Doctor 022

Biochemistry

Sheet no. 13

Writer: Al-Razi Node Team

Corrector: Al-Razi Node Team

Doctor: Dr. Diala, Dr. Mamoun

Ionization of Amino Acids

Why do Amino Acids get Ionized?

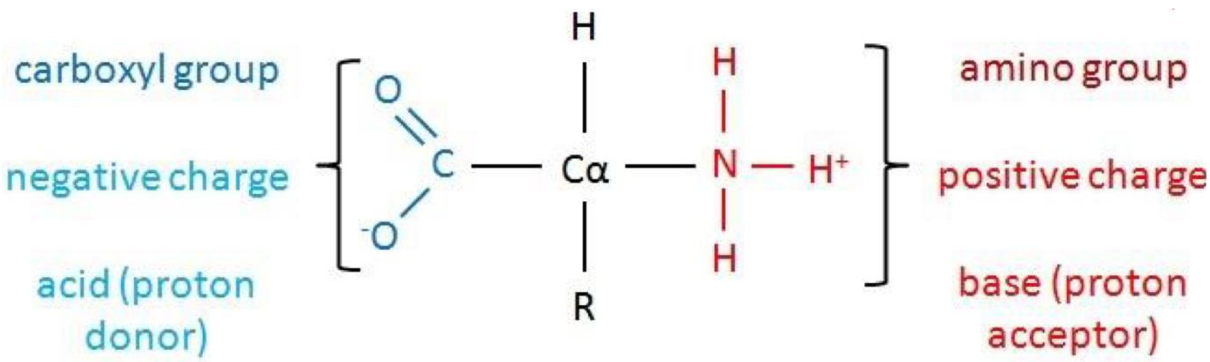
As we know, an amino acid has an amine group and a carboxylic acid group and both can accept or lose a proton.

So what happens to these groups at different pH values?

Let's start with the physiological pH:

At the physiological pH, which is around 7.4 in humans, the carboxyl group (COOH) of an amino acid is deprotonated and has a negative charge. The amino group (NH2) is protonated and has a positive charge. This is due to their pK_a values. The pK_a of the carboxyl group is around 2 at physiological pH, which is lower than the pH of 7.4, so the group is deprotonated (there are not enough H^+ ions so it will lose its proton). On the other hand, the pK_a of the amino group is around 9, which is higher than the physiological pH, so the group is protonated (there are too many ions for this group so it will be associated with H^+ ions).

****Remember: pK_a is the pH value at which a molecule is half-protonated and half-deprotonated. Other terms used to describe this state include half-charged and half-uncharged, half-ionized and half-un-ionized, and half in acid form and half in conjugate base form. The pK_a value is a measure of the strength of an acid or base, and it is related to the equilibrium constant for the acid-base reaction.**



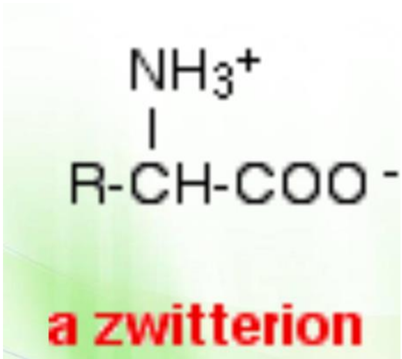
The Zwitterion and the Isoelectric Point:

A <u>zwitterion</u> is a compound that contains both a positive and a negative charge, but the total net charge is zero. This occurs when a molecule has both acidic and basic functional groups, such as amino acid, and the charges of the ions on the acidic and basic groups neutralize each other, resulting in a net charge of zero (when the -ve charge = +ve charge).

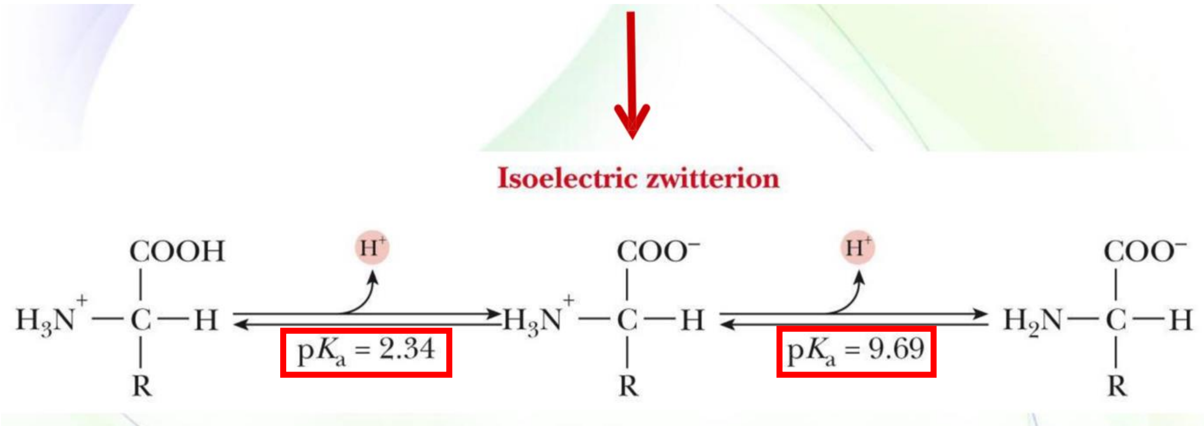
At physiological pH, non-polar and polar uncharged amino acids (without ionizable/charged groups) are electrically neutral.

Zwitterion: a molecule with two opposite charges and a net charge of zero.

The <u>isoelectric point</u> is the pH value when the molecule is in its zwitterionic form (when it has an equal number of positive and negative charges), resulting in a net charge of zero. This is the pH at which the acidic and basic groups of the molecule are deprotonated and protonated respectively, and the molecule exists as a <u>neutral zwitterion.</u>



The ionization state of an amino acid molecule is determined by the dissociation of its carboxylic acid and amino groups, which are influenced by the pH of the surrounding environment.



At low (acidic) pH values, all the functional groups in an amino acid molecule will be protonated. In such circumstances, both the carboxyl and amino groups will be in their acidic form, capable of donating a proton (H^+).

Explanation:

At very low pH values, the solution is rich in H^+ ions, causing all functional groups in an amino acid molecule to become protonated.

Notice that: The carboxyl group, upon protonation, becomes neutral, while upon deprotonation it acquires a negative charge. Conversely, the amino group, upon protonation, acquires a positive charge, while upon deprotonation it becomes neutral.

As the pH is gradually increasing, the carboxylic acid group will begin to lose its proton. At a pH equal to its pK_a value, which is typically around 2, half of the carboxylic acid groups in the amino acid molecule will be protonated and the other half will be deprotonated.

As the pH is increasing, all the carboxylic acid groups in the amino acid molecule will eventually lose their protons.

At a pH of 8, the amino group begins to gradually lose its protons.

When the solution reaches a pH of around 9 (which is the same as the pKa value of the amino group), half of the amino groups in the amino acid will be protonated and the other half will be deprotonated.

We conclude as follows: At very high pH values, all functional groups in the amino acid molecule will be deprotonated, resulting in a negatively charged carboxyl group and a neutral amino group.

To sum up:

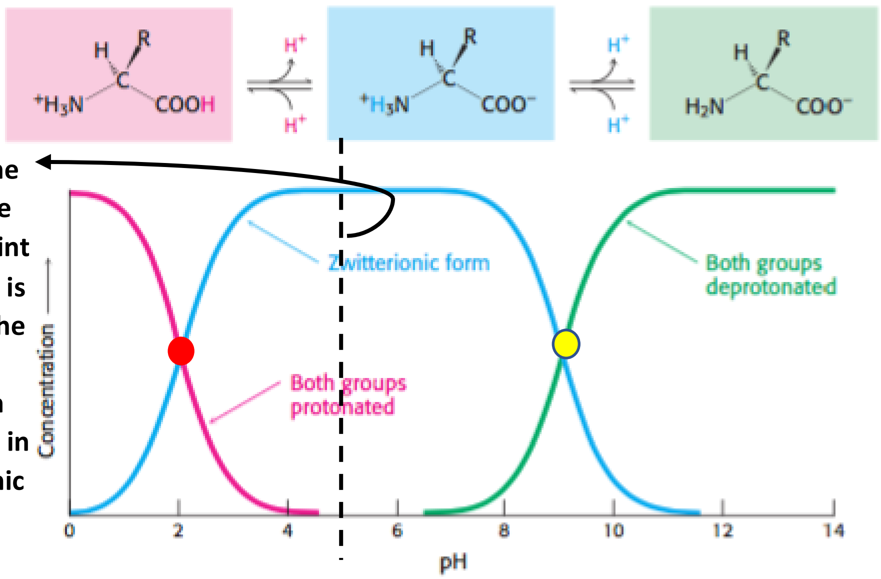
At very low (acidic) pH values, the amino group in the amino acid molecule will have a positive charge, and the carboxyl group will be neutral, resulting in a net charge of +1.

At very high (basic) pH values, the carboxyl will have a negative charge, and the amino group will be neutral, resulting in a net charge of -1.

At a pH where the negative and positive charges are equal, the amino acid molecule will have a net charge of zero, and it will be in the zwitterion state.

It's important to note that all of these pH values are specific to the functional groups in the amino acid molecule and not to the amino acids themselves.

The dotted line represents the isoelectric point (≈ 5.5), which is the pH with the highest concentration of amino acid in the zwitterionic form.



The concentrations of the fully protonated and the zwitterionic forms are equal (50:50) when the pH is equal to the pK_a of the carboxyl group (≈2).

The concentrations of the zwitterionic and the fully deprotonated forms are equal (50:50) when the pH is equal to the pK_a of the amino group (≈9).

This diagram once again illustrates the ionization of amino acids

Pink box = Pink line, Blue box = Blue line, Green box = Green line

As it is shown in the graph above, in an acidic environment (low pH) both the amino and carboxylic group are protonated (high concentration for pink).

If the acidic environment gradually changes into a more basic one, the concentration of the amino acid in the fully protonated form will decrease (decrease for pink) while the concentration of the neutral zwitterionic form increases (increase for blue). This is because as the pH of the environment becomes greater than the pK_a value of the carboxyl group (-COOH), the more the concentration of its conjugate base ($-COO^-$) increases, and after a certain point, all of the amino acids will be in the zwitterionic form.

If the pH of the environment of the amino acid increases even further and becomes even more basic, the amino groups of the amino acids will lose their proton and thus, lose their positive charge. The more basic the pH becomes, the more amino acids will be completely deprotonated (green increases) and the concentration of amino acids in the zwitterionic form decreases (blue decreases).

The pH where the concentrations of the fully protonated and the zwitterionic amino acids were the same, is equal to the pK_a value of the carboxyl group.

The pH where the concentrations of the zwitterionic and the fully deprotonated amino acids were the same, is equal to the pK_a value of the amino group.

We have previously mentioned that the isoelectric point is the pH value at which nearly all amino acid molecules are in the zwitterionic (neutral) form.

A more accurate definition would be:

The isoelectric point (pI) is the pH where the net charge of a molecule such as an amino acid or a protein is zero.

We have also mentioned that the isoelectric point (pI) for amino acids with nonpolar or polar uncharged side chains is 5.5. But how did we reach to this value?

Let's take the nonpolar amino acid Alanine as an example. Alanine is considered nonpolar because its side chain only consists of a methyl group, and therefore it only contains 2 ionizable groups in its structure (the amino and the carboxyl groups).

It's important to note that when we talk about pK_a, it's only concerning the ionizable groups (carboxylic or amino) in the amino acid, but when we talk about the isoelectric point, we are talking about the amino acid as a whole.

Therefore, in order to calculate the pI of the entire molecule, the pK_a's of all ionizable must be taken into consideration.

Since Alanine and all the other nonpolar amino acids (and some polar uncharged amino acids) only contain 2 ionizable groups, they only have 2 pK_a's. Therefore, their pI is calculated by taking the numerical average of the carboxyl group pK_a (≈ 2) and the amino group pK_a (≈ 9):

(2 + 9)/2 = 5.5

$$\mathrm{pI} = \frac{\mathrm{p}K_{a1} + \mathrm{p}K_{a2}}{2}$$

In the case of nonpolar and some polar uncharged amino acids, pK_{a1} and pK_{a2} are those of the carboxyl and amino groups.

This would mean that when pH is 5.5, almost 100% of the the amino acid will be in its zwitterionic form, with an unprotonated ionized carboxylic group and a protonated ionized amino group (the total net charge is zero).

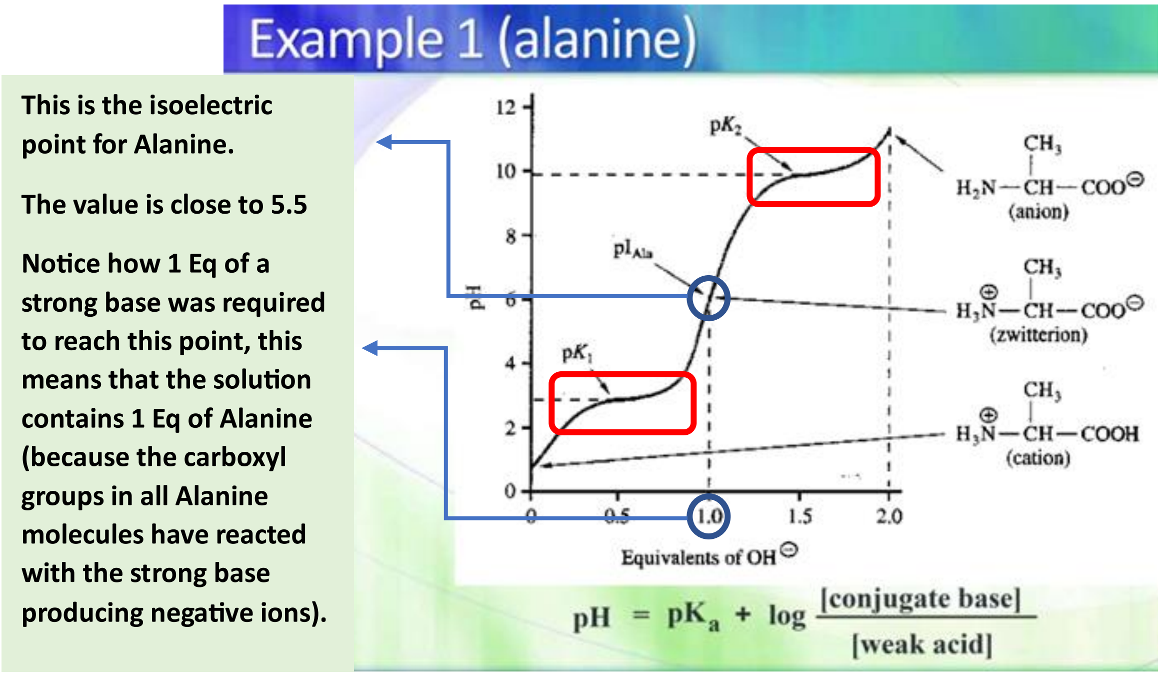
This is how the form of Alanine changes as the pH of its environment changes:

NOTE: (هاي الملاحظة الدكتور اهتم فيها كثير)

When the <u>pH is less than the pK_a</u> of the group, the <u>group will be protonated</u>.

If <u>pH is greater than the pK_a</u> of the group, the <u>group will be un-protonated</u>.

(To be exact, when the pH is less than the pK_a of the group, this group will be protonated in more than 50% of the amino acids present, and vice versa).



It is important to emphasize the fact that the protonated form of both the amino and the carboxyl group have the characteristics of weak acids (although the protonated amino group is much less acidic than the carboxyl).

This means that amino acids with 2 ionizable groups such as Alanine can act as weak diprotic acids, and can therefore be titrated and even used as buffers

-Notice the red rectangles around the buffer regions of both groups, which shows how these groups help resist the change in pH when a strong base is added, just as buffers do. This allows amino acids to act as buffers in the body at different pH levels.

-The zwitterionic form of the amino acid is found as a majority in the region between the pK_a's of both groups, and it is only at the isoelectric point (around 5.5 in this case) that only the zwitterionic form is present.

The Ionization of Side Chains:

Not all amino acids have only two ionizable groups in their structures found in their backbones, some amino acids have other ionizable groups on their side chains.

In fact, nine of the 20 amino acids have ionizable side chains that act as weak acids and thus help in buffering.

These amino acids are:

Tyrosine, Serine, Threonine, and Cysteine (which are polar amino acids)

Lysine, Arginine, and Histidine (which are positively charged amino acids)

Aspartic and Glutamic Acid (both of which are negatively charged)

***Very Important: Each side chain has its own pK_a value for ionization.**

The pI of Amino Acids

Memorize all these pK_a values

Side chain: R group, pI: isoelectronic point

Let's consider pK_a of $-NH_3^+$= 9 and pK_a of –COOH = 2 (for the backbones of all amino acids).

*Tip: try to revise the amino acids while studying this table (abreviations, R-groups...), such as:

What is the R group for Arginine? Is it positively or negatively charged amino acid? → Guanidinium, positively charged

What is the charge of sulfur in cysteine when losing its proton? → negatively charged

Amino Acid	Side Chain pK_a	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

-These R groups can play a role in buffering, an even more important role than the that of the carboxylic and amino groups of the backbone; when discussing peptides and proteins there will be no carboxyl group and amino group for amino acids except the first one and the last one.

(This means that only the pK_a of the first amino group, the last carboxyl group, and the pK_a's of all the side chains will affect the isoelectric point of peptides and proteins).

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

-In this case, to achieve the zwitterionic form, the total charge on the groups with similar charges must equal one (1) so that the one (1) opposite charge present on the molecule can balance them.

*Understand the concepts, don't memorize them.

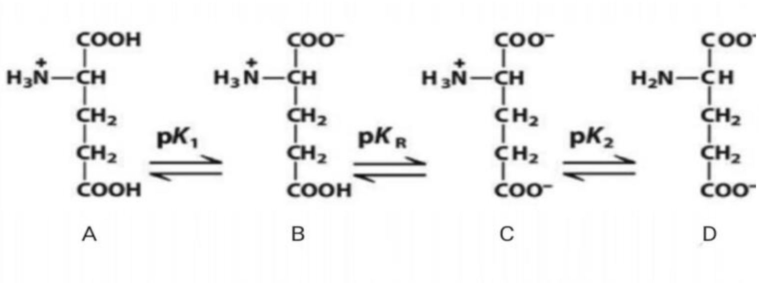
What type of questions will we face in the exam?

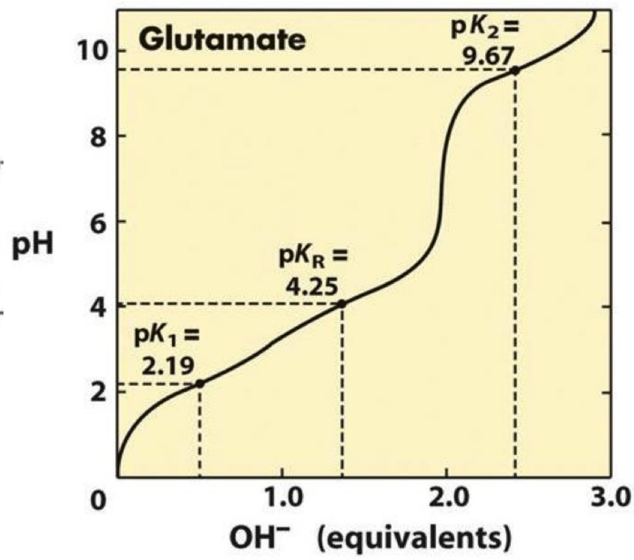
We will be given a titration curve and will be asked about the amino acid.

Example: Glutamate

Glutamate has an acidic amino group, and therefore has 3 ionizable groups (a carboxyl and amino group in its backbone and an R group that contains a carboxylic group as well); so it can carry 2 negative charges and one positive charge.

Does it have a zwitterionic form?...yes!!
What is its isoelectric point?! lets understand ☺, Use the curve!!





- At a very low pH (pH=1), all the groups are protonated,
  - The main carboxyl group has no charge.
  - The carboxyl group of the R group has no charge.
  - The amino group has positive charge.
  - The total charge = +1

- By gradually increasing pH, we will reach pH = $pK_{a1} \approx 2$:
  The carboxyl group pK_a of the backbone will act as a buffer specifically the main carboxyl group

- Reaching pH = $pK_{aR} \approx 4$: the R group pK_a, the R group is half protonated and half unprotonated (½ COO- and ½ COOH,* recall the term of pk_a):
  - The main carboxyl group is deprotonated, and has a negative charge.
  - The amino group is protonated ,has positive charge
    *Recall when pH is lower than pK_a the group is protonated
  - What is the buffering region of the Glutamate <u>R group,</u> not the main carboxyl group?
    Answer: pH : (3, 5) = (4-1, 4+1)

By adding more OH^- / strong base, the pH increases dramatically reaching the 3rd region (amino group region):

- pH = 9 = $pK_{a2} \approx 9$: the amino group is half protonated and half deprotonated:
  - The buffering range of the amino group of the backbone's amino acid: (9-1, 9+1) = (8, 10)

What is the Isoelectric Point of Glutamate?

Let's think together: ☺ Hint: use the figures

- When the pH = 7

○ Main carboxyl group → pH is higher than pK_a → deprotonated.
→has negative charge.
○ Side chain carboxyl group → pH is higher than pK_a → deprotonated.
→has a negative charge.
○ Amino group → pH is lower than pK_a → protonated.
→has positive charge.
The net charge = -1

- pH = 1

○ Main carboxyl group → pH is lower than pK_a → protonated.
→neutral.
○ Side chain carboxyl group → pH is lower than pK_a →protonated.
→neutral.
○ Amino group→ ph is lower than pka →protonated.
→has positive charge.
The net charge = +1

- pH = 3

○ Main carboxyl group → pH is higher than pK_a → deprotonated.
→has negative charge.
○ Side chain carboxyl group → pH is lower than pK_a → protonated.
→neutral.
○ Amino group→ pH is lower than pK_a → protonated.
→has positive charge.
The net charge = 0

THIS IS THE ISOELECTRIC POINT AND FIGURE B IS THE ZWITTERIONIC FORM!!

To calculate the isoelectric point of Glu, the pK_a's of the two carboxyl groups are averaged.

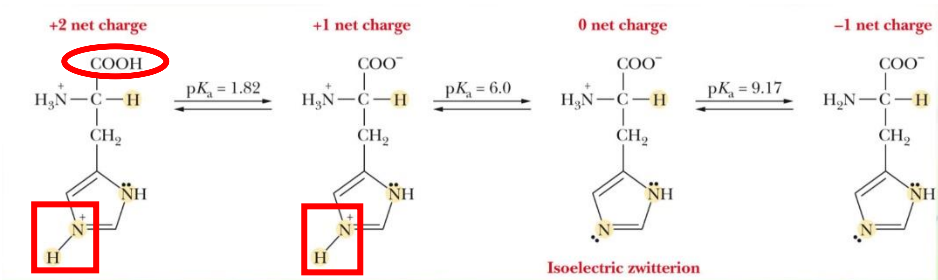
General Rule: Take the average pK_a of the 2 groups that have the same charge.

In the exam, they may bring a hypothetical molecule, not an actual amino acid, which is why we need to understand the concept.

(A student asked: between which two points we take the average? Doctor's answer: If we had two groups of opposite charges we take the average between them, if we had three groups of different charges, two of them are negative and one positive we take the average for the negatively charged molecules.)

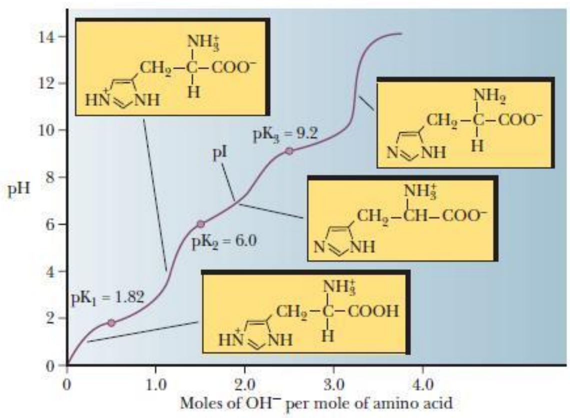
Another Example, Histidine:

Histidine is a positively charged amino acid, which means that if the Nitrogen in its side chain (in the red square) is protonated, it will be carrying a positive charge, and if it was deprotonated it won't carry any charge (neutral).



The pK$_a$ of its R group is 6, which is near physiological pH.

In the following plot:



The pH at the beginning is extremely low, but as we added a strong base, the pH will rise and will enter the buffering range of the backbone's carboxylic group ($pK_{a1} \approx 2$). As the pH reaches 3, the buffering abilities provided by the carboxylic group decrease, which increase the rate at which the pH is rising. This rapid increase continues until the pH level enters the buffering range of Histidine's R group ($pK_{aR} \approx 6$, buffering region: 5-7) and the same process occurs until it reaches the third region ($pKa2 \approx 9$) where the backbone's amino group gets deprotonated.

Therefore, we have three buffering ranges for the three ionizable groups (with different midpoints).

To sum up, Histidine can act as a buffer around pH=2, pH=6 and pH=9.

So, what is the isoelectric point of histidine?

When pH=1:

The carboxyl group will be protonated (no charge), the R group and amine group are protonated (positive charge), so the total net charge is +2.

When pH=4:

The carboxyl group is deprotonated (negative charge), the R group and amine group are both protonated (positive charge), so the total net charge= +1 .

When pH = 8:

The carboxyl group is deprotonated (negative charge), the R group is deprotonated (no charge), and the amine group is protonated because pH is less than pK_{a2} (positive charge), so the total net charge = 0 → zwitterionic.

This means that the isoelectric point is precisely (6+9)/2 = 7.5

(Note: 6 and 9 are the pK_a values of the positive charges).

Since the pK_a of Histidine's R group is near physiological pH, it will play a very important role in buffering physiological environment in cells, blood, and so on…

In addition to the amino groups and carboxyl groups of proteins and peptides, the R groups of amino acids and especially Histidine's Imidazole group are all very important.

Some might wonder that pK_{aR} = 6 is not close to physiological pH (7.4), so why do we considered it to be near to the physiological pH?

It's because we have large amounts of Histidine in proteins, which is why it won't be 6, and instead, it will be 7.3 (it's influenced by the surrounding environment which there are lots of R groups and molecules. We'll find van der waals, hydrophobic and electrostatic interactions and hydrogen bonding).

Meaning that although the pK_{aR} of an individual Histidine is 6, it won't remain that way in the protein systems of our bodies, increasing it to about 7.3.

An example of a protein buffer is Albumin, which is the predominant plasma protein (~60%), has 16 His/mole, and is thought to account for 95% of non-carbonate buffering action in plasma.

In our blood we have millions of Albumin molecules which means that we have a huge amount of R groups of Histidine, but it is still unknown whether the role of albumin in buffering blood is important or not, but our textbook considers it important.

Practice Questions:

1) **What is the ratio of conjugate base / acid of glutamate at pH = 4.5?**
2) **What is the total charge of Lysine at pH 7?**

Answers:

1) **pK_{aR} for Glutamate is 4.1, which means that at pH 4.5, the solution would be in the buffering region of Glutamate's R group.**

   To find the ratio of conjugate base / acid we must apply the Henderson-Hasselbalch equation:

   pH = pK_{aR} + log conjugate base/acid → 4.5 = 4.1 + log conjugate base/acid → log conjugate base/acid = 0.4 → conjugate base/acid = 2.5

2) **The pK_{aR} value for Lysine is 11, which means that Lysine's backbone amino group ($pK_{a2} \approx 9$) will get deprotonated before the amino group of its R group.**
   At pH 7:
   The backbone's carboxyl group will carry a negative charge, the backbone's amino group will be mostly carrying a positive charge, and the amino group on Lysine's side chain will carry a positive charge.
   Net charge: +1

Formation of Polypeptides

Definitions and concepts:

A residue: each amino acid in a (poly) peptide. They could also be building blocks (monomers) of larger molecules (such as glucose residues making up glycogen).

So if we say "amino acid residue", we mean that the amino acid is part of a larger protein or polypeptide, but if we say "the amino acid Histidine" for example, we are referring to a single Histidine molecule.

****Dipeptide, tripeptide, tetrapeptide, etc:**

Dipeptide: contains 2 amino acids. Tripeptide: contains 3 amino acids.

****Oligopeptide: a short chain of (20-30) amino acids.**

****Polypeptide (liner): a longer peptide with no particular structure.**

****Protein: polypeptide chain or chains with 3D structure + functions.**

The average molecular <u>weight of an amino acid residue</u> is about <u>110</u>.

The molecular <u>weights of most proteins</u> are <u>between 5500 and 220,000</u>.

no. of amino acids per one protein almost between 50 and 2000 amino acids:

(5500/110 = 50 and 220,000/110 = 2000).

Ex: If we say we have polypeptide (100 amino acids), the size of the polypeptide will be 100*110 Da

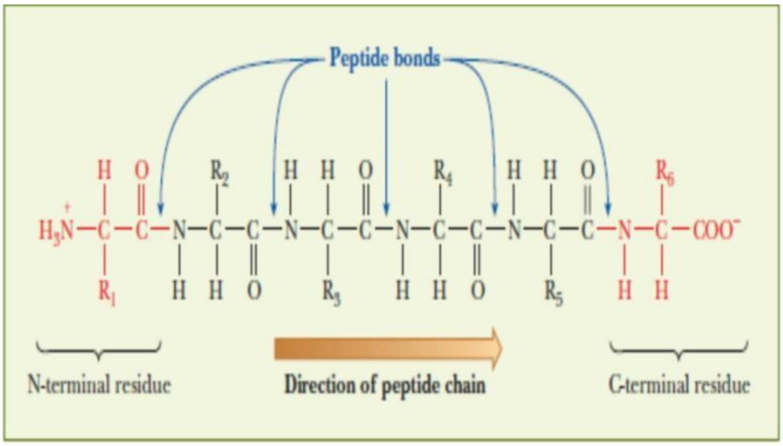
- **We refer to the mass of a polypeptide in units of Daltons (Da) or (kDa = 1000 Da). A 10,000 MW protein has a mass of 10,000 Da or 10 kDa.**

Peptide bonds:

The bond that connects two amino acids together. In organic chemistry, we call them amide bonds, but inside polypeptides we call them peptide bonds.

Amino acids are joined together by condensation chemical reactions (dehydration reactions). The peptide bond is formed between a protonated amino group in the backbone of one amino acid (loses two hydrogen atoms), and a deprotonated carboxyl of the backbone of another one (loses an oxygen atom). R groups have nothing to do in this reaction.

As it is shown in this figure, the beginning of the molecule is the amino group (N-terminus) and the end of the molecule is the carboxyl group (C-terminus), when we add third amino group we add it to the carboxyl group (forming another peptide bond).



Features of peptide bonds:

**** The peptide bond has a resonance structure:**

The double bond between the C and O can be shifted to be between the C and N, leaving the oxygen negatively charged and the nitrogen positively charged so the peptide bond can be charged.

****A Zigzag structure:**

****Because of its resonance structure, it forms a (semi) Double Bond between two amino acids:**
Resulting in the polypeptide being planar, charged, rigid, and un-rotatable (“fixed in space").
Instead, the R groups are the ones able to rotate up and down.

****Hydrogen bonding except in Proline (Pro).**