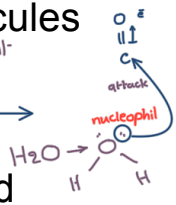
Water and pH

Water → ideal biological solvent

- Water is the predominant chemical component of living organisms.

- Its unique physical properties include:
  - The ability to solvate a wide range of organic and inorganic molecules by its exceptional capacity for forming hydrogen bonds. → Universal solvent
  - An excellent nucleophile, water is a reactant or product in many metabolic reactions. → eletron rich — cata — Ana
  - Water has a slight propensity to dissociate into hydroxide ions and protons. $H_2O + H_2O \rightarrow OH^- + H_3O^+$

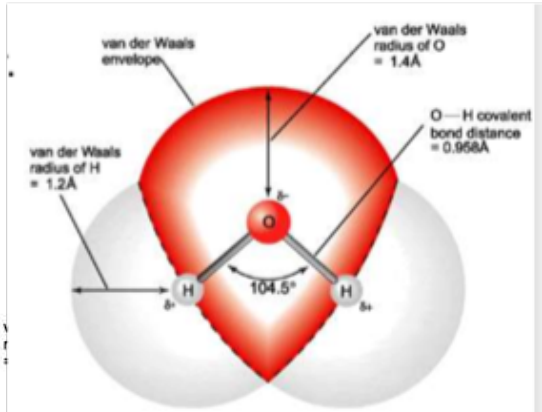


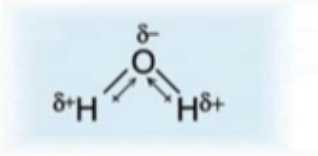
- Normal blood pH ranges from 7.35-7.45 — physiological pH = 7.4
  - Acidosis (blood pH < 7.35) include diabetic ketosis and lactic acidosis.
  - Alkalosis (pH > 7.45) may, for example, follow vomiting of acidic gastric contents.

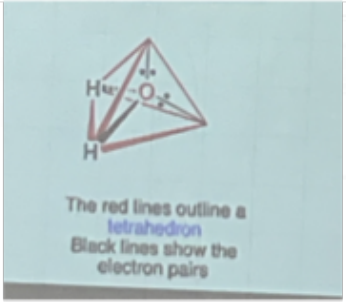
Water Is an Ideal Biologic Solvent

Water Molecules Form Dipoles

- A water molecule is an irregular, slightly skewed tetrahedron with oxygen at its center.
- Water is a **dipole,** a molecule with electrical charge distributed asymmetrically about its structure.
- The strongly electronegative oxygen atom pulls electrons away from the hydrogen nuclei, leaving them with a partial positive charge, while its two unshared electron pairs constitute a region of local negative charge.





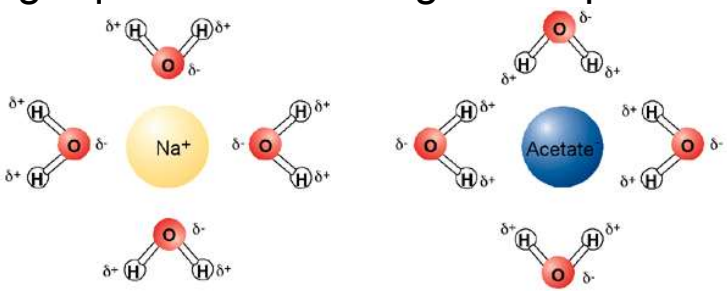


Water Is an Ideal Biologic Solvent

- Water, a strong dipole, has a high **dielectric constant** .As described quantitatively by Coulomb's law, the strength of interaction between oppositely charged particles is inversely proportionate to the dielectric constant of the surrounding medium.

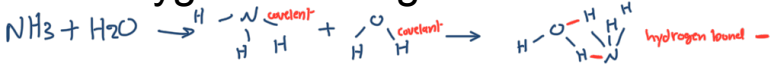
- The dielectric constant for a vacuum is unity; and for water it is 78.5. Water therefore greatly decreases the force of attraction between charged and polar species relative to water-free environments with lower dielectric constants.

- Its strong dipole and high dielectric constant enable water to dissolve large quantities of charged compounds such as salts



Water Molecules Form Hydrogen Bonds

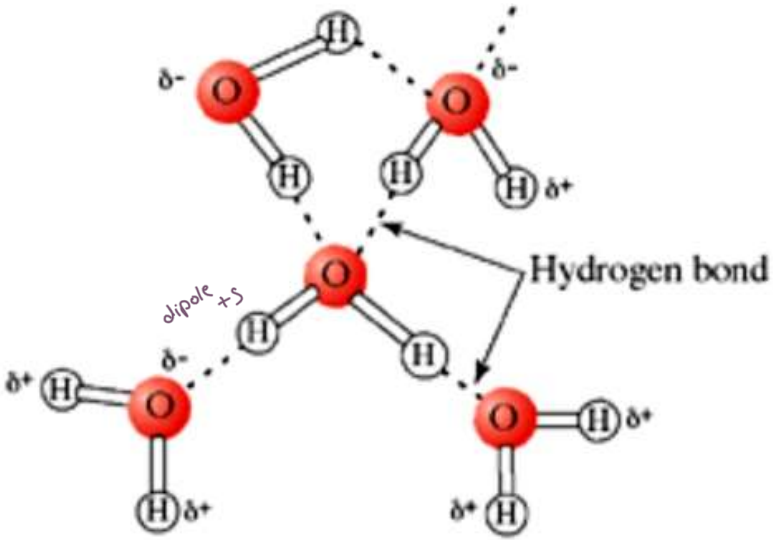
- A hydrogen nucleus covalently bound to an electron-withdrawing oxygen or nitrogen atom can interact with an unshared electron pair on another oxygen or nitrogen atom to form a **hydrogen bond .**

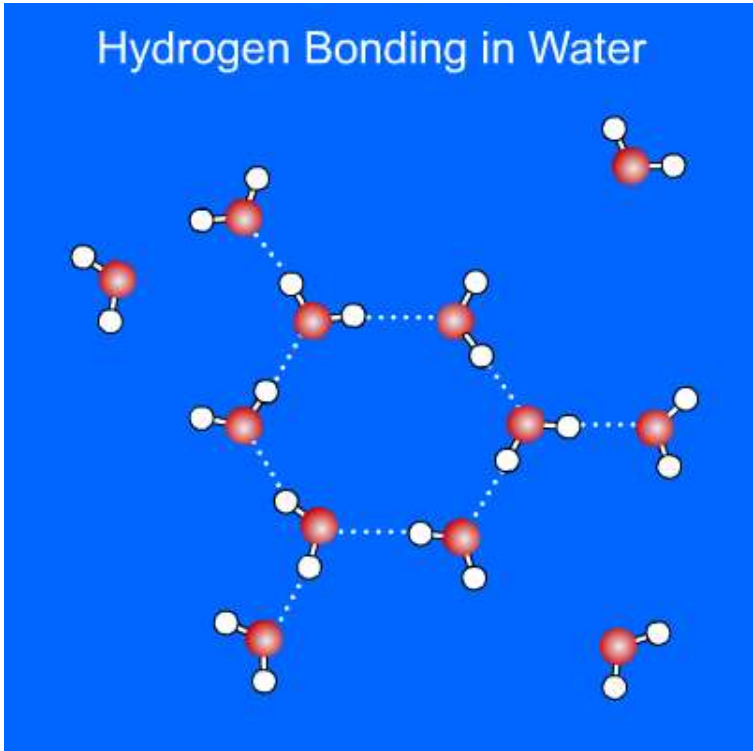


- Hydrogen bonding favors the self-association of water molecules into ordered arrays.

- Hydrogen bonding influences the physical properties of water and accounts for its exceptionally high viscosity, surface tension, and boiling point.

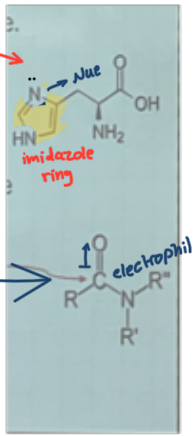
- These bonds are both relatively weak and transient, with a half-life of about one microsecond. Rupture of a hydrogen bond in liquid water requires only about 4.5 kcal/mol, less than 5% of the energy required to rupture a covalent O-H bond.


Hydrogen bond
dipole


Hydrogen Bonding in Water

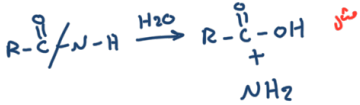
Water Is an Excellent Nucleophile

- Metabolic reactions often involve the attack by lone pairs of electrons on electron-rich molecules termed **nucleophiles** on electron-poor atoms called **electrophiles** .Nucleophiles and electrophiles do not necessarily possess a formal negative or positive charge.
- Water, whose two lone pairs of electrons bear a partial negative charge, is an excellent nucleophile. ✓
- Other nucleophiles of biologic importance include the oxygen atoms of phosphates, alcohols, and carboxylic acids; the sulfur of thiols; the nitrogen of amines; and the imidazole ring of histidine.
- Common electrophiles include the carbonyl carbons in amides, esters, aldehydes, and ketones and the phosphorus atoms of phosphoesters.



- Nucleophilic attack by water generally results in the cleavage of the amide, glycoside, or ester bonds that hold biopolymers together. This process is termed **hydrolysis** .

break



- Conversely, when monomer units are joined together to form biopolymers such as proteins or glycogen, water is a product

Dehydration

Water Molecules Exhibit a Slight But Important Tendency to Dissociate

- The ability of water to ionize, is of central importance for life.

- Water can act both as an acid and as a base, its ionization may be represented as an intermolecular proton transfer that forms a hydronium ion (H_3O^+)and a hydroxide ion (OH^-)

- The transferred proton is actually associated with a cluster of water molecules. Protons exist in solution not only as H_3O^+ but also as multimers such as $H_5O_2^+$ and $H_7O_3^+$

- Since hydronium and hydroxide ions continuously recombine to form water molecules, an *individual* hydrogen or oxygen cannot be stated to be present as an ion or as part of a water molecule. At one instant it is an ion; an instant later it is part of a molecule.

Water Molecules Exhibit a Slight But Important Tendency to Dissociate

- Hydrogen ions and hydroxide ions contribute significantly to the properties of water.

- For dissociation of water

  $Kw = [H_3O^+]\,[OH^-]$ → Molar conc.

  10^{-14}

- where brackets indicates the molar conc. of ions, Kw is the dissociation constant of water and = $1x10^{-14}$ so

$-\log Kw = -\log [H_3O^+] + -\log [OH^-]$

$14 = pH + pOH$

- **pH Is the Negative Log of the Hydrogen Ion Concentration**

pH = -log $[H_3O^+]$

Example: If the concentration of H_3O^+in solution is $1x10^{-7}$ calculate pH

$pH = -\log [H_3O^+]$

$= -\log (1x10^{-7}) = 7$

pka = −log ka

pKa → imp

- Many biochemicals possess functional groups that are weak acids or bases.
- Carboxyl groups, amino groups, and phosphate esters, whose second dissociation falls within the physiologic range, are present in proteins and nucleic acids, most coenzymes, and most intermediary metabolites. → have functional groups → ① weak acid ② weak base
- pKa is important for understanding the influence of intracellular pH on structure and biologic activity.
- Charge-based separations such as electrophoresis and ion exchange chromatography also are best understood in terms of the dissociation behavior of functional groups.
- We term the protonated (gained proton) species (eg, HA or R-NH_3^+) the **acid** and the unprotonated species (eg, A^- or R-NH_2) its **conjugate base.**

The Henderson-Hasselbalch Equation Describes the Behavior of Weak Acids

- For a weak acid:

$$HA \rightleftharpoons H^+ + A^-$$

$$HA \rightarrow \underset{x}{H_3O^+} + \underset{x}{A^-} \rightarrow K_a = \frac{[H_3O^+][A^-]}{[HA]} = \frac{[H_3O^+]^2}{[HA]}$$

$$[H_3O^+] = [A^-]$$

$$\log \frac{\text{Ionized concentration}}{\text{Unionized concentration}} = \text{pH} - \text{p}K_a$$

$$pK_a = -\log K_a \rightarrow K_a \propto \text{Acidity} \propto \frac{1}{pK_a}$$

$(H_3O^+ \uparrow, A^- \uparrow, HA \downarrow)$

- For a weak base:

$$pH_{w.A} = pK_a + \log \frac{\text{ionized } [A^-]}{\text{Unionized } [HA]}$$

$$RNH_2 \rightleftharpoons RNH_3^+ + OH^-$$

$$pH_{w.B} = pK_a + \log \frac{\text{Unionized } [NH_2]}{\text{ionized } [NH_3^+]}$$

$$\log \frac{\text{Unionized concentration}}{\text{Ionized concentration}} = \text{pH} - \text{p}K_a$$

Values of the pKa depend Properties of the Medium

- The medium may either raise or lower the p*K*a depending on whether the undissociated acid or its conjugate base is the charged species. → pka of acid or base → effected by the medium of that acid / base

- The effect of dielectric constant on p*K*a may be observed by adding ethanol to water. The ability of ionize the compound (break down bonds)

ka↓, acidity↓ pka↑

$COOH \rightarrow COO^- + H_3O^+$ → ka↓ → pka↑

- The p*K*a of a carboxylic acid *increases*, *w*hereas that of an amine *decreases* because ethanol decreases the ability of water to solve a charged species.

$NH_2 \rightarrow NH_3^+ + OH^-$

→ base↓ → acidt

ka↑ → pka↓

$$\frac{\text{Basic strength}}{\text{Acidic strength}} \propto pka$$

- The p*K*a values of dissociating groups in the interiors of proteins thus are profoundly affected by their local environment, including the presence or absence of water.

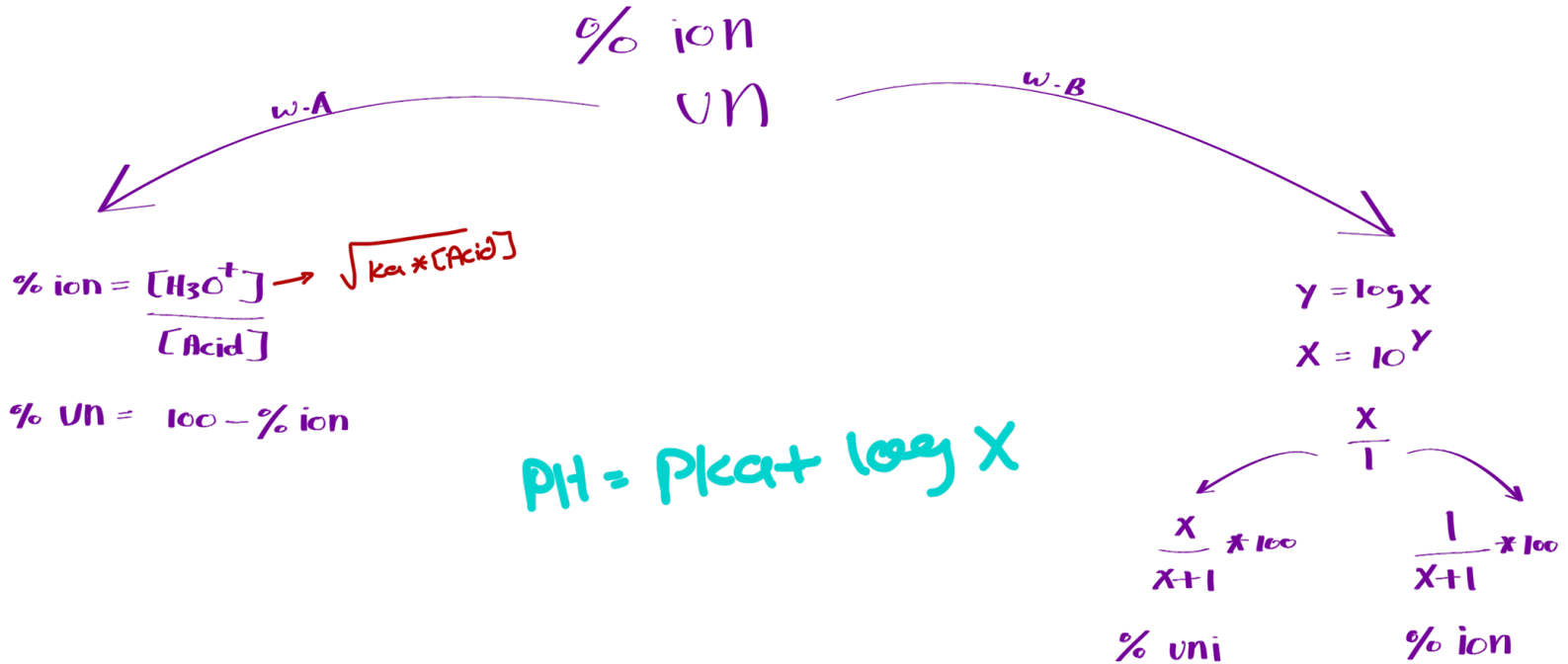
$$\mathrm{pH} = -\log\sqrt{10^{-\mathrm{pKa}} \times [\mathrm{Acid}]}$$

$$\mathrm{pOH} = -\log\sqrt{10^{-\mathrm{pKb}} \times [\mathrm{Base}]}$$

ومنه نحسب pH باستخدام العلاقة:

$$\mathrm{pH} = 14 - \mathrm{pOH}$$

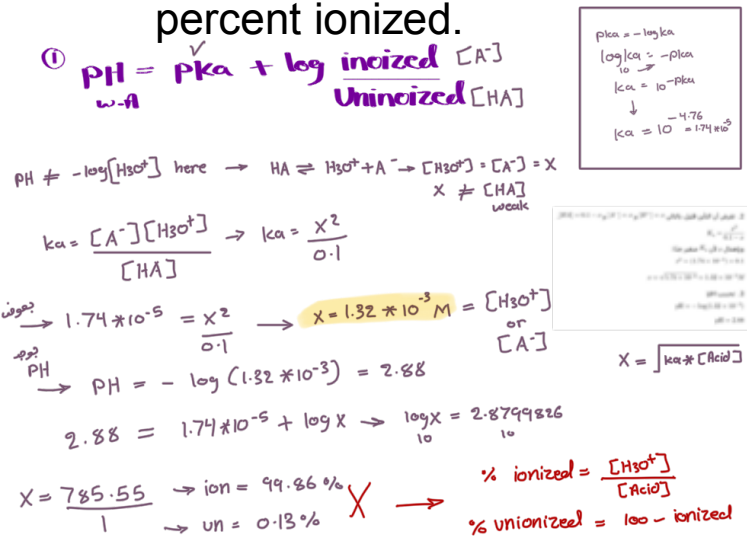
$$pH = pka + \log \begin{cases} \text{w.A} & \frac{\text{ion}}{\text{un}} \\ \text{w.B} & \frac{\text{un}}{\text{ion}} \end{cases}$$



Question

1. Calculate the pH of 0.1 M solution of acetic acid, pKa = 4.76, calculate the percentage of ionized and unionized forms.

2. Calculate the percetage of ionized and unionized for histidine (basic) in hemoglobin at physiological pH knowing that pKa for the side chain of histidine is 6.0. If the pH of blood decreased to 7.1, calculate the percent ionized.



الفرق بين السؤالين هو نوع الحساب المستخدم وطبيعة المادة (حمض أو قاعدة) التي تتعاملين معها.

الفرق الرئيسي بين السؤالين:

1. **السؤال الأول (حساب نسبة التأين لحمض الأسيتيك)**

- هنا تتعاملين مع **حمض ضعيف**، واستخدمتِ **ثابت تفكك الحمض** K_a لحساب تركيز H^+، ثم نسبة التأين.
- الطريقة الصحيحة لحساب نسبة التأين في الأحماض الضعيفة هي:

$$\%\text{ionization} = \left(\frac{[H^+]}{\text{initial concentration}}\right) \times 100$$

- حسابك السابق كان خاطئًا لأنك استخدمت معادلة **هندرسون-هاسلباخ** بطريقة غير صحيحة في تحديد النسبة بين المتأين وغير المتأين.

2. **السؤال الثاني (حساب نسبة التأين للقاعدة الضعيفة)**

- هنا تتعاملين مع **قاعدة ضعيفة**، واستخدمتِ **معادلة هندرسون-هاسلباخ** لحساب نسبة الأشكال المتأينة وغير المتأينة.
- القاعدة الضعيفة تتبع العلاقة:

$$\text{pH} = \text{pKa} + \log\left(\frac{\text{Unionized}}{\text{Ionized}}\right)$$

- هنا لا تحتاجين إلى K_b أو حساب OH^- كما في حالة الحمض، بل يمكنك مباشرةً إيجاد نسبة **المادة غير المتأينة إلى المتأينة**.

الفرق في الاستخدام:

- في **الأحماض الضعيفة**، لا يمكن استخدام $\frac{\text{unionized}}{\text{ionized}}$ بشكل مباشر، لأنك بحاجة إلى حساب $[H^+]$ باستخدام K_a.
- في **القواعد الضعيفة**، يمكنك استخدام النسبة بين الشكل غير المتأين والمتأين مباشرةً من خلال معادلة هندرسون-هاسلباخ.

لهذا السبب، طريقتك كانت صحيحة في السؤال الثاني (القاعدة الضعيفة) لكنها خاطئة في السؤال الأول (الحمض الضعيف).

الخطأ في حساب نسبة التأين

الطريقة الصحيحة لحساب نسبة التأين هي:

$$\%\text{ionization} = \left(\frac{[H^+]}{\text{initial concentration}}\right) \times 100$$

حيث:

- $[H^+] = 1.32 \times 10^{-3} M$
- $\text{initial concentration} = 0.1 M$

التطبيق الصحيح:

$$\%\text{ionization} = \left(\frac{1.32 \times 10^{-3}}{0.1}\right) \times 100$$

$$= 1.32\%$$

الخطأ في حساب النسبة غير المتأينة

نسبة الحمض غير المتأين تُحسب كالتالي:

$$\%\text{unionized} = 100 - \%\text{ionization}$$

$$= 100 - 1.32 = 98.68\%$$

أين كان خطؤك؟

يبدو أنك استخدمت معادلة خاطئة في حساب التأين، مما أدى إلى حصولك على 99.86% بدلاً من 1.32%. هذا غير منطقي لأن حمض الأسيتيك **حمض ضعيف** وتأينه لا يكون بهذه النسبة العالية.

الإجابة الصحيحة:

- **النسبة المتأينة (Ionized) = 1.32%**
- **النسبة غير المتأينة (Unionized) = 98.68%**

حل المسائل مع الخطوات:

المسألة الأولى: حساب pH لمحلول 0.1 M من حمض الأسيتيك وحساب نسبة التأين

المعطيات:

- **تركيز حمض الأسيتيك** = 0.1 M
- **pKa** لحمض الأسيتيك = 4.76

الخطوات:

1. نستخدم **معادلة هندرسون-هاسلباخ** لحساب pH:

$$\mathrm{pH} = \mathrm{pKa} + \log\left(\frac{[A^-]}{[HA]}\right)$$

- حيث $[A^-]$ هو تركيز القاعدة المترافقة (الأسيتات)، و $[HA]$ هو تركيز الحمض غير المتأين.
- عند إذابة حمض ضعيف في الماء، نحسب تركيز H^+ باستخدام:

$$K_a = \frac{[\mathrm{H}^+][A^-]}{[HA]}$$

حيث $K_a = 10^{-4.76}$.

2. نفرض أن التأين قليل، بالتالي $[H^+] = x$ و $[A^-] = x$ و $[HA] = 0.1 - x$.

$$K_a = \frac{x^2}{0.1 - x}$$

وبإهمال x لأن K_a صغير جدًا:

$$x^2 = (1.74 \times 10^{-5}) \times 0.1$$

$$x = \sqrt{1.74 \times 10^{-6}} = 1.32 \times 10^{-3} M$$

3. نحسب pH:

$$\mathrm{pH} = -\log(1.32 \times 10^{-3})$$

$$\mathrm{pH} = 2.88$$

4. **حساب نسبة التأين:**

$$\%\text{ionization} = \left(\frac{[\mathrm{H}^+]}{\text{initial concentration}}\right) \times 100$$

$$= \left(\frac{1.32 \times 10^{-3}}{0.1}\right) \times 100 = 1.32\%$$

المسألة الثانية: حساب نسبة التأين لهيستيدين عند pH = 7.4 و pH = 7.1

المعطيات:

- **pKa** لسلسلة الهيستيدين الجانبية = 6.0
- **pH** الدم الطبيعي = 7.4
- **تعيد الحساب pH عند انخفاض إلى 7.1**

الخطوات:

1. نستخدم معادلة هندرسون-هاسلباخ:

$$\mathrm{pH} = \mathrm{pKa} + \log\left(\frac{[\mathrm{A}^-]}{[\mathrm{HA}]}\right)$$

$$\log\left(\frac{[\mathrm{A}^-]}{[\mathrm{HA}]}\right) = \mathrm{pH} - \mathrm{pKa}$$

2. **عند pH = 7.4:**

$$\log\left(\frac{[\mathrm{A}^-]}{[\mathrm{HA}]}\right) = 7.4 - 6.0 = 1.4$$

$$\frac{[\mathrm{A}^-]}{[\mathrm{HA}]} = 10^{1.4} = 25.12$$

نحسب النسبة المئوية للمادة المتأينة (القاعدة A^-):

$$\%A^- = \frac{25.12}{25.12 + 1} \times 100 = \frac{25.12}{26.12} \times 100 = 96.2\%$$

والنسبة غير المتأينة:

$$\%HA = 100 - 96.2 = 3.8\%$$

3. **عند pH = 7.1:**

$$\log\left(\frac{[\mathrm{A}^-]}{[\mathrm{HA}]}\right) = 7.1 - 6.0 = 1.1$$

$$\frac{[\mathrm{A}^-]}{[\mathrm{HA}]} = 10^{1.1} = 12.59$$

النسبة المئوية للمتأين:

$$\%A^- = \frac{12.59}{12.59 + 1} \times 100 = \frac{12.59}{13.59} \times 100 = 92.6\%$$

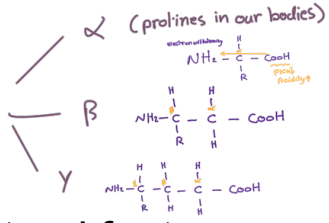
النسبة غير المتأينة:

$$\%HA = 100 - 92.6 = 7.4\%$$

Protein structure and function

Amino acids

Structure of amino acids (aa)

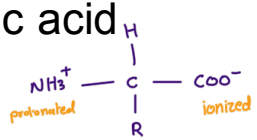


- Proteins are diverse in function but share common structural feature of being linear polymers of α-amino acids (20 aa in nature)
- Amino acids are very small biomolecules with an average molecular weight of about 135 daltons.
- Each a.a. has a carboxyl group, an amino group (except proline) and distinctive side chain

  → il· has imino group

  PH = 7.4
- They exist naturally in a zwitterion state where the carboxylic acid moiety is ionized and the basic amino group is protonated

  → nel· charge = zero



- In amino acids, COOH has a pKa (about 2) lower than that of normal carboxylic group (4-5) due to the presence of nitrogen which acts as electron withdrawing group

COOH alone : pka = 4-5

COOH in amino acid : pka = 2 decreases

why? → NH2 withdrawing group → it pulls the ē of·

As a result· :

Acidity ↑

ka↑ , Pka↓

4-5 → 2

So let me check if I get it right. So the string of the acid depends on how stable that acid is. So when the carboxylic acid links with a molecule or a compound that contains a withdrawing group, it has to lose a proton. When it loses that proton, it becomes more negative. So more negative, it's hard to keep the electrons because the electrons are negative too. So when the withdrawing group pulls the electron, that makes the carboxylic acid group more stable and thus more acidic, right? Stronger acid.

Yes, you've got it! Here's a quick recap to confirm:

1. **Acidity of an acid** depends on how **stable** its conjugate base (the ion after losing a proton) is. In the case of carboxylic acids, the conjugate base is the **carboxylate anion ($RCOO^-$)**.
2. When the carboxylic acid (COOH) loses a proton (H^+), it becomes negatively charged (COO^-). Negative charges are harder to hold onto because like charges repel each other. This is where the **stability** of the negative charge matters.
3. If the carboxylic acid is attached to a **w drawing group** (like a nitro group, halogen, etc.), the withdrawing group pulls electron density away from the carboxylate anion.

Structure of amino acids

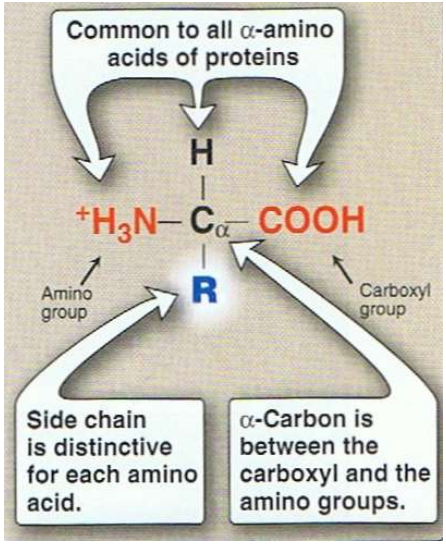
- They are classified as α, β, γ, *etc*. amino acids according the carbon that bears the nitrogen.

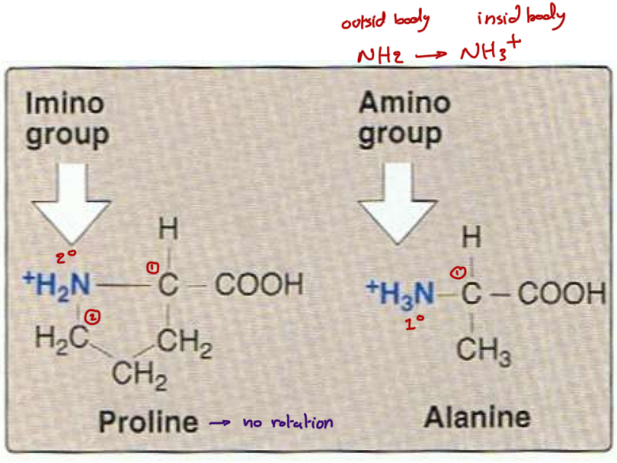
حسب اي كربونه متصله بال NH2

- Amino acids are devided into: essential and non-essential

must take of external sourse (9) — produced inside the body

The essential are Ile, Leu, Lys, Met, Phe, Thr, Trp, His and Val. while the rest can be synthesized in our bodies





classification of Amino acid

Base polar
BAPAN non-polar
Acid Aromatic

BA → charged polar
(Art loving guy)

B: Basic
Loves His Art
Lysine
Histidine,
Argenine

A: Acidic
As God
aspartic acid,
Glutamic acid

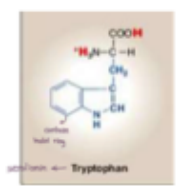
P (uncharged)
(Mad sera)

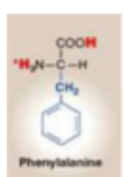
SeraThrow her Cyster
to Glu Asprin
Tyrosine

Serine
Threonine
Cysteine
Glutamine,
Asparagine,
Tyrosine

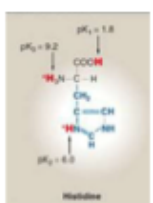
A
(Fat tire)

Phat Tire

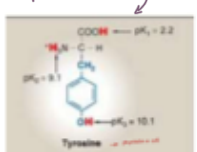




non-polar



Basic



phenylal. کا مشتق
polar

N
(prof. Lue and peresident)

prof Leu Arrived at valet Ground to Met the phenylalanine but it was a Tryp

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

1. Nonpolar amino acids

polar
non polar
Acidic
Basic

- Include: Alanine, Glycine, Isoleucine, Leucine, Methionine, Phenylalanine, Proline, Tryptophan, Valine

- Nonpolar amino acids share only in hydrophobic interaction (No hydrogen or ionic bonds) which stabilize the protein structure

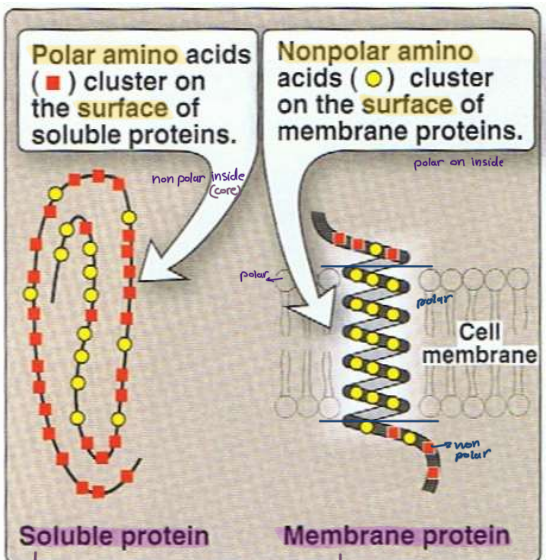
- Determine the three dimensional shape and their location in the cell.

depends polar / non polar aa order on the protin (Surface , inside)

helices breaker

- Proline contains imino so it interrupts the α-helices in globular proteins and contributes to the formation of fibrous proteins

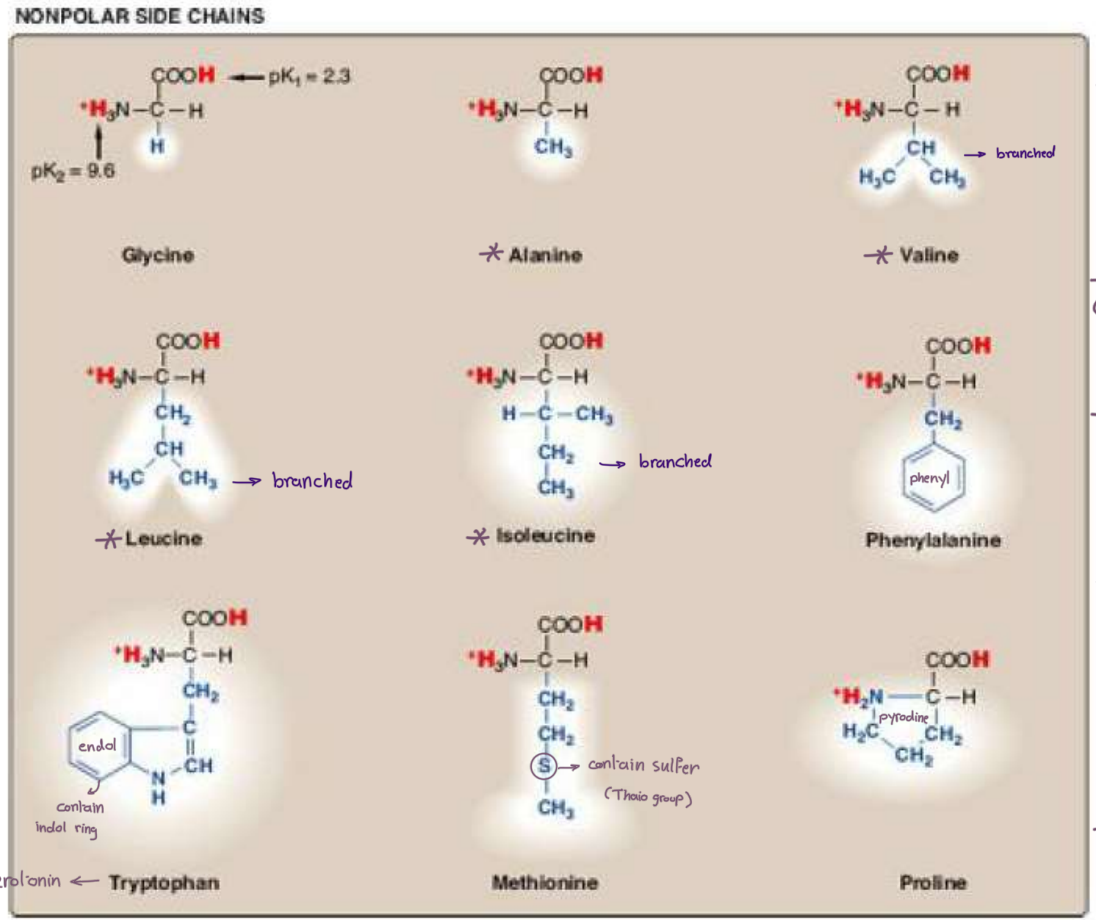
↳ doesn't involves folding



↳ intracellular (cystol) → hydrophilic
∴ it must be hydrophilic in the cytoplasm fluid (cystol)

↳ plasma membrane hydrophopic protine

1. Nonpolar amino acids



→ pka :

Coo^- = 2.3

NH_3^+ = 9.6

→ All Amino acids (aa) are chiral expect : Glycine (achiral)

→ optical activity related to chiral

optical activity : light rotation

→ phenylalanine —(phenylalanine hydroxylase)→ Tyrosine

Epn · nor · epn Dopamine

→ * aa are hydrocarbon (C and H side chain)

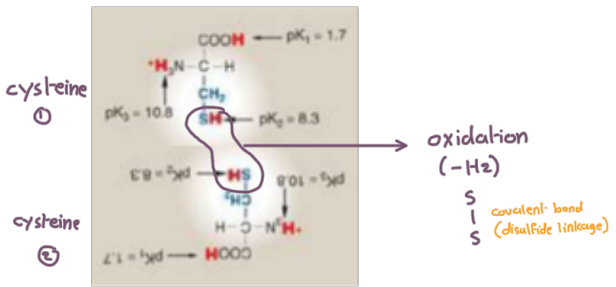
Aliphatic Amino Acids

→ aa that contain S Methionine , cysteine

(thioether bond) (sulphydral group)

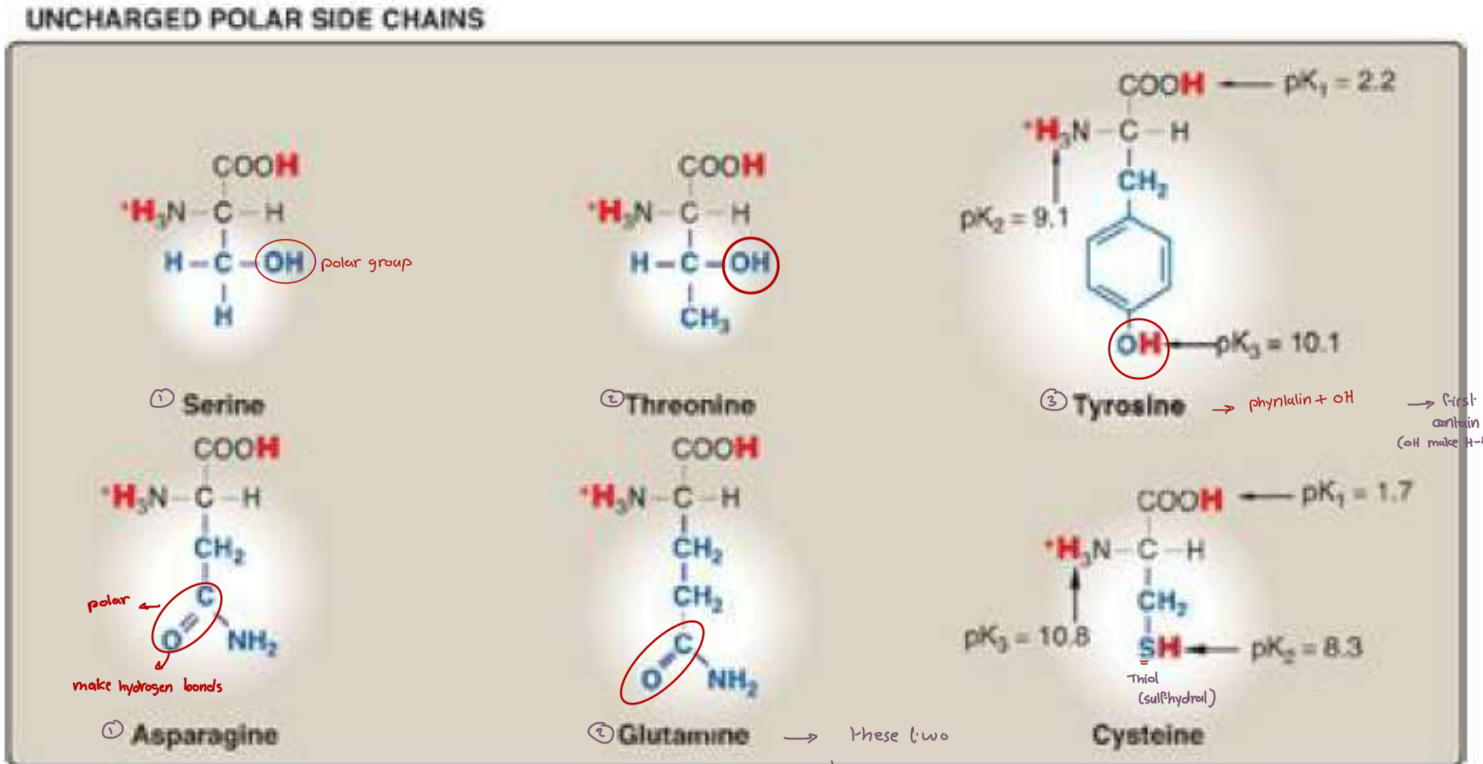
2. Uncharged polar amino acids

- Include: Asparagine, Glutamine, Serine, Threonine, Tyrosine and Cysteine
- form hydrogen bond with other polar aa
- Present outside of the proteins that function in aqueous environment and in interior of membrane associated proteins.
- Cysteine has sulfhydryl group which can be oxidized to form a dimer, Cystine (C-S-S-C)



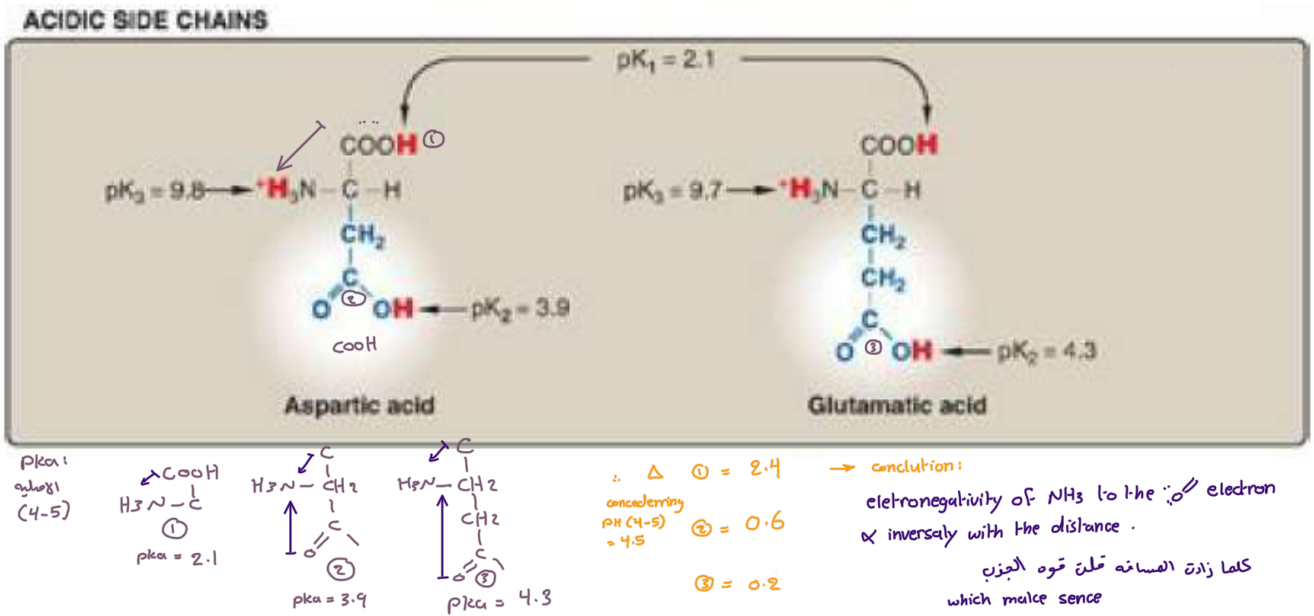
cystine Dimer

2. Uncharged polar amino acids



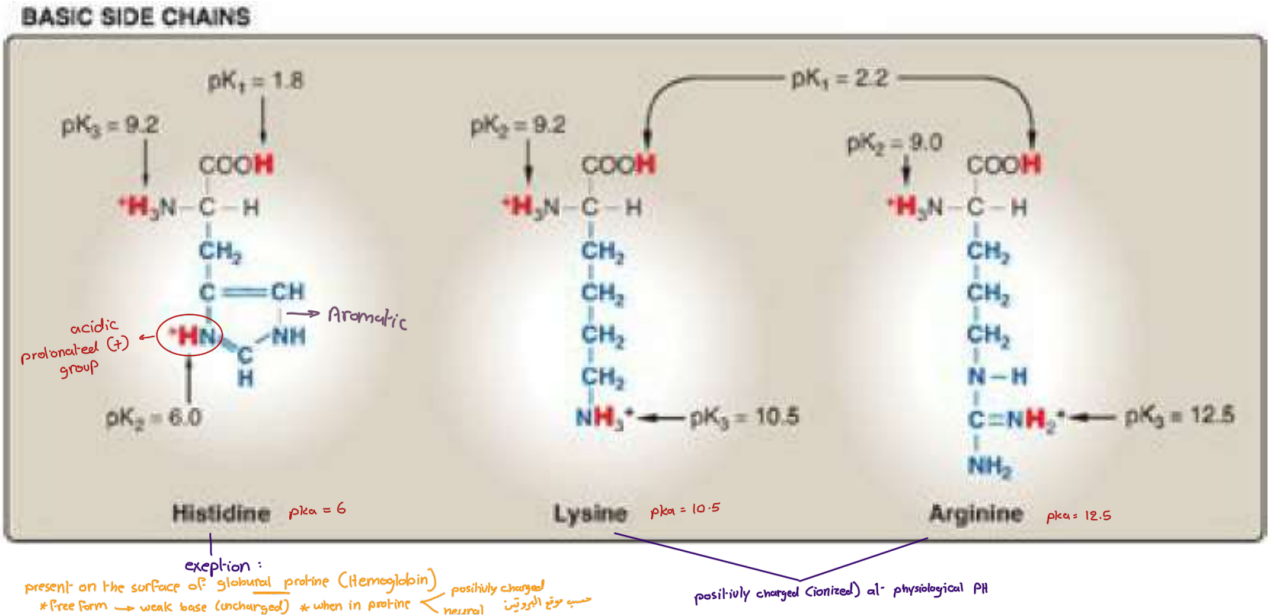
3. Acidic amino acids (Tol· charge = posil·ive)

- Include: aspartic acid, Glutamic acid
- The side chain dissociate to COO^- at physiological pH



4. Basic amino acids (Tol· charge = Negative)

- Include Histidine, Lysine and Argenine
- Side chain is protonated and generally has a positive charge at physiological pH.



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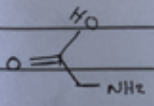
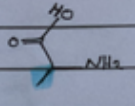
Non - polar

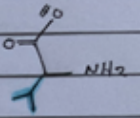
Glycine



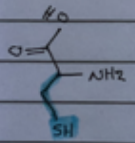
Alanine



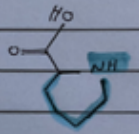
Valine



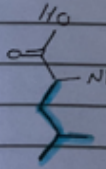
cysteine



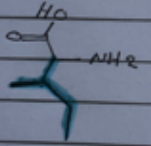
Proline



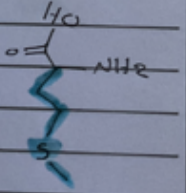
Leucine



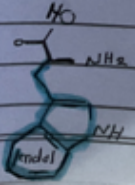
Isoleucine



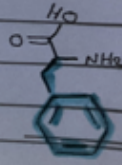
Methionine



Tryptophan



phenylalanine



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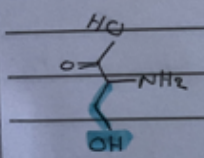
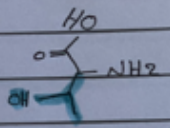
polar (uncharged)

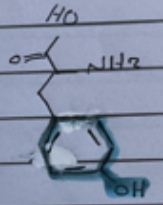
Serine



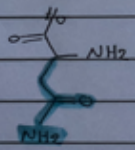
Threonine



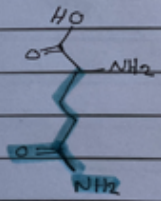
Tyrosine



Asparagine



Glutamine



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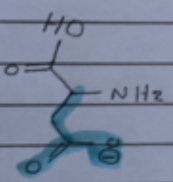
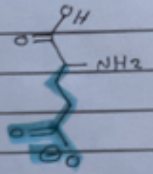
Charged ($\bar{A}$. $\overset{+}{B}$)

Acids (negative)

① Aspartic Acid

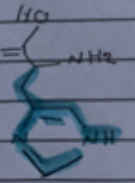


② Glutamic Acid

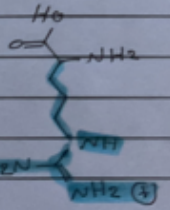


Bases (positive)

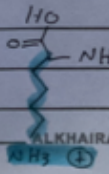
① Histidine



② Arginine

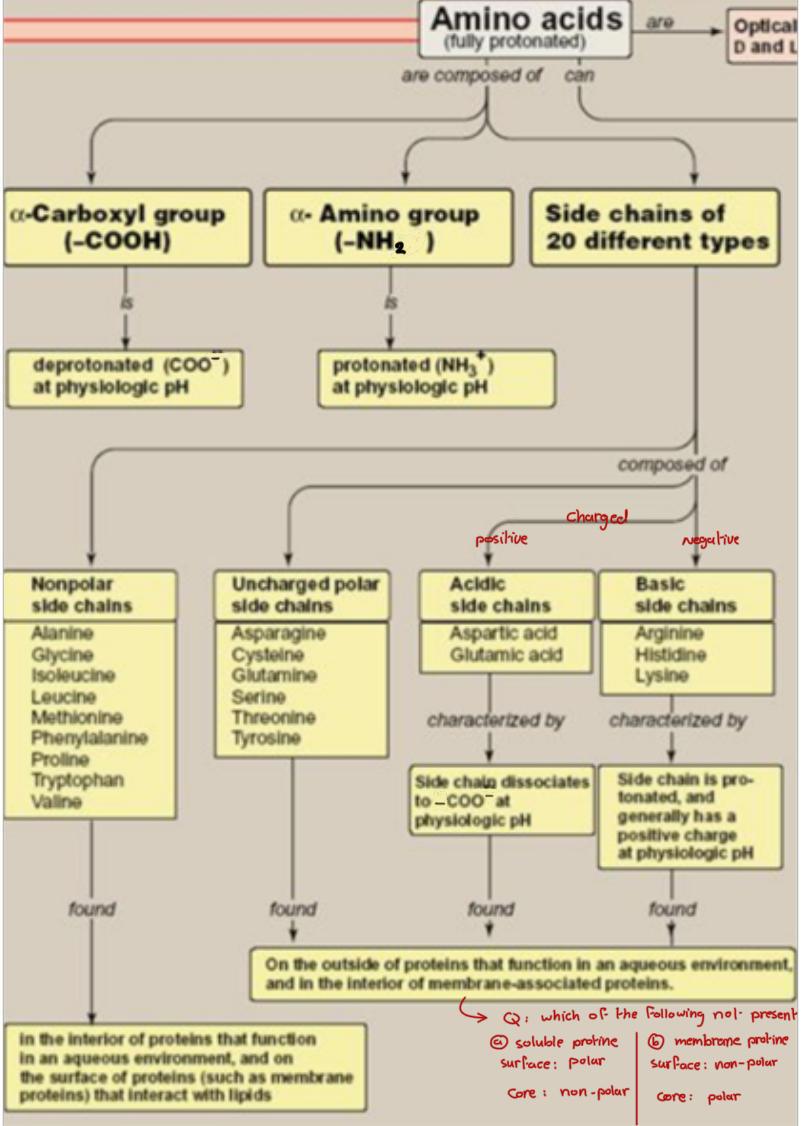


③ Lysine



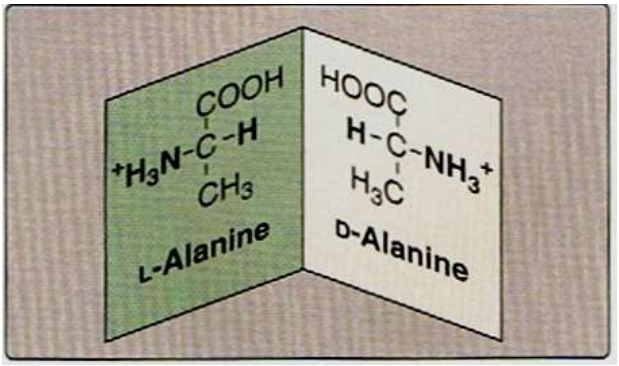
Abbreviations and symbols of amino acids

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

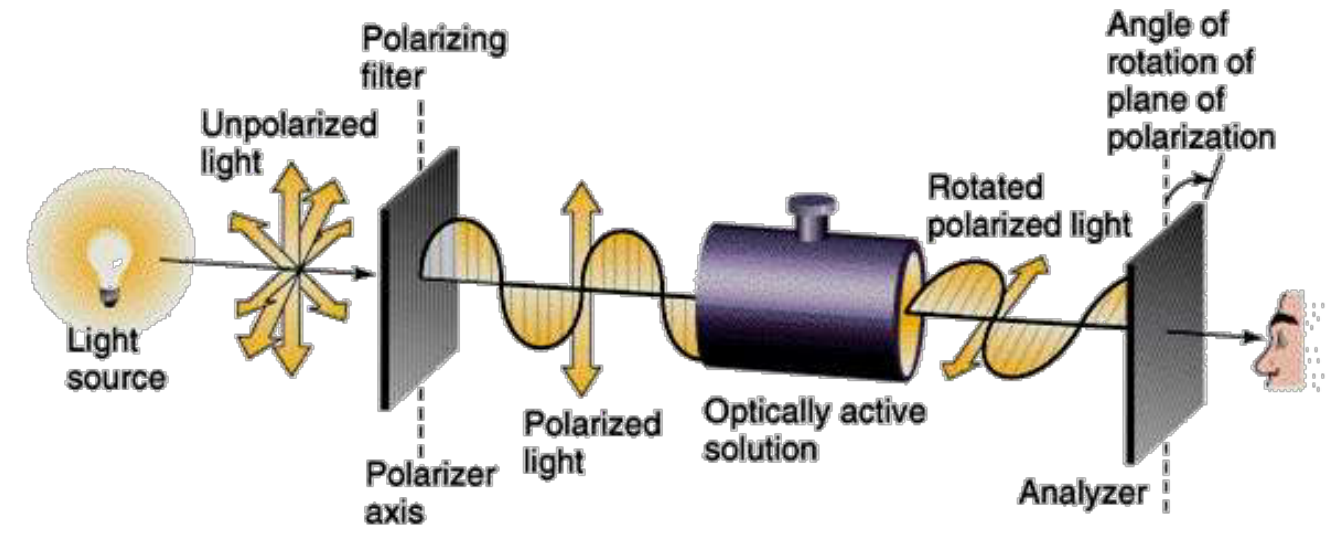

Amino acids
(fully protonated)
are
Optical
D and L
are composed of
can
α-Carboxyl group (–COOH)
α- Amino group (–NH2)
Side chains of 20 different types
is
is
deprotonated (COO⁻) at physiologic pH
protonated (NH3⁺) at physiologic pH
composed of
Charged
positive
negative
Nonpolar side chains
Alanine
Glycine
Isoleucine
Leucine
Methionine
Phenylalanine
Proline
Tryptophan
Valine
Uncharged polar side chains
Asparagine
Cysteine
Glutamine
Serine
Threonine
Tyrosine
Acidic side chains
Aspartic acid
Glutamic acid
Basic side chains
Arginine
Histidine
Lysine
characterized by
characterized by
Side chain dissociates to –COO⁻ at physiologic pH
Side chain is protonated, and generally has a positive charge at physiologic pH
found
found
found
found
On the outside of proteins that function in an aqueous environment, and in the interior of membrane-associated proteins.
Q: which of the following not present
in the interior of proteins that function in an aqueous environment, and on the surface of proteins (such as membrane proteins) that interact with lipids
(a) soluble protine
surface: polar
core: non-polar
(b) membrane protine
surface: non-polar
core: polar

Optical properties of amino acids

- With the exception of glycine, the α-carbon of all aa‘s is optically active (chiral)
- a.a. Exist in two forms, L and D, which are mirror images
- All amino acids found in proteins are of the L-configuration
- D- amino acids are found in some antibiotics and in bacterial cell walls

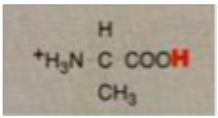


Optical properties of amino acids

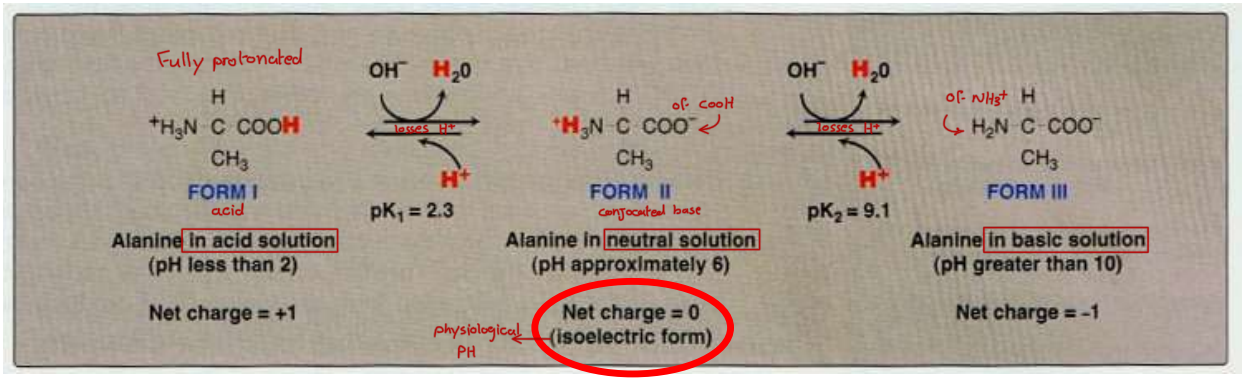


Acidic and basic properties of amino acids

- Amino acids can act as buffers



multiprotic acid (have more than one H to donate it)



- The quantitative relationship is described by Henderson-Hasselbalch equation:

$$pH = pK_1 + \log \frac{[II]}{[I]}$$

product / reactant

Titration curve for alanine

$$pH = pK_1 + \log \frac{[II]}{[I]}$$

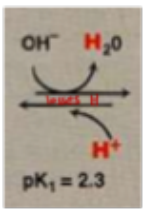
(product / reactant)

Region of buffering : (maximum buffer capicity)

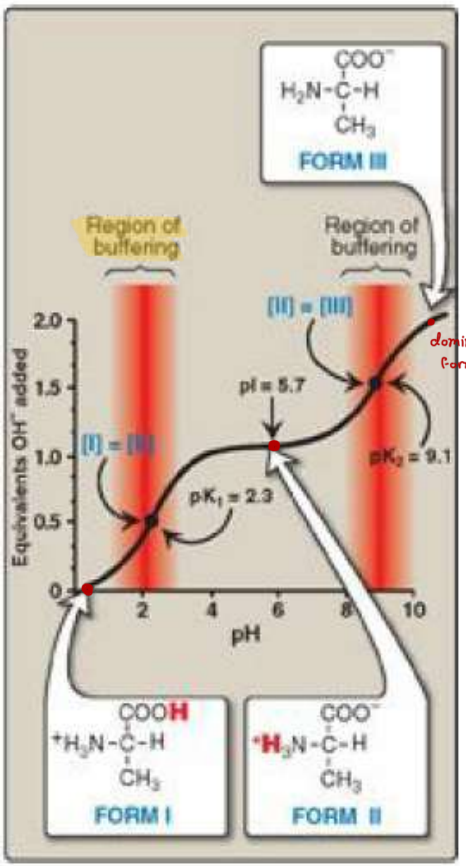
pka = 2.3 → pH = pka + log [form two] / [form one]

50 / 50 = 1 log 1 = zero

∴ Pka = 2.3 → PH = Pka one buffering region

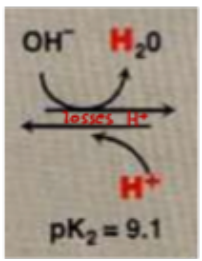


pka 2.3 contain 50% form one and 50% form two



Pka = 9.1

same ther PH = Pka



Pka 9.1 have 50% form two 50% form three

isoelectric point here is :

$$\frac{2.3 + 9.1}{2} = 5.7$$

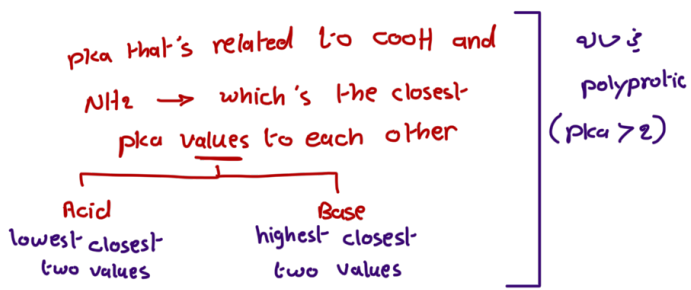
net charge = zero ← # Isoelectric point (pI)

- At Its Isoelectric pH (pI), an Amino Acid Bears No Net Charge
- The isoelectric pH is calculated as the pH midway between p*K*a values on either side of the isoelectric species.
- Example: alanine has only two dissociating groups, p*K*a (R-COOH) is 2.35 and p*K*a (R-NH_3^+) is 9.69. The isoelectric pH (pI) of alanine is

$$pI = (pKa1 + pKa2)/2 = 6.02$$

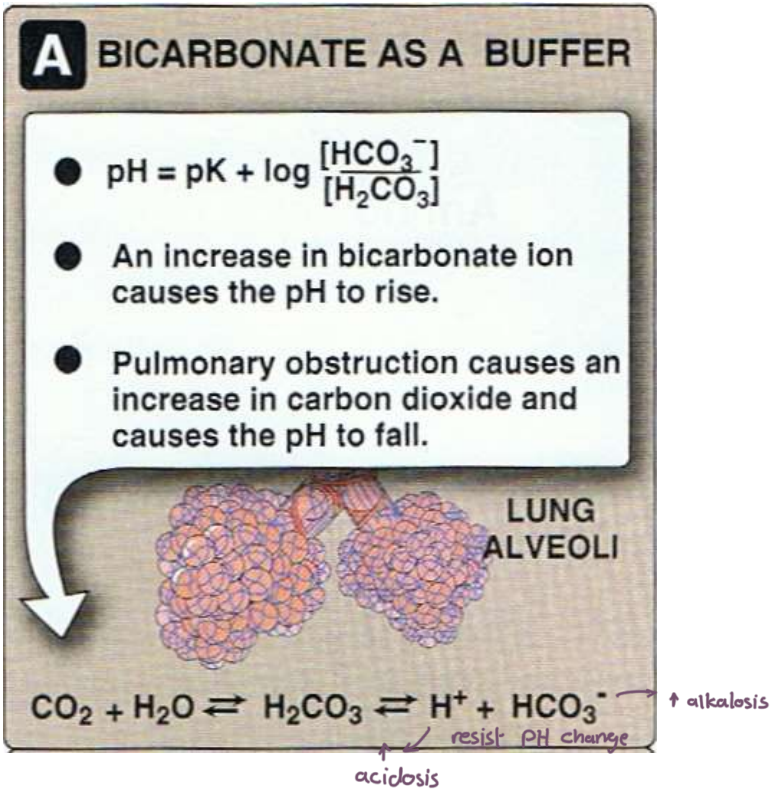
- For polyfunctional acids, pI is also the pH midway between the p*K*a values on either side of the isoionic species. For example, the pI for aspartic acid is

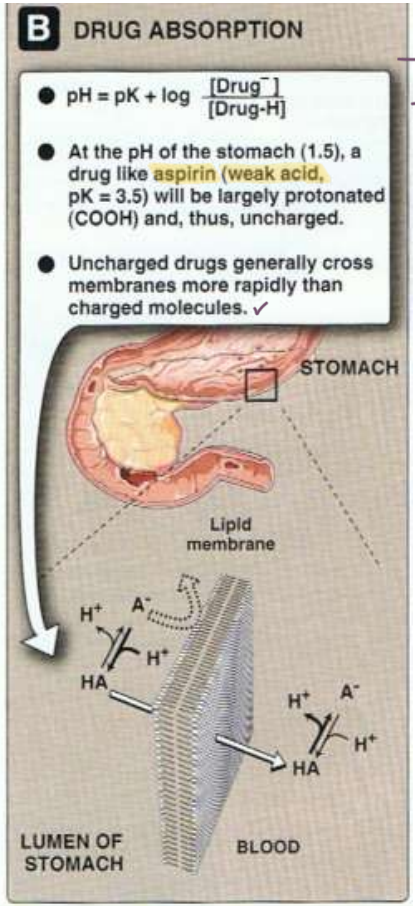
$$pI = (pKa1 + pKa2)/2$$
$$(2.09 + 3.96)/ 2 = 3.02$$



Application on buffer effect

our blood is buffered by :
- Bicarbonate
- AA
- Hemoglobine





→ most drugs :
- weak acids / bases

Biological importance of proteins

1. Proteins are essential component of membranes.
2. Plasma membrane proteins regulate the transfer of various substances across the cell membrane or act as *receptors*.
3. All enzymes are protein in nature.
4. All antibodies (immunoglobulins) are protein in nature, play an important role in the bodies' defensive mechanisms.
5. Some hormones are proteins in nature e.g. insulin, glucagon, and growth hormone.
6. *Hemoglobin* carries oxygen in the blood and *myoglobin* stores O_2 in muscles. Both are proteins in nature.

Biological importance of proteins

7. Some proteins are protective e.g. *keratins* make the skin resistant to chemicals. Others have supportive functions e.g. *collagen and elastin*.

8. Amino acids are converted to other substances of great physiologic importance e.g. creatine, heme, histamine, serotonin, purines and pyrimidines.

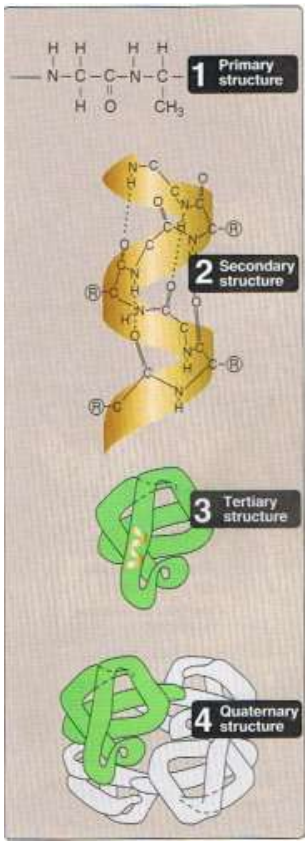
9. *Actin* and *myosin* are contractile proteins found in muscle cells and are responsible for muscular contraction.

10. Plasma proteins can carry: lipids forming lipoprotein complexes; hormones (e.g. thyroid and steroid hormones) and minerals (e.g. calcium and copper).

Structure of proteins

- The are four levels of protein structures:

1. Primary structure: the amino acid sequence of proteins. → linear
2. Secondary structure: α-helices and β-sheets.
3. Tertiary structure: the three dimensional structure of protein (3D)
4. Quaternary structure: arrangement of polypeptide subunits → two or more piptide chain



- The folding of the protein ranges from simple combinations of α -helices and β-sheets forming small motifs to the complex folding of polypeptide domains of multifunctional proteins.

simple folding → small motifs

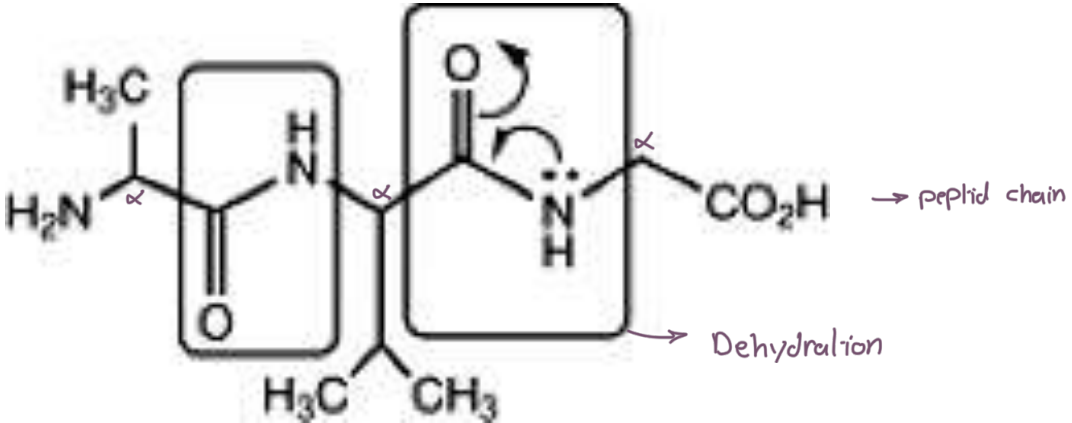
Complex folding

Classification of proteins

aa≤15 ➤ **Oligopeptides**: Peptides with **fewer than 15** residues e.g., gonadotropin-releasing hormone [GnRH] contains **10** residues

15-50 ➤ **Polypeptides**: Peptides consisting of **15 to 50 residues** e.g., adrenocorticotropin hormone consists of **39** residues.

50≤aa ➤ **Protein**: polypeptide that contains **more than 50 amino acid** residues e.g., parathyroid hormone contains **84** residues



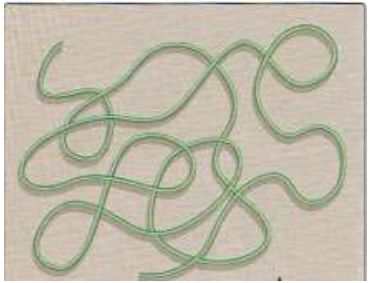
1. Primary strucure

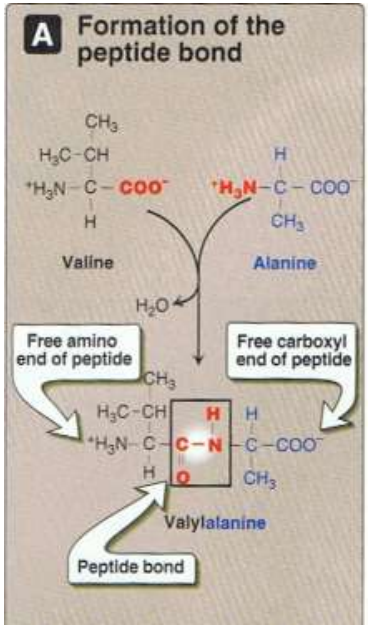
- The amino acid sequence of proteins

- Genetic diseases (mutations) occurs due to defect in the amino acid sequence leading to improper folding and impairment of function. (weakness)

- In proteins, amino acids are joined covalently by peptide bonds, which are amide linkages between the α-carboxyl group of one amino acid, and the α-amino group of another. bond: peptide linkage (Amide) which is a covalent bond

- The peptide bond of the protein can be hydrolyzed by prolonged (معتدل) exposure (تعرض) to acid or base at high temperature (1) or enzymatically (2). break down

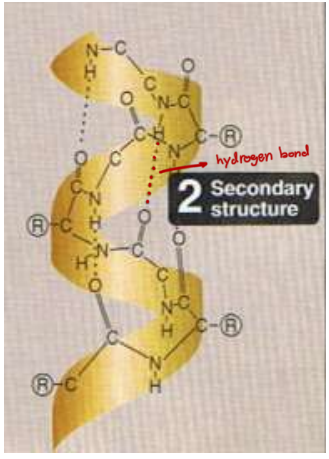


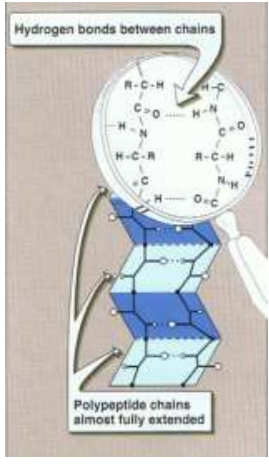


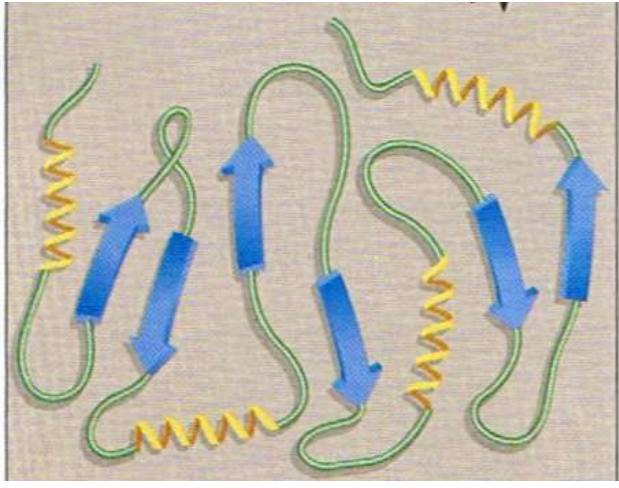
2. Secondary structure

- The secondary structure of protein is stabilized by hydrogen bonding
- Folding of the protein to:

1. α-helix:
2. β-pleated sheats: can be parallel or antiparallel
3. β-turns: usually at the surface of the protein, contains usually proline which causes a kink the structure in addition to glycine.



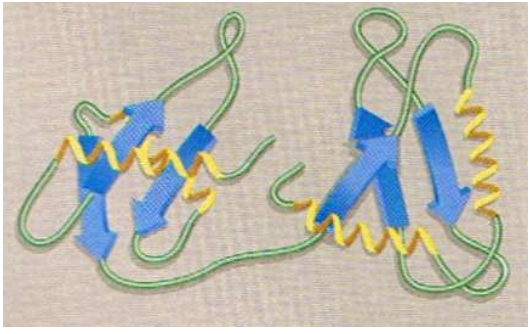




3. Tertiary structure

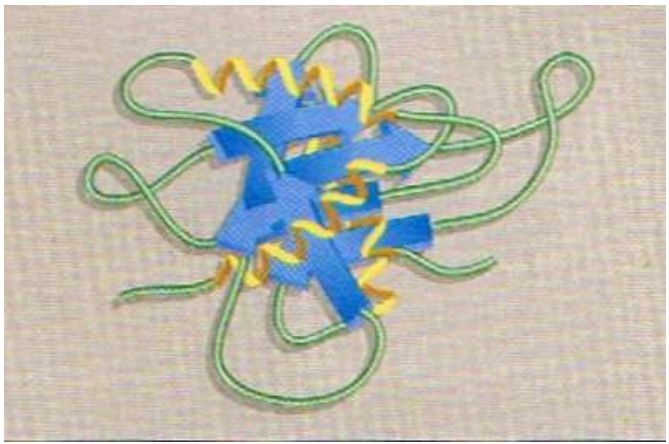
- The tertiary structure is the three dimensional structure of proteins (folding of the domains)
- The protein tends to fold correctly with a low energy state.
- Interactions stabilizing the tertiary structure:

1. Disulfide bond: in presence of Cysteine which forms a covalent bond (-S-S-)
2. Hydrophobic interactions
3. Hydrogen bonding
4. Ionic interactions



4. Quaternary structure

- The polypeptide can be one domain (monomer), dimer, trimer depends on the number of subunits.
  ↳ Domain
- A specialized group of proteins (called chaperones) are required for the proper folding of the protein.



كعب

Denaturation of the proteins

- Unfolding of protein: occurs due to different factors:
  - Urea
  - Extreme pH and temperature
  - Organic solvents

معمه

- Leads to loss of secondary and tertiary structure and hence, loss of function.

- Most of proteins can‘t refold upon removal of the denaturant (irreversible denaturation)

Diseases related to denaturation of proteins

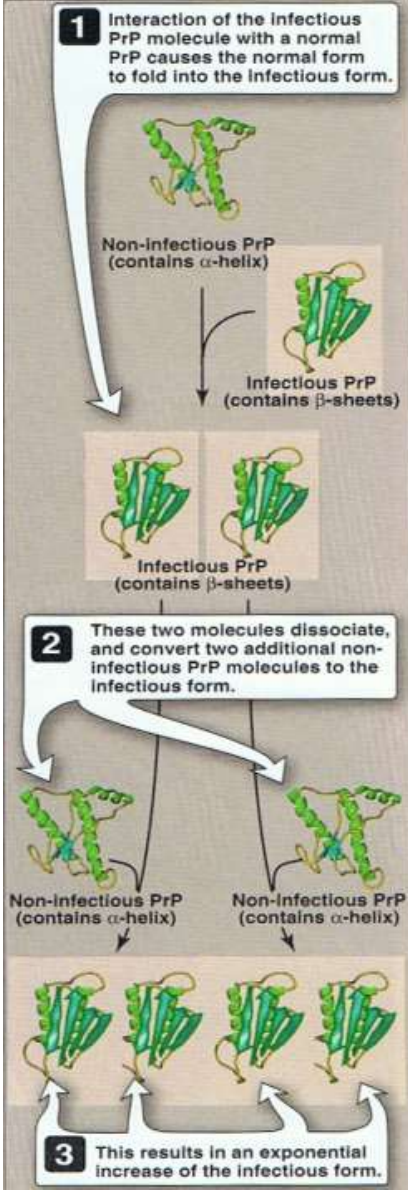
- Alzheimer disease:

  Normal proteins,after abnormal chemical processing, take on a unique conformational state that leads to the formation of neurotoxic amyloid protein assemblies consisting of β-pleated sheets.

  Another protine → Toxic

- In Transmissable Spongiform Encephalopathy 5 (Mad cow disease):

  The infective agent is an altered version of a normal prion protein that acts as a "template" for converting normal protein to the pathogenic conformation.



Biosynthesis of protein

- The information that tells a cell how to build the proteins it needs to survive is coded in the structure of the DNA in the nucleus of that cell.

- Because there are only four nucleotides and there are 20 amino acids that must be coded. the nucleotides are grouped in threes, however, there are 64 possible triplets, or **codons**

- DNA only stores the genetic information, while RNA is responsible of its translation to protein

Biosynthesis of protein

1. Transcription → in Nucleus

- Before the information in DNA can be decoded, a small portion of the DNA double helix must be uncoiled
- A strand of RNA is then synthesized that is a complementary (مكمله) copy of one strand of the DNA using RNA polymerase.
- RNA uses U where T would be found in DNA and base pairing occurs between two chains that run in *opposite directions*. The RNA complement of this DNA should therefore be written as follows.
- 3´ T-A-C-A-A-G-C-A-G-T-T-G-G-T-C-G-T-G... 5´ *DNA*
  (opposite direction)
- 5´ A-U-G-U-U-C-G-U-C-A-A-C-C-A-G-C-A-C... 3´ *mRNA*
- Since this RNA strand contains the message that was coded in the DNA, it is called **messenger RNA**, or **mRNA**.

Biosynthesis of protein

2. Translation → in cystol

- The messenger RNA now binds to a ribosome, where the message is translated into a sequence of amino acids.
- The amino acids that are incorporated into the protein being synthesized are carried by relatively small RNA molecules known as **transfer RNA**, or **tRNA**.
- There are at least 60 tRNAs, which differ slightly in their structures, in each cell. At one end of each tRNA is a specific sequence of three nucleotides that can bind to the messenger RNA. At the other end is a specific amino acid.
- each three-nucleotide segment of the messenger RNA molecule codes for the incorporation of a particular amino acid.

Biosynthesis of protein
2. Translation

- The signal to start making a polypeptide chain in simple, prokaryotic cells is the triplet AUG, which codes for the amino acid methionine (Met). The synthesis of every protein in these cells therefore starts with a Met residue at the *N*-terminal end of the polypeptide chain. After the tRNA that carries Met binds to the start signal on the messenger RNA, a tRNA carrying the second amino acid binds to the next codon. A dipeptide is synthesized when the Met residue is transferred from the first tRNA to the amino acid on the second tRNA.

- The mRNA now moves through the ribosome, and a tRNA carrying the third amino acid (Val) binds to the next codon. The dipeptide is then transferred to the amino acid on this third tRNA to form a tripeptide.

- This sequence of steps continues until one of three codons is encountered: UAA, UGA, or UAG. These codons give the signal for terminating the synthesis of the polypeptide chain, and the chain is cleaved from the last tRNA residue

Biosynthesis of protein
2. Translation

- The sequence of DNA described in this section would produce the following sequence of amino acids.

Met-Phe-Val-Asn-Gln-His-...

- This polypeptide is not necessarily an active protein. All proteins in prokaryotic cells start with Met when synthesized, but not all proteins have Met first in their active form.

- It is often necessary to clip off this Met after the polypeptide has been synthesized to give a protein with a different *N*-terminal amino acid

Biosynthesis of protein

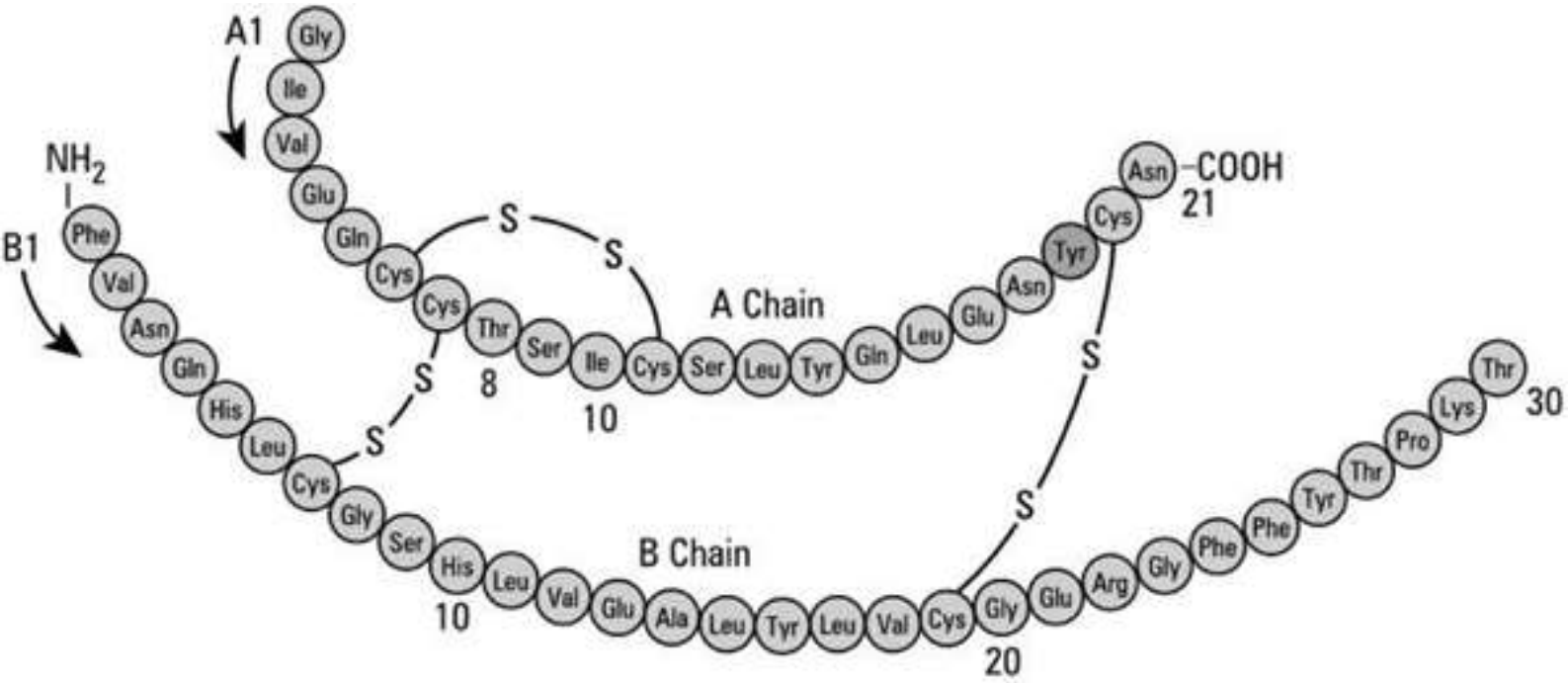
3. Post-translational modification

- Modifications to the polypeptide often have to be made before an active protein is formed

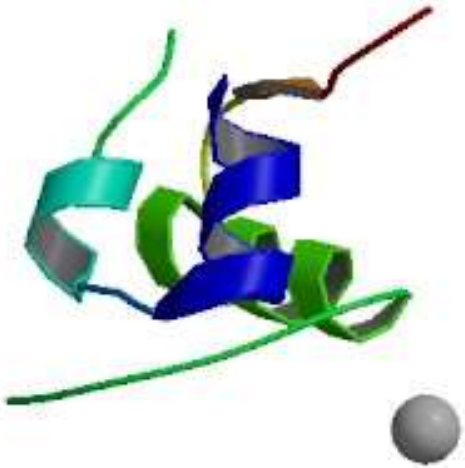
- Example:
  - Insulin consists of two polypeptide chains connected by disulfide linkages. In theory, it would be possible to make these chains one at a time and then try to assemble them to make the final protein
  - The polypeptide chain that is synthesized contains a total of 81 amino acids.
  - All of the disulfide bonds that will be present in insulin are present in this chain. The protein is made when a sequence of 30 amino acids is clipped out of the middle of this polypeptide chain

Primary structure of human insulin

Chains A and B, including the interchain disulfide bonds A7-B7 and A20-B19 and intrachain disulfide bond A6-A11



Tertiary and quaternary structure of insulin



كرويّه

Globular proteins

Hemeproteins → (contain heme)

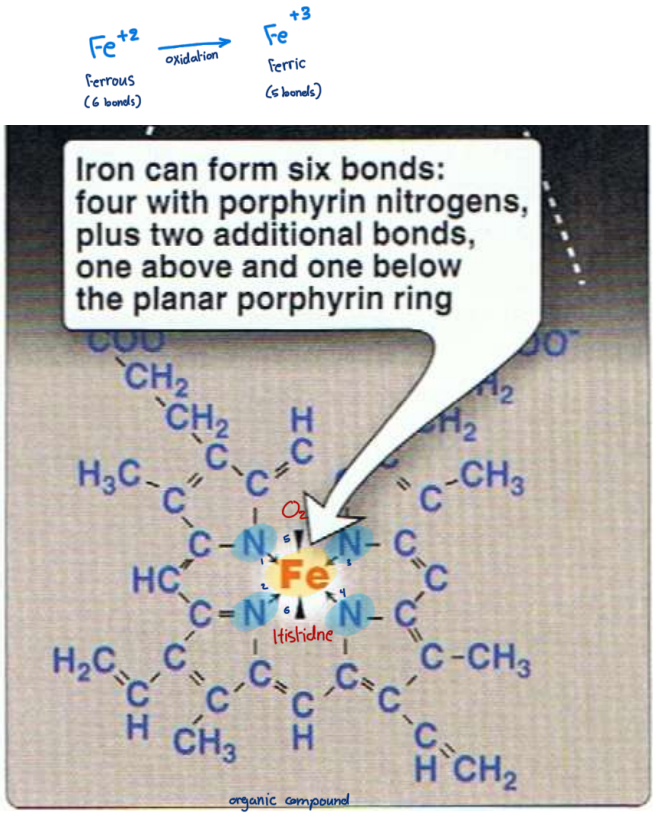
- Hemeproteins are group of specialized proteins that contains heme as tightly bound prosthetic group
  non-polypiptide unit

- Function in
  - Oxygen binding
  - Electron transport
  - Oxygen transport
  - Photosynthesis

- The most abundant hemeproteins are ① Myoglobin and ② hemoglobin

Structure of heme

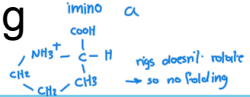
- Function according to the protein it binds
- Heme is a complex of protoporphyrin IX and ferrous iron (Fe^{+2}). The iron is held in the center of the heme molecule by bonds to the four nitrogens of the porphyrin ring.
- The heme Fe^{+2} can form two additional bonds, one on each side of the planar porphyrin, in myoglobin and hemoglobin, one of these positions is coordinated to the side chain of a histidine residue of the globin molecule, whereas the other position is available to bind oxygen



Structure of myoglobin (monomer)

- In heart and skeletal muscle (needs ATP)
- Functions as reservoir for oxygen and as oxygen carrier
- Consists of single polypeptide that is structurally similar to hemoglobin subunit.
- Consist of eight α-helices which represent 80% of the structure which are ended by proline or β-bends stabilized by ionic and hydrogen bonding

imino α



rigs doesn't rotate → so no folding

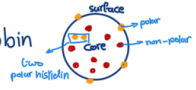
b ↓ glycine and proline → helixes breakers

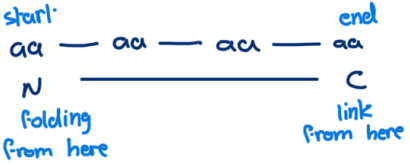
I want it soluble

- Polar and charged aa are present on the surface of the molecule which form hydrogen bonding with water
- with the exception of two histidines, nonpolar aa's form the interior structure

The only polar molecules inside

∴ Myoglobin





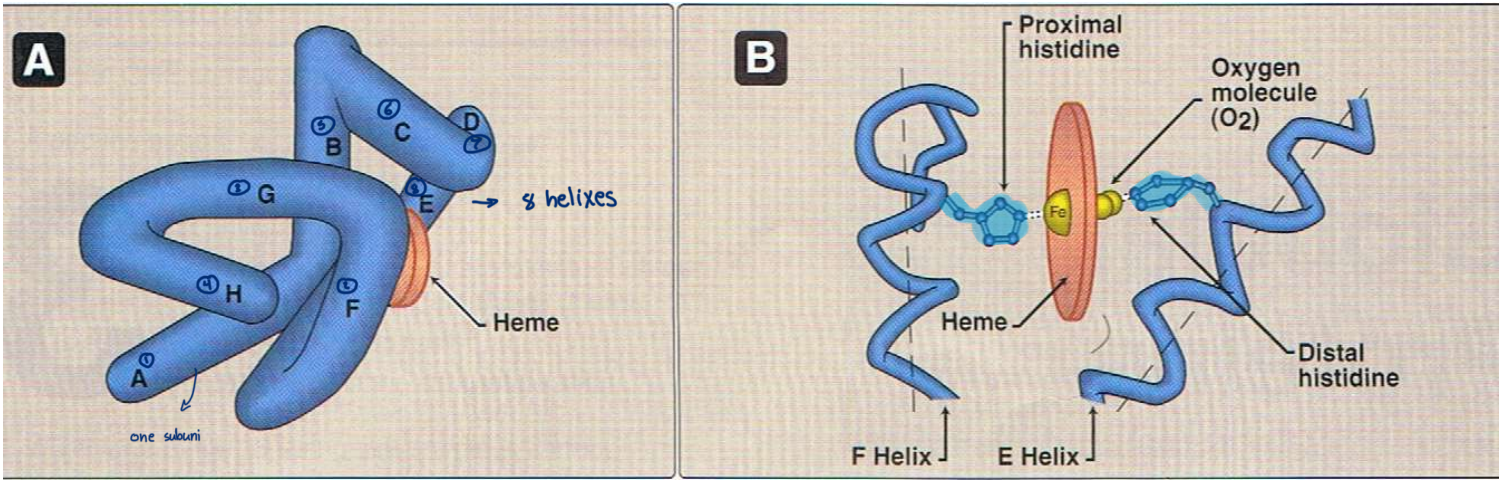
Binding of O2 to myoglobin

- The proximal histidine binds the iron of the heme while the distal histidine helps to stabilize the binding of oxygen to the ferrous iron.

Helix (F, E)

Function :

- The globin portion of myoglobin thus creates a special microenvironment for the heme that permits the reversible binding of one oxygen molecule (oxygenation).



Hemoglobin

- In red blood cells
- Transport oxygen from lung to the capillaries of tissues as well as CO_2 in the reverse direction
- Hemoglobin A, mainly in adults, composed of four polypeptide chains (2α and 2β) held together non-covalently

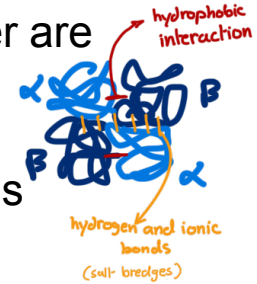
- Oxygen binding is regulated by interaction with allosteric effectors
- The subunits occupy different relative positions in deoxyhemoglobin compared with oxyhemoglobin

more than one → Quntirtiary

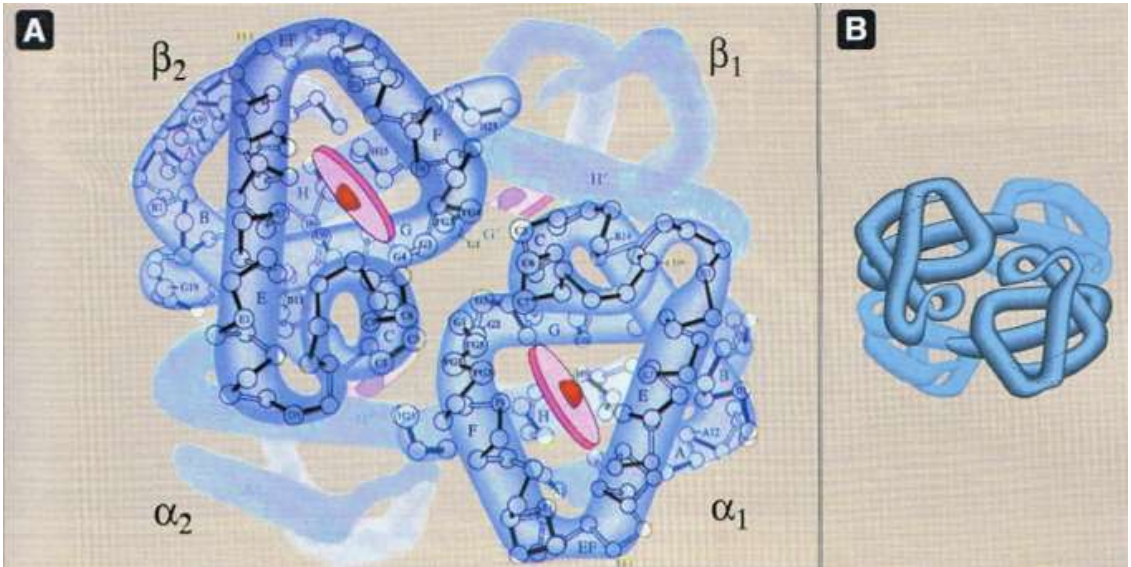
subunits
Domains

Structure of hemoglobin

➢The hemoglobin tetramer composed of two identical dimers, (αβ1) and (αβ2. The two polypeptide chains within each dimer are held tightly together, primarily by hydrophobic interactions



➢Interchain hydrophobic interactions form strong associations between α-subunits and β-subunits in the dimers, ionic and hydrogen bonds also occur between the members of the dimer.



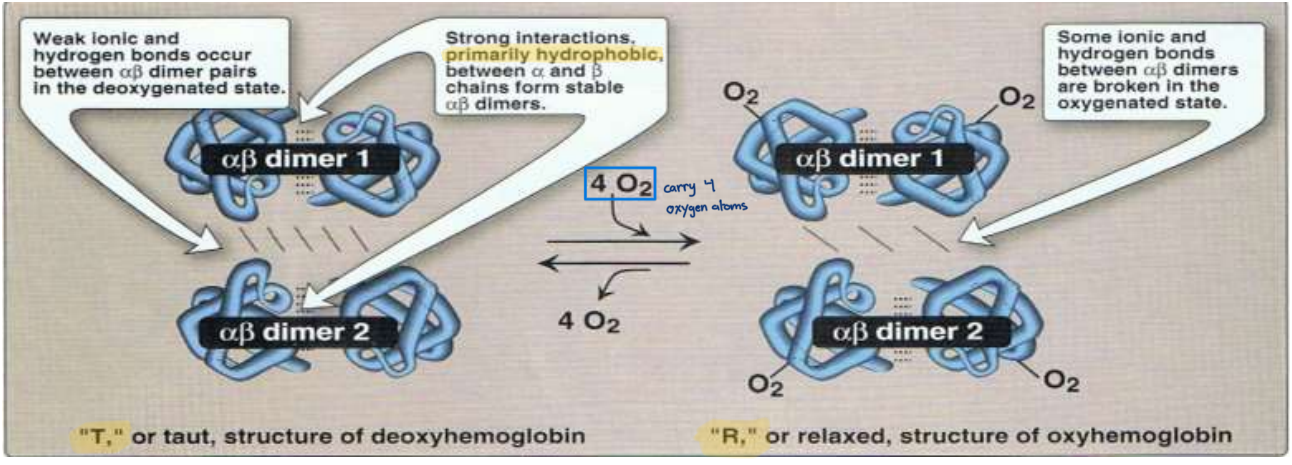
Hemoglobin

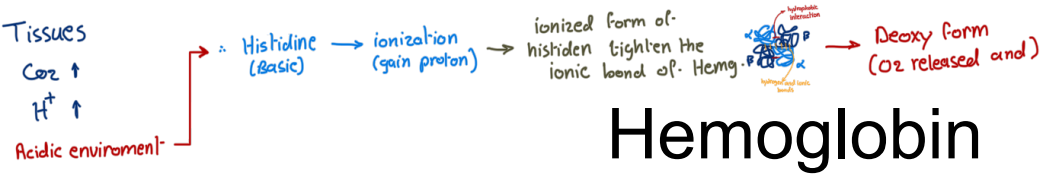
Two forms — determain by the region the hemoglobin present in

- Deoxy T : taut (tense) form → no oxygen binding involves
- oxy R : relaxed form → oxygen binding occur

- The deoxy form of hemoglobin is called the "T or taut (tense) form, a constrained structure that limits the movement of the polypeptide chains, has a low oxygen-affinity.

- The binding of oxygen to hemoglobin causes rupture of some of the ionic and hydrogen bonds. This leads to a structure called the 'R' or relaxed form, in which the polypeptide chains have more freedom of movement.





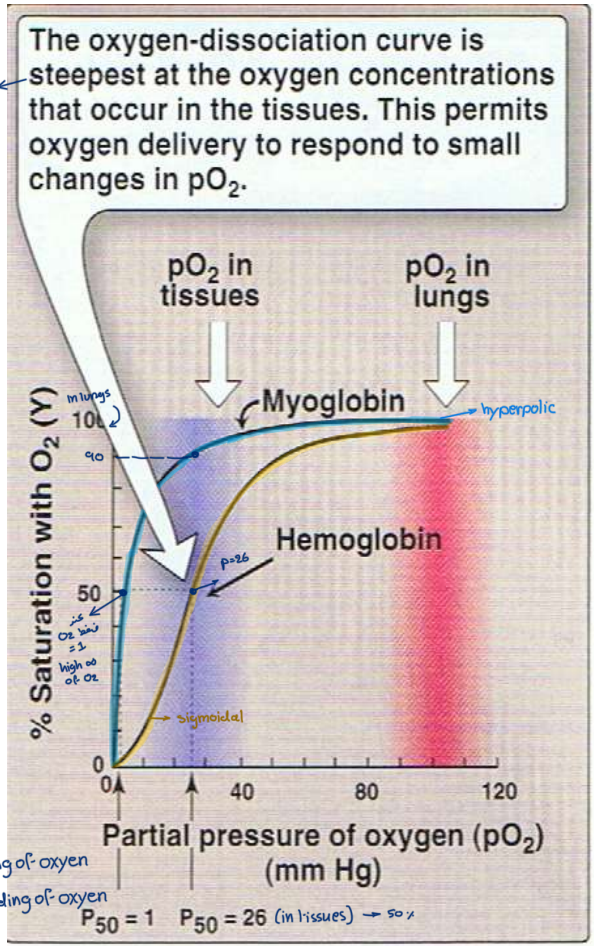
Hemoglobin

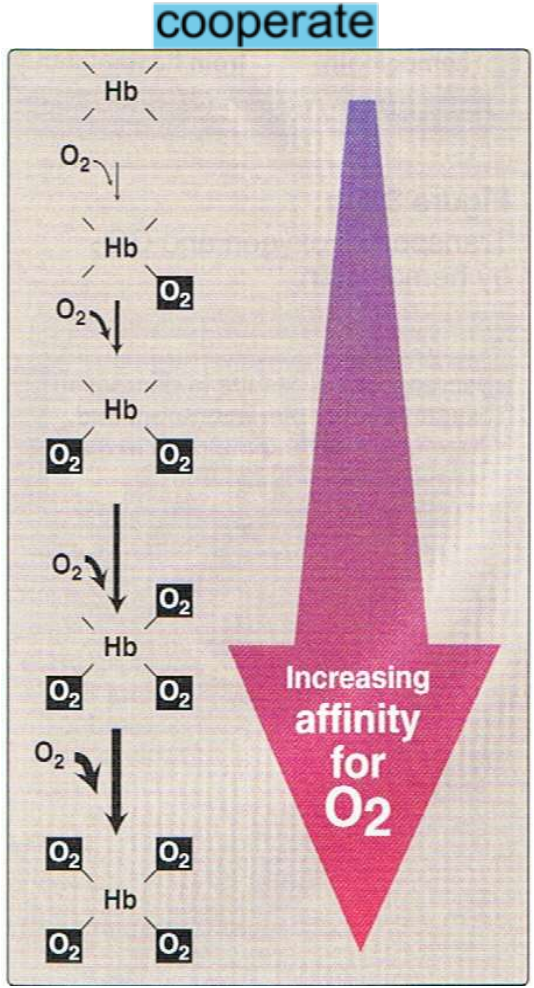
- The R form is the high oxygen affinity form of hemoglobin.
- The oxygen- dissociation curve for hemoglobin is sigmoidal in shape (in contrast to that of myoglobin, which is hyperbolic), indicating that the subunits cooperate in binding oxygen.

Just heard of first

- Cooperative binding of oxygen by the four subunits of hemoglobin means that the binding at one heme group increases the oxygen affinity
- The steep slope of the oxygen-dissociation curve permits hemoglobin to carry and deliver oxygen efficiently from sites of high to sites of low pO2.
- A molecule with a hyperbolic oxygen-dissociation curve could not achieve the same degree of oxygen release within this range of partial pressures of oxygen. Instead, it would have maximum affinity for oxygen throughout this oxygen pressure range and so would deliver no oxygen to the tissues.

Sigmoidal O_2-dissociation curve





Allosteric effector

→ regulation of O_2 - CO_2 exchange by determain the form of hemog.

- The pH of the environment (the decrease in the pH enhances the release of O_2 from Hb)
- The pCO_2 (increase in pCO_2 enhances the release of O_2 (Bohr effect)
- The availability of 2,3-bisphosphoglycerate. 2,3-BPG binds to the Hb and decreases its oxygen affinity and shifts the oxygen-dissociation curve to the right
- The oxygen-dissociation curve of Hb is shifted to the right to cope long-term with the effects of chronic hypoxia or anemia
- Carbon monoxide (CO) binds tightly (but reversibly) to the hemoglobin iron, forming carbon monoxyhemoglobin, HbCO.

Effect of pH

- Source of the protons that lower the pH:
  - The concentration of both CO_2 and H^+ in the capillaries of metabolically active tissues is higher than that observed in alveolar capillaries of the lungs, where CO_2 is released into the expired air. (on the oppisite)
  - Organic acids, such as lactic acid, are produced during anaerobic metabolism in rapidly contracting muscle (لاهوائي)
  - In the tissues, CO_2 is converted by carbonic anhydrase to carbonic acid:

$$CO_2 + H_2O \rightleftarrows H_2CO_3$$

- which spontaneously loses a proton, becoming bicarbonate (the major blood buffer):

$$H_2CO_3 \rightleftarrows HCO_3^- + H^+$$

- This differential pH gradient (lungs having a higher pH, tissues a lower pH) favors the unloading of oxygen in the peripheral tissues, and the loading of oxygen in the lung which makes hemoglobin a more efficient transporter of oxygen. (فعّال)

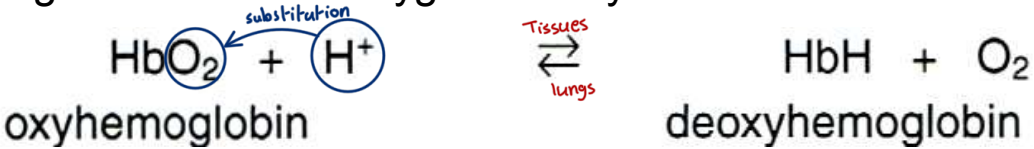
Effect of pH
Mechanism of the Bohr effect

(Co2 gas, Acidity)

- The Bohr effect reflects the fact that the deoxy form of hemoglobin has a greater affinity for protons than does oxyhemoglobin. This effect is caused by ionizable groups, such as the N-terminal α-amino groups, and specific histidine side chains that have higher pKas in deoxyhemoglobin than in oxyhemoglobin.

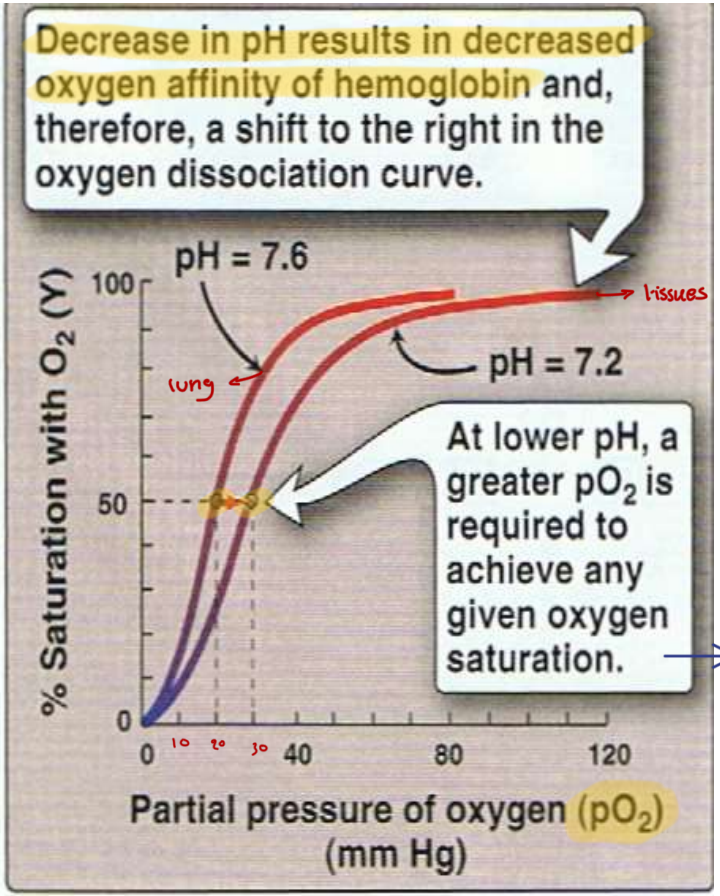
(strong base pKa↑)
شدة قبل استقبال البروتون

- Therefore, an increase in the concentration of protons (resulting in a decrease in pH) causes these groups to become protonated (charged) and able to form ionic bonds (also called salt bridges). These bonds preferentially stabilize the deoxy form of hemoglobin, producing a decrease in oxygen affinity.



- where an increase in protons (or a lower pO2) shifts the equilibrium to the right (favoring deoxyhemoglobin), whereas an increase in pO2 (or a decrease in protons) shifts the equilibrium to the left.

Effect of pH



Hemog (Dex)
Hypoxia

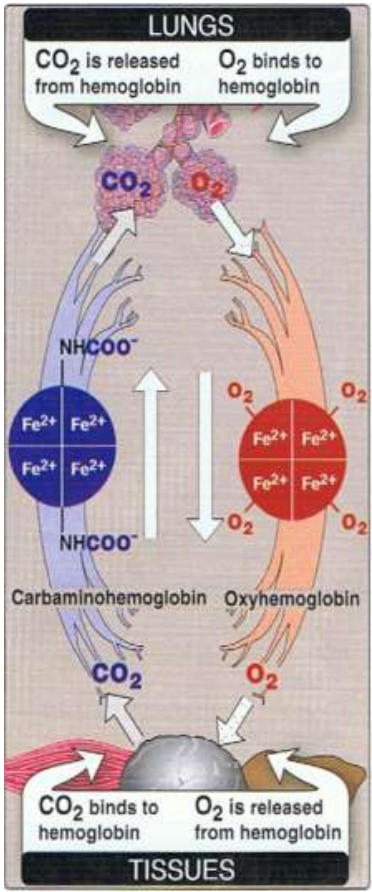
Effect of CO_2

- some CO_2 is carried as carbamate bound to the uncharged a-amino groups of hemoglobin → on N terminal (carbamino-hemoglobin)

$$Hb\text{–}NH_2 + CO_2 \rightleftarrows Hb\text{–}NH\text{–}COO^- + H^+$$

- The binding of CO_2 stabilizes the T (taut) or deoxy form of hemoglobin, resulting in a decrease in its affinity for oxygen.

- In the lungs, CO_2 dissociates from the hemoglobin, and is released in the breath.



Effect of 2,3-bisphosphoglycerate on oxygen affinity

a product of glycoloses processes

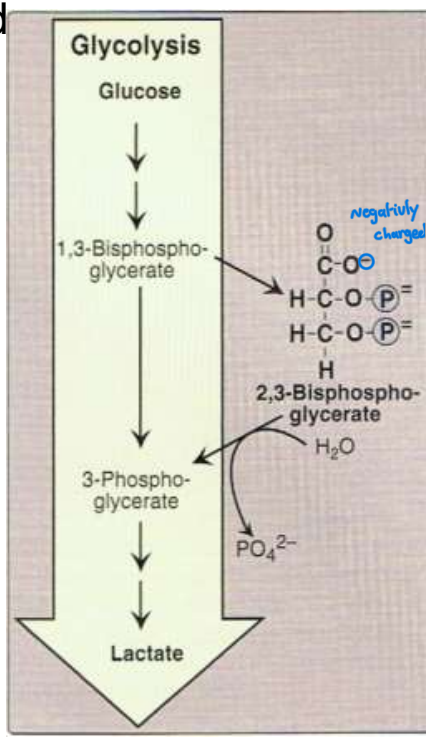
- 2,3- Bisphosphoglycerate (2,3-BPG) is an important regulator of the binding of oxygen to hemoglobin.
- It is the most abundant organic phosphate in the red blood cell, where its concentration is approximately that of hemoglobin.

Function

- 2,3-BPG decreases the oxygen affinity of hemoglobin by binding to deoxyhemoglobin but not to oxyhemoglobin.
- This preferential binding stabilizes the taut conformation of deoxyhemoglobin.

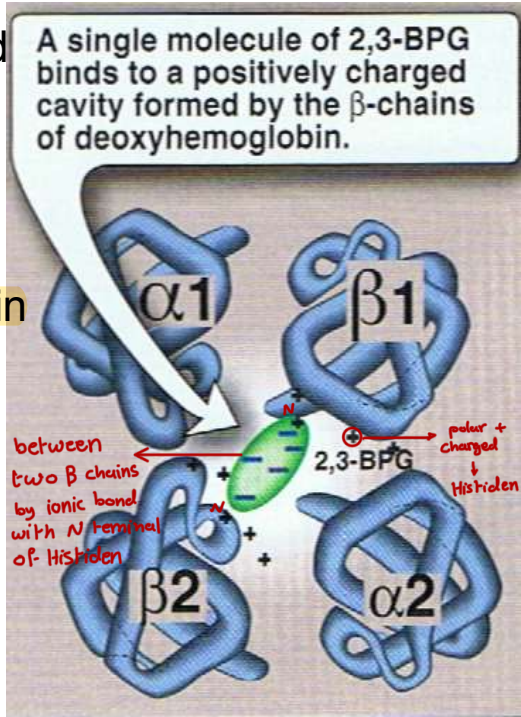
$$HbO_2 + \text{2,3-BPG} \rightleftarrows \text{Hb–2,3-BPG} + O_2$$

oxyhemoglobin — deoxyhemoglobin



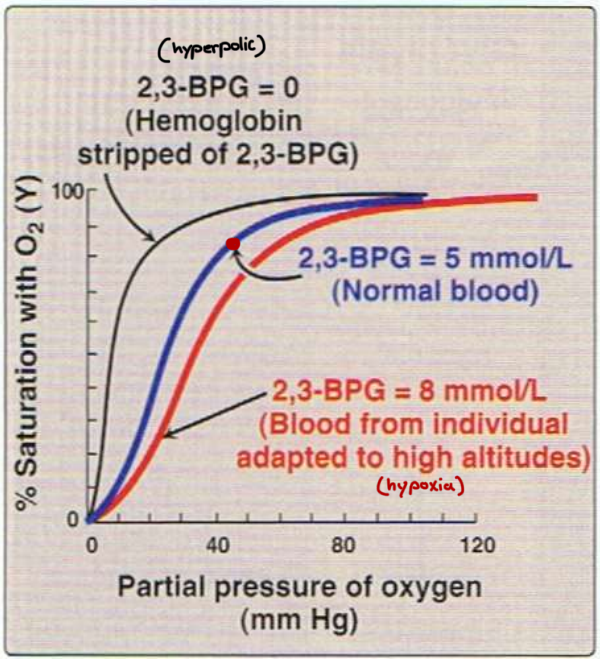
Effect of 2,3-BPG

- One molecule of 2,3-BPG binds to a pocket, formed by the two β-globin chains, in the center of the deoxyhemoglobin tetramer .
- This pocket contains several positively charged amino acids that form ionic bonds with the negatively charged phosphate groups of 2,3-BPG.
- A mutation of one of these residues can result in hemoglobin variants with abnormally high oxygen affinity
- 2,3-BPG is expelled on oxygenation of the hemoglobin.



Effect of 2,3-BPG

- In RBC's, the presence of 2,3- BPG significantly reduces the affinity of hemoglobin for oxygen, shifting the oxygen-dissociation curve to the right.
- This reduced affinity enables hemoglobin to release oxygen efficiently at the partial pressures found in the tissues.
- The concentration of 2,3-BPG in the red blood cell increases in response to chronic hypoxia (obstructive pulmonary emphysema, or at high altitudes), where circulating hemoglobin may have difficulty receiving sufficient oxygen



2,3-BPG in transfused blood

مواد حافظة للدم

- storing blood in acid-citrate-dextrose, leads to a decrease of 2,3-BPG in the red cells. Such blood displays an abnormally high oxygen affinity, and fails to unload its bound oxygen properly in the tissues. Hemoglobin deficient in 2,3-BPG thus acts as an oxygen "trap" rather than as an oxygen transport system.

نحبس

- Transfused red blood cells are able to restore their depleted supplies of 2,3-BPG in 24 to 48 hours. However, severely ill patients may be seriously compromised if transfused with large quantities of such 2,3-BPG-"stripped" blood. The decrease in 2,3-BPG can be prevented by adding substrates such as inosine to the storage medium.

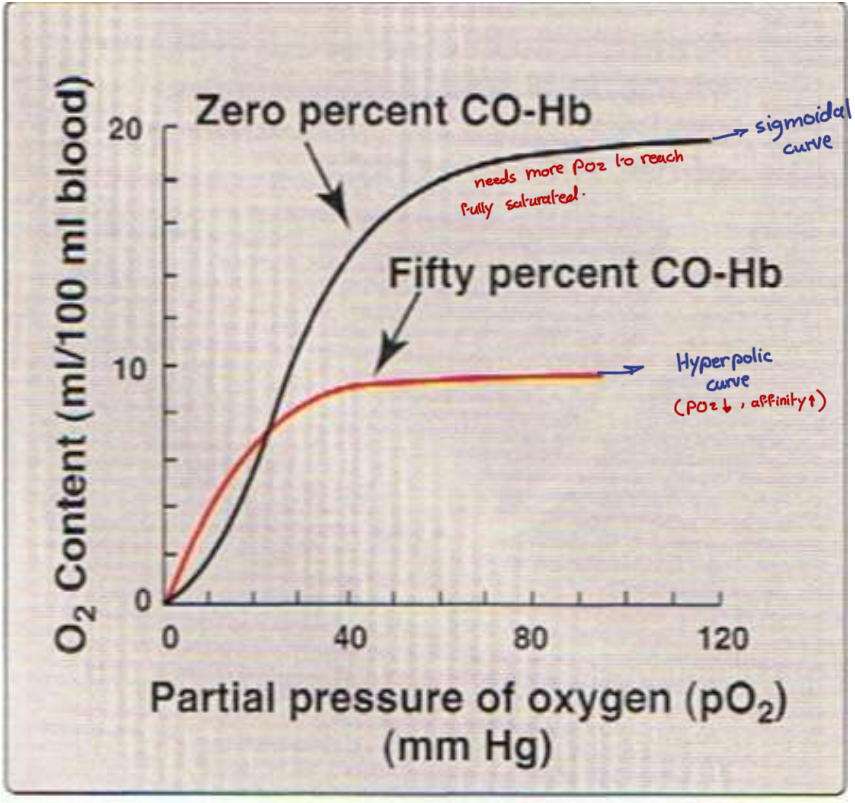
- Inosine, an uncharged molecule, can enter the red blood cell, where its ribose moiety is released, phosphorylated, and enters the hexose monophosphate pathway eventually being converted to 2,3-BPG.

Binding of CO

- Carbon monoxide (CO) binds tightly (but reversibly) to the hemoglobin iron (HbCO) with affinity 220 times more than O_2.
- When carbon monoxide binds to one or more of the four heme sites, hemoglobin shifts to the relaxed conformation, causing the remaining heme sites to bind oxygen with high affinity.
- This shifts the oxygen saturation curve to the left, and changes the normal sigmoidal shape toward a hyperbola makes hemoglobin unable to release oxygen to the tissues
- Carbon monoxide toxicity appears to result from a combination of tissue hypoxia and direct CO-mediated damage at the cellular level which is treated with 100 percent oxygen therapy (facilitates the dissociation of CO from the hemoglobin).

Effect of CO →

Hemog won't reach 100% saturated bc Co bind to O2 place on the Hemog.

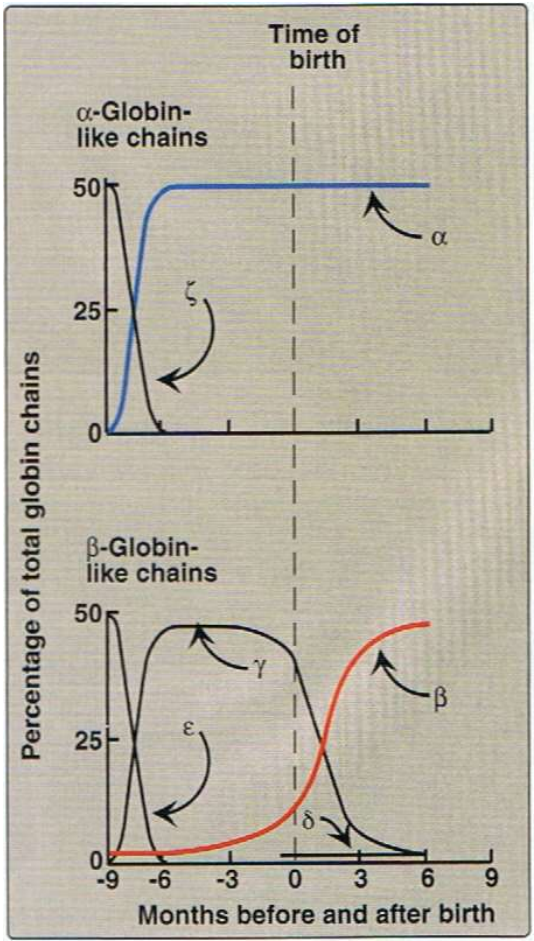


- All of the above is about Hb type A (most abandant in our body)
But we have other type

Minor Hemoglobins

1. HbF: in infants
2. HbA2: After 12 weeks after birth — began to apper
3. HbA1c: under unphysiologic conditions (glycosylated)

Form	Chain composition	Fraction of total hemoglobin
HbA	$\alpha_2\beta_2$	90%
HbF	$\alpha_2\gamma_2$	<2% (new borns)
HbA_2	$\alpha_2\delta_2$	2–5%
HbA_{1c}	$\alpha_2\beta_2$-glucose	3–9%



Fetal hemoglobin (HbF)

$\alpha_2 \gamma_2$ — β like subunit

- HbF is a tetramer consisting of two α chains identical to those found in HbA, plus two gamma (γ) chains ($\alpha_2\gamma_2$). The γ chains are members of the globin gene family
- HbF synthesis during development: In the first few weeks after conception, embryonic hemoglobin composed of two zeta chains and two epsilon chains (ζ2ε2), is synthesized by the embryonic yolk sac.

Gower 1 (3 months)

- Within a few weeks, the fetal liver begins to synthesize HbF in the developing bone marrow. HbF is the major hemoglobin found in the fetus and newborn, accounts for about 60% of the total Hb in the erythrocytes during the last months of fetal life.
- HbA synthesis starts in the bone marrow at about the eighth month of pregnancy and gradually replaces HbF → but it's not the major yet
- Under physiologic conditions, HbF has a higher affinity for oxygen than does HbA, so HbF's binding only weakly to 2,3-BPG.
- In contrast, if both HbA and HbF are stripped of their 2,3-BPG, they then have a similar affinity for oxygen. The higher oxygen affinity of HbF facilitates the transfer of oxygen from the maternal circulation across the placenta to the red blood cells of the fetus.

sigmoidal ← HbA and HbF → Hyperbolic

Hemoglobin A2 (HbA2)

- HbA2 is a minor component of normal adult hemoglobin, first appearing about twelve weeks after birth and, ultimately, constituting about two percent of the total hemoglobin.

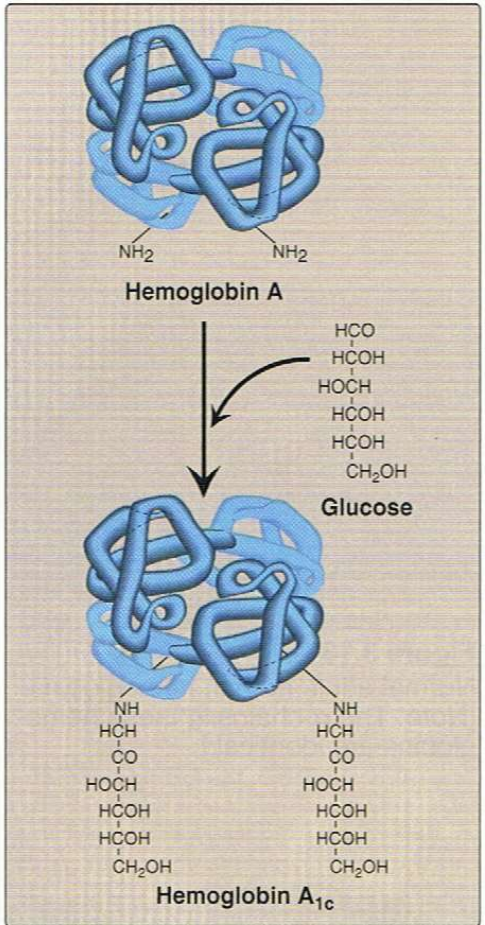
- It is composed of two α-globin chains and two delta- (δ) globin chains (α2δ2)

Hemoglobin A1c

- Under physiologic conditions, HbA is slowly and nonenzymically glycosylated, the extent of glycosylation being dependent on the plasma concentration of a particular hexose. The most abundant form of glycosylated hemoglobin is HbA1.

- It has glucose residues attached predominantly to the NH_2 groups of the N-terminal valines of the β-globin chains

- HbA1c are found in red blood cells of patients with diabetes mellitus

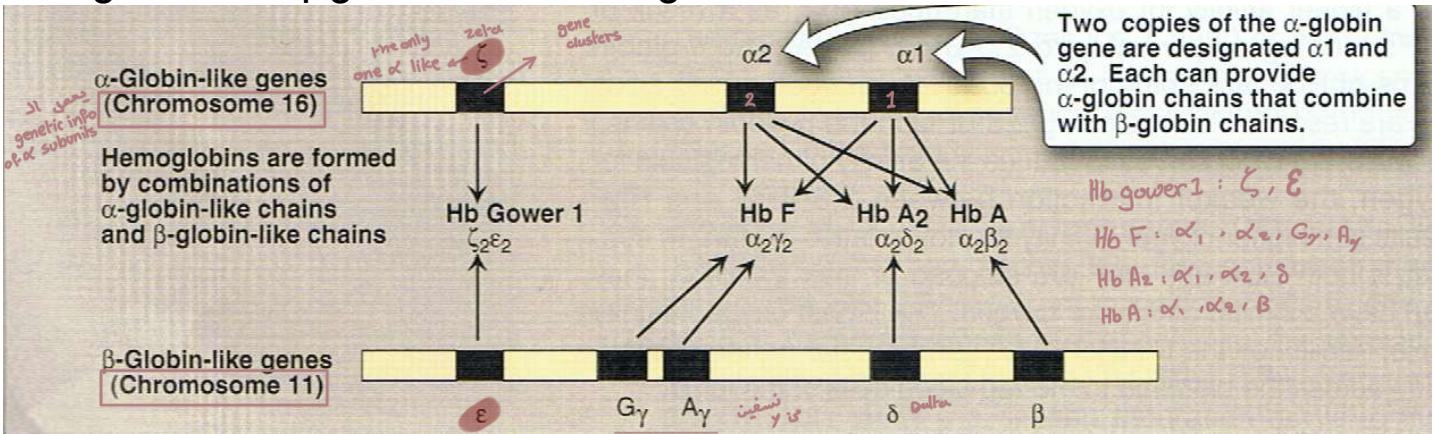


Organization of the globin genes
Gene family

- The genes coding for the α-globin-like and β -globin-like subunits of the hemoglobin chains occur in two separate gene clusters (or families) located on two different chromosomes

- The α -gene cluster on chromosome 16 contains two genes for the α -globin chains. It also contains the zeta (ζ) gene

- A single gene for the β -globin chain is located on chromosome 11. There are an additional four β -globin-like genes: the epsilon (ε) gene, two γ genes and the δ gene



RBC — premature → contain nuclies , in bone narrow
mature → doesn't contain one , out of it

Steps in globin chain synthesis → in bone narrow

- Expression of a globin gene begins in the nucleus of red cell precursors, where the DNA sequence encoding the gene is transcribed.
- The RNA produced by transcription is actually a precursor of the messenger RNA (mRNA) that is used as a template for the synthesis of a globin chain.
- Before it can serve this function, two noncoding stretches of RNA (introns) must be removed from the mRNA precursor sequence, and the remaining three fragments (exons) reattached in a linear manner
- The resulting mature mRNA enters the cytosol, where its genetic information is translated, producing a globin chain

hemoglobinopathies

- Hemoglobinopathies are disorders caused by:
  - a ➢ Production of a structurally abnormal hemoglobin molecule
  - b ➢ Synthesis of insufficient quantities of normal hemoglobin subunits
  - c ➢ or, rarely, both.

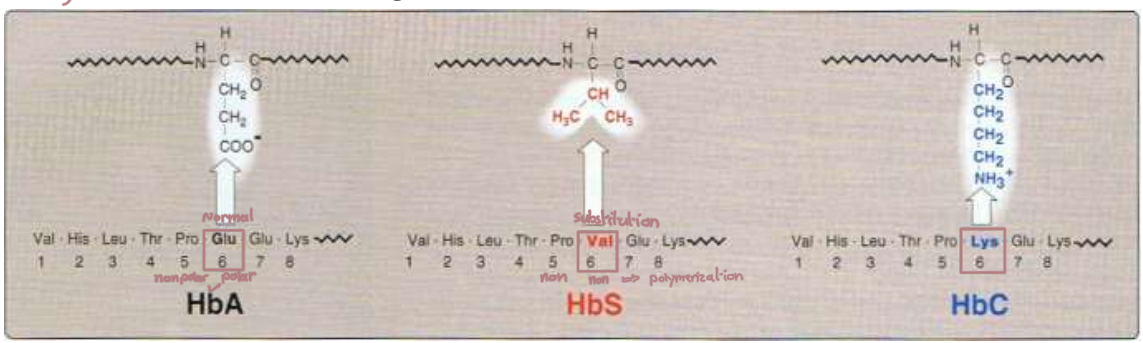
Examples:

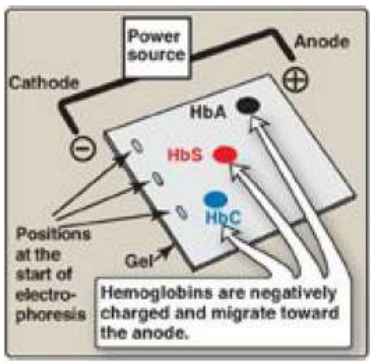
abnormal structure of Hemoy
- ➢ Sickle cell disease (HbS disease) point mutation in β chain (E6V)
- ➢ hemoglobin C disease (HbC disease) mutation in β chain (E6K)

insufficient
- ➢ thalassemia syndromes (α and β thalassemia)

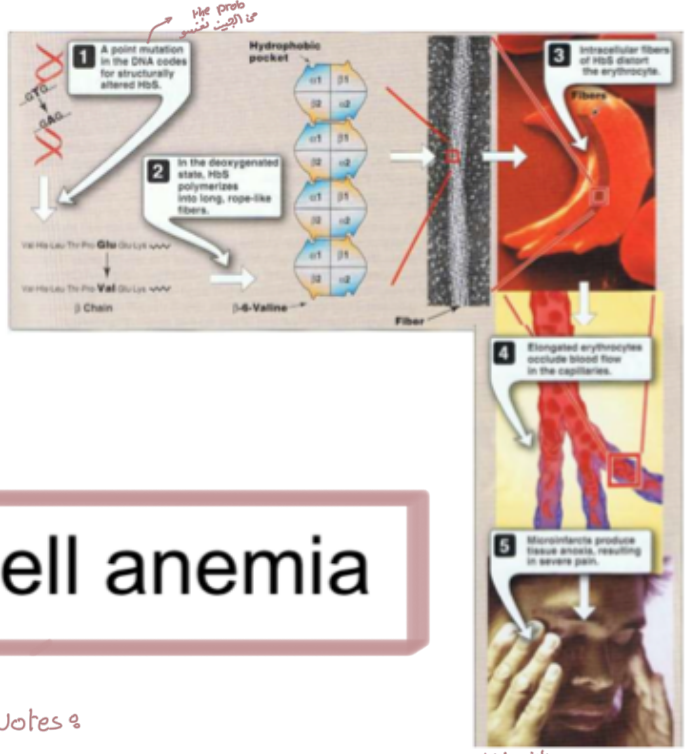
nothing
- ➢ methemoglobinemia

↳ lysine





- The substitution of the nonpolar valine for a charged glutamate residue forms a protrusion on the β-globin (polar → non polar)
- At low oxygen tension, HbS polymerizes inside the red blood cells, first forming a gel, which assembled later to fibrous polymers ; producing rigid, misshapen erythrocytes.
- Sickled cells block the flow of blood in the narrow capillaries leading to localized anoxia (oxygen deprivation) in the tissue, causing pain and eventually death (infarction) of cells in the vicinity of the blockage.



factors increase sickling

- Sickling and the severity of disease is enhanced by:
  - decreased oxygen tension as a result of high altitudes or flying in a nonpressurized plane
  - increased pCO2
  - decreased pH ↓ → Acidity ↑ → CO2 ↑
  - An increased concentration of 2,3-BPG in erythrocytes.

Sickle cell anemia

Treatment

1. Adequate hydration
2. Analgesics (pain killers)
3. Aggressive antibiotic therapy if infection is present
4. transfusions in patients at high risk for fatal vasocculsions. Intermittent transfusions with packed red cells reduce the risk of stroke
5. Hydroxyurea (an anti- tumor drug) decreases the frequency of painful crises and reduces mortality. The mechanism of action is not understood, but it may increase HbF that decreases sickling

high risk

Notes :

1. The high frequency of the HbS gene among black Africans, despite its damaging effects in the homozygous state (two gens are مطفرة)
1. heterozygotes for the sickle cell gene are less susceptible to malaria, caused by the parasite *Plasmodium falciparum*. (just one of the gens — Hemog 120 — Hbs < 120 day)
2. This organism spends an obligatory part of its life cycle in the red blood cell. Because these cells have a shorter life span than normal, the parasite cannot complete the intracellular stage of its development
3. This fact may provide a selective advantage to heterozygotes living in regions where malaria is a major cause of death.

Hemoglobin C disease

- Like HbS, HbC is a hemoglobin variant that has a single amino acid substitution in the sixth position of the β-globin chain
- In this case, however, a lysine is substituted for the glutamate (as compared with a valine substitution in HbS
- Patients homozygous for hemoglobin C generally have a relatively mild, chronic hemolytic anemia. These patients do not suffer from infarctive crises, and no specific therapy is required.

Hemoglobin SC disease

- In this disease, some β-globin chains have the sickle cell mutation, whereas other β-globin chains carry the mutation found in HbC disease
- Patients with HbSC disease have both of their β-globin genes abnormal, although different from each other
- Compared to sickle cell disease, hemoglobin levels tend to be higher in HbSC disease (may be at the low end of the normal range)
- Patients with HbSC disease to remain well (and undiagnosed) until they suffer an infarctive crisis (beginning in childhood)
- This crisis often follows childbirth or surgery and may be fatal.

Methemoglobinemias

- Oxidation of the heme component of hemoglobin to the ferric (Fe^{+3}) state forms methemoglobin, which cannot bind oxygen.
  → only 5 bond (instead of 6) no bond left. of oxygen

- caused by:
  - The action of certain drugs, such as nitrates → vasodilators
  - Endogenous products, such as reactive oxygen intermediates
  - Inherited defects, for example, certain mutations in the α- or β-globin chain promote the formation of methemoglobin (HbM)
  - Deficiency of NADH-cytochrome b_5 reductase which is responsible for the conversion of methemoglobin (Fe^{+3}) to hemoglobin (Fe^{+2}), leads to the accumulation of methemoglobin

- newborns are particularly susceptible to the effects of methemoglobin-producing compounds

- The methemoglobinemias are characterized by "chocolate cyanosis" (a brownish-blue coloration of the skin and membranes) and chocolate colored-blood, as a result of the dark-colored methemoglobin
  Fe^{+3} color ←

- Symptoms are related to tissue hypoxia, and include anxiety, headache, and dyspnea. In rare cases, coma and death can occur.

Thalassemia

- The thalassemias are hereditary hemolytic diseases in which an imbalance occurs in the synthesis of globin chains (either the α- or β-globin is defective)
- As a group, they are the most common single gene disorders in humans
- Normally, synthesis of the α- and β-globin chains are coordinated, so that each α-globin chain has a β-globin chain (formation of α2β2 (HbA)
- A thalassemia can be caused by a variety of mutations, including entire gene deletions, or substitutions or deletions of one to many nucleotides in the DNA.
- It can be classified as either a disorder in which no globin chains are produced (α°- or β°-thalassemia), or one in which some chains are synthesized, but at a reduced rate (α+- or β+-thalassemia)

β-Thalassemias

- In these disorders, synthesis of β -globin chains is decreased or absent, whereas α-globin chain synthesis is normal

- α-Globin chains cannot form stable tetramers which precipitate, causing the premature death of cells initially destined to become mature red blood cells

in blood

- Accumulation of $\alpha_2\gamma_2$ (HbF) and γ_4 (Hb Bart's) also occurs. There are only two copies of the β-globin gene in each cell (one on each chromosome 11)

normal

but we can't find HbA / HbA1C

- Individuals with β-globin gene defects have either β-thalassemia trait (β-thalassemia minor) if they have only one defective β-globin gene, or β-thalassemia major if both genes are defective

حامل للمرض فقط

β-Thalassemias

- Because the β-globin gene is not expressed until late in fetal gestation, the physical manifestations of β-thalassemias appear only after birth. Those individuals with β-thalassemia minor make some β-chains, and usually do not require specific treatment

- Infants born with thalassemia major have the sad fate of being seemingly healthy at birth, but becoming severely anemic during the first or second year of life (require regular transfusions of blood)

- Although this treatment is lifesaving, the cumulative effect of the transfusions is iron overload (a syndrome known as hemosiderosis), which typically causes death between the ages of 15 and 25 years

- The increasing use of bone marrow replacement therapy has been a boon to these patients

α-Thalassemia

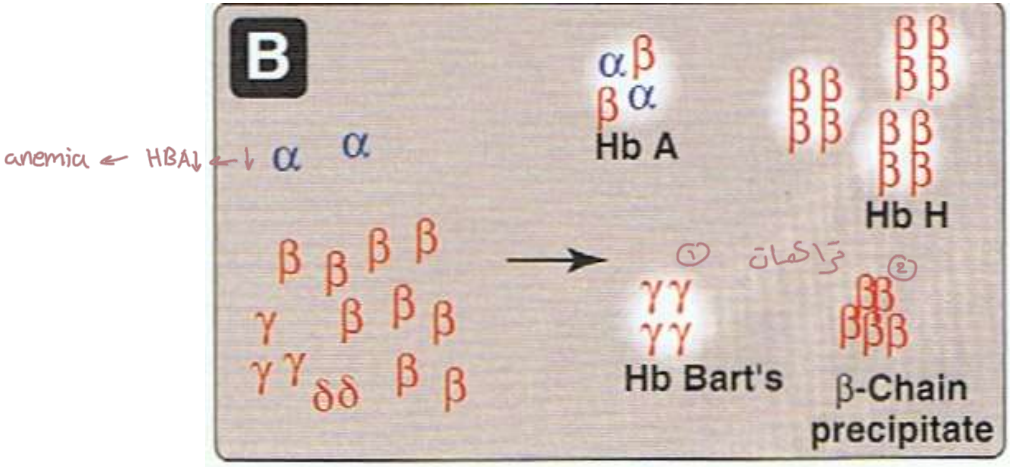
- These are defects in which the synthesis of α-globin chains is decreased or absent.

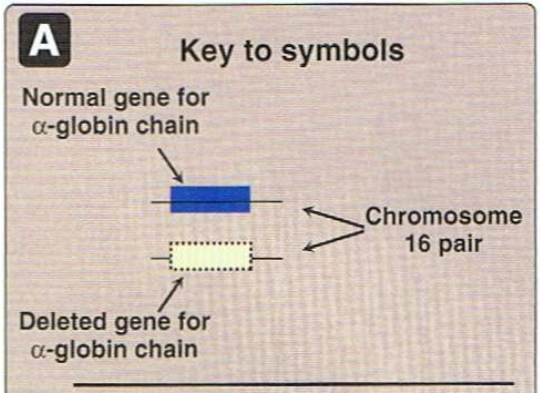
- Because each individual's genome contains four copies of the α-globin gene (two on each chromosome 16), there are several levels of α-globin chain deficiencies:
  - If one is defective, the individual is termed a silent carrier of α-thalassemia (no physical manifestations of the disease)
  - If two are defective, the individual is designated as having α-thalassemia trait
  - If three α-globin genes are defective, the individual has hemoglobin H (HbH) disease, a mildly to moderately severe hemolytic anemia

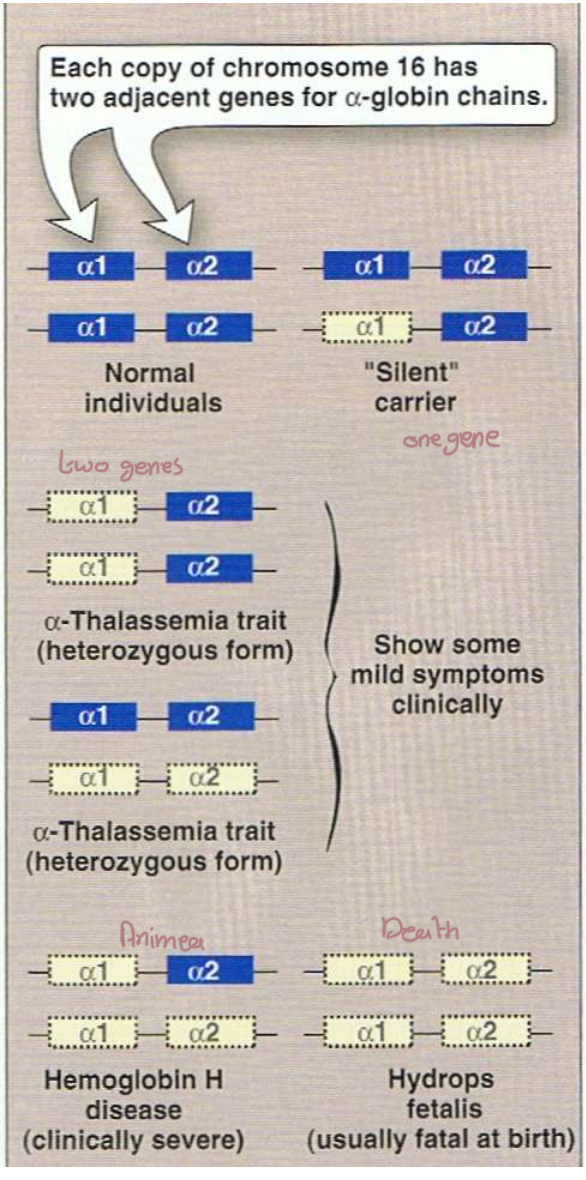
- The synthesis of unaffected γ- and then β-globin chains continues, resulting in the accumulation of γ tetramers in the newborn (γ_4, Hb Bart's) or β tetramers (β_4, HbH)

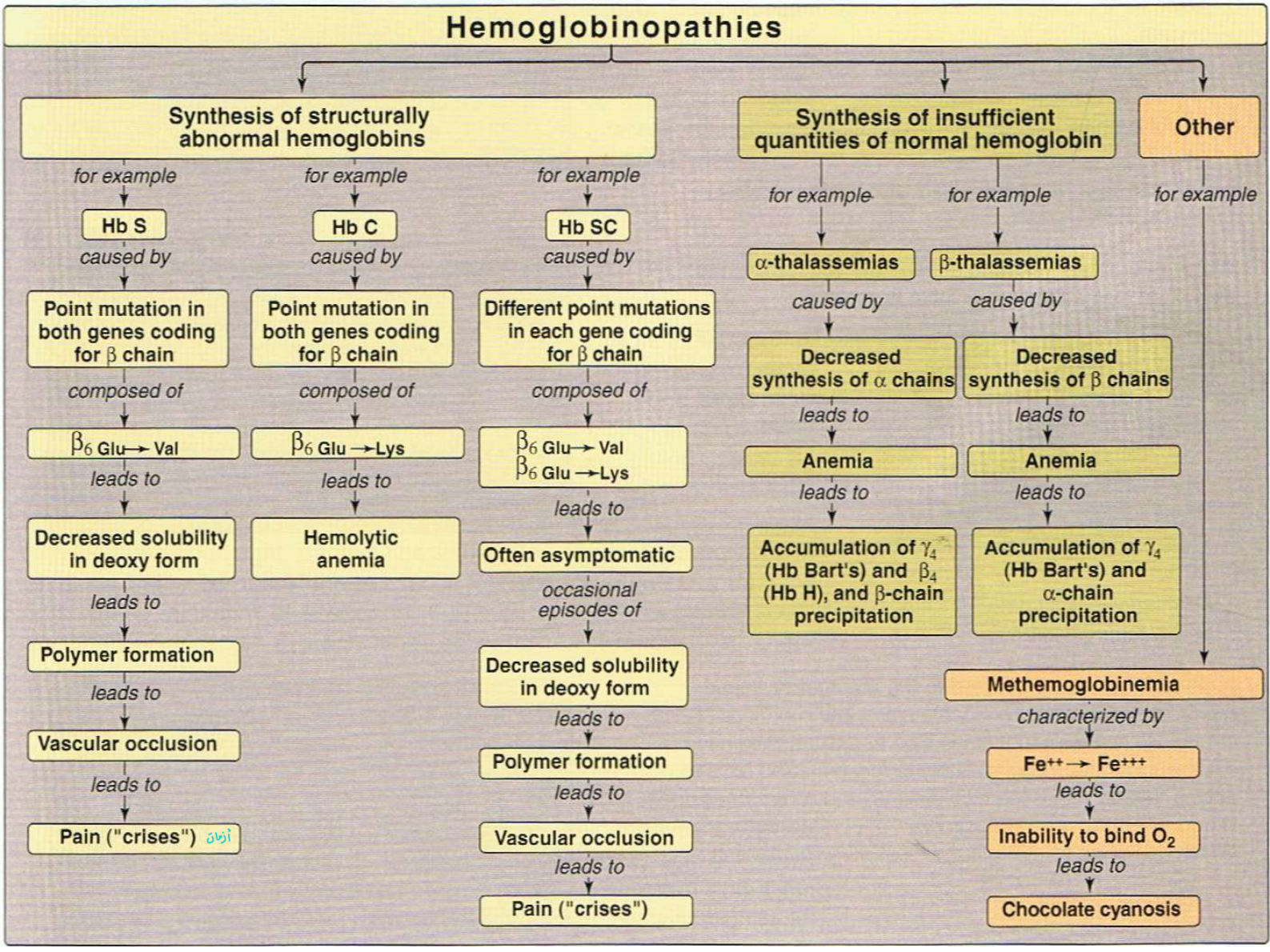
α-Thalassemia

- Although these tetramers are fairly soluble, the subunits show no heme-heme interaction.
- Their oxygen dissociation curves are almost hyperbolic, indicating that these tetramers have very high oxygen affinities (useless as oxygen delivers to the tissues)
- If all four α -globin genes are defective, fetal death result, because α-globin chains are required for the synthesis of HbF.




A
Key to symbols
Normal gene for α-globin chain
Chromosome 16 pair
Deleted gene for α-globin chain


Each copy of chromosome 16 has two adjacent genes for α-globin chains.
α1 α2
α1 α2
Normal individuals
α1 α2
α1 α2
"Silent" carrier
one gene
two genes
α1 α2
α1 α2
α-Thalassemia trait (heterozygous form)
Show some mild symptoms clinically
α1 α2
α1 α2
α-Thalassemia trait (heterozygous form)
Primera
α1 α2
α1 α2
Hemoglobin H disease (clinically severe)
Death
α1 α2
α1 α2
Hydrops fetalis (usually fatal at birth)


Hemoglobinopathies
Synthesis of structurally abnormal hemoglobins
Synthesis of insufficient quantities of normal hemoglobin
Other
for example
Hb S
caused by
Point mutation in both genes coding for β chain
composed of
β6 Glu→ Val
leads to
Decreased solubility in deoxy form
leads to
Polymer formation
leads to
Vascular occlusion
leads to
Pain ("crises")
for example
Hb C
caused by
Point mutation in both genes coding for β chain
composed of
β6 Glu →Lys
leads to
Hemolytic anemia
for example
Hb SC
caused by
Different point mutations in each gene coding for β chain
composed of
β6 Glu→ Val
β6 Glu →Lys
leads to
Often asymptomatic
occasional episodes of
Decreased solubility in deoxy form
leads to
Polymer formation
leads to
Vascular occlusion
leads to
Pain ("crises")
for example
α-thalassemias
caused by
Decreased synthesis of α chains
leads to
Anemia
leads to
Accumulation of γ4 (Hb Bart's) and β4 (Hb H), and β-chain precipitation
for example
β-thalassemias
caused by
Decreased synthesis of β chains
leads to
Anemia
leads to
Accumulation of γ4 (Hb Bart's) and α-chain precipitation
for example
Methemoglobinemia
characterized by
Fe++ → Fe+++
leads to
Inability to bind O2
leads to
Chocolate cyanosis

Quick Mnemonics

- **Sickle Cell: S = Substitution of Valine (E6V)**
- **C disease: C = Change to Lysine**
- **SC disease = S + C mutations**
- **β-Thalassemia = β missing → α excess → anemia**
- **α-Thalassemia = more genes missing = more severe**

collagen

elastic

(gives shape)

Structural proteins

Fibrous proteins

ليفيّ

→ no Folding

Just H bond

للتثبيت

Fibrous proteins

- Serve structural function in the body
- Collagen is a component of skin, connective tissue, blood vessel wall, sclera and cornea of the eye.

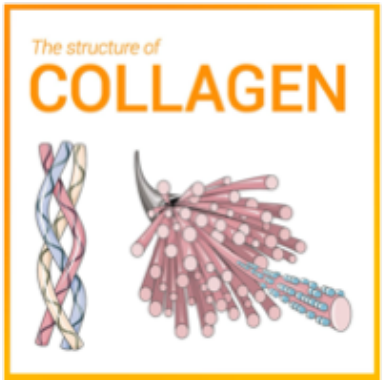
بتعصى لأي ضغط ؟

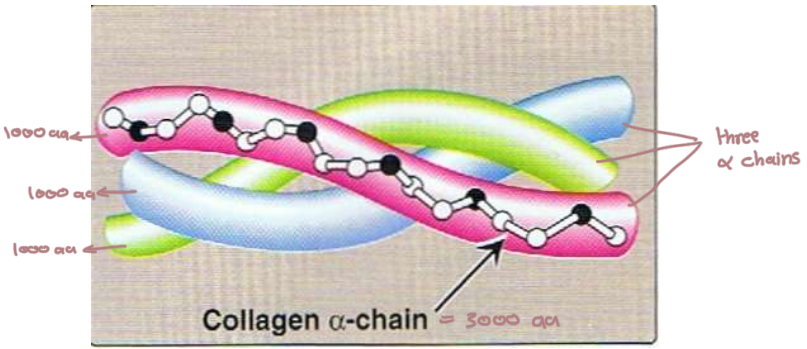
- Exhibit special mechanical properties, resulting from its unique structure, which are obtained by combining specific amino acids into regular, secondary structural elements
- Collagen and elastin are examples

Collagen

- Has long rigid structure with three α-chains wound around each other in a triple helix (1000 aa each)

- Their types and organization depend on the tissue:
  - May be dispersed as a gel to give support to the structure as in vetreous humer of the eye
  - May be bundled in tight parallel fibers that provide strength as in tendons
  - Collagen of bone occurs as fibers arranged at an angle to each other so as to resist mechanical shear from any direction





Types of Collagen

- Collagen can be organized into three types depending on their locations and functions.

1. Fibril-forming collagen: type I, II and III have rope-like structure

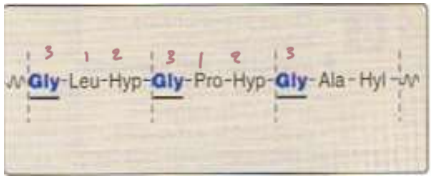
2. Network forming collagen: type IV and VII form a three dimensional mesh that constitute a major part of basement membrane

3. Fibril associated collagen: type IX and XII bind to the surface of collagen fibril

TYPE	TISSUE DISTRIBUTION
	Fibril-forming
I	Skin, bone, tendon, blood vessels, cornea
II	Cartilage, intervertebral disk, vitreous body
III	Blood vessels, fetal skin
	Network-forming
IV	Basement membrane
VII	Beneath stratified squamous epithelia
	Fibril-associated
IX	Cartilage
XII	Tendon, ligaments, some other tissues

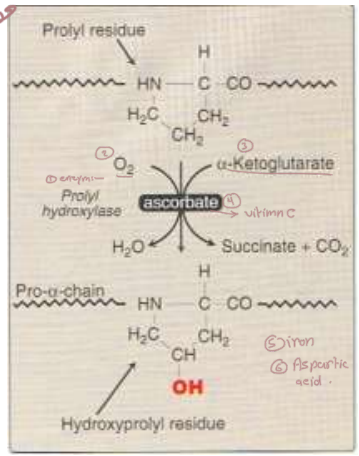
Structure of Collagen

- Amino acid sequence: it is rich in proline and glycine. Glycine is present in every third position

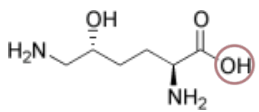


- Triple helical structure: elongated, triple helical structure

- Hydroxyproline and hydroxylysine: come from the hydroxylation of proline and lysine residues (posttranslational modification) necessary for the stabilization of the triple-helical structure



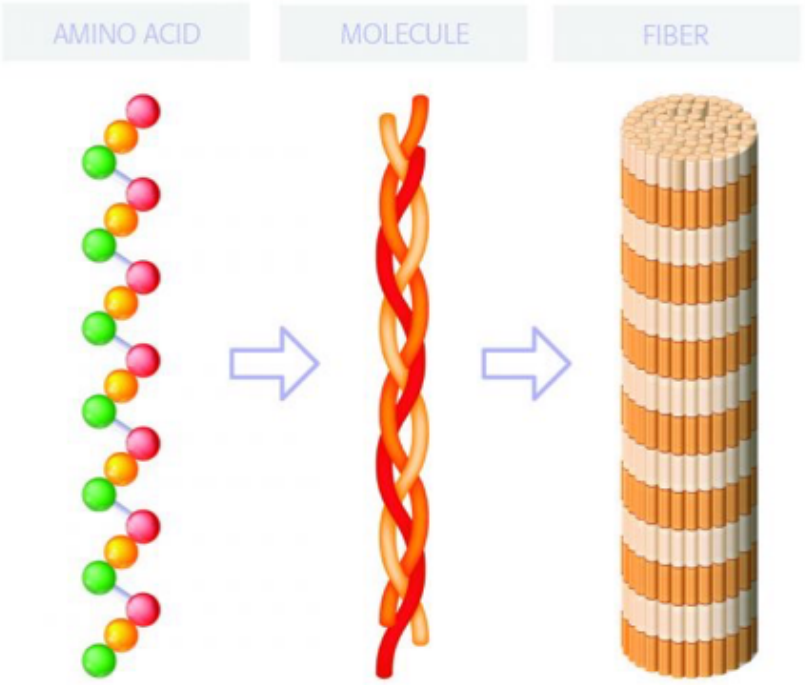
- Glycosylation: enzymatic glycosylation of the hydroxyl group of hydroxylysine. Mainly by glucose and galactose.



hydroxylysine

What Is Collagen?

The Structure Of Collagen



Triple helical structure: elongated, triple helical structure

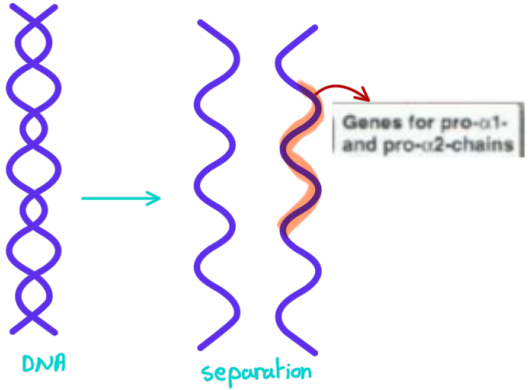
Biosynthesis of collagen

بتجمعو وبجوتو مع الخلية ←

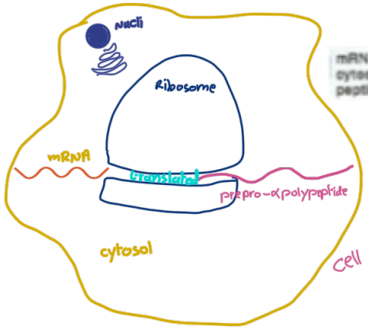
- Precursors of collagen are formed in fibroplast, secreted into the extracellular matrix after enzymatic modification, the mature collagen monomers aggregate and become crosslinked to form collagen fibrils

→ in connective tissue

1. Formation of pro-α-chain ✓
2. Hydroxylation : performed by prolyl hydroxylase and lysyl hydroxylase, requires molecular oxygen and vitamin C. → needs 6 things مهمة
3. Glycosylation ✓
4. Assembly and secretion ✓
5. Extracellular cleavage of procollagen molecule
6. Formation of collagen fibrils
7. Cross-link formation (bundle of collagen fibers)

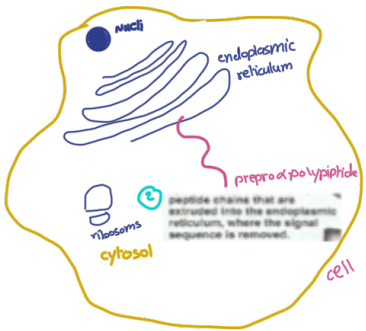


are transcribed into mRNAs.

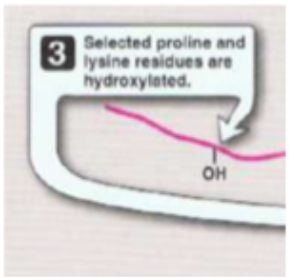


mRNA is translated in the cytosol into prepro-α polypeptide chains that are

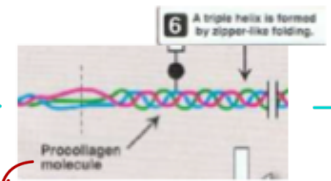
①

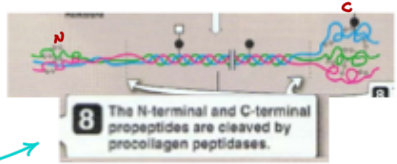


② peptide chains that are extruded into the endoplasmic reticulum, where the signal sequence is removed.



three of these assemble together



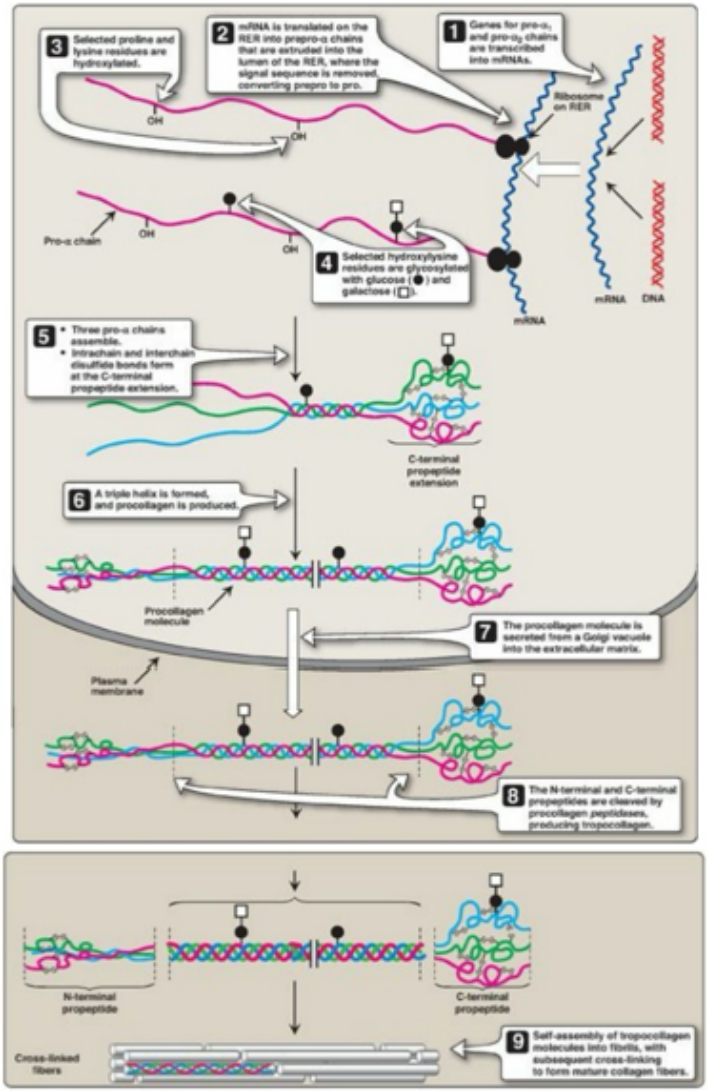


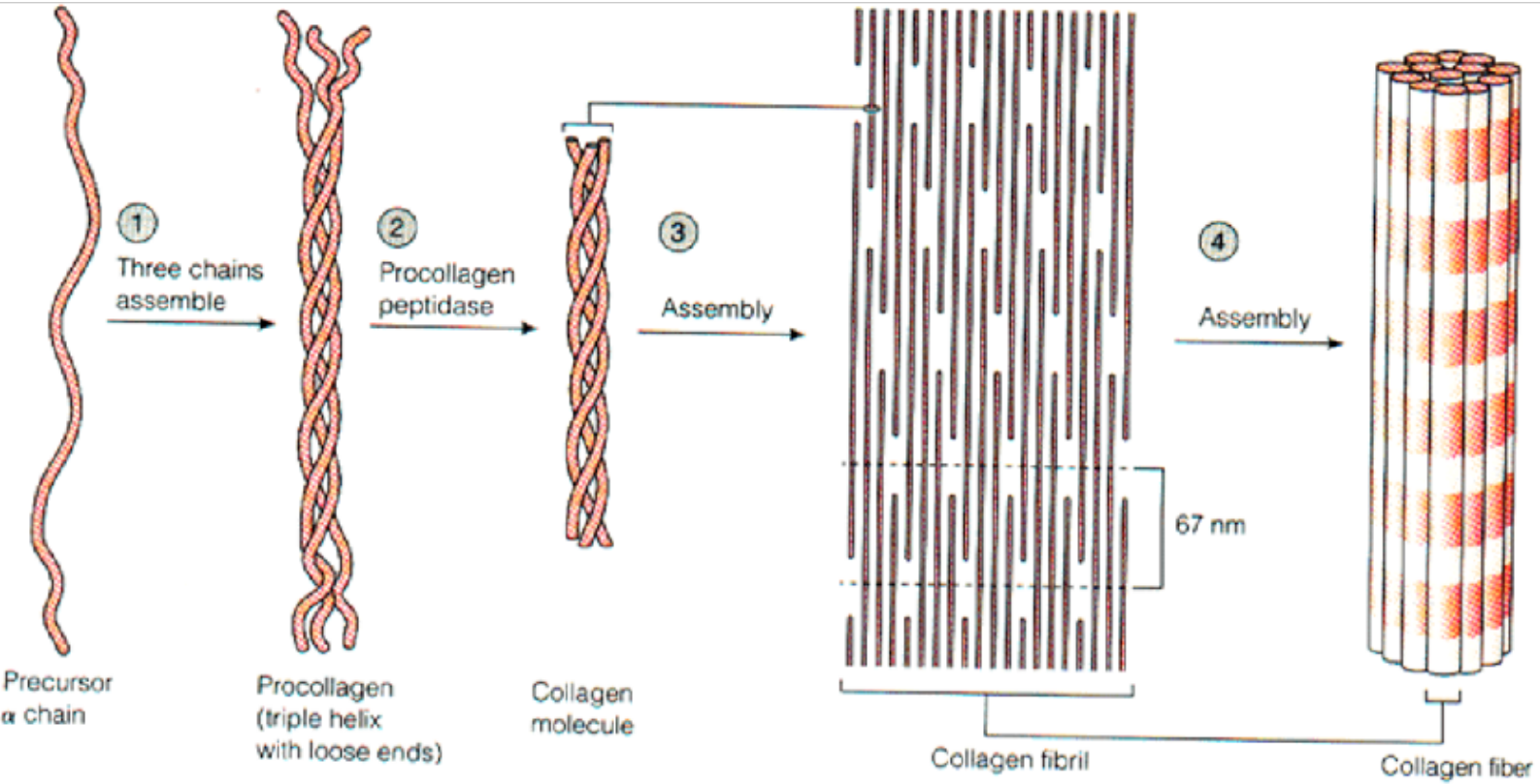
The result

⑤ Intrachain and interchain disulfide bonds form at the C-terminal propeptide extension.

7 The procollagen molecule is secreted from a Golgi vacuole into the extracellular matrix.

Finally


1 Genes for pro-α1 and pro-α2 chains are transcribed into mRNAs.
2 mRNA is translated on the RER into prepro-α chains that are extruded into the lumen of the RER, where the signal sequence is removed, converting prepro to pro.
3 Selected proline and lysine residues are hydroxylated.
4 Selected hydroxylysine residues are glycosylated with glucose (●) and galactose (□).
5 • Three pro-α chains assemble. • Intrachain and interchain disulfide bonds form at the C-terminal propeptide extension.
6 A triple helix is formed, and procollagen is produced.
7 The procollagen molecule is secreted from a Golgi vacuole into the extracellular matrix.
8 The N-terminal and C-terminal propeptides are cleaved by procollagen peptidases, producing tropocollagen.
9 Self-assembly of tropocollagen molecules into fibrils, with subsequent cross-linking to form mature collagen fibers.
Ribosome on RER
mRNA
DNA
Pro-α chain
OH
C-terminal propeptide extension
Procollagen molecule
Plasma membrane
N-terminal propeptide
C-terminal propeptide
Cross-linked fibers


1
Three chains assemble
2
Procollagen peptidase
3
Assembly
4
Assembly
67 nm
Precursor α chain
Procollagen (triple helix with loose ends)
Collagen molecule
Collagen fibril
Collagen fiber

Degradation of Collagen

break down

- Normal collagen are highly stable molecules
- As response to growth or injury, the breakdown of collagen is mainly due to collagenase
- For type I collagen the cleavage is specific, generating three-quarter and one quarter

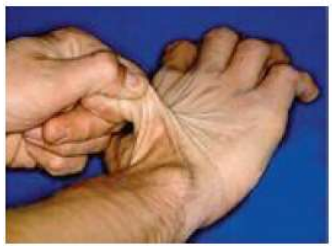
α_1 chain → $\frac{3}{4}$

α_2 chain → $\frac{1}{4}$

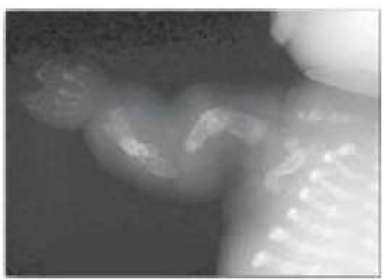
- Further degradation to amino acids occurs by other matrix proteinases

Collagen diseases

- Ehlers- Danlos syndrome
  - Results from a defficiency in lysyl hydroxylase or procollagen peptidase enzymes or amino acid mutation of collagen I, III or V
  - In collagen III mutation (present in arteries), collagen is not secreted so lethal vascular problems occur, in addition to stretchy skin and loose joints

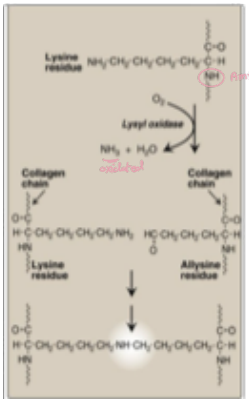


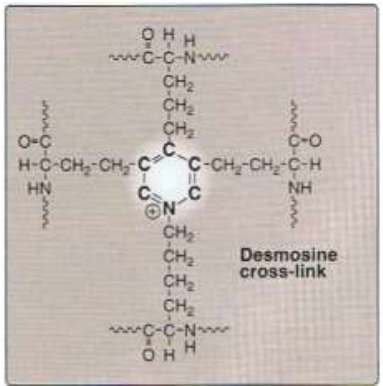
- Osteogenesis imperfecta
  - Inherited disorder, characterized by bones that easily bend and fracture
  - Humped back is a common feature of the disease
  - There are two types:
    - Osteogenesis imperfecta tarda: early infancy with fractures secondary to minor trauma
    - Osteogenesis imperfecta congenita: more severe, pateints die before birth.

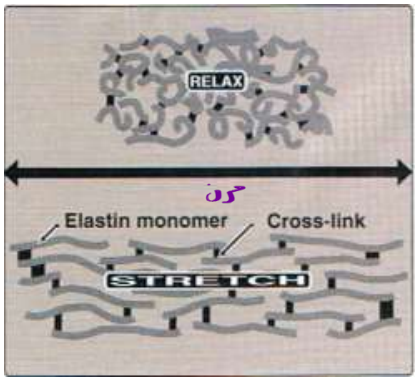


Elastin

- Is a connective tissue protein with rubber like properties which can stretch and bend in any direction when stressed.
- Found in lung, walls of large arteries and elastic legaments
- Structure of elastin
  - Protein polymer synthesized from a precursor (tropoelastin)
  - 700 aa of small, nonpolar aa, rich in proline and lysine
  - Secreted and deposited onto fibrillin bunch of elastine
  - Oxidative deamination of lysine by lysine oxidase produces allysine which forms the desmosine cross-link gives elasticity

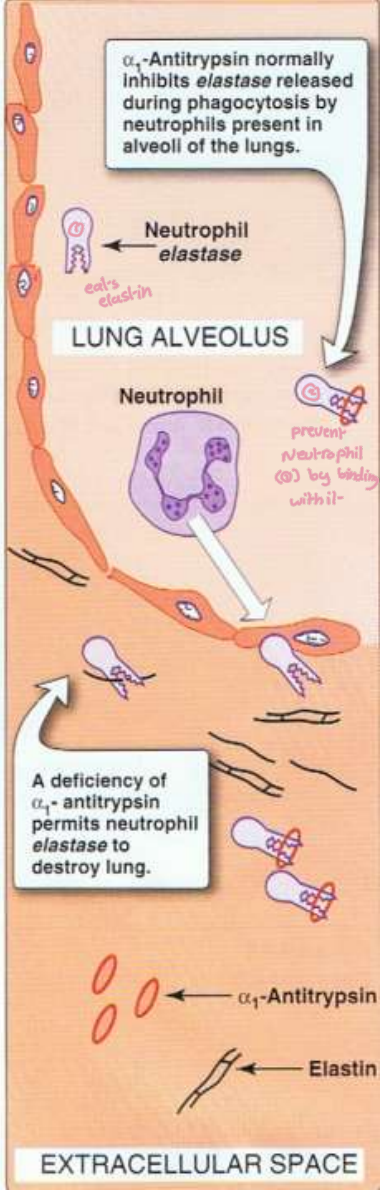






Elastin

- Role of α1 antitrypsine in elastin degradation
  - Produced by liver and other tissues as monocytes and alveolar macrophages
  - Inhibit no. of the proteolytic enzymes including trypsin and neutrophil elastase so prevents elasin degradation in the alveoli
- α1 antitrypsine defficiency
  - In the alveoli: elastase released by activated and degenerating neutrophils is normally inhibited by α1 antitrypsin



α1 antitrypsine deficiency

most common

- different mutations are known, but one single purine base mutation (GAG –AAG) resulting in the substitution of lysine for glutamic acid at position 342 of the protein is clinically the most widespread

- An individual must inherit two abnormal α1-AT alleles to be at risk for the development of emphysema. In a heterozygote, lung produces α1-AT sufficient to protect the alveoli from damage

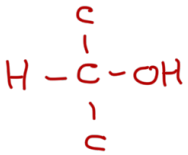
- A specific α1-AT methionine is required for the binding of the inhibitor to its target proteases.
- Smoking causes the oxidation and inactivation of that methionine residue, rendering the inhibitor powerless to neutralize elastase.

  - Smokers with α1-AT deficiency, therefore, have a considerably elevated rate of lung destruction and a poorer survival rate than nonsmokers with the deficiency
  - The deficiency of elastase inhibitor can be reversed by weekly intravenous administration of α1-AT

Carbohydrates

General characteristics

- The term came from the hydrate (H_2O) of carbon (C)



- It has the general formula $(CH_2O)_n$ → no. C
- The most abundant compounds found in nature
- Used as source of energy and energy storage
- Can be converted into fats and proteins
- Important in the formation of genes, vitamins and drugs
- Participate in biological transport
  - transporter (ex: ion channles) : involves suger in il-'s strucrel

Classification of carbohydrates

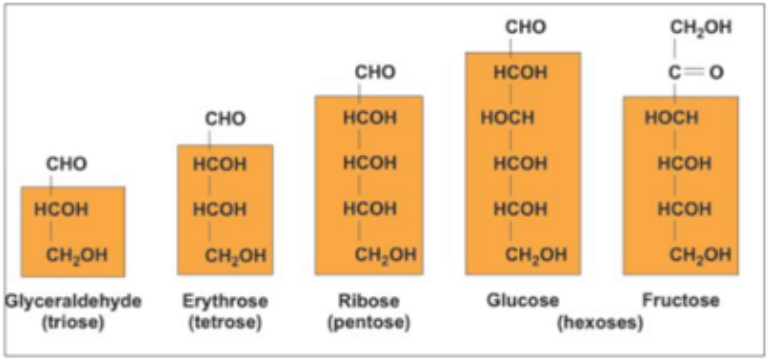
- Monosaccharides:
- Disaccharides
- Oligosaccharides:
- Polysaccharides or glycans

Classification of carbohydrates

1

- Monosaccharides: (one suger unit.)
  - Trioses: glyceraldehyde. (3C)
  - Tetroses: erythrose. (4C)
  - Pentoses: ribose. (5C) → RNA
  - Hexoses: glucose, galactose, mannose, fructose. (6C)

Classification (according to carbons No.)



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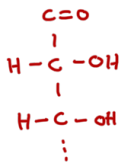
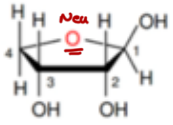
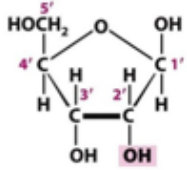
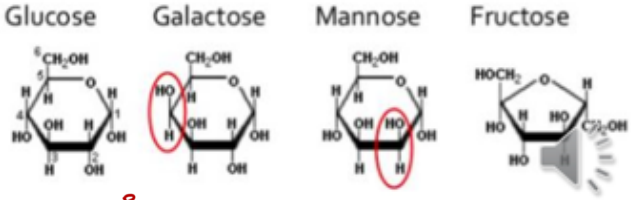


in solutions →





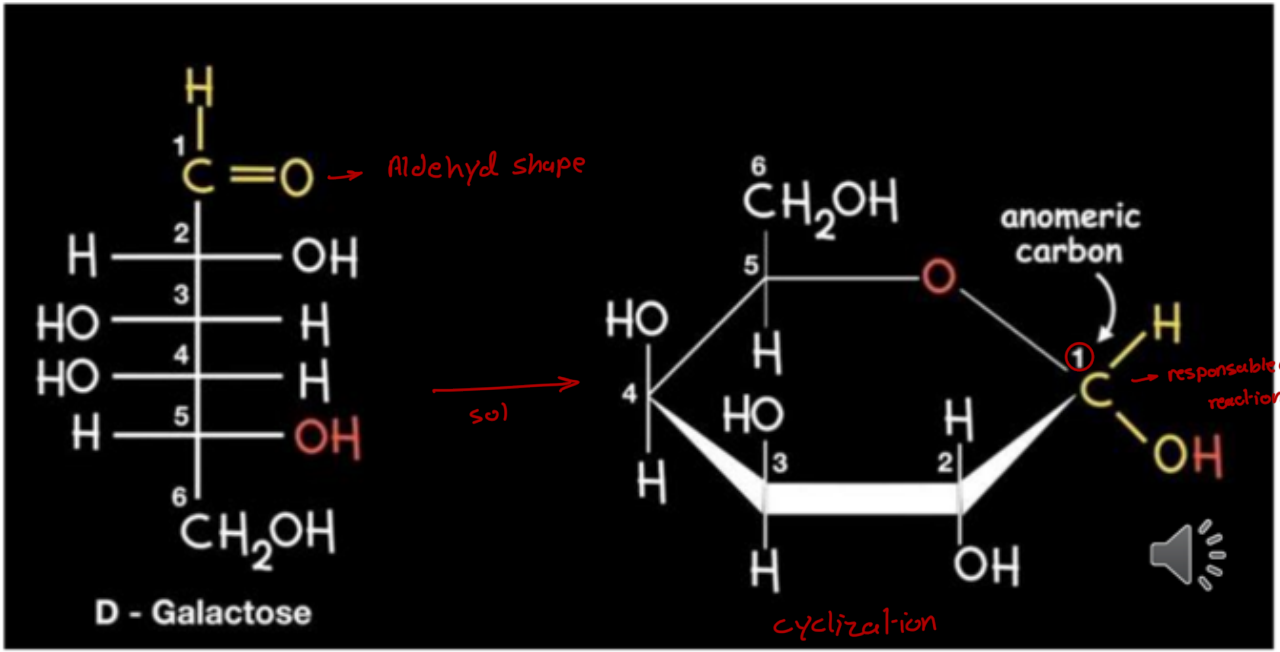


- Monosaccharide are either aldose or ketose

(according to suger type) — A: C=O (on the first carbon) — B: C=O (middle)

Monosaccharides

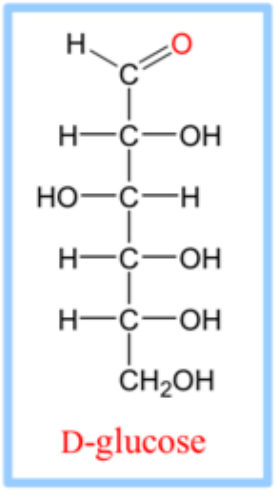
- Numbering starts from anomeric carbon



Monosaccharides

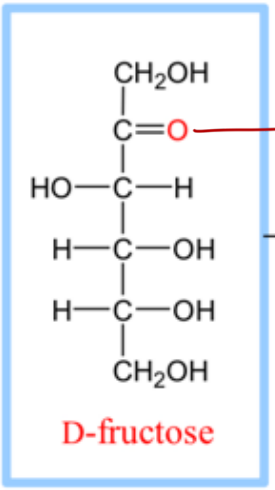
- Either aldose or ketose

Aldose



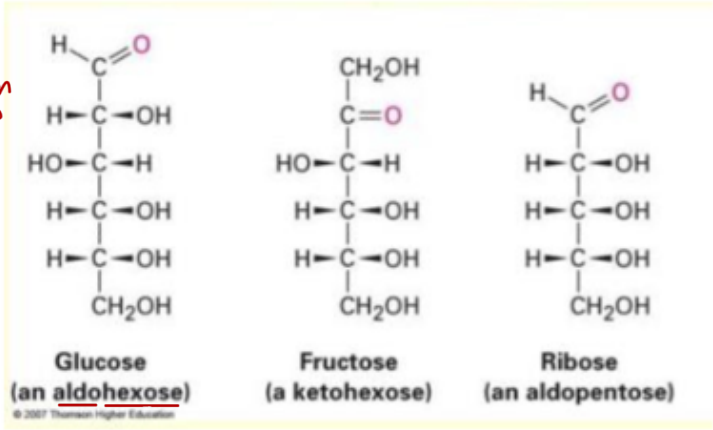
D-glucose

Ketose



D-fructose

keton shap



Classification of carbohydrates

2

- Disaccharides: 2 monosaccharides covalently linked (e.g. Sucrose, maltose, lactose)

3

- Oligosaccharides: (3–10) sugar units
  - Tri, tetra, penta up to 9 or 10 units covalently linked

4

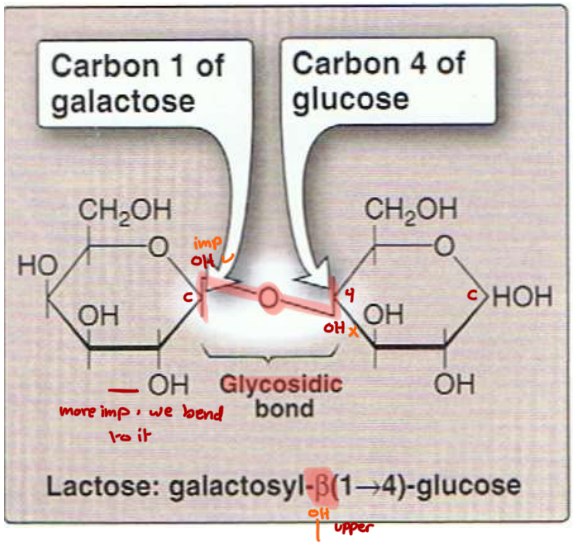
- Polysaccharides or glycans
  - a. Simple polysaccharides (starch, glycogen, amylopectin) → All the units are the same mono sugar
  - b. Complex carbohydrates (nucleic acid, glycoproteins, glycolipids, ...etc)
    → (diff. sugars)

Glycosidic bond

(covalent)

- For di- and polysaccharides
- Can form O- or N-glycosidic bond

N terminal of aa bond with OH group of sugar



Disaccharides

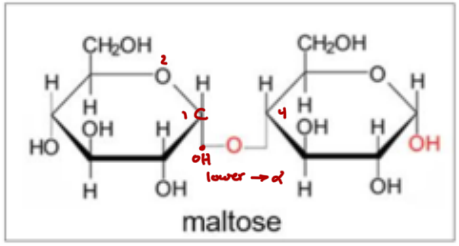
* ex :

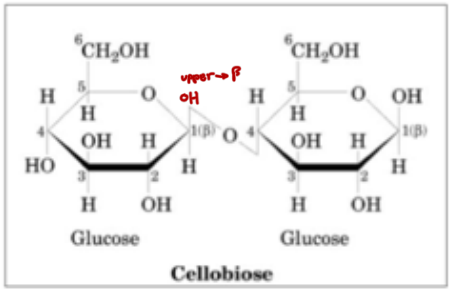
- **Maltose**: is a disaccharide with an $\alpha(1 \rightarrow 4)$ glycosidic link between C1 - C4 OH of 2 glucoses.

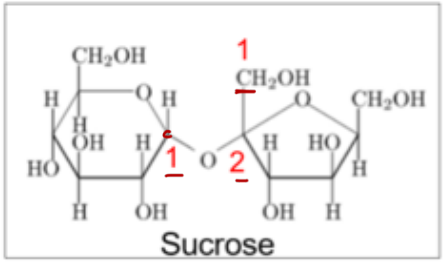
- **Cellobiose**: is the otherwise equivalent β anomer (O on C1 points up) linked by $\beta(1 \rightarrow 4)$ glycosidic linkage

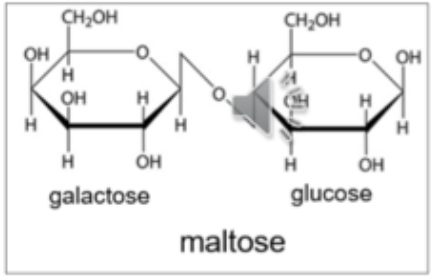
- **Sucrose**, common table sugar, has a glycosidic bond linking the anomeric hydroxyls of glucose & fructose. the linkage is $\alpha(1 \rightarrow 2)$

- **Lactose**, milk sugar, is composed of galactose & glucose, with $\beta(1 \rightarrow 4)$ linkage from the anomeric OH of galactose.



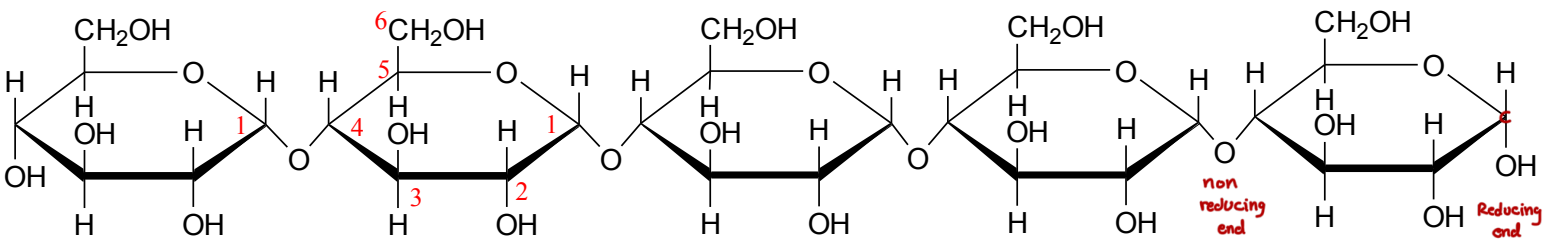






Polysaccharides

- **Plants** store glucose as **amylose** or **amylopectin**, glucose polymers collectively called starch.
- Glucose storage in **polymeric** form **minimizes osmotic effects**.
- **Amylose** is a glucose polymer with **α(1→4)** linkages.
- The end of the polysaccharide with an anomeric C1 not involved in a glycosidic bond is called the **reducing end**.



amylose

Sugar isomers

All sugers have same chemical formula

#1

- Compounds with the same chemical formula are called isomers.

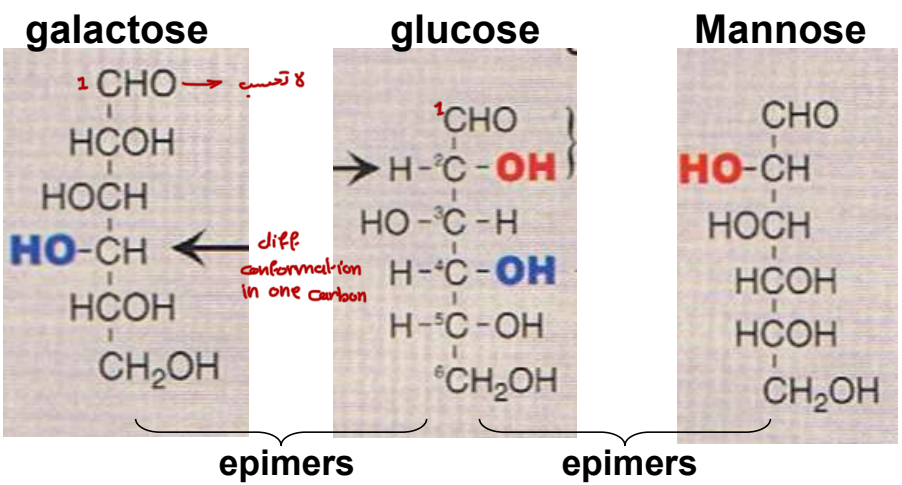
#2

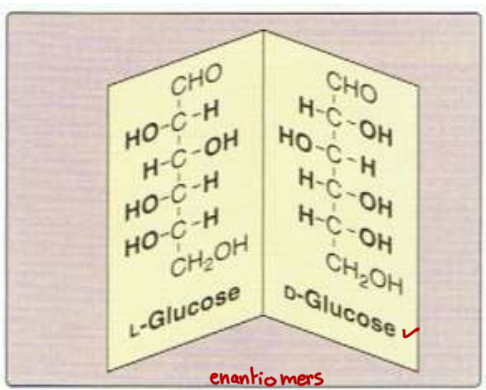
$(CH_2O)_n$

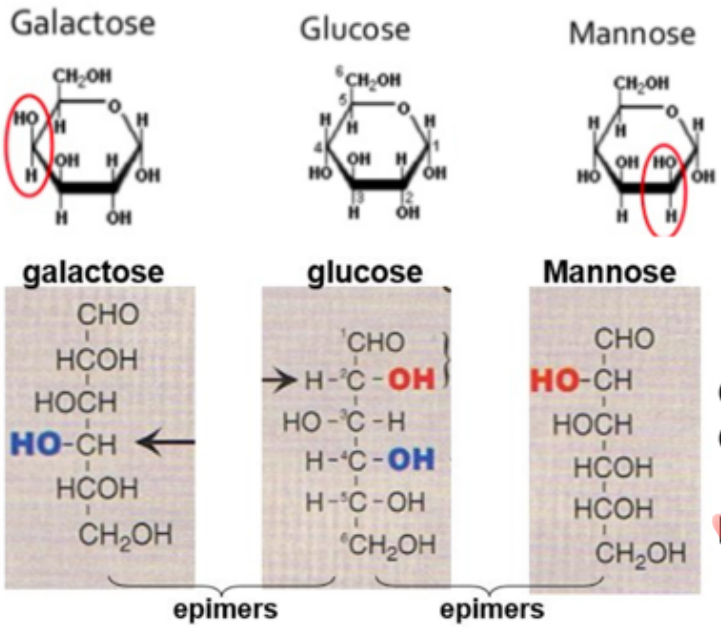
- **Epimers**: If two monosaccharide isomers differ in configuration around one specific carbon atom (with the exception of the carbonyl carbon), they are defined as epimers of each other.

#3

- If a pair of sugars are mirror images of each other (enantiomers), the two members of the pair are designated as D- and L-sugars.

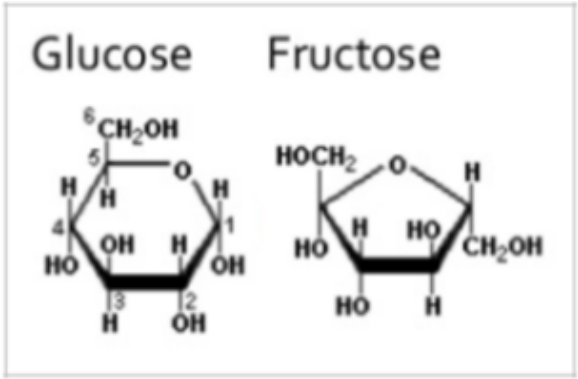






Glucose and galactose are C4 -epimers.
Glucose and mannose are C2-epimers.

Mannose and Galactose are not epimers.



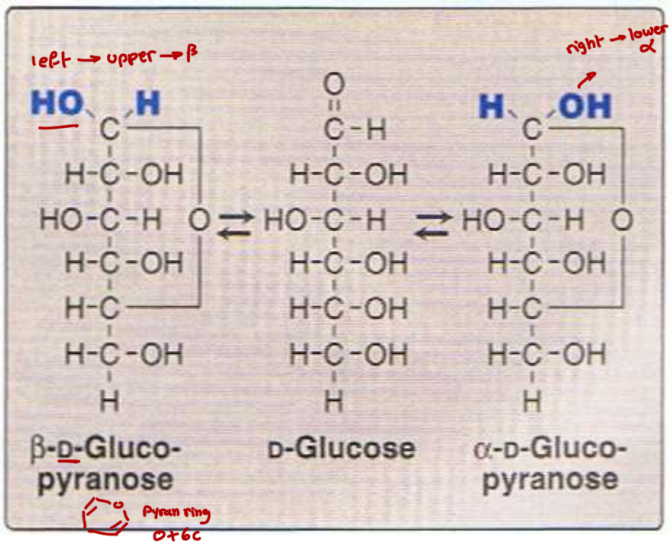
Glucose and fructose are isomers, but not epimers

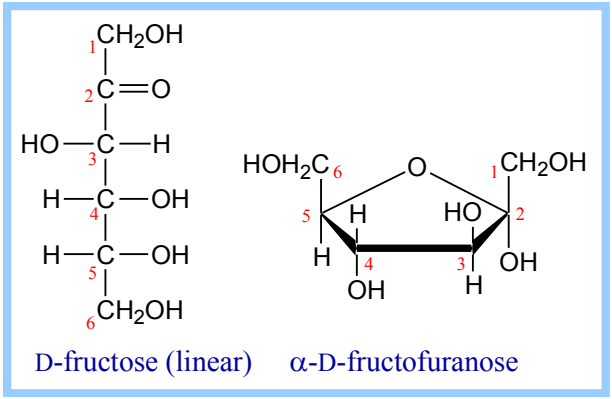
α and β sugars

- When a sugar cyclizes, an anomeric carbon is created from the aldehyde group of an aldose or keto group of a ketose.

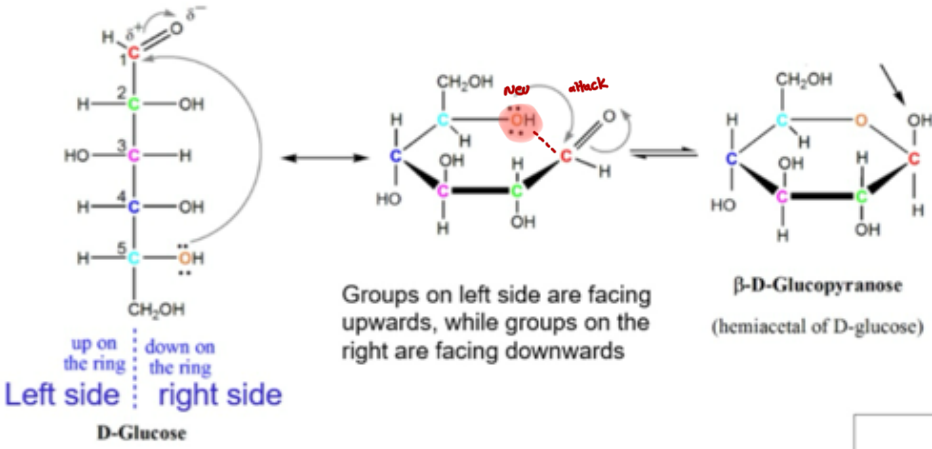
- Glucose forms an intra-molecular hemiacetal, as the C1 aldehyde & C5 OH react, to form a 6-member pyranose ring, named after pyran

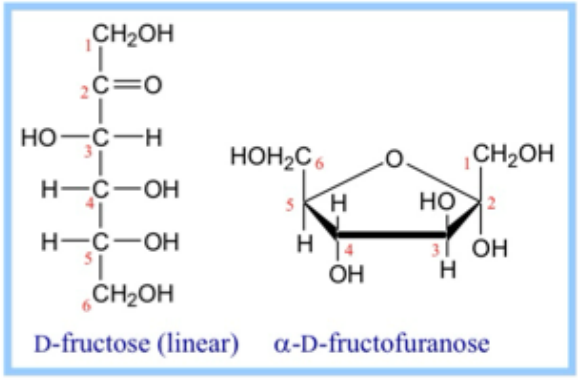
- This carbon can have two configuation, α or β. If the oxygen on the anomeric carbon is not attached to any other structure, that sugar is a reducing sugar
  - α (OH below the ring)
  - β (OH above the ring).

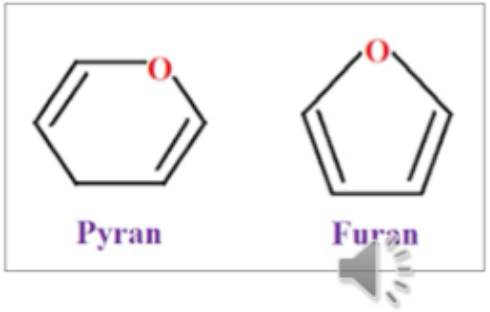




α and β sugars







Digestion of carbohydrates

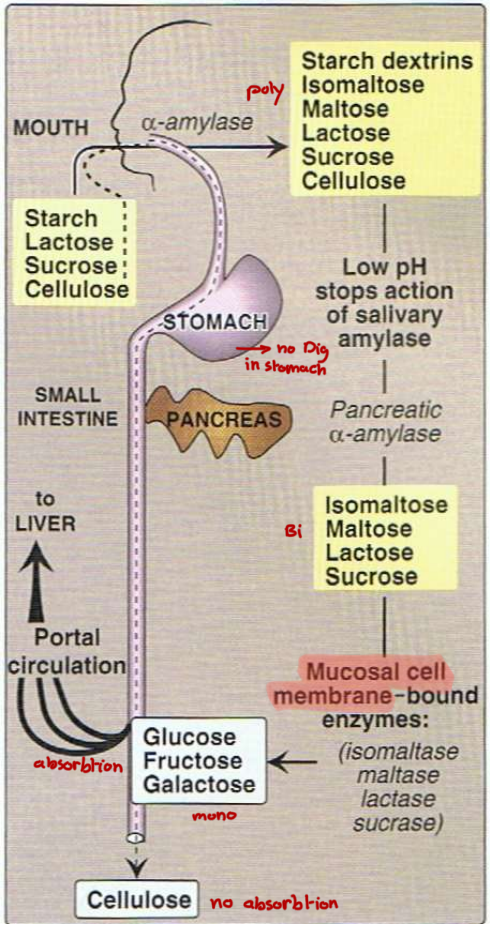
- Digestion of carbohydrates begins in the mouth by salivary α-amylase enzyme which breaks α-1,4 glycosidic bond

  β can't be breaks

  ↓ Enzymes break sugar with α bonds only

- The digestion stops in the stomach because the amylase is inactivated by the high acidity

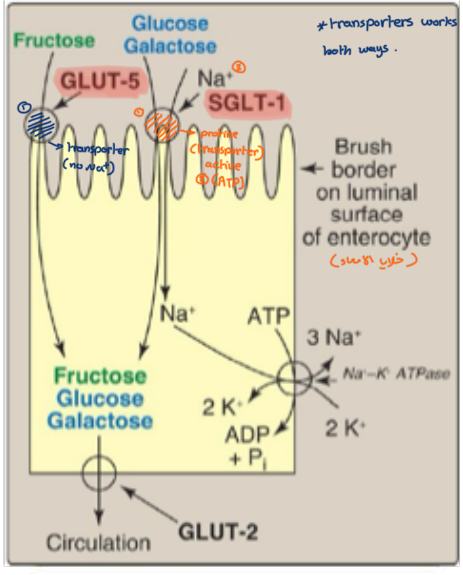
- further digestion of carbohydrates by pancreatic enzymes occurs in the small intestine by pancreatic amylase



Absorption of monosaccharides

- The duodenum and upper jejunum absorb the bulk of the sugars.
- Insulin is not required for the uptake of glucose by intestinal cells.
- galactose and glucose are transported to the mucosal cells by an active, energy-requiring process that involves a specific transport protein and requires a concurrent uptake of sodium ions.
- Fructose uptake requires a sodium-independent monosaccharide transporter (GLUT-5) for its absorption

معمان



Abnormal degradation of disaccharides

- Because predominantly monosaccharides are absorbed, any defect in a specific disaccharidase activity of the intestinal mucosa causes the passage of undigested carbohydrates into the large intestine.

- As a consequence of the presence of this osmotically active material, water is drawn from the mucosa into the large intestine, causing osmotic diarrhea.

- This is reinforced by the bacterial fermentation of the remaining carbohydrate to two- and three-carbon compounds (which are also osmotically active) producing large volumes of CO_2 and H_2 gas, causing abdominal cramps, diarrhea, and flatulence,

Abnormal degradation of disaccharides

- Digestive enzyme deficiency
- Lactose intolerance: lactase deficiency

مضاوعه الاكتوز سه تراكم

- Isomaltase-sucrase deficiency: defect in sucrose degradation (10% of eskimos)

الاسقلاب

مزيته الفحص

- Measurement of hydrogen gas in the breath is a reliable test for determining the amount of ingested carbohydrate not absorbed by the body