Lecture : 2

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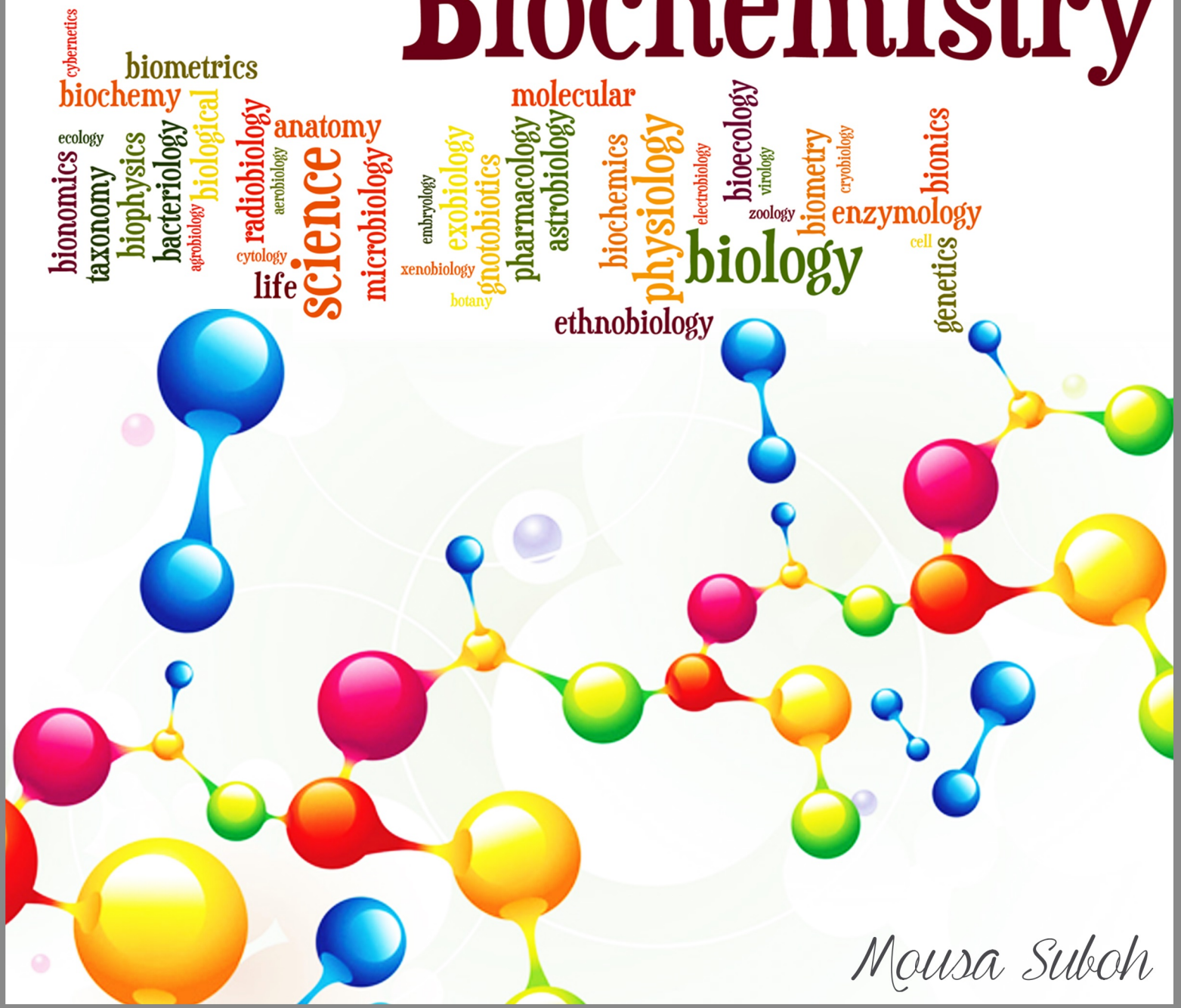
Medical Committee
The University of Jordan

Biochemistry



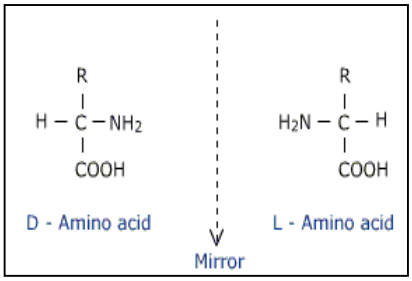
Mousa Suboh

AMINO ACIDS (2)

Questions from the previous lecture :

1-How can we distinguish between D-amino acid and L-amino acid?

According to the location of the amino group in the amino acid, the R group is above, the carboxylic group is below , then you look at the amino group if it is at the right "D amino acid" and if it is at the left "L amino acid ".



2- if the β carbon was branched , how do we name the other carbons ? Choose the longest chain bound toβ carbon and name them γ,δ , ...etc , and the shorter chain don't name it .

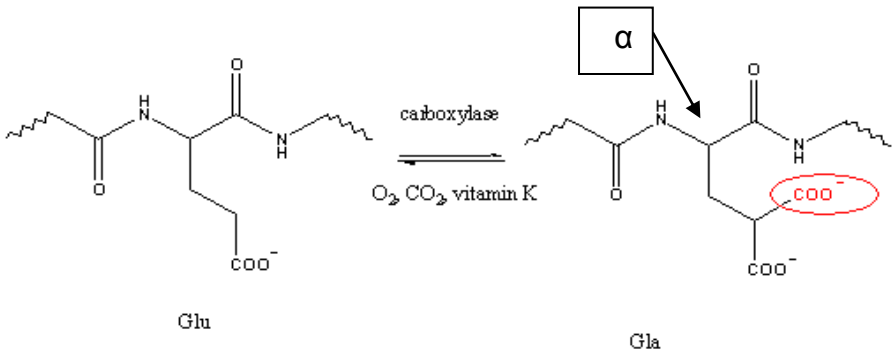
3- if we have a ring, how do we name them? It is not so much important

4- if we have 3 carbons in R group , what is the name of the last carbon? Omega ω ... but in lysine is not omega because it ends with NH_2.

Notes :1)it is important to know every amino acid to which class belongs , but it is not necessary to distinguish between amino acids in the same class structurally .

2)γ- carboxyglutamate, why is it calledγ ?

Because the carboxyl group is linked with γ carbon of this amino acid .



Notice here that the amino acid is not free , it is carboxylated as residue .

Ionization of amino acid

A very important thing about amino acids that they can act as buffers.

Buffers:- solutions that resist changes in PH, HOW ? by donating or accepting protons . ---Whenever the PH of solution goes up ,the buffer releases protons to compensate and vice versa.

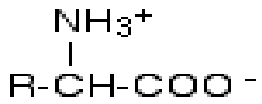
-Amino acids can act as buffers because they have both a carboxyl group and an amino group , both of them have their own (Pkas) , carboxyl group is a weak acid and the amino group is a weak base.

#At physiological PH , amino acids are charged "doubly charged", both of these groups will be ionized ; meaning that the carboxyl group will lose a proton and has a negative charge (unprotonated) while the amino group will get a proton and has a positive charge (protonated) .

#As a result , the net charge of an amino acid is zero-except the ones that have R group that can be charged- for example nonpolar amino acid as Alanine ,Valine,leucine , isoleucine...etc, which have only have two groups(amino group , carboxyl group) that can be protonated or unprotonated, ionized or unionized, charged or neutral.

#The net charge of such amino acid at physiological PH equals ZERO, because the positive charge will cancel the negative charge (neutral).

#Because they are charged and because they are still neutral we call them ***ZWITTERIONS***



a zwitterion

What is the effect of PH on amino acid ionization ?

What's really important and you must memorize is the pka of the carboxyl group" equals 2" and the Pka of the amino group " equals 9". "Forget about fraction in the values"

 # What does Pka mean ?

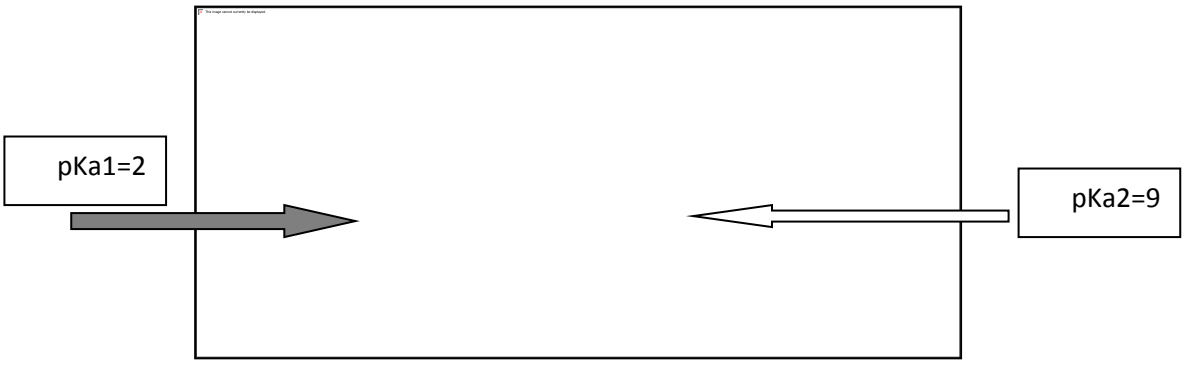
Pka is the PH when an acid or a molecule is half dissociated , also it is PH when the conjugate base concentration equals the acid concentration.

-50% protonated, 50%unprotonated.

-50%acidic form ,50% conjugate base form.

-so, PH=Pka

#Pka of carboxyl group is 2 means :at PH=2 50% protonated (COOH) ,50% unprotonated and ionized (COO-)



1-if we have the amino acid and very low PH, let's say at 1 it means that the carboxyl group will be protonated (unionized , neutral) ,the amino group will be protonated (ionized, positively charged) ,the net charge of amino acid at PH 1 is +1 .

2-The PH increases and when it reaches 2→ pKa of the carboxyl group : 50% of thecarboxyl group will be pronated and 50% of the carboxyl group will be unprotonated, in other words 50% are +1 charged and 50% are zero.

3-The PH increases more and more so the amino group is still protonated and ionized ,the carboxyl group is unprotonated and ionized, the total net charge is zero ,this is ZWITTERIONIC FORM

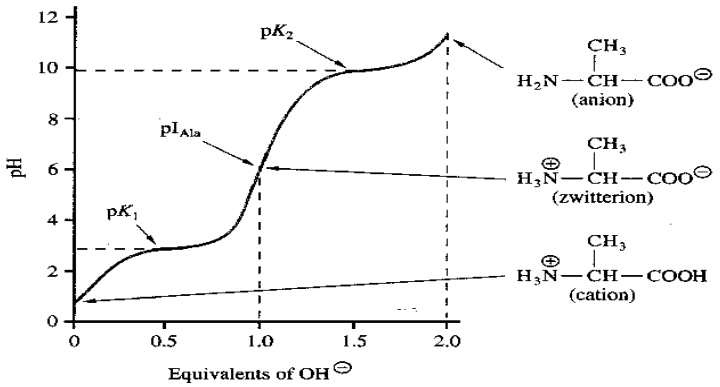
4-When PH reaches 9 → pka of amino group : it means that the amino group is half protonated and half unprotonated ,half ionized (positively charged)and half unionized (no charge).

5-when PH is high 11,12,13 the total net charge of amino acid is -1.

*<u>Returning to the plot above:</u>
- X axis is the pH and y axis is the ratio , at very low pH , all the amino acid will be in the red form(the first curve).
- increasing pH will lead to decreasing the ratio of this form and start having the 2nd form of this amino acid, charge will change from +1 to zero,until we reach the point "look at the arrow "that represents the pka of the carboxyl group.(when the carboxyl group is half dissociated)
-By increasing the pH further ,the first form will be less and the second will be more until reach a point where all the amino acid is in zwitterionic form.

-By increasing the PH further , another form will start to appear as the amino group will start losing its proton and become unionized until the pH reaches 9 where the amino group is half dissociated,after 9 the amount of the form of -1 charge will be more .

Titration curve of one of these amino acids(Alanine)



At PH = PI ….> 100% of amino acid is in the zwittrionic for

-We have the amino acid with zero equivalenceof hydroxyl group, we keep adding strong base to the amino acid and we have atitration curve , why do we have it ? because amino acids act as buffers (we'll get to a point where the change in pH will be small).

-We reach the 1st buffering capacity of Alanine that is given by the carboxyl group , after thatpH increases dramatically; it goes up to the 2nd buffering capacity that is provided by the amino group of the amino acid .

#These are the three forms :

*a) cationic (pH <2) ,b) zwitterionic (pH >2) c)anionic "pH >9"

- you can calculate the ratio of the amino acid (ionizedor unionized) per group (carboxyl group or amino group).

-you can calculate the ratio of the conjugate base and the acid using the Henderson-Hasselbalch Equation.

#you must know that the Pka of carboxyl group is 2, for amino group is 9.

Isoelectric point:(PI)

The PH when the net charge of a molecule such as amino acid or a protein is zero but it is charged ." The amino acid is charged yet the net is zero"

$$pI = \frac{pK_{a1} + pK_{a2}}{2}$$ ⟶ For amino acids that don't have an ionizable R group.

PI of Alanine =(2+9)/2=5.5.

#some amino acids have R groups that can be ionized and you must know what these amino acids are .As a result ,these amino acids will have 3 buffering capacities, one for the carboxyl group, another for the amino group and the third for the R group.

#these amino acids are :

1)Tyrosine has hydroxyl group ,aromatic amino acid with phenol

2) Cysteine has thiol (-SH) group (reactive)

3)Arginine positively charged amino acid as well as lysine and histidine.

4)serine , it has hydroxyl group ,polar amino acid as well as threonine .

5) Aspartic acid and Glutamic acid which are negatively charged .

#You have to memorize the pkas of the side groups of these amino acids, we will remove the tyrosine, serine and threonine because their pka is very high(13.5).

Amino acid	Pka of the side chain	PI
Arginine	12.5	10.8
Aspartic Acid	4	3
Cysteine	8	5
Glutamic acid	4.1	3.2
histidine	6	7.5
Lysine	11	10

*considering the table above :

when PH = pka of R group Arginine ... it means that at this PH ... the R group is half dissociated .

#Cysteine will be negatively charged (ionized) above 8 pH.

the pka of R group of Histidine is close to the physiological pH , as a result ,when we talk about proteins, enzymes and they have histidine ,they can play a very important biological function by accepting or donating protons.

* *How do we calculate the PI of the amino acid with ionizable side chain ?*

by taking the average of the pKa's of the groups with same charge when ionized .

Examples:

Glutamic acid (-ve R group) ,we calculate the average of the carboxyl group of the amino acid with the carboxyl group of R group

PI = (2+4)/2=3

Another example : Arginine ,positively charged amino acid

PI= (12.5+9)/2=10.75

Histidine:(+ve R group) ....> PI= (6+9)/2=7.5

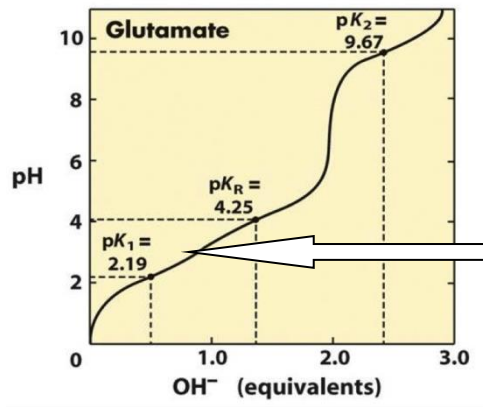
You have to draw the titration curve for every single amino acid of those six A.A.

EX: titration curve of Glutamic acid , 3 pkas , 3 buffering capacities.

1-Pka 1 for carboxylic group

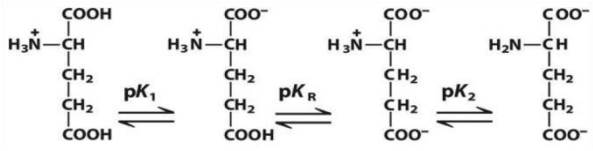
2-Pka 2 for R group

3-Pka 3 for amino group



How do we calculate the isoelectric point of Glutamic acid?

pI = $(pK_R + pK_{COOH})/2$ = (2+4)/2=3



*What does it mean ?

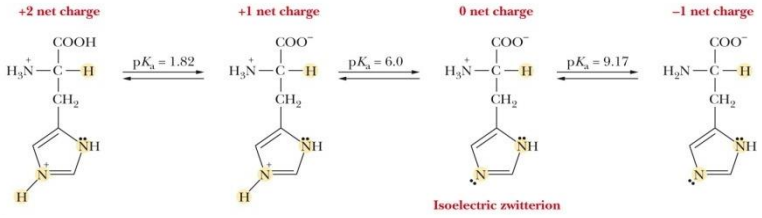
-It is the PH when the molecule has zero charge even though it's ionized.

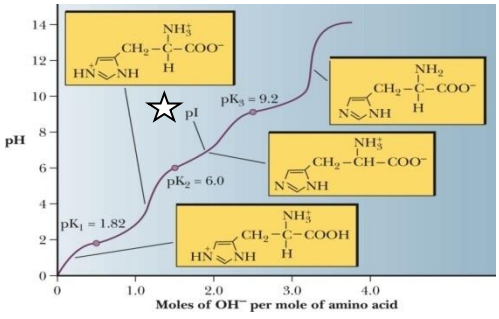
-At this PH ... COOH is ionized but the R group is not ionized.

*What is the net charge when the PH is 7? -1

Histidine :

The pka of the R group is 6 and that's why it's biologically important





At very low PH, amino group is ionized and protonated ,carboxyl group protonated and unionized, Nitrogen atom of the imidazole ring is protnated and ionized . The total charge is +2

pH increases : +1+1-1=+1

#PH increases more and reaches the pka of the R group so its start to lose its proton, it will become unprotonated and unionized , +1-1=0 (zwitterionic form)

#when pH equals 9 , the amino group starts to lose its proton, total net charge is -1.

Questions

1.Draw the titration curves of the six aminoacids.

*when you draw , you need to know the 3 pKas , and remember that the change in Ph in the vicinity of pKa is small ☺

2. what is the ratio of conjugate base/acid of glutamate at pH 4.5 ?

*pka must be given ☺

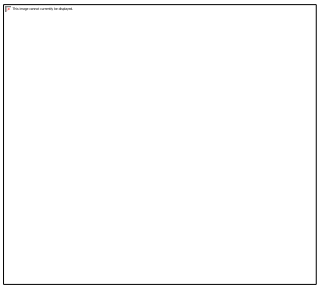
3. what is the total charge of lysine at PH 7 ? +1

What do you need to know ?

1.the names of amino acids.
2. The special structural features of amino acids

EX:

Q : the structure of this amino acid is:



1.lysine

2.arginine

3.histidine

Polypeptides

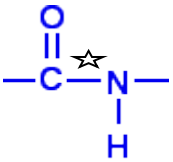
-Proteins are made up of amino acids .

-Amino acid +amino acid +amino acid = protein (wrong)

-amino acids make peptides, when the peptide has a 3D structure it becomes protein otherwise it just peptide like a rope and has no structure.

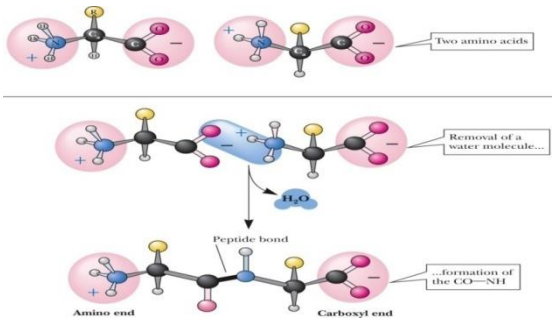
How do we form a peptide ?

By formation of apeptide bond between two amino acids or amide bond.it's called peptide bond in peptides and amide bond in any organic molecule.



#The carboxylic group of one amino acid is linked covalently with the amino group of the second amino acid so they form a peptide bond ,very important to know that *water* comes out because this reaction is called condensation reaction which gives off steam (H_2O).

#Also ,we can separate between two linked amino acids by adding water , so what we do is we lysethe peptide bond and this reaction is called hydrolysis reaction, hydro :water , lysis : break,, you break using water.



Definitions and concepts

1)amino acid : one free molecule

2)Amino acid residue: amino acid that is part of a peptide.

3)Dipeptide (has 2 A.A),tripeptide (3 A.A),tetrapeptide (4 A.A)

4) oligopeptide (peptide) :short peptide that is composed of small number of amino acids. (20-30 A.A)

5)polypeptide : long peptide that is composed of large number of amino acids.

6)protein :polypeptide with a 3D structure.

7)the average molecular weight of an amino acid residue is 110 Dalton .

-For example if there is a polypeptide which is composed of 100 amino acid , what is the molecular weight of this polypeptide ?

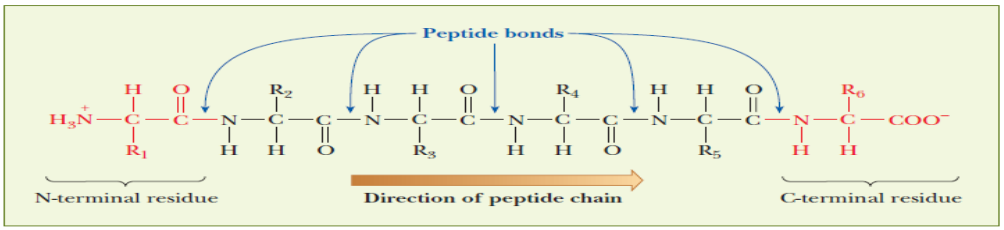
Mwt =100*110=11,000 Daltons , 11 KDa(kilo Daltons).

Directionality of reading

*It's important when we talk about peptides, carbohydrates, lipids .

*there is a starting point and ending point.

*if we have two amino acids andwe put them together ,the result is dipeptide, if we put another amino acid ,the amino group of the 3rd amino acid will react with the carboxylic acid of the 2nd amino acid in this direction. *the amino group of the third amino acid is added on the carboxyl group of the second amino acid .



#Always the first amino group will be free , we start reading from amino terminus to the carboxylterminus .

HOW can we distinguish the amino acids from each other in a peptide ?by their side chains R groups.