



Sheet

Slides

Number

13

Done by:

Tala Saleh

Corrected by:

Tamer Barakat

Doctor

Diala Abu-Hassan

Biochemical application of monosodium glutamate MSG

MSG is a glutamic acid derivative, used as a **flavor enhancer** in Asian food.



Chinese restaurant syndrome is a set of symptoms (chills, headaches and dizziness) that some people have after eating **Chinese food** where **MSG** is used a lot.

Questions on amino acids:

1) What is specific about proline?

Proline is an **imino** acid unique in that it contains a **secondary nitrogen** in its backbone resulted from the bonding of the amino group to the R group, thus it is a **secondary amine** rather than a primary amine like in other amino acids.

2) Which amino acids are found charged at physiological conditions?

5 amino acids have a side chain which can be charged at physiological conditions. **2** are **negatively charged**: aspartic acid and glutamic acid (*acidic side chains*), and **3** are **positively charged**: lysine, arginine and histidine (*basic side chains*).

3) Name amino acids that share the same functional group in their side chain.

Glutamine and Asparagine → Share the **amide group**.

Serine, Threonine and Tyrosine → Share **the hydroxyl group**.

Amino acid and protein molecular weight

The **average molecular weight** of an amino acid residue is about **110**. The molecular weights of most proteins are between **5,500** and **220,000**.

We refer to the **mass** of a polypeptide in the unit of **Daltons (Da)** or **kilo Daltons (kDa)**.

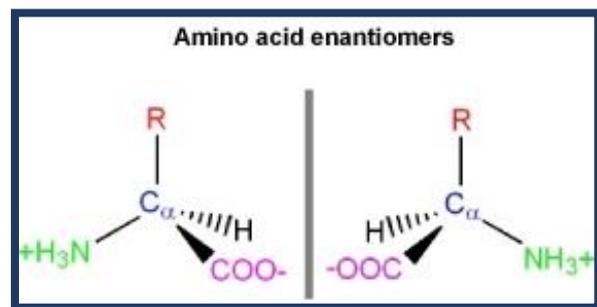
→ A 10,000 MW protein has a mass of 10,000 Da / 10 kDa.

Amino acids stereoisomers / optical isomers

Because the alpha carbon has **4 different groups**, amino acids can exist as **stereoisomers** in proteins (*except glycine since its alpha carbon is attached to 2 H atoms*).

The molecules are **mirror image** to each other and **cannot be superimposed**, they rotate polarized light in **opposite directions**.

(Right D- dexter, Left L-laevus)

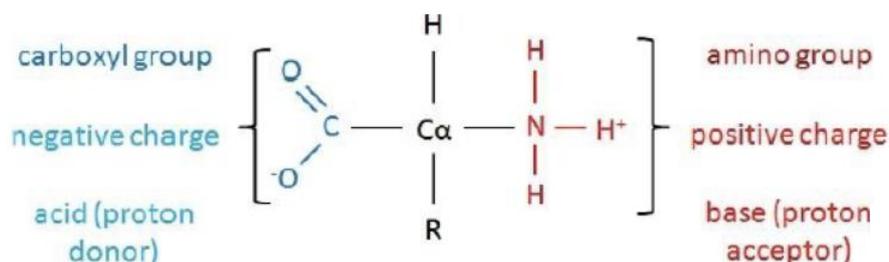


Both isomers (D-, L-) are found in nature however, all amino acids in proteins have **L- configuration**. D- Amino acids are found in bacteria cell walls and in some antibiotics.

Remember: we have D-sugars in our bodies.

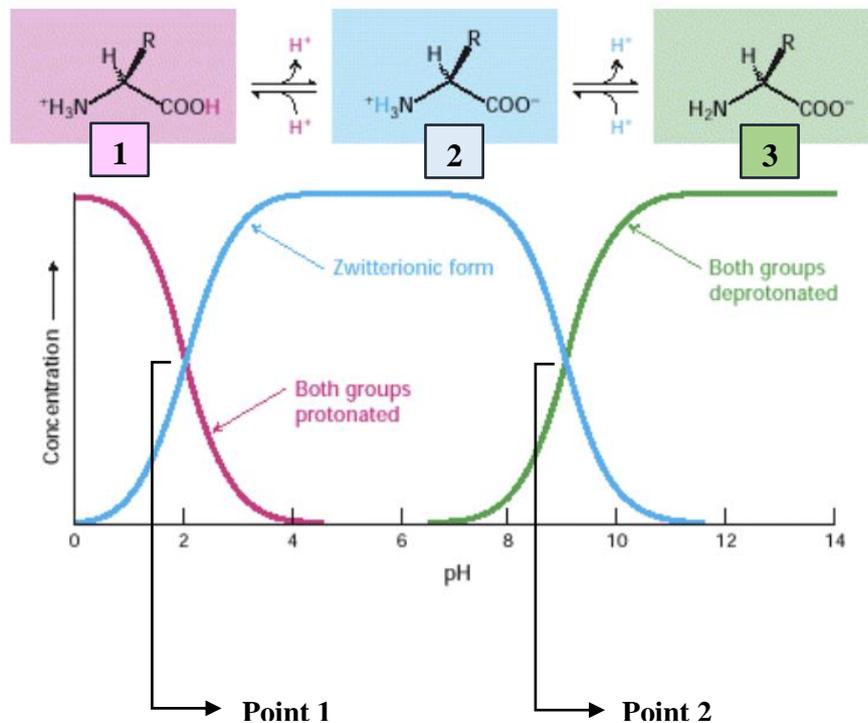
Ionization of amino acids

The main structure of amino acid contains a **carboxyl group** and an **amino group**, these 2 groups can be **ionized**.



Note the following curve and the numbered structures:

- At **very low pH** all the amino acids will be **1**.
- As the pH increases, the carboxyl group will start losing its proton.
1 **Decreases** while **2** **increases**.
- At physiological pH, all amino acids will be in their **zwitterionic** form **2**.
- At high pH the amino group will start losing its proton.
2 **Decreases** while **3** starts appearing.
- At **very high pH** all the amino acids will be **3**.



Point 1: It is the pK_a of the **carboxyl group**, when half of the amino acids carboxyl group is **de-protonated** and **charged** while the other half is **protonated** and **uncharged**.

Point 2: It is the pK_a of the **amino group**, when half of the amino acids amino group is **protonated** and **charged** while the other half is **de-protonated** and **uncharged**.

Amino acids as buffers

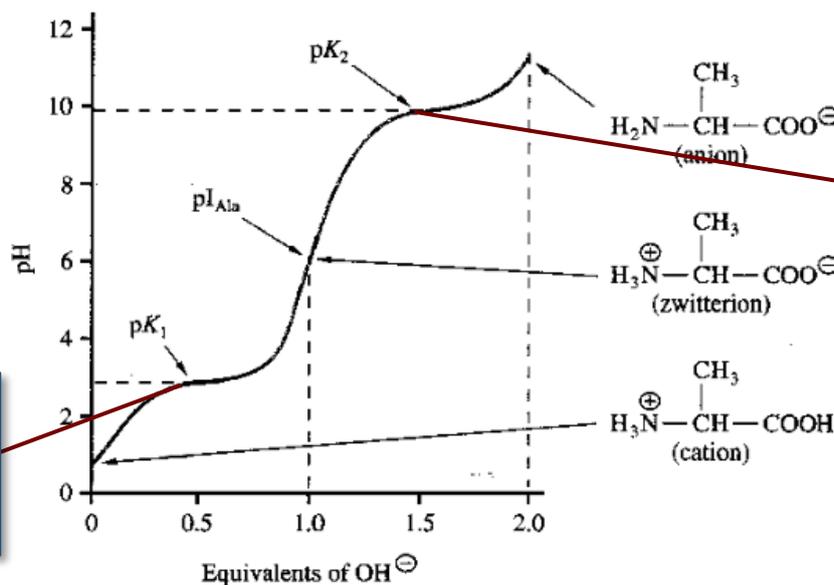
Amino acids contain a -COOH group which acts as a **weak acid**, and a -NH_2 group which acts as a **weak base**, hence amino acids acting as buffers.

- The curve is not shown as a one large step because of the **different** pK_a values of these 2 groups.
- -COOH undergoes titration **before** -NH_2 because -COOH is a **stronger acid** compared to -NH_2 .

Amino acids have a minimum of 2 buffering capacities:

- 1) For the **carboxyl** group at low pH.
- 2) For the **amino** group at high pH.
- 3) For the **R group** if it's ionizable.

Check this titration curve of alanine (*its R group is not ionizable*). In this example, **OH** is **added** to the amino acid, therefore **deprotonation** will be taking place:



This midpoint "where $\text{pH}=\text{p}K_a$ " is the $\text{p}K_a$ of the carboxyl group ≈ 2

This midpoint "where $\text{pH}=\text{p}K_a$ " is the $\text{p}K_a$ of the amino group ≈ 9

- 1) As the **pH increases** (*H atoms decrease*), the **carboxyl group loses a proton** thus **resisting the change in pH** acting as a buffer which is the **1st plateau** in the graph.
- 2) When we reach the end of the 1st buffering capacity, a slight addition of OH causes a **sharp increase in the pH**, which stops after reaching the **2nd buffering capacity of the amine group**.
- 3) The **amino group** of the zwitterion **loses a proton** thus **resisting the change in pH** acting as a buffer again which is the **2nd plateau** in the graph. Any further addition of OH will increase the pH **sharply** with no more buffer zones.

The Henderson-Hasselbalch equation can be used to calculate and predict the ratio of **base to acid**.

$$\text{pH} = \text{pK}_a + \log \frac{[\text{conjugate base}]}{[\text{acid}]}$$

Example: which form of amino acid is dominant at a pH of 8 "using the graph before"?

From the graph we can tell that both the **zwitterion "acid"** and the **anion "conjugate base"** **exist at the pH of 8**. The deprotonation of **NH₃** is involved so its pK_a, which equals 9, is used.

Thus, from the equation the **base to acid ratio** will be **-1** → The **Acid** is more abundant than the **base (10 folds)** → **Zwitterion** is more abundant at the **pH of 8**.

*Note that in both cases we didn't care about the **carboxyl group** because its 100% in its deprotonated (charged form)*

Isoelectric Point (pI)

The pH where the **net charge** of molecules such as an amino acid or protein is **zero** is known as **the Isoelectric Point**.

- The *isoelectric point* for polar and non-polar amino acids with **2 pKa values** can be calculated using this equation:

$$pI = \frac{pK_{a1} + pK_{a2}}{2}$$

- From the alanine graph above we can see that the pI value is the *average* between both **pKa1 and pKa2**, which almost equals using the equation above:

$$pI = \frac{2 + 9}{2} = 5.5$$

- We can also deduce the pI from the graph, it's in equal distances away from both the pKa values.

Amino Acids with more than 2 pKa values

Some amino acids "*9 in total*" have **ionizable R group**, therefore there are **3 different pKa values** (The R groups' pKa will be referred to as pK_R).

Such as: tyrosine, cysteine, arginine, lysine, histidine, serine, threonine, and aspartic and glutamic acids.

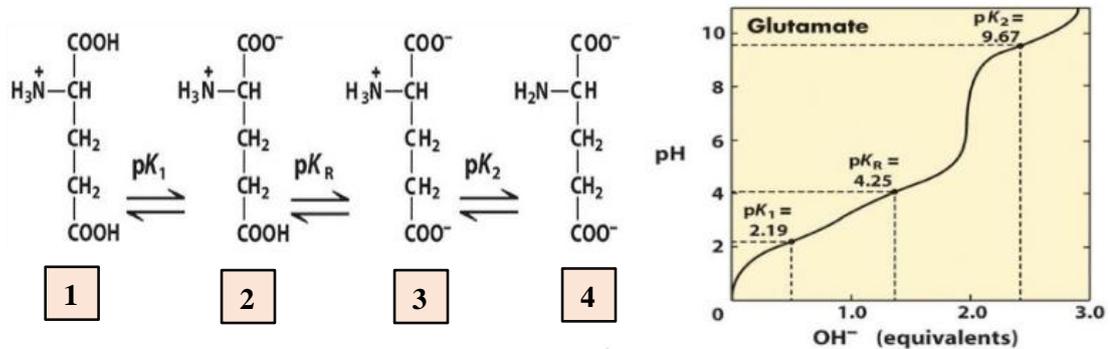
The **pI** will be calculated in a different method by taking the **average of the pKa values** of the amino acids forms that have the **opposite charges** (So that the net charge is 0).

Amino Acid	Side Chain pK_a^3	pI
Arginine	12.5	10.8
Aspartic Acid	4.0	3.0
Cysteine	8.0	5.0
Glutamic Acid	4.1	3.2
Histidine	6.0	7.5
Lysine	11.0	10

Considering pKa for $-NH_2 = 9$ and the pKa for $-COOH = 2$ in all amino acids.

Example 1: Glutamic Acid

Glutamic acid has a **carboxyl group**, which is **ionizable**, in its side chain.



As the pH increases the following happens:

- 1) The **backbone carboxyl** group will be **deprotonated**. At a pH of 2, half of the amino acid will be of the fully protonated form and the other half will be a zwitterion.
- 2) Then the **carboxyl group of the R group** will be **deprotonated**. A pH of 4 will be its midpoint. So, the pK_R = 4.
- 3) At a high pH, the **amino group** will be **deprotonated**. Giving the fully deprotonated form of glutamic acid.
- 4) To calculate the **isoelectric point**, we must take pKa values of 2 amino acids where their **net charges = 0**. So, we calculate the total charge of each form and pick 2 forms that have equal and opposite charges (form **1** and **3**) (pK_R and pK₁).

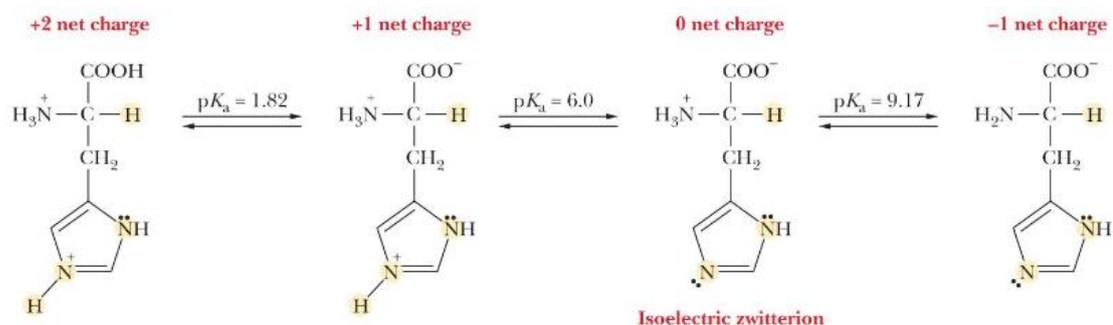
$$pI = \frac{2 + 4}{2} = 3$$

In other words, we take the average of the pKa values around the zwitterion form. This is not the same method mentioned by the doctor.

Example 2: Histidine

Histidine has an amine group in its ring, so it has a **basic ionizable R group**.

Thus, as you increase the pH the following happens:



- 1) The **carboxyl group** loses a proton. ($pK_{a1} = 2$)
- 2) The **amine group in the R group** loses a proton. ($pK_{a2} = 6$)
- 3) The **original amine group** loses a proton. ($pK_{a3} = 9$)

Note how the amino acid in the R group is a stronger acid compared to the original amino acid.

- 4) **Calculating the pI** by taking pK_{a2} and pK_{a3} (pK_a values around the zwitterion):

$$pI = \frac{6 + 9}{2} = 7.5$$

So for now what we are required to know is:

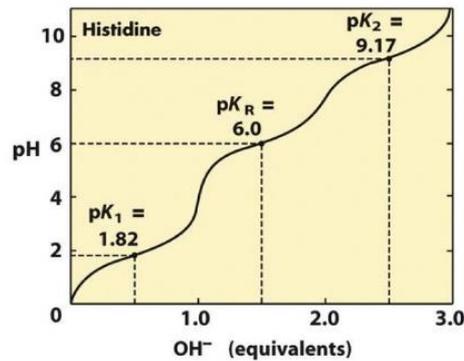
- 1) *Understanding any titration curves given .*
- 2) *Determining which form is predominant at a certain pH.*
- 3) *Calculating the isoelectric point of different amino acids.*
- 4) *Names of amino acids, their special features and their 3 letter abbreviation.*
- 5) *Identifying different amino acid structures.*
- 6) *The uncommon amino acids, their precursors and their functions.*
- 7) *The R groups whether they are acidic, basic or near neutral.*

Questions:

1) Draw the titration curve of histidine.

Using the pKa values: pKa1= 1.82 , pKr= 6 and pKa2= 9.17

Keeping in mind that the buffering capacity= pKa ± 1

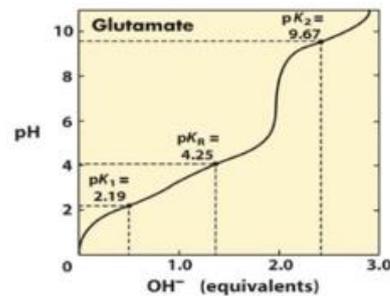


2) What is the ratio of conjugate base to acid of glutamate at pH= 4.5.

$$pH = pKa + \log \frac{\text{base}}{\text{acid}}$$

$$4.5 = 4.25 + \log \frac{\text{base}}{\text{acid}}$$

$$\rightarrow \text{Base to acid ratio} = 1.78$$



Note that we used the pka value that is in range of the pH. Since pH is 4.5, the pka must be between 3.5 and 5,5

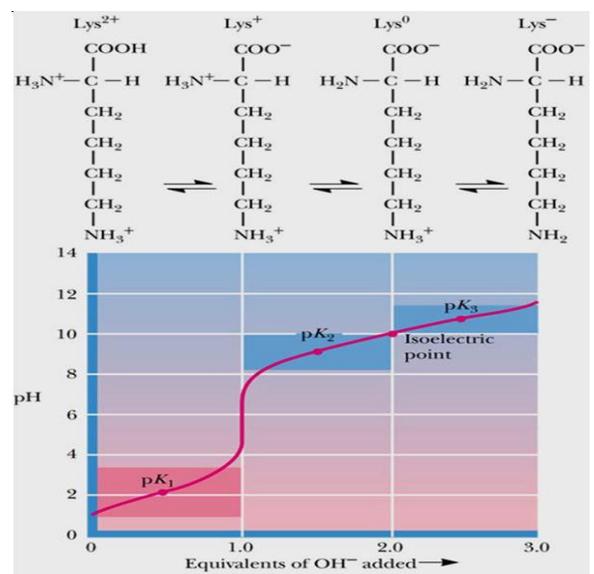
3) What is the total charge of lysine at pH= 7

In amino acids the pI is defined as the pH at which the amino acid has no net charge.

Therefore, when pH > pI, an amino acid has a negative net charge and when pH < pI, an amino acid has a positive net charge.

In this question, pH= 7 and pI= 9.74

→ Total charge of lysine is +1



Peptides

Important definitions:

- **Residue:** a subunit that is part of a larger molecule (such as glucose in glycogen, amino acid in a polypeptide).
We use the prefix (Di, Tri, Tetra...) to designate the **number of amino acids** linked together (Dipeptide, Tripeptide, Tetrapeptide...)
- **Oligopeptide:** a short sequence of amino acids connected to each other (short chain of 20-30 amino acids).
- **Polypeptide:** a longer peptide with **no particular structure**.
- **Protein:** a polypeptide chain with an **organized 3D structure** and a **specific function** (usually more than 100 residues).

The average molecular weight of an amino acid= 110 Daltons

Example 1: a protein has a MW = 5500, how many amino acid residues does it contain?

$$\text{Number of amino acids} = \frac{\text{proteins' MW}}{\text{amino acid MW}}$$

$$\text{Number of amino acids} = \frac{5500}{110} = 50 \text{ amino acids}$$

Example 2: a protein composed of 100 amino acid, what is the MW of this protein?

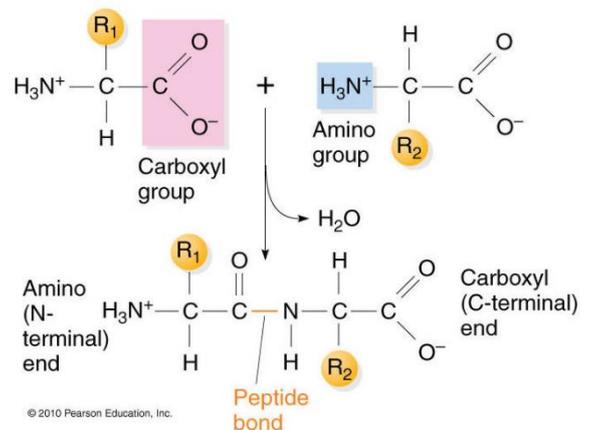
$$\text{Proteins' MW} = \text{amino acid MW} * \text{number of amino acids}$$

$$\text{Proteins' MW} = 110 * 100 = 11 \text{ kDa}$$

Peptide Bond

The **linkage** between **two** amino acids (between the alpha carboxyl group and the amine group of another amino acid) as a result of a **condensation** reaction where a **H₂O molecule is eliminated**, is known as the **Peptide bond**.

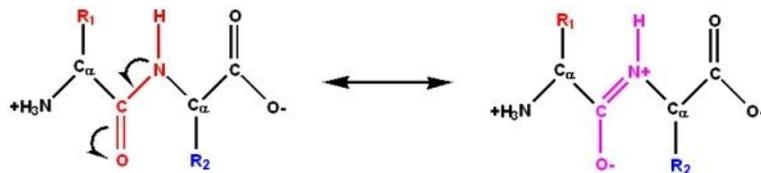
Also known chemically as an *amide bond*.



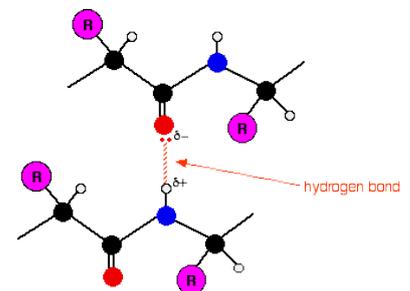
Features of the peptide bond

- It is a **zigzag** structure
- It has a **double bond resonance** making the peptide bond: **rigid, planar** and **charged**.

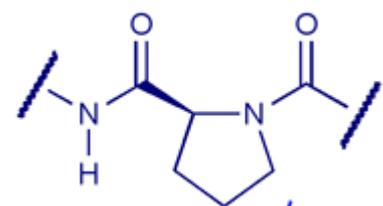
Resonance of this double bond is found because the double bond between the C and O moves to the peptide linkage of C and N.



- **Hydrogen Bonds** are formed between amino acids. (*Oxygen of the carbonyl group is the **H bond acceptor** and the **H atom** of the amino group is the **H bond donor***).

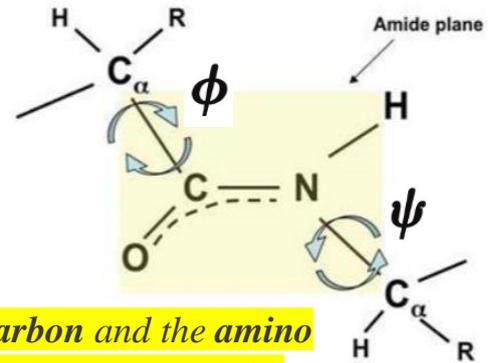


*Except proline, due to its **cyclic structure** the Nitrogen has only **1 H atom** which is used in forming the peptide bond. So, proline **can't** be a hydrogen bond **donor**, however it **can** be a hydrogen bond **acceptor**.*



Question: the protein has to be flexible in order to take different forms, but the peptide bond is **rigid** (due to the resonance hybrid, it cannot be rotated), how does the protein make its structure possible?

The peptide bond itself is rigid and can't be rotated, but the bonds within the amino acid itself (**phi and psi**) are flexible and can be rotated, therefore the protein can be folded into its 3D structure.

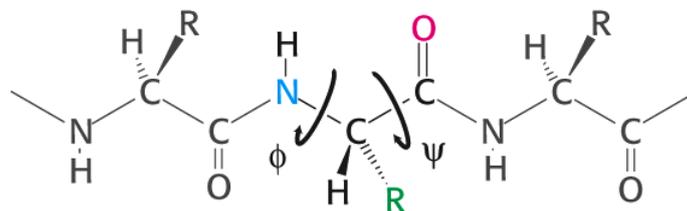


Note that the **phi** is the bond between the **alpha carbon** and the **amino nitrogen** while the **psi** is the bond between the **alpha carbon** and the **carboxyl carbon**, both are **flexible** unlike the peptide bond.

The Peptide Chain

- The backbone of a peptide chain consists of:

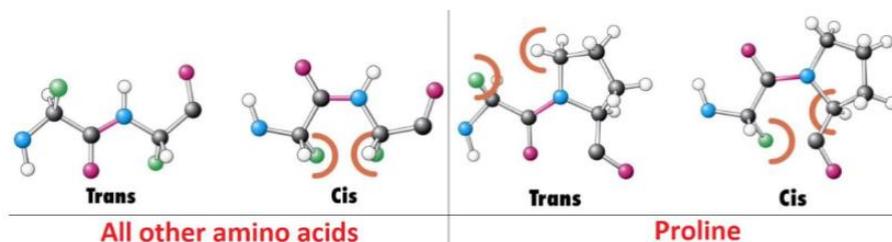
- 1) α -amide nitrogen
- 2) α -carbon
- 3) α -carbonyl carbon atom



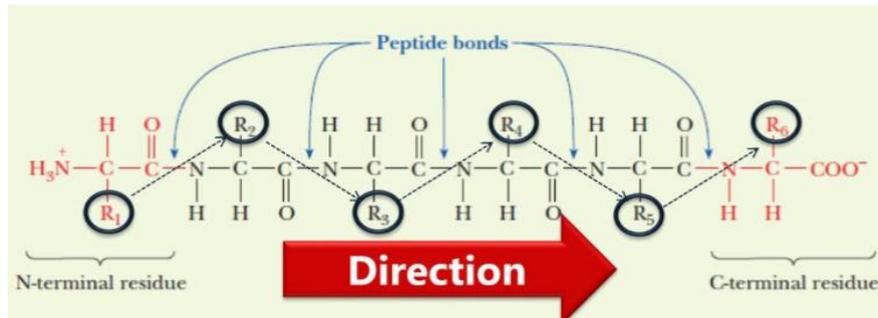
- The R groups in the peptide chain are **branched**, orientated toward the **outside** and in a **trans configuration**.

(Which provides **less steric hindrance** between the functional groups attached to the alpha carbon, the **steric hindrance** would be **greater** if it was in a **cis configuration**).

- Except in **proline**, where both cis and trans conformations have **equivalent energies** (repulsion will occur anyway). Thus, **proline** is found in **the cis configuration** more frequently than other amino acids.



- The peptide chain **starts** with the **N- terminus** and **ends** with the **C- terminus**. The amino acids are **added** to the **C- terminus** (*C-terminus can be modified while the N- terminus can't be modified*).



- The polarity of the peptide backbone is due to the **N- terminus** (*positive end*) and the **C- terminus** (*negative end*).

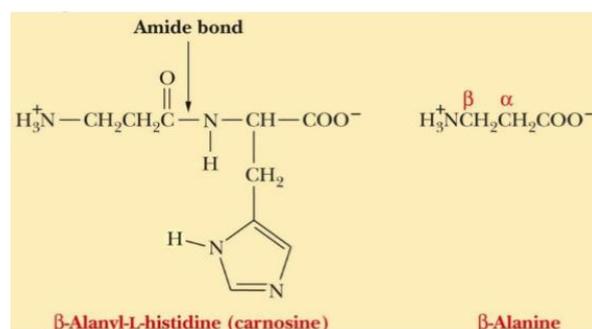
Examples of functional and exceptional peptides

- Carnosine
- Glutathione
- Enkephalins
- Oxytocin and vasopressin(ADH)
- Gramicidin S and Tyrocidine A
- Aspartame

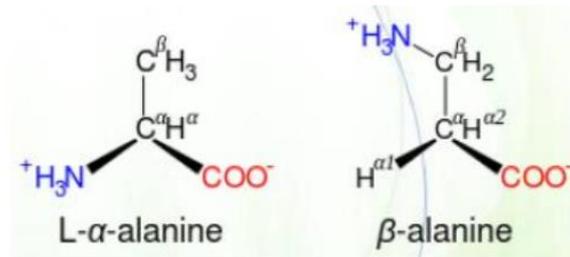
1) Carnosine (β -alanyl-L-histidine)

It is a **dipeptide** constructed of two amino acids (*β -alanine and L-histidine*).

The amine group is bonded to the β carbon of alanine.



The difference between β -alanine and α -alanine is that the amine group is linked with the α -carbon in α -alanine.



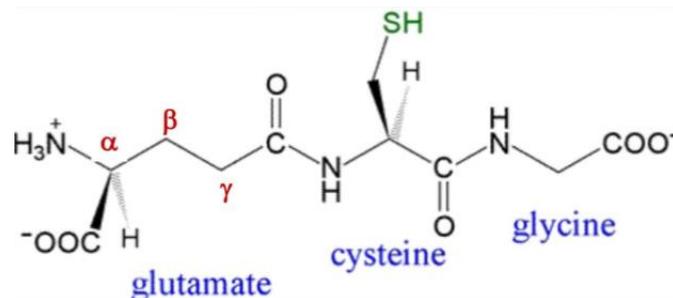
Functions:

- Protection of the cells from **ROS** (radical oxygen species) and **peroxides**.
- Contraction of **muscles**.

It is highly concentrated in muscle and brain tissues.

2) Glutathione (γ -glutamyl-L-cysteinylglycine)

It is a **tripeptide**, made up of 3 amino acids (*γ -Glutamate-L-cysteine-Glycine*)



Note that it is a γ -glutamyl amino acid because γ -carboxyl group of the glutamic acid is involved in the peptide bond with the amine group of cysteine, which is bonded to the amine group of glycine.

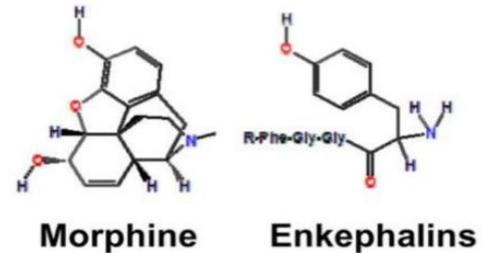
Function:

- **Glutathione** has a major role as it functions as an **anti-oxidant**. It removes oxidizing agents like **reactive oxygen species**.

Enkephalin and morphine

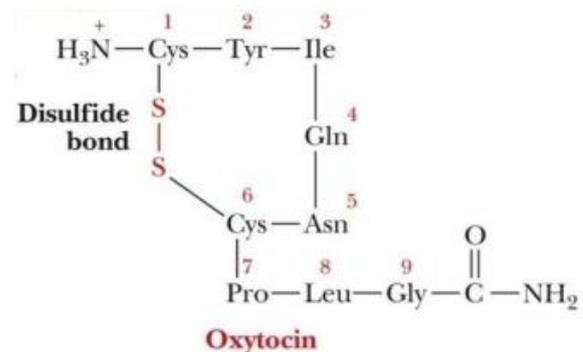
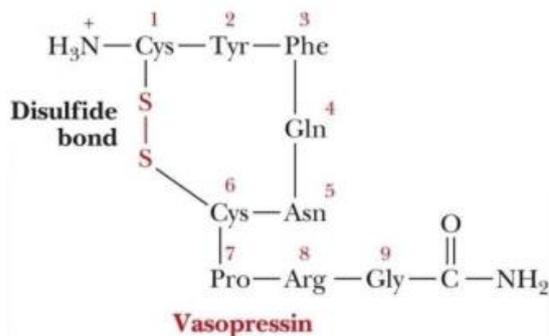
There are similarities between the 3D structures of **opiates** (which are drugs derived from opium) such as **morphine** and **enkephalin**.

Morphine is an **addictive** pain reliever that is wildly used in hospitals. Due to the similar 3D shape of it to that of the **enkephalin**, **it binds to the same receptors that enkephalin binds to**.



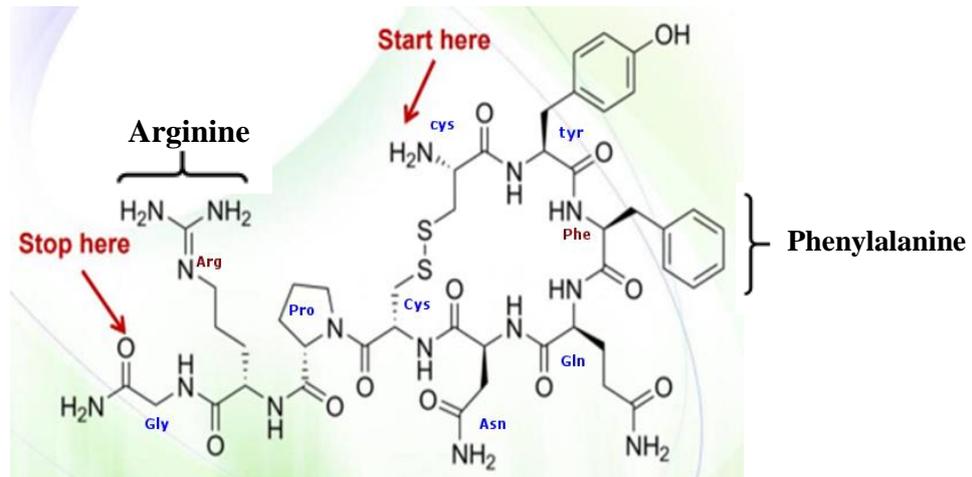
4) Oxytocin and Vasopressin

- They are **hormones** with a **cyclic structure** due to the (**S-S link**) between **Cys** amino acids of the same peptide.
- Both have a modification of an **amide group** at the **C-terminus**.
- Both are **nona-peptides** (made up of 9 amino acids).



Name of the Hormone	The different amino acids	Functions
Oxytocin	8Leucine, 3Isoleucine	<ol style="list-style-type: none"> 1) Regulates the contraction of the uterine muscles when delivering the baby (labor contraction) 2) It is present in males and have a pleasant feeling
Vasopressin (ADH)	8Arginine, 3Phenylalanine	<ol style="list-style-type: none"> 1) Regulates the contraction of smooth muscle 2) Increases water retention 3) Increases blood pressure 4) Renal function 5) Maintain salt concentration

Question: *what is the primary structure of the figure below?*



Try to spot the special groups, here we can see arginine and phenylalanine → Is it oxytocin or vasopressin?

5) Gramicidin S & Tyrocidine A

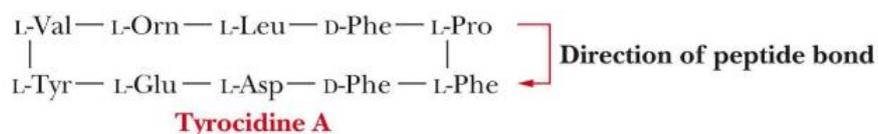
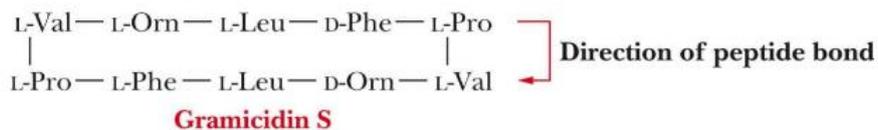
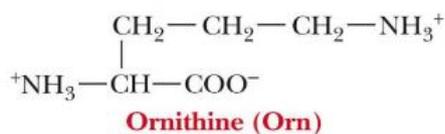
They are **cyclic deca-peptides** (*made up of 10 amino acids*).

Produced by: Bacterium Bacillus brevis and acts as an antibiotic.

Features: Both contain **D-and L-amino acids**.

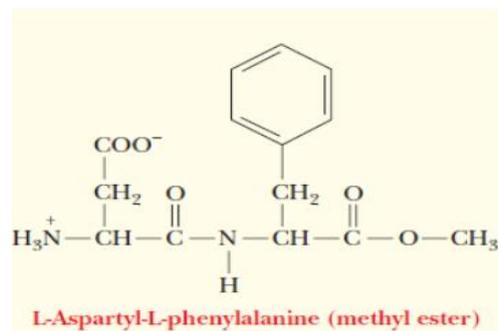
Both contain the amino acid **ornithine** (Orn).

Ornithine doesn't occur in proteins. The 20 amino acids that we studied are the only ones that occur in protein synthesis.



6) Aspartame (L-Aspartyl-L-phenylalanine / methyl ester)

- It is a **dipeptide** (*L-aspartic acid and L-phenylalanine*).
- The carboxyl end of phenylphthaline is modified by a **methyl group**.
- It is **200 times sweeter than sugar** (*used as a sweetener.*)
- If the **D-amino acid** is substituted for either one of the amino acid or for both of them, the resulting derivative is **bitter** rather than sweet.

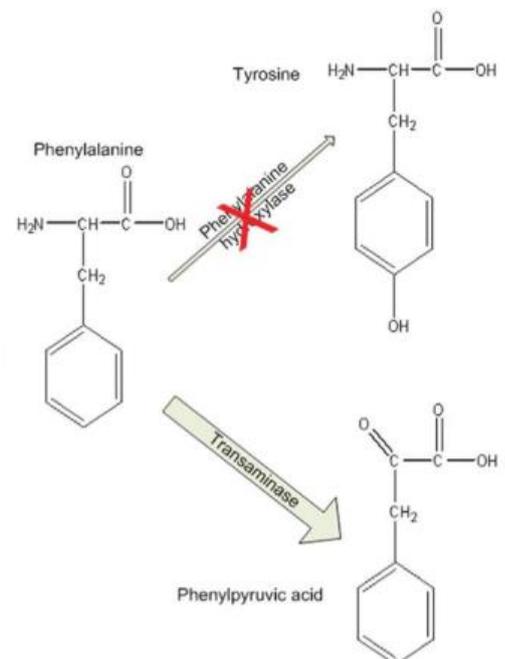


Phenylketonuria (PKU)

PKU is a hereditary inborn error of metabolism, caused by defection of the enzyme **phenylalanine hydroxylase**. It causes accumulation of phenyl-pyruvate, which **causes mental retardation**.

→ Sources of phenylalanine such as **aspartame** and milk must be **limited**, and as a substitution of aspartame, alatame is used which contains **alanine** rather than **phenylalanine**.

Normally in the body, **phenylalanine** has to be converted to **tyrosine**, but when **phenylalanine hydroxylase** enzyme is **defected**, phenylalanine gets converted to **phenyl-pyruvate** instead, and it gets **accumulated**. Its accumulation is dangerous because it can get to the **CNS** and damage the brain causing **retardation**.



Good Luck 😊