), attack α(1 -->4) linkages.
  - β-amylase is an exoglycosidase that cleaves from the nonreducing end of the polymer.

- α -amylase, is an endoglycosidase, which can hydrolyze a glycosidic linkage anywhere along the chain to produce glucose and maltose.

- Amylose can be completely degraded to glucose and maltose by the two amylases, but amylopectin is not completely degraded because the branching linkages are not attacked. However, debranching enzymes occur in both plants and animals; they degrade the α(1 -->6) linkages. When these enzymes are combined with the amylases, they contribute to the complete degradation of both forms of starch.

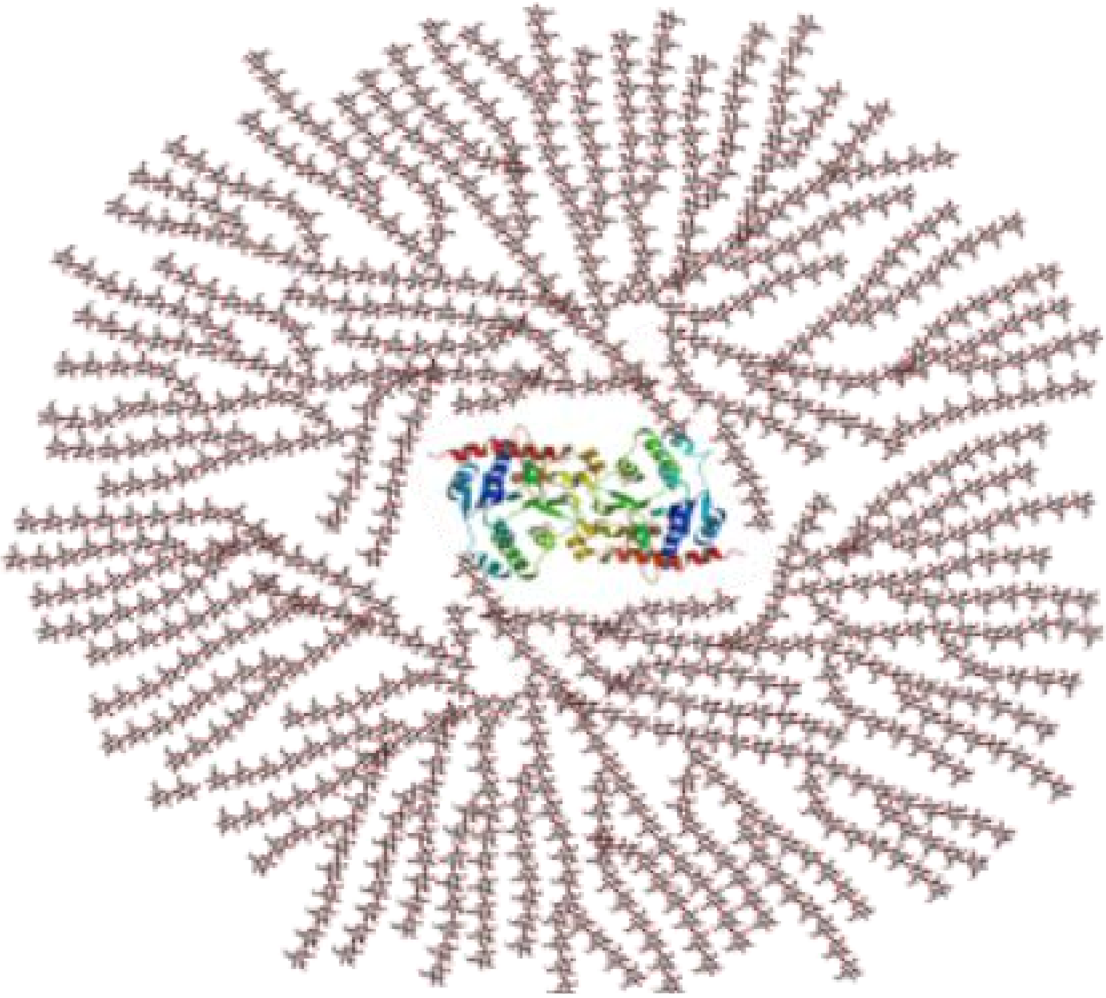


(a) Two forms of starch

Glycogen

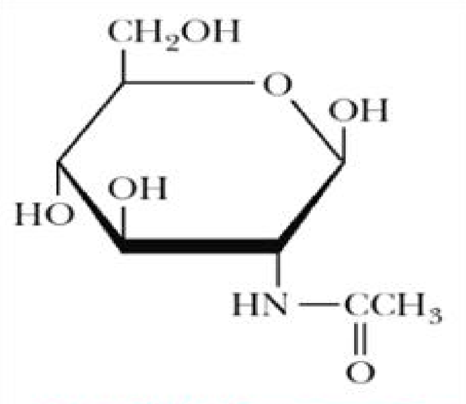
- Glycogen is the major form of stored carbohydrate in animals. This crucial molecule is a homopolymer of glucose in α–(1,4) linkage; it is also highly branched, with α–(1,6) branch linkages occurring every 8-10 residues.
- Glycogen is a very compact structure that results from the coiling of the polymer chains. This compactness allows large amounts of carbon energy to be stored in a small volume, with little effect on cellular osmolarity.

- In humans, ***glycogen*** is made and stored primarily in the cells of the liver and skeletal muscle.

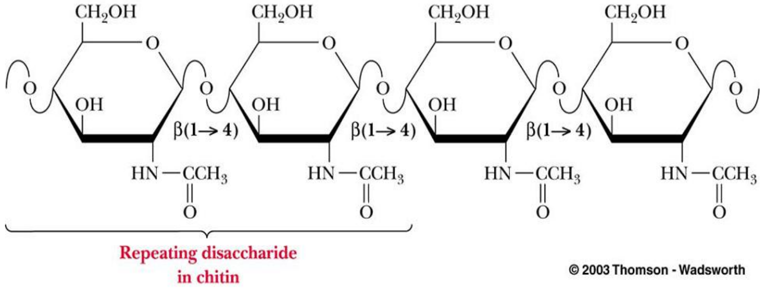


Chitin

- A polysaccharide that is similar to cellulose in both structure and function is chitin, which is also a linear homopolysaccharide with all the residues linked in β(1 -->4) glycosidic bonds. Chitin differs from cellulose in the nature of the monosaccharide unit; in cellulose, the monomer is β-D-glucose; in chitin, the monomer is *N-acetyl-β-D-glucosamine*.



N-Acetyl-β-D-glucosamine

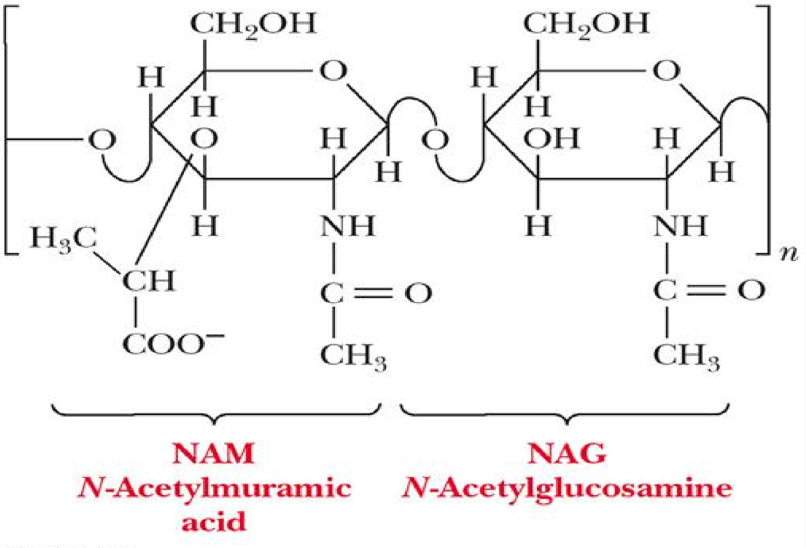


© 2003 Thomson - Wadsworth

- Chitin is the major structural component of the exoskeletons of invertebrates, such as insects and crustaceans; also occurs in cell walls of algae, fungi, and yeasts .

What role do polysaccharides play in the structure of cell walls?

- *Heteropolysaccharides* are major components of bacterial cell walls. A distinguishing feature of prokaryotic cell walls is that the polysaccharides are crosslinked by peptides. The repeating unit of the polysaccharide consists of two residues held together by β(1 -->4) glycosidic links, as was the case in cellulose and chitin.
    - One of the two monomers is ***N-acetyl-D-glucosamine***, which occurs in chitin.
    - The other monomer is ***N-acetylmuramic acid*** .



© 2006 Brooks/Cole - Thomson

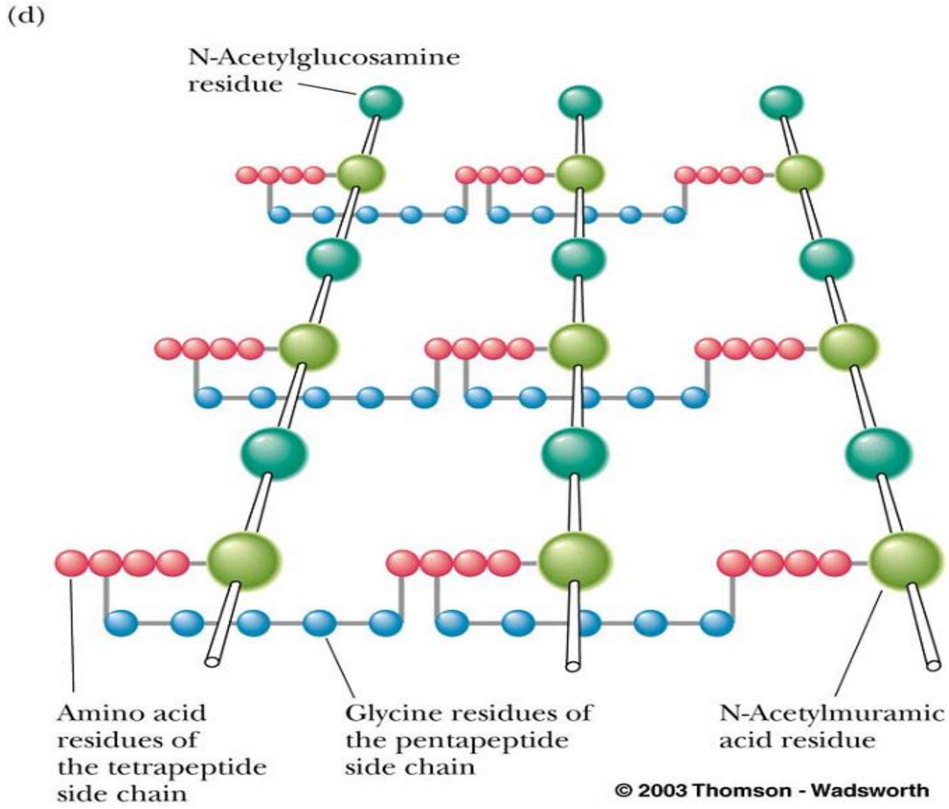
- The cross-links in bacterial cell walls consist of small peptides.
    - Ex:

      In *Staphylococcus aureus*, an oligomer of four amino acids (a tetramer) is bonded to N-acetylmuramic acid, forming a side chain. The tetrapeptides are themselves cross-linked by another small peptide, in this case consisting of five amino acids.

      This tetrapeptide is unusual in that it contains two amino acids of the D-series, namely **D-Ala** and **D-Gln**

      < Recall that bacterial cell walls are one of the few places where D-amino acids occur in nature.>

- The material that results from the crosslinking of polysaccharides by peptides is a *peptidoglycan*, so named because it has both peptide and carbohydrate components. (Shown in the figure below)



© 2003 Thomson - Wadsworth

اللهمَّ تقبل العَمل مع قلّته، والجُهد مع ضالتِه والسَّعي مع شوائبه

وَآخِرُ دَعْوَاهُمْ أَنْ الْحَمْدُ للهِ رَبِّ الْعَالَمِينَ