BIOCHEMISTRY

Faculty of medicine – JU2018

◉ Sheet ○ Slides

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Revision

- In the last lecture we talked about amino acids -Which are the precursors of Proteins-

-Remember that you have to memorize the classification, three letter abbreviation, -and a little bit of the structure – for example in the exam you'll be given the structure of Proline for example and you have to know that this is Proline or you'll be given a structure of a certain polar amino acid and you have to know that it's polar and to differentiate between it and other non-polar amino acids-

- We said that the structure of Proline is very important because it has a lot of effects on other proteins.

-Now some questions to revise the important notes from the last lecture:

- What is the smallest amino acid? Glycine
- What is the only amino acid that is achiral? Glycine
- What are the 2 amino acids that have sulfur in their side chains? Methionine & Cysteine
- What is the name of the bond that characterizes Cysteine? Thiol -Remember that it's terminal, and it's reactive to form Disulfide bond-
- What is the name of the molecule that Methionine can form? S-Adenosyl-L-Methionine (which is a derivative of Methionine)
- Mention the 3 amino acids containing Hydroxyl groups -OH- in their R-groups? Serine, Threonine, Tyrosine
- The Hydroxyl groups in Serine, Threonine and Tyrosine can be modified, what is the name of this modification? Phosphorylation (Addition of Phosphate groups, and this modification is important because it regulates Proteins and their structures.
- What are the 3 amino acids that are aliphatic non-polar? Valine, Leucine, Isoleucine -Remember the branching pattern of leucine and isoleucine (in isoleucine the branching is at the β-Carbon, whereas in leucine the branching is at the γ-Carbon)-
- There are 3 amino acids that are positively charged, what are they? Arginine, Lysine, Histidine -Histidine is the precursor of an important neurotransmitter and signaling molecule which is Histamine-

- What is the amino acid that has a double ring structure? Tryptophan (Trp)
  - Tryptophan is the precursor of 2 important molecules: Melatonin (For day-night cycle), and Serotonin (A neurotransmitter that gives the feeling of happiness)-
- What is the difference in structure between Phenylalanine and Tyrosine? The Presence of a Hydroxyl group in Tyrosine

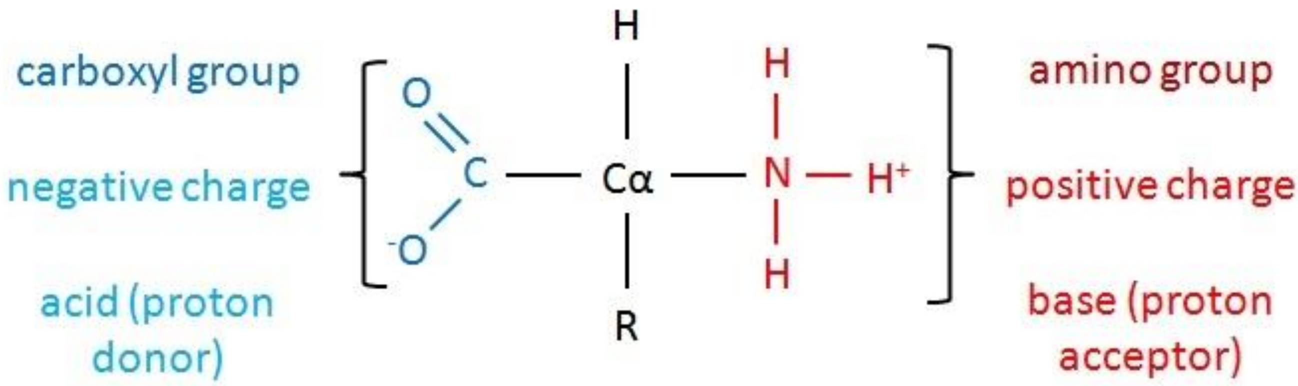
That was the most important notes, now let's start with our lecture which is:

Ionization of Amino Acids

Amino acids have 2 groups which can be ionized (protonated or deprotonated):

1-The carboxyl group (COOH) can act as a weak acid and loses a proton (deprotonation) to form the conjugate base carboxylate (COO^-)

2-The amino group (NH_2) which can act as a weak base and gains a proton (protonation) to form the conjugate acid (NH_3^+)



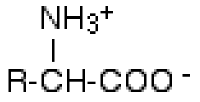
As the carboxyl group act as a weak acid so it has its own Pka as well as the amino group

Remember: The Pka is the PH when the concentration of the acid equals the concentration of the conjugate base, or when the concentration of the base equals the concentration of the conjugate acid

So, when the PH of the solution reaches the Pka of the Carboxyl group, then you can say that 50% of the acid (carboxylic acid) is now deprotonated, and if the PH of the solution had reached the Pka of the base(the amino group), then you can say that 50% of the amino groups are now protonated.

Now let's talk about some new terms

-Zwitterion: Molecule with two opposite charges and a net charge of zero (dipolar ion)



a zwitterion

-When a molecule exists in the PH whereby its net charge is equal to zero, we say that this molecule is in the Zwitterionic Form.

-Isoelectric Point (PI): The PH when a molecule is ionized or charged but the net charge is zero (the PH when the zwitterion is formed)

Now Dear doctor Notice that

If the amino group was → protonated → It is positively charged

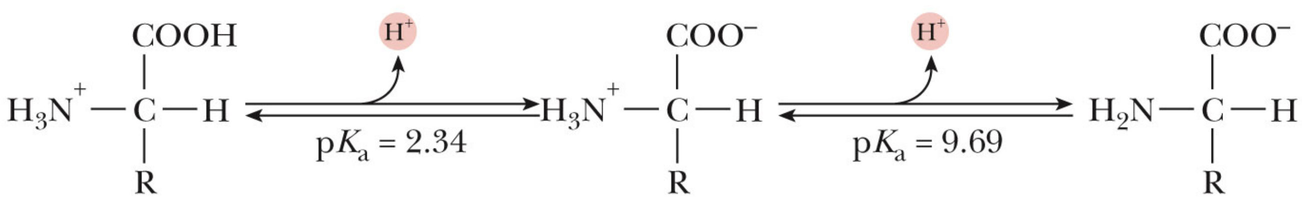
→Deprotonated →It is with no charge

If the carboxyl group was →Protonated → It is with no charge

→ Deprotonated → it is negatively charged

Effect of PH

Isoelectric zwitterion



-At a very low PH both groups (the carboxylic group and the amino group) will be protonated (The net charge is +1)

****Remember that decreasing the PH requires increasing the concentration of the free protons in the solution so at a very low PH there is abundance of free protons, so the two groups can be easily protonated**

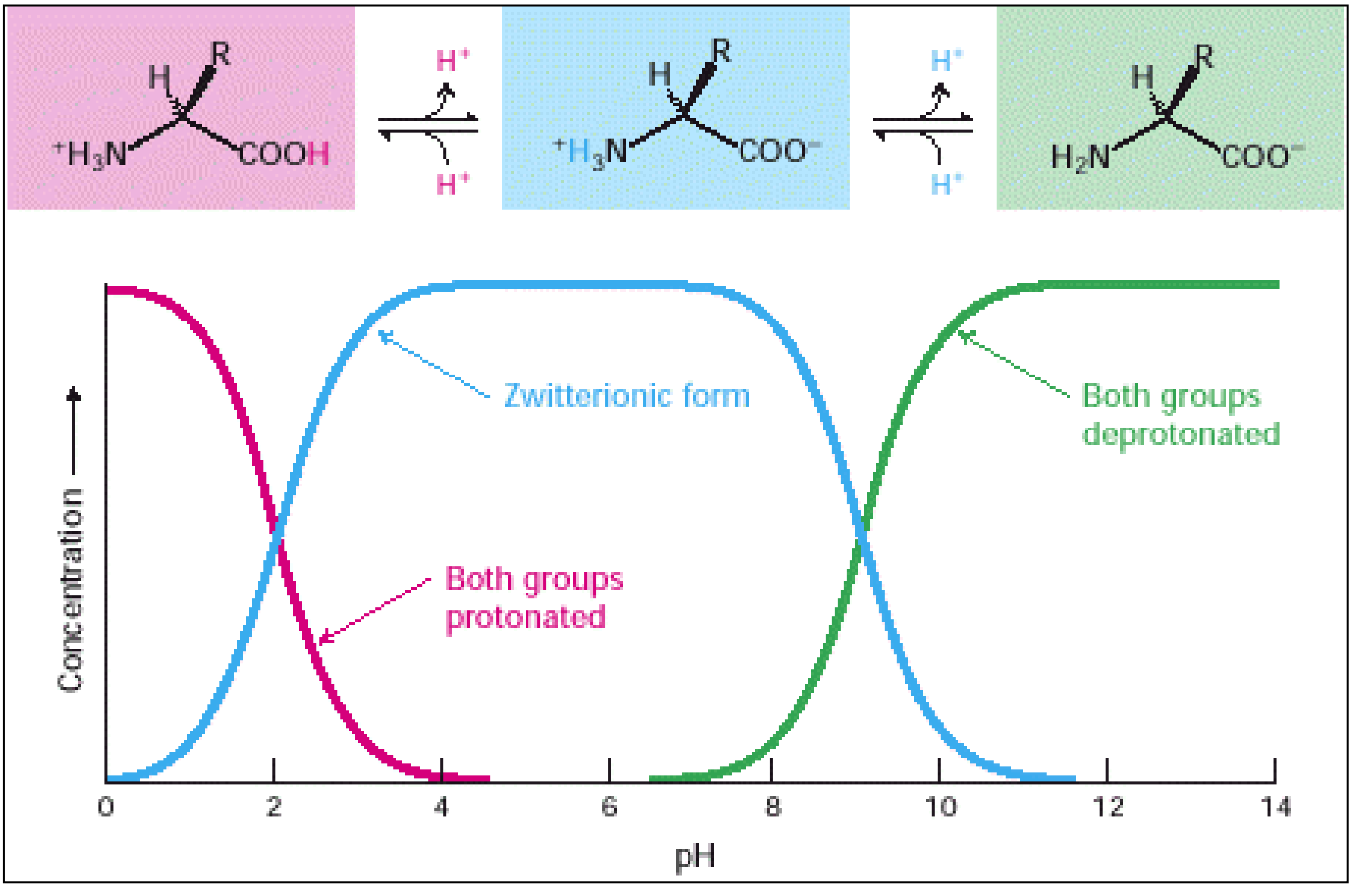
-As we increase the PH and get closer to the Pka of the carboxyl group (which approximately equals to 2) it starts to lose its proton and 50% of its protons is lost when the PH reaches its Pka (The net charge is +1 due to the protonated)

- If the PH reaches the isoelectric point, so 100% of the amino acid is now in the Zwitterion form (The net charge is zero as the carboxyl group is 100% deprotonated and the amino group protonated)

- if the PH goes higher than the PI and closer to the Pka of the amino group (Which approximately equals 9), it starts to lose its proton (The net charge is -1 due to the negative charge on the carboxyl group)

-When the PH equals the Pka of the amino group, 50% of the amino groups are now deprotonated

-At very high PH, all the amino groups will be deprotonated as well as the carboxyl groups (The net charge is -1)



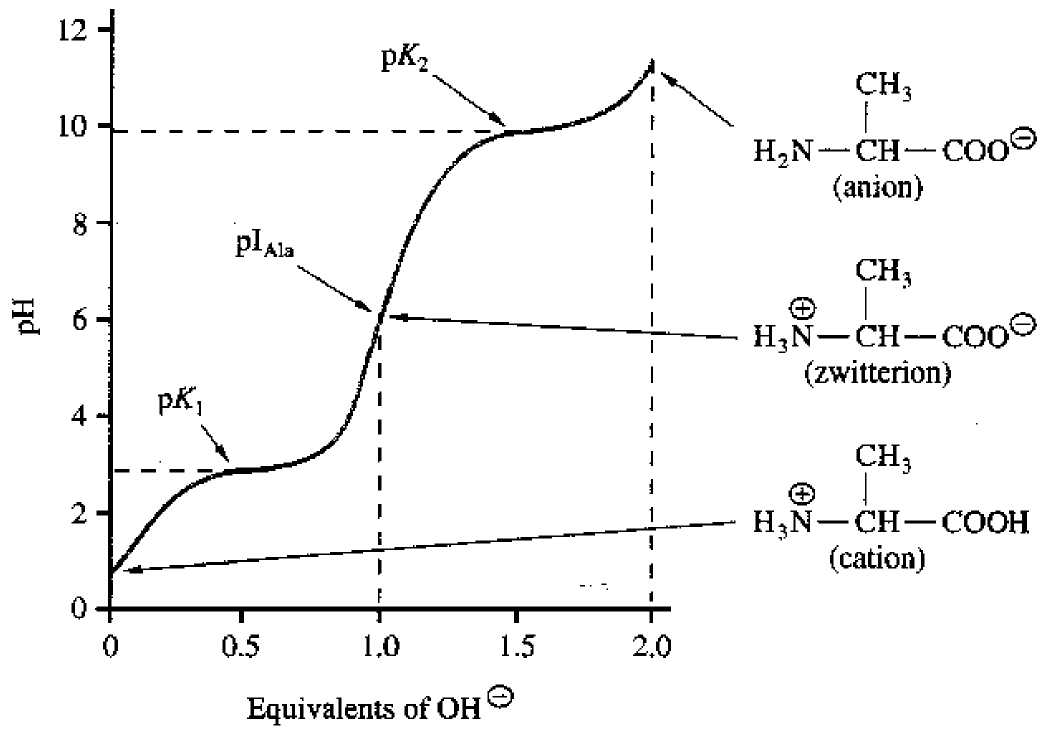
Each amino acid has at least 2 Pka values according to its structure, so each amino acid can work as a buffer with 2 buffering capacities

-The first cross point is the Pka where 50% of the carboxyl group is protonated and 50% is Deprotonated (COO^-), and as we said if we increase the PH more we'll have all the carboxyl groups being deprotonated and we reach the zwitterionic form where the net charge is 0 .

-The second cross point is the Pka where 50% of the amino group is deprotonated and 50% is protonated (NH_3^+), and if we increase the PH more we'll have all the amino groups being deprotonated and the net charge will be -1 .

Some amino acids have 3 Pka values and the third one is related to their side chains (will be discussed later)

Example: Alanine



-The first Pka is for the carboxyl group and the second the one is for amino group

-Always remember that the Pka is the point where we have 50%of the anion or the cation and 50%of the zwitterionic form.

-Dear Doctor, just try to apply the previous steps in this titration curve of Alanine

-To calculate the PI for such amino acid, we take the average of the carboxyl group Pka and the amino group Pka

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

So, by applying this equation to the previous example you can say

PI = (2+9)/2= 5.5

The isoelectric point of the alanine is 5.5

Ionization of side chains

-Nine of the 20 amino acids have ionizable side chains, these amino acids are tyrosine, cysteine, arginine, lysine, histidine, aspartic acid, and glutamic acid

-Remember that the ability of the protonation or deprotonation is not determined by the presence of a charge on the molecule, so among these nine amino acids there is 4 neutral side chains, 3 positively charged side chains in (Arginine, lysine, and Histidine), and 2 negatively charged side chains in (Aspartic acid and Glutamic acid)

-As these side chains can be protonated or deprotonated, they have their own Pka values also

-The table below shows some amino acids and their Pka and PI values

***Note: This table must be memorized, and it is very important**

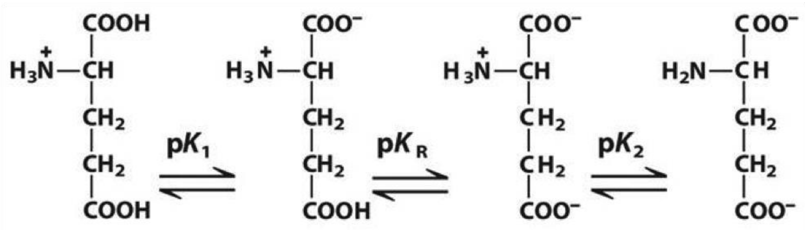
Amino Acid	Side Chain pK_a [3]	pI
Arginine	12.5	10.8
Aspartic Acid	4.0	3.0
Cysteine	8.0	5.0
Glutamic Acid	4.1	3.2
Histidine	6.0	7.5
Lysine	11.0	10

Calculation of pI of amino acids with ionizable R groups

-As there is a difference in the Pka values of the amino groups and the carboxyl groups among the backbones of the different amino acids, let 's consider Pka of (NH2) = 9 and the Pka of the (COOH) = 2 for all amino acids

-The isoelectric point for these amino acids is calculated by taking the average of the pKa's of the groups with same charge when ionized

Example: Glutamate



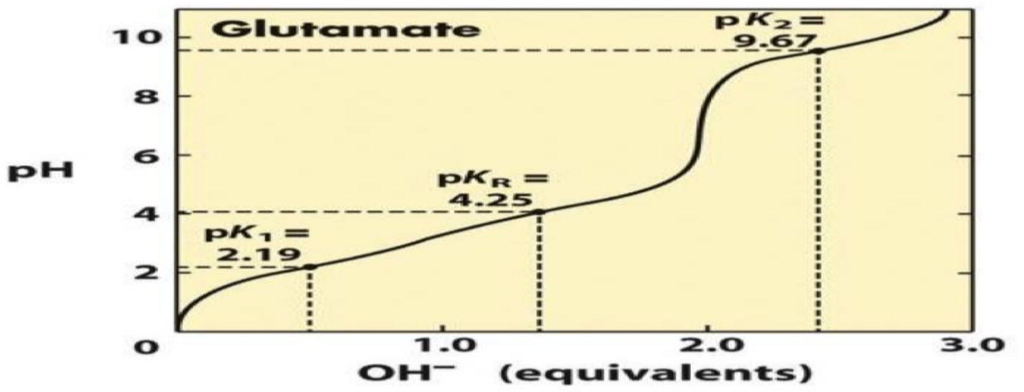
-To calculate the isoelectric point of Glu the Pka's of the 2 carboxylic groups are averaged (they have the same charge when ionized)

-So what happens here is exactly the same as what we have mentioned earlier, first when we increase PH the carboxyl group of the α-carbon start to deprotonate until we reach the Pka of this carboxyl group where the net charge equals to 0 (Zwitterionic form), then at higher PH values the carboxyl group of the side chain starts to deprotonate until we reach the Pka of this carboxyl group where the net charge equals to -1, then when we increase the PH more and more the amino group starts to deprotonate until we reach its Pka value where the net charge equals to -2 .

-The total charge of glutamate at a very high PH is (-2) instead of (-1), this due to the deprotonation of the carboxyl group in the side chain in addition to the carboxyl group of the backbone in such PH

-The second form represent the Zwitterion form of the glutamate

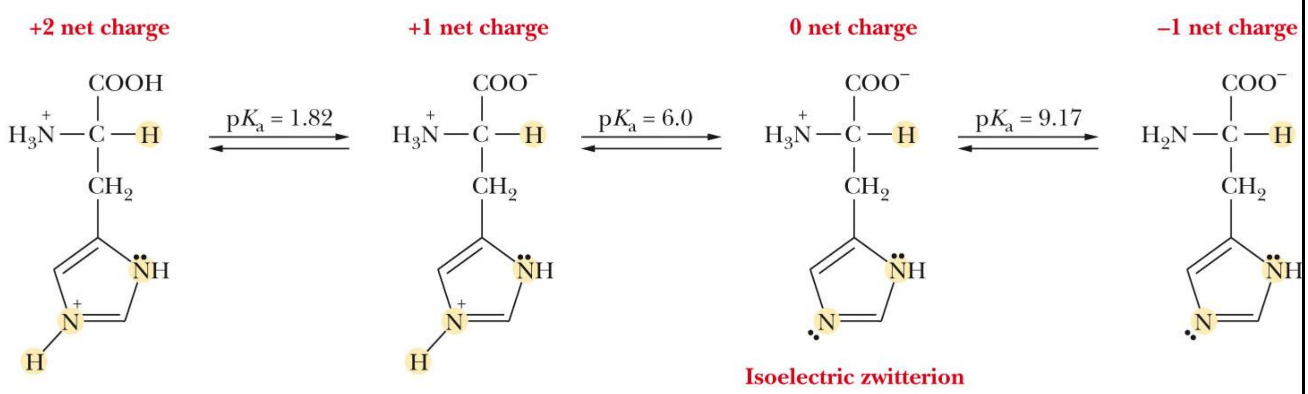
-Notice that the Glutamate can act as buffer with 3 buffering capacities



Example: Histidine

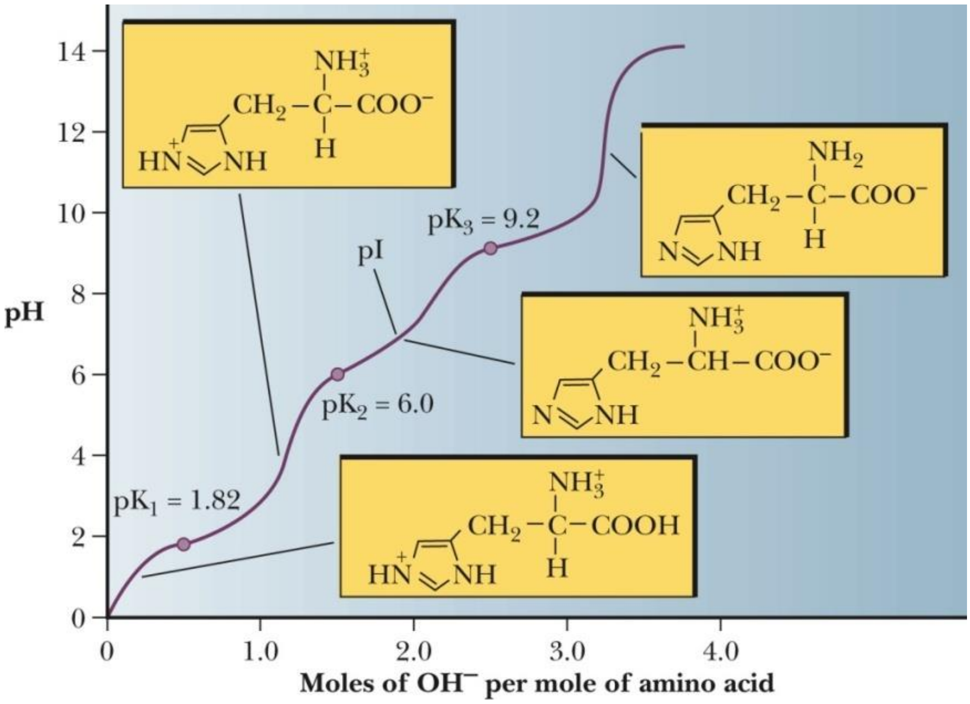
-Histidine is a very important amino acid in the body and the only amino acid which can act as a biological buffer in our body and this is because its Pka is close to the physiological PH of the blood

- **-PI = ~7.5 (The imidazole group can be uncharged or positively charged near neutral pH).**



-To calculate the PI of the Histidine you must take the average of Pka's of the amino group and the imidazole group (they have same charge when ionized)

-The third molecule of the equation represents the Zwitterion form of the Histidine



-Note the histidine has a net charge of (+2) instead of (+1) at a very low PH, this is because that the imidazole in addition to the amino group will be protonated at such PH, the carboxyl group will be also protonated, but it has no effect as its charge when protonated is zero

Questions (From the slides)

Q1: Draw the titration curve of Histidine

Q2: What is the ratio of conjugate Base/ acid of glutamate at PH 4.5

Q3: What is the total charge of lysine at PH 7?!

-What do you need to know from amino acids slides

The names of amino acids

The special structural features of amino acids

Their abbreviations or designations

The uncommon amino acids, their precursor and function (if any)

The Pka of groups ,not exact numbers, but which ones are acidic, basic, or near neutral