Lecture : 3

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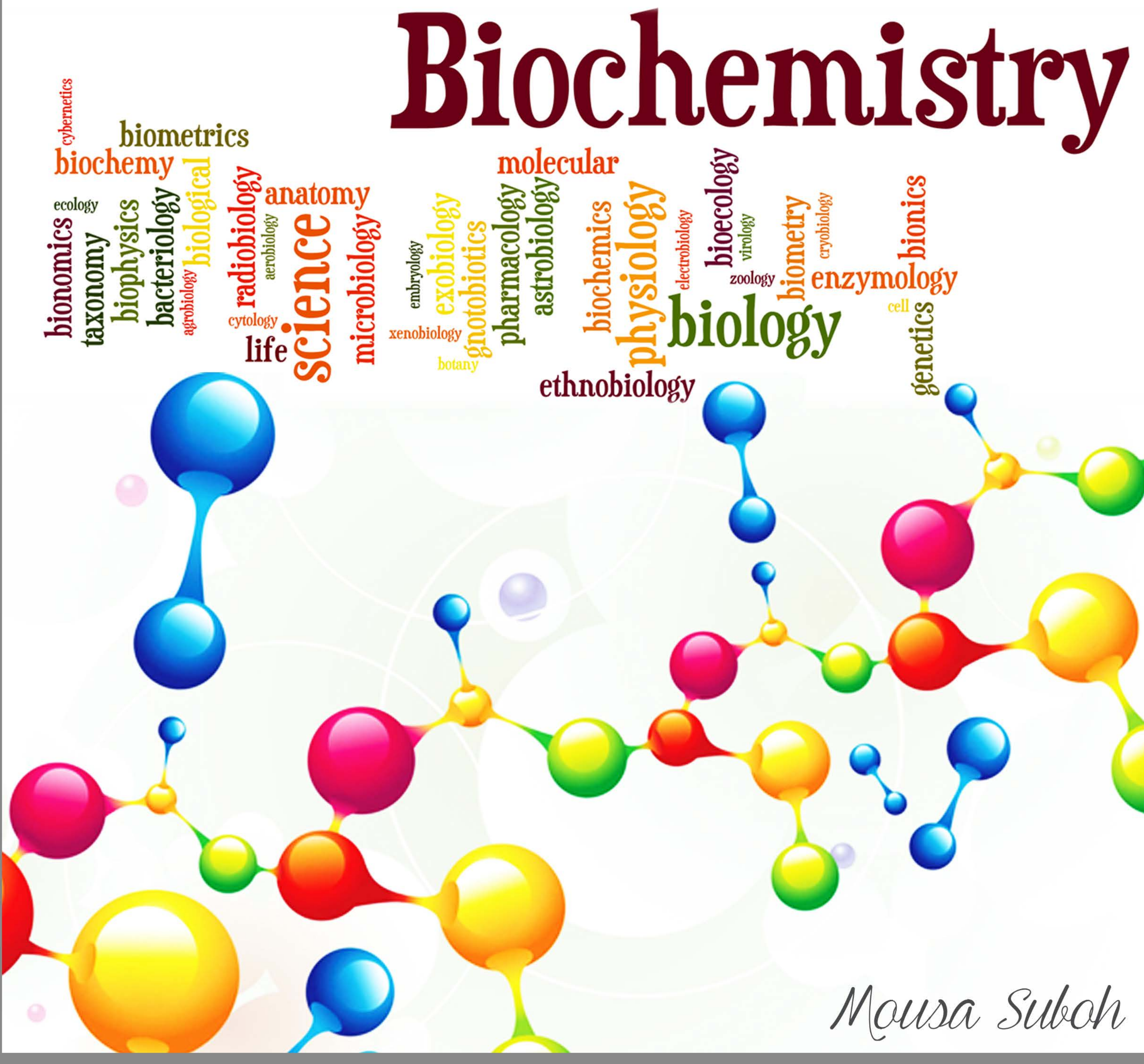
Medical Committee
The University of Jordan



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Amino Acids and Peptides

Lecture#3

Ionization of Amino Acids :

* All amino acids have at least two ionizable groups (The carboxylic group and the amino group) , both of them can be ionized , can be either protonated or deprotonated and this is determined by the PH of the solution .

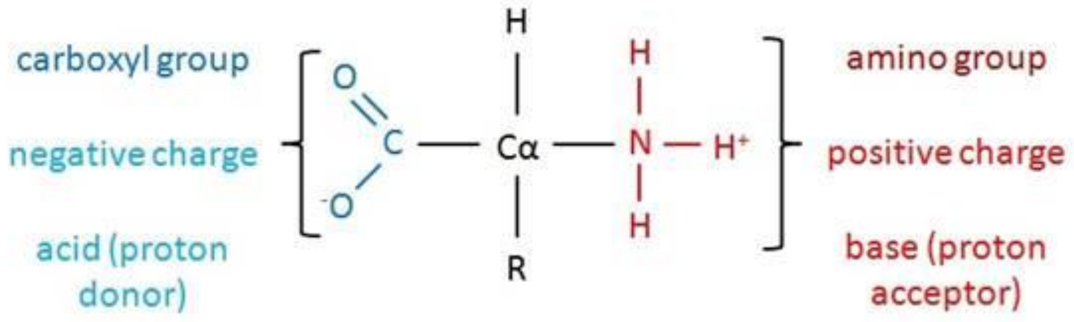
How does the PH determine this ?

PH value represents the H+ concentration in the solution . So when the H+ concentration is high(pH is low) , the ionizable group will not give H+ and will be protonated , and when the H+ concentration decreases in the solution(pH increase) , the ionizable groups will give H+ to the solution and be deprotonated

How can the hydrogen atoms be lost from any compound ?

1) Oxidation – Reduction reaction ; but this needs enzymes .

2) Acid – base reaction ; without the need of enzymes and instead it depends on the differences of PH value in the solution .



* as we mentioned , each amino acid has at least two ionizable groups . In addition to them , many amino acids have ionizable R-groups.

Rules :

☺ Non-polar amino acids don't have ionizable R-group .

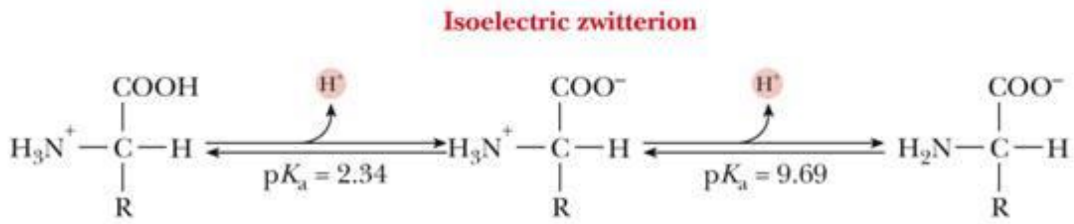
☺ Amino acids that can participate in the ionization process are 9 types out of 20 :

- Three of them are the polar amino acids which have hydroxyl group (-OH) in their side chains "Hydrogen atom in –OH can be lost when rising the pH leaving oxygen atom with a negative charge that's why it's an ionizable group", These three amino acids are (Serine , Threoeine and Tyrosin)
- Cysteine which has –SH on its side chain which is considerable as ionizable group .
- Charged amino acids : they are 5 in number , 3 of them are positively charged (Lysine ,Arginine and Histidine) and 2 of them are negatively charged (Glutamate and Aspartate).

- **Isoelectric point** : PH value when the charge of the molecule equals to zero .
- **Zwitterion** : The molecule which has a net charge equals to zero .

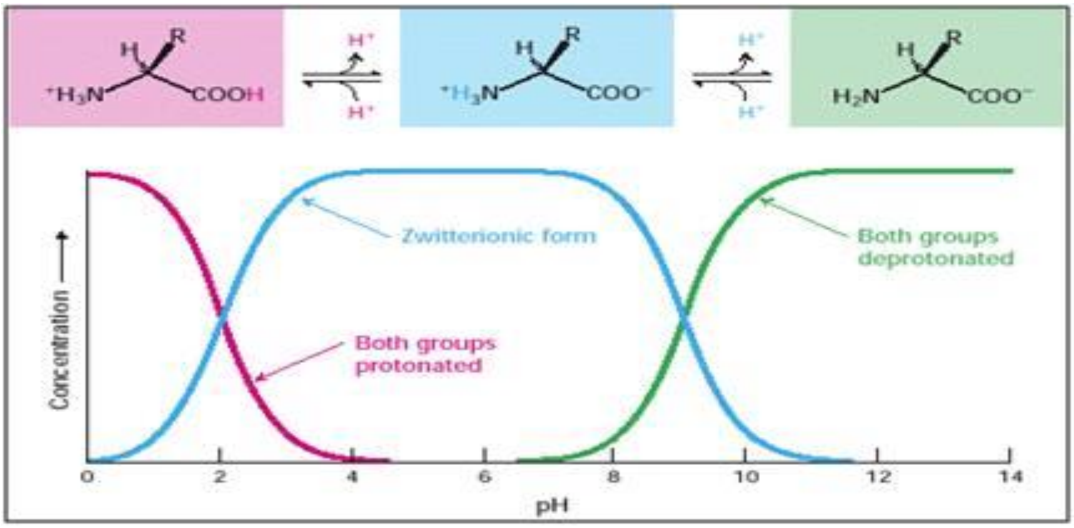
****So the PH at which the molecule become zwitterions is called Isoelectric point .***

Effect of PH :



In the example above there is a general structure of an amino acid with (α-carbon , H atom , side chain , carboxyl group and amino group).
Firstly , when the PH of the solution is below (2) , this means that the solution is very acidic "has high [H+] " , so ionizable groups will not lose H+ , because the solution is saturated with H+ that's why both ionizable groups will be protonated
After that , when we start increasing the PH value of the solution " and thus become less acidic" , the [H+] will decrease so the ionizable groups will start donating protons to the solution that's why when pH increase above 2 the carboxylic group will be deprotonated and when pH reaches 9 or more the Amino group will be deprotonated as well .
Returning back to the example above , we see that at the start when the pH is below 2 both ionizable groups are protonated and the molecule has a net charge of +1 now upon increasing Pka of the solution the donating of protons happens by two steps :-
1) In the first step the carboxyl group of the backbone will donate the proton and become –COO- and the net charge of the molecule will be (zero) .

2) In the second step , by increasing the PH , the amino group will lose its proton and thus , the net charge of the molecule will be (-1) .
* These two steps happen when the amino acid is non-polar ; which means it doesn't have ionizable group in its side chain .



In the Figure above :

* When the PH is below (2) :

- Both ionizable groups (carboxyl and amino groups) are protonated .
- Net charge of the molecule = (+1)

* When PH becomes 2 or more :

- The carboxyl group become deprotonated and the amino group is still protonated .

but the carboxyl group will not be deprotonated in one step ; when PH = 2 , 50% of the carboxyl group are protonated and 50% are deprotonated (50% have a charge of +1 and 50% have a charge of zero , respectively) , then when PH becomes more than (2) all carboxyl group become deprotonated and the net charge of the molecule will be zero (Zwitterionic form) .this means upon increasing the Pka above 2 the percentage of Zwitteronic form increase until reaching Isoelectric point in which all the molecules will be in Zwitterionic form.**

* When the PH becomes = 9 :

of the amino group will be deprotonated " thus have net 50% - charge of (-1) " , and 50% are protonated " have net charge of . " (zero

: When Ph becomes more than 9 *

-Amino group will be totally deprotonated .

- The net charge = -1

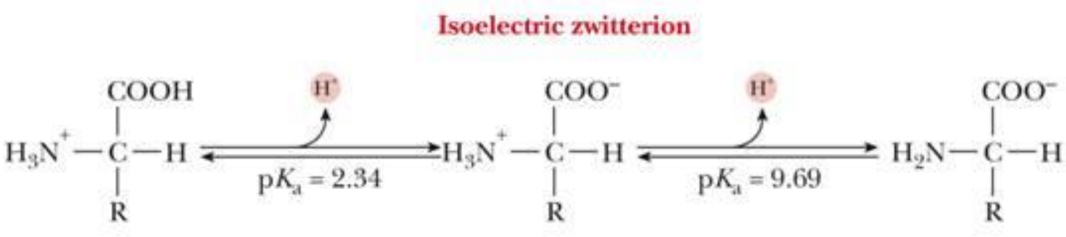
Henderson-Hasselbalch Equation :

- We use this equation to calculate the ratio of the acid to its conjugate base for an carboxyl group and amino group , and thus we can know how mauch of each of them are protonated and deprotonated .
- We calculate the ratio as the inverse of the log of the ratio of the acid to its conjugate base as we are given the values of PH and Pka

$$pH = pK_a + \log \frac{[\text{conjugate base}]}{[\text{weak acid}]}$$

How to calculate the isoelectric point (PI) :

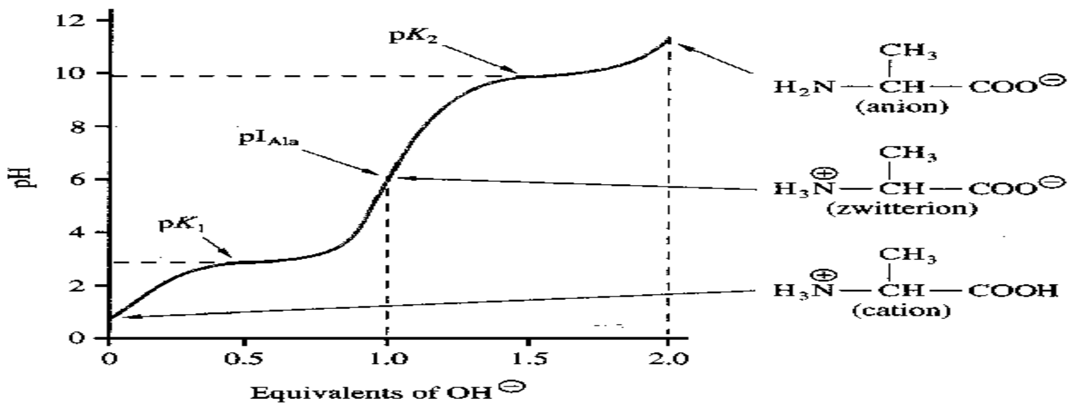
In the example below , at the first Pka (2.34) : the two ionizable groups are protonated and the total charge is (+1) WHILE on the second Pka (9.69) , the two ionizable groups are deprotonated and the total charge is (-1).



- We want to calculate the Isoelectric point (The PH when the total charge = zero)
- The Isoelectric zwitterion is on the middle between the two Pka so we use the law below .

pI=(pKa1+pKa2)/2

- The diagram below is called (The titration curve of Alanine) which shows how the ionizable groups are changing while increasing of PH of the medium .



- At the first titration , one H+ has been lost , and in the second titration another one has been lost .

<u>- We should be able to recognize the number of the titrations in the curve in order to determine the type of the amino acid the curve belongs to .</u>

- In this curve , there is two titrations so we should know that this amino acid has only two ionizable groups "titratable groups" , so it should be a non-polar amino acid .

Number of titrations(number of Pka) = Number of ionizable groups in the amino acid

In the exam we may be asked to know the type of the amino ** acid based on the number of titrations in the titration curve . For example we may get the diagram above and have 5 options one of them is non-polar amino acid which represents the correct .

<u>Titration curve for amino acids with ionizable side chains :-</u>

- Each ionizable group has its special Pka at which it loses its proton at .

Amino Acid	Side chain Pka3	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**

- Depending on these values we can calculate the PI for each amino acid **so we don't need to memorize it**

- This table is required , but we don't need to memorize the exact numbers , we should know that :

* The basic ones have Pka > 9

* Pka of Histidine is around 6

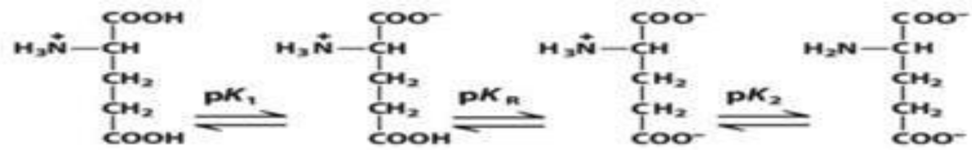
* The acidic ones have Pka <6

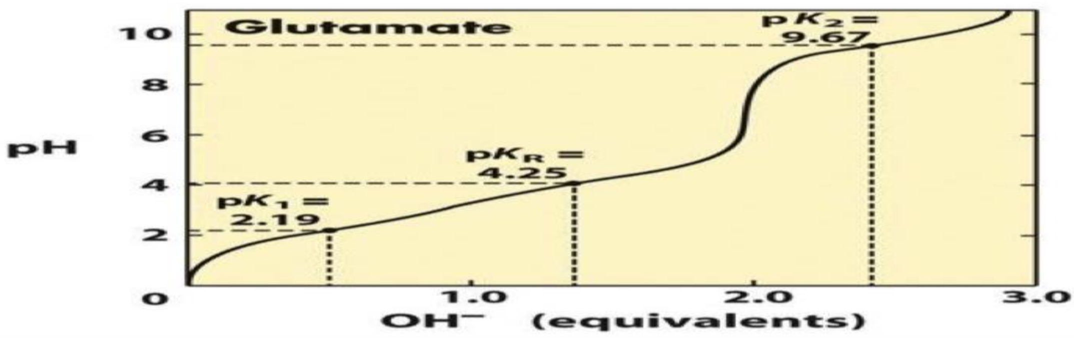
Now we will talk about how to calculate the PI for the amino acids with ionizable side chain group (using a titration curve consists of 3 titrations).

- The Philosophy of the calculation of PI is to know the point at which the net charge of the molecule = zero , so the method we used for the non-polar amino acids can't be applied here .

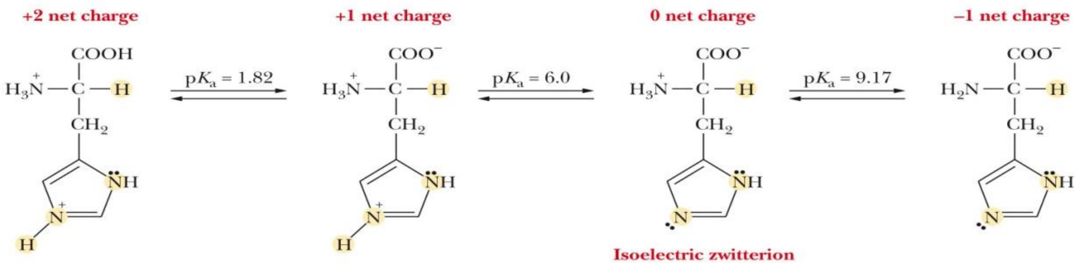
- As mentioned before , the PI is the PH where the net charge of the molecule = zero .

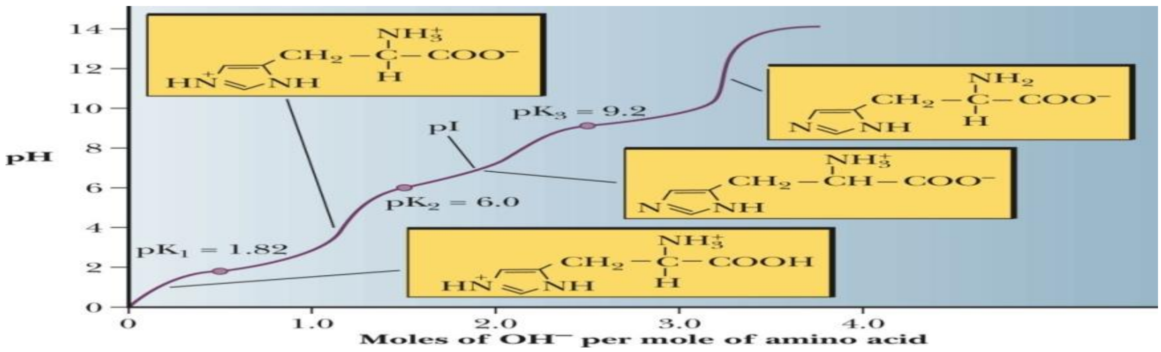
-In this example below we have glutamate which has 3 pKa values one for COOH which is 2 and one for The side chain which is 4 and one for the Amino group which is 9 , and we have 4 molecules with different net charge at first +1 then 0(Zwitterion) Then -1 then -2 now in order to calculate pI we need to consider our calculation between the pKa of the positive form and the negative form that are located around the Zwitterion molecule and calculate their pI





pI = (pKR+ pKCOOH)/2 = (4.25+2.19)/2=3.22





* The figure in above represents the titration curve of the Histidine , which has positively charged side chain and the total charge of the molecule is (+2) . We start in a very acidic medium then we start increasing PH until we reach PH=2 the carboxyl group will be deprotonated so the net charge of the molecule will be (+1) , after that ,when we reach PH = 6 (at which the ionizable side chain of Histidine loses its H+) , so the net charge of the molecule will be zero . Later on , when we reach PH=9 , the H+ of the backbone amino group will be lost and thus the total charge of the molecule will be (-1) .

- Between the (-1) and the (+1) we got the zwitterionic form , so the PI will be between these two Pka values (6.0 and 9.17)

PI = (6+9)/2 = 7.5

- **This method can be applied also on the peptides which have more amino acids and also can be applied on proteins .**

General Rules :

- The carboxyl group of the backbone will lose its proton on PH=2 .

- The acidic groups in the side chain "if present" will lose its proton at 6>PH>2 ; (PH around 4)

- At PH = 6 , Histidine (aromatic amine) will lose its proton .

- The back bone amino group will lose its proton at 9>PH>6 .

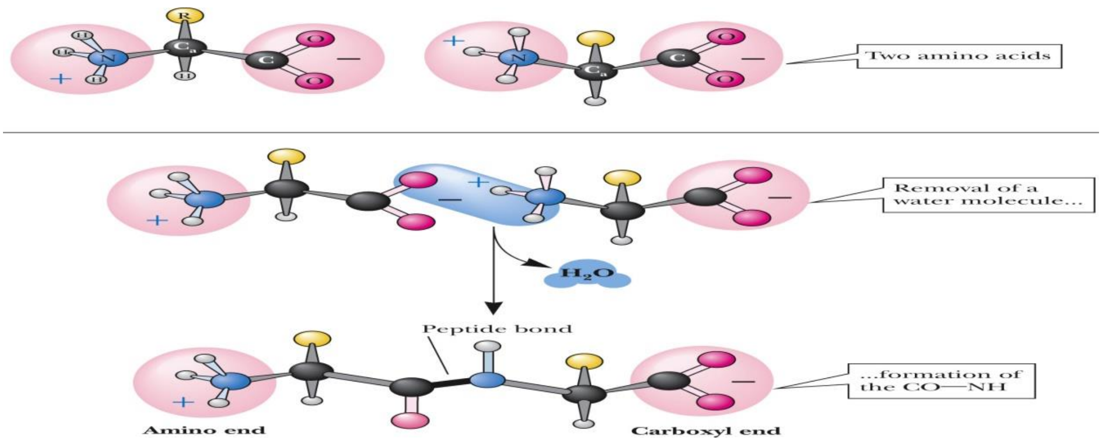
- The Side chain amino group will lose its proton at PH >9 like in Lysin And Arginine

The arrangement of the steps of loosing of protons from the ionizable groups in the amino acid :

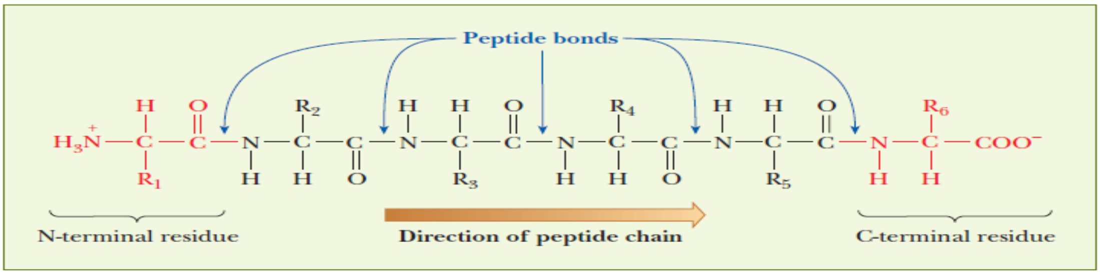
- Backbone carboxylic acid .
- Side chain carboxylic acid .
- Aromatic amine side chain , ex : Histidine .
- Backbone amino group .
- Side chain amino group

Peptides

- Peptide bond chemically is called amide bond and it is a covalent bond between a carbonyl group and N atom .
- This bond is formed by condensation reaction(dehydration reaction) in which water molecule will be lost. (-OH from the carboxyl group and –H from the amino group).



Each amino acid has one free amino group (N-terminal) and one free carboxyl group (C-terminal) . And the direction of peptide chain is always from N-terminal toward cC-terminal and so we name the peptide bond depending on this direction



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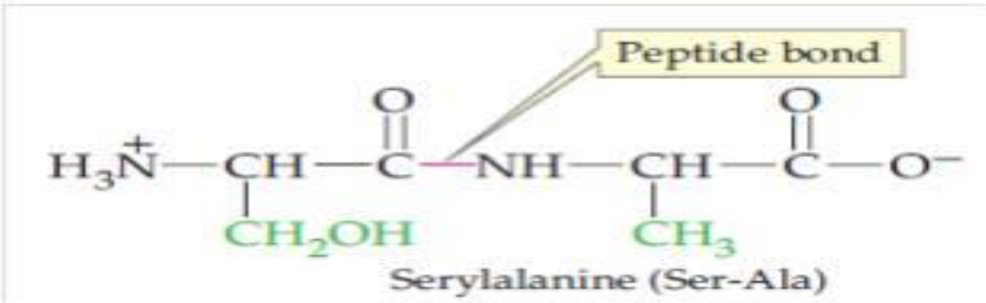
Example : If we want to make a peptide bond between Alanine and serin we have two methods :
1-we put Alanine firstly then serine so the amino group of The Alanine will be free and the carboxylic group of the Serine will be free and we call this peptide bond (Alanylserine) Ala- Ser
2-We put serine first then Alanine so the amino group of Serine will be free and the carboxylic group of the Alanine will be free and we call this peptide bond (Seryalanine) Ser-Ala .



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** **Regardless of the length of the polypeptide , it has just one free carboxylic group (C-terminal) and one free amino group (N-terminal) , and all other groups are engaged in peptide bond formation .**
****The different arrangement of amino acids in the polypeptide will result in different names , structures and functions .**

Definitions and concepts :

- A residue : each amino acid in a poly peptide . " inside the sequence of amino acids each of them is called residue" .
- Dipeptide : consists of two amino acids .
- Tripeptide : consists of three amino acids .
- Tetrapeptide :consists of four amino acids .
- Oligopeptide (peptide): a short chain of 20-30 amino acids .

- Polypeptide: a longer peptide with no particular structure .
-Protein: a polypeptide chains with an organized 3D structures.
- The average molecular weight of an amino acid residue is about 110 .
- The molecular weights of most proteins are between 5500 and 220,000 (*calculate how many amino acids*).
-We refer to the mass of a polypeptide in units of Daltons .
- A 10,000-MW protein has a mass of 10,000 Daltons (Da) or 10 kilodaltons (kDa).

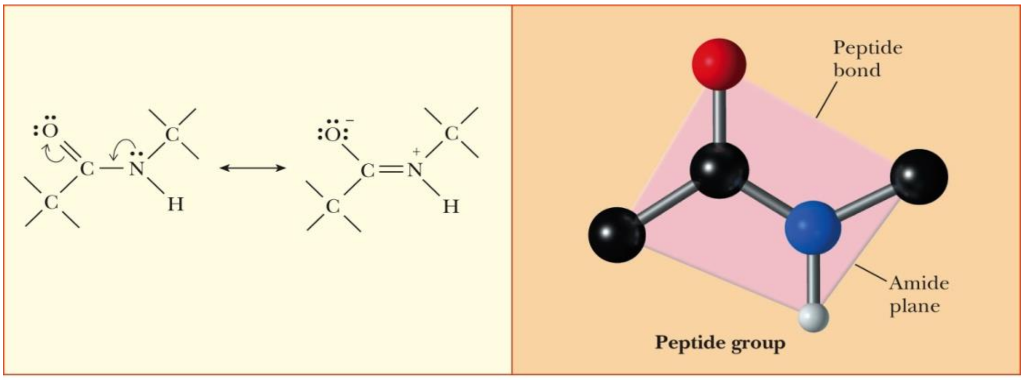
Features of the peptide bond :

- Peptide bond is formed between carbonyl group and N atom .

- There will be resonance between the carbonyl group and the N atom .

** This resonance structure makes peptide bond :

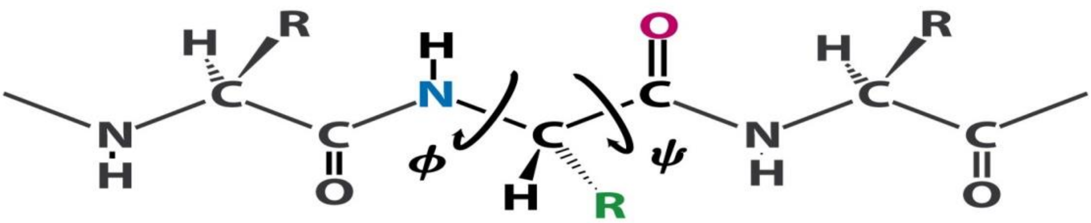
1) Zigzag structure
2) Planar
3) uncharged



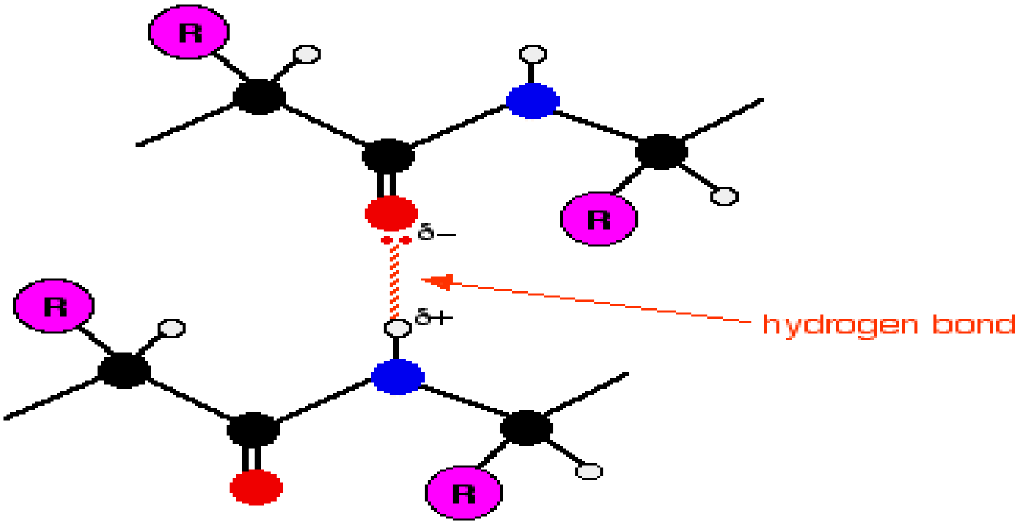
A Resonance structures of the peptide group.

B The planar peptide group.

4) rigid (double bond) : so the will be no rotation around the peptide bond and also this peptide bond will be planar " because it is rigid" . BUT ; the other bonds inside the polypeptide will be rotatable , ex: (C-C) bond



5) Hydrogen bonding : because it has carbonyl group -C=O , the oxygen can be engaged in the H- bonding and the N atom can also be engaged in H – bonding . **Except** for Proline because its N atom forms a ring and attached to the α-carbon , so it can't make H-bond



Why all peptide bonds are on the trans configuration ?

Because trans is more stable as it decreases the steric repulsion , because there is rotation in the bonds in the polypeptide . **Except** for Proline it makes no difference to be on cis or trans conformation (they both have about equivalent energies" , **Proline is thus found in the cis configuration more frequently than other amino acid residues.**

For all amino acids :-

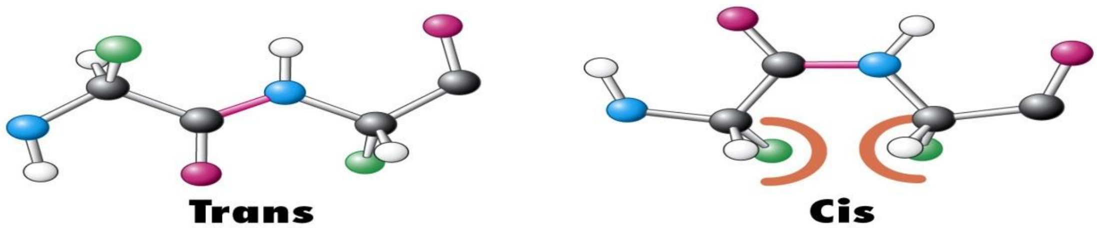


Figure 2-25
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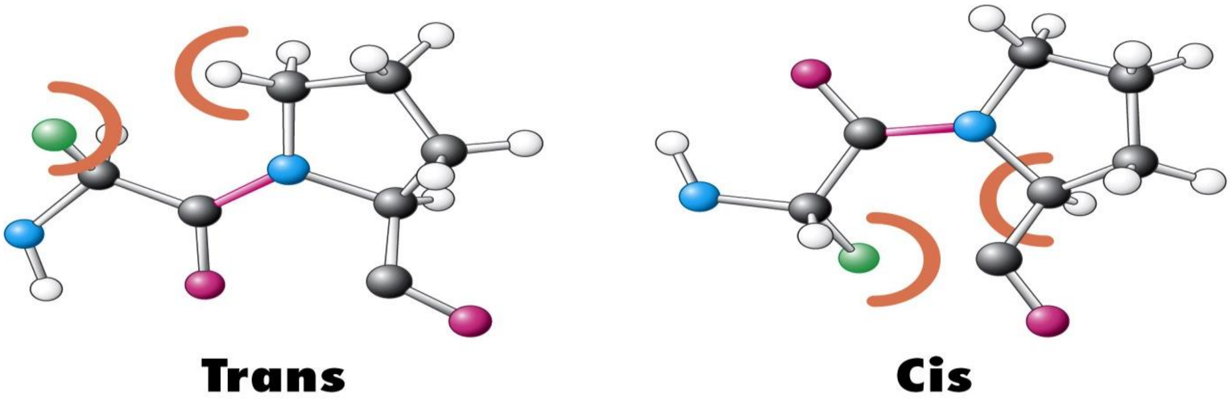
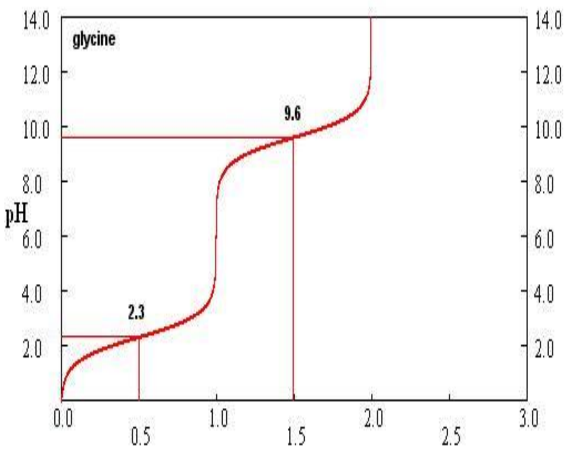
for prolin :-



Quiz :-

1-The isoelectric point for glycin in this titration curve is ?

A-7 B- 3 C- 6 D-4 E- 8



2-In Lysin amino acid the net charge of the molecule at Pka= 9 is :

A-+1 B- 0 C- +2 D- -2 E- 0.5

3-In Cystine amino acid the net charge of the molecules at Pka = 8 is :

A -1 B- +1 C- 0 D- (-0.5) E- +2

4- For arginine amino acid if you knew the Pka1 = 2 for carboxylic group and Pka2 = 9 for amino group and Pka 3 for the side chain is 12.5 the Isoelectiric point is :

A -5.5   B-7.25   C-9.7   D- 10.8   E- 11.9

5-one of the following amino acids has an can exist In the Cis conformation when forming a polypeptide

A - Glycin
B – Lysin
C- serine
D- Valine
E- Prollin

Answers : 1- C the isoelectric point of nonpolar amino acid is between the 2 Pka values : (9.6+ 2.3) /2 =6
2- E At PH = 9, 50% of the molecules will have a net charge of +1 and 50% will have a net charge of
zero, thus the net charge of the solution is 0.5
3- D At PH = 8, The carboxylic group will be deprotonated, while 50% of the solution amino acids
will have the SH group protonated with a net charge of zero and 50% will have it deprotonated
with a net charge of -1 . Thus at PH = 8 the net charge of the solution will be -0.5
4- D if you follow the titration curve you will find the arginine become in the zwitterionic form between pKa2 and PKa 3
So IP = (12.5 +9)/2 =10.8

5- E
Done by : Salsabeela Bani hamad
Corrected by : Ali Khresat
"Keep going , each step may get harder but don't stop the view is beautiful at the top "