

Bio chemistry of protein

1.)

Parameter	Cow	Buffalo
1. Diameter of Hair pores.	Uniform	Non-uniform. Both wide and narrow hair pores are present.
2. No. of hair pores.	So many	less
3. Distribution of hair pores.	Uniform	Random (no sequence)
4. Surface.	Smooth & flat	greasy & uneven because of presence of so many nerve papillae or projections.

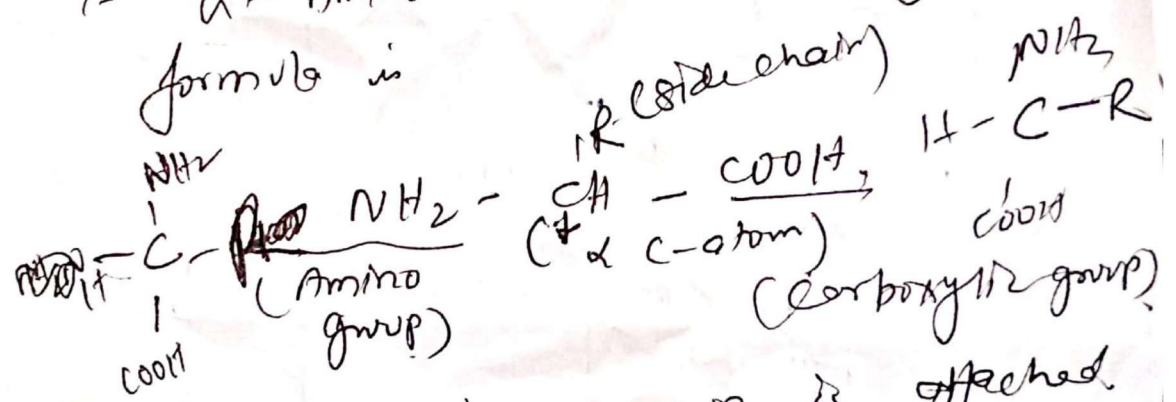
2.

Parameter	Goat	Sheep
1. Grain surface	Smooth & flat	uneven.
2. Hair/wool pores.	Uniform distribution.	non-uniform distribution.
3. Types of hair/wool pores.	Coarse hair pores (less) Fine hair pores (more)	Coarse wool pores & fine wool pores (diff in their diameter is not so distinct as in case of goat skin)
	Coarse hair pores are arranged in groups of three (two groups followed by fine hair pores)	

2) Classification of Amino acids

~~Aliphatic Amino Acids~~

The Amino acids are classified according to their side chain (R) or alkyl group. Amino Unit or mer of protein is α -Amino acids. whose general formula is



Basic amino group is attached to α C-atom, that's why it is called α -amino acid.

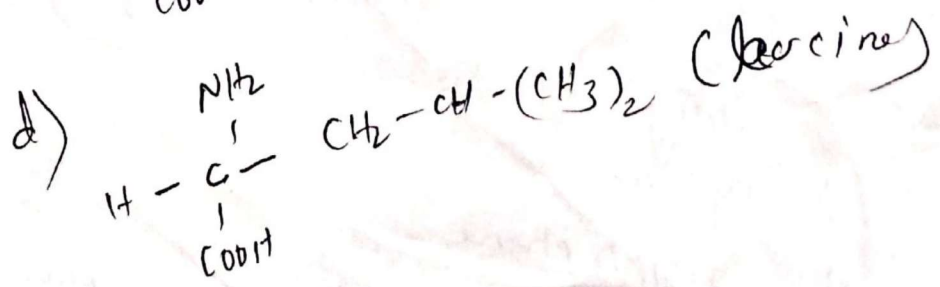
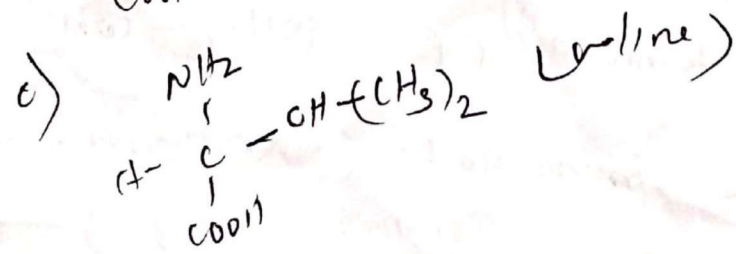
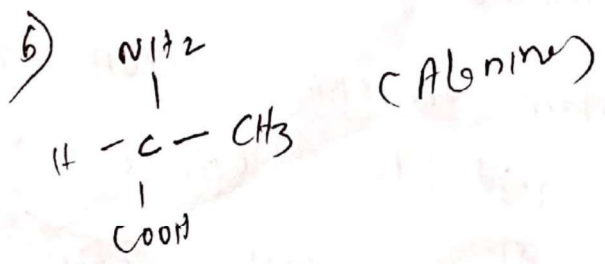
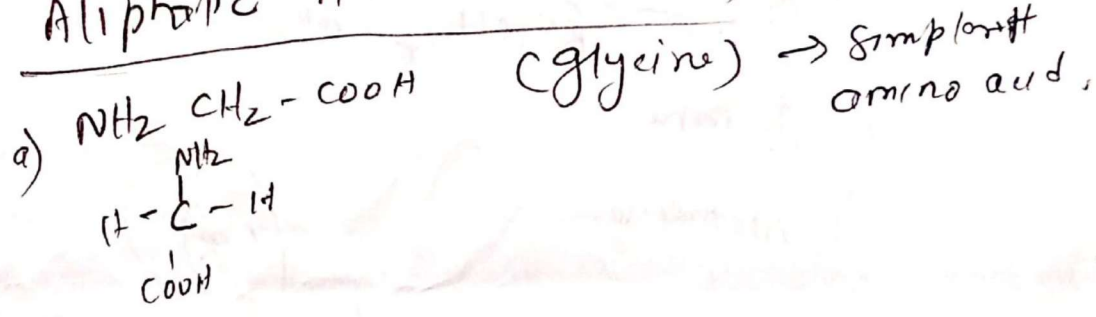
- The group "R" in an amino acid is called side chain.
- One amino acid differs from another amino acid by their side chain (R) only.
- These side chains (R) range from a single H-atom to complex hydrocarbon group.

There are 20 amino acids present in collagen. R can be aliphatic, aromatic, basic, acidic, sulphur containing, etc. on individual amino acids.

The physical and chemical properties of amino acids differ from one another. They are classified based upon the type of "R" group.

The following is the classification of amino acids

a) Aliphatic Amino acids



5) Proline

b) ~~Hydroxy~~ Amino acids containing hydroxyl group

1) Serine (R - CH₂OH)

2) Tyrosine (R - CH₂ -  - OH)

3) Hydroxy proline

c) Basic Amino acids → (3) (R group contains an extra - NH₂ groups)

1) Ex. - lysine (R → (CH₂)₄ - NH₂)

2) Arginine

3) Histidine

d) Acidic amino acids → (2) R groups contain an extra - COOH groups

1) Aspartic acid (R - CH₂COOH)

2) Glutamic acid (R - (CH₂)₂ - COOH)

e) Aromatic Amino acids (R - contain an aromatic group.) → (3)

1) Phenyl alanine

2) Tyrosine

3) Tryptophan

f) Amino acids containing sulphur group (1) cysteine

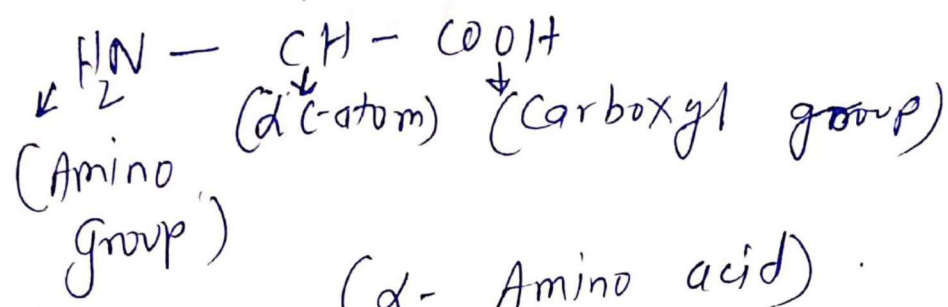
Hydrogen
C_α

(3)

Define Polypeptides. Detail the three dimensional structure of protein

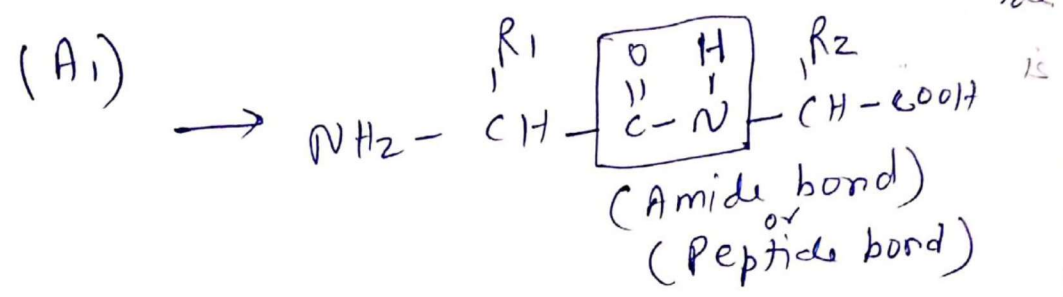
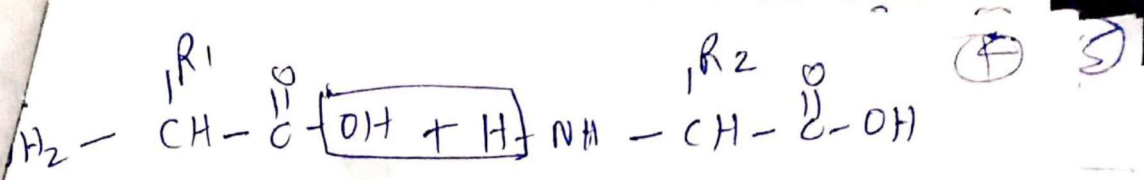
Unit of protein is an amino acid.

The structure of amino acid can be represented by general formula



Since -NH₂ group is attached to α C-atom; that's why it is called α-amino acid.

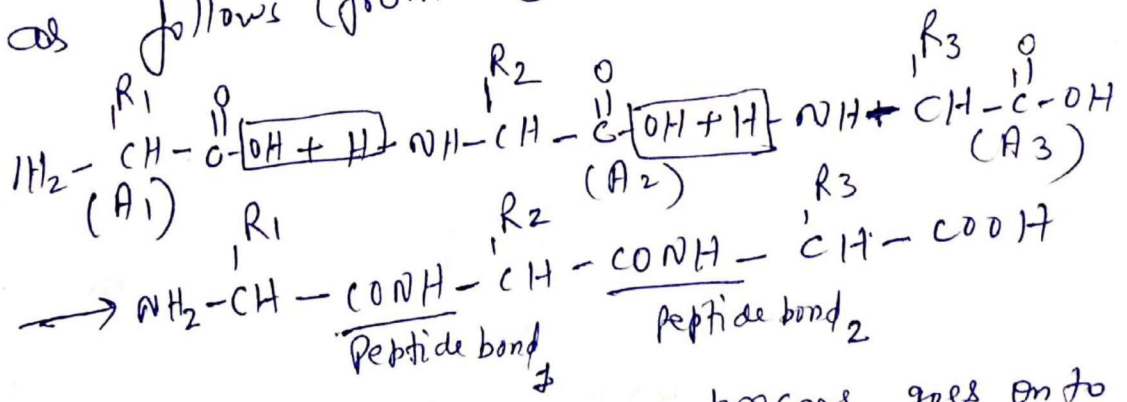
When one Amino acid joins with another amino acid the H₂O group from the carboxyl group of one amino acid and the H-atom from amino group of another amino acid reacts together to form an amide (-CONH-) bond, with the removal of one H₂O molecule. This amide bond is



In a structure of protein, many amino acids react with one another to form many peptide bonds with the removal of H₂O molecules, to form a long chain. This long chain is called polypeptide chain due to presence of many peptide bonds.

Polypeptides \rightarrow Poly + Peptides
 (many)

as follows (from 3 amino acids A₁, A₂ and A₃). The reaction is as



and like wise the process goes on to form a long polypeptide chain.

The Three dimensional structure of protein (collagen)

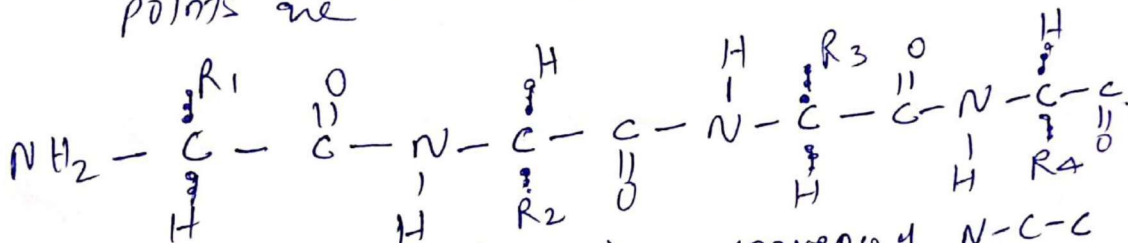
we see that in the above polypeptide chain, so many the group repeats that is

$$\text{NH CHR}_1 \text{CO NH CHR}_2 \text{CO NH CHR}_3 \text{CO}$$

When $R_1, R_2, R_3 \dots R_n \approx R$

this can be represented as NH CHR CO and it is called amino acid residue.

→ In a long polypeptide chain, the main points are



In the main chain, there is a sequence of $\text{N}-\text{C}-\text{C}$.

In a peptide bond, the O-atom of $>\text{C}=\text{O}$ group and H-atom of $>\text{NH}$ group are present on the alternate side of main chain.

They are not on the same side of main chain.

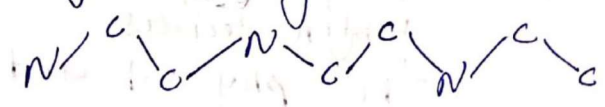
The O-atom of one amino acid residue ($\text{NH CHR}_1 \text{CO}$) and the O-atom of another amino acid residue ($\text{NH CHR}_2 \text{CO}$) are present on the alternate side of the main chain.

They are also not on the same side of main chain.

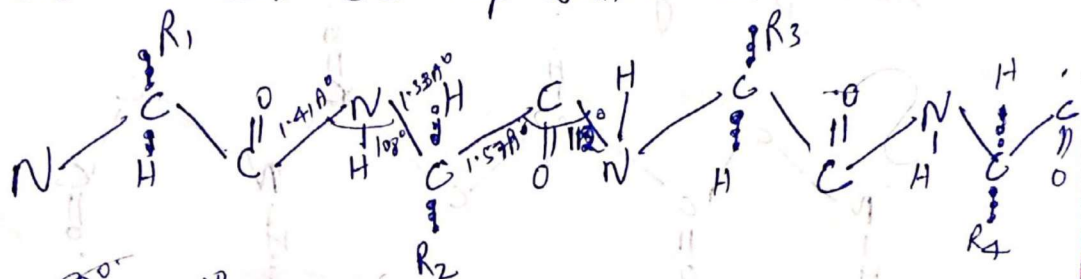
side chain (R_1) of one

side chain (R_2) of another amino acid residue (adjacent) $(\text{CNHCH}_2\text{CO})$ are present on the alternate side of main $\text{N}-\text{C}-\text{C}$ chain.

The main $\text{N}-\text{C}-\text{C}-\text{N}-\text{C}-\text{C}-\text{N}-\text{C}-\text{C}$ chain is also not straight. They are in zig-zag position.



The final structure of a single polypeptide chain can be represented as.



$\text{N} \begin{array}{c} \diagup \\ \diagdown \end{array} \text{C} \begin{array}{c} \diagdown \\ \diagup \end{array} \text{N} \begin{array}{c} \diagup \\ \diagdown \end{array} \text{C} \begin{array}{c} \diagdown \\ \diagup \end{array} \text{N} \begin{array}{c} \diagup \\ \diagdown \end{array} \text{C}$ represents x-axis sequence.

peptide bond $\left[\begin{array}{c} \text{O} \\ \parallel \\ -\text{C}-\text{N}- \\ | \\ \text{H} \end{array} \right]$ represents y-axis

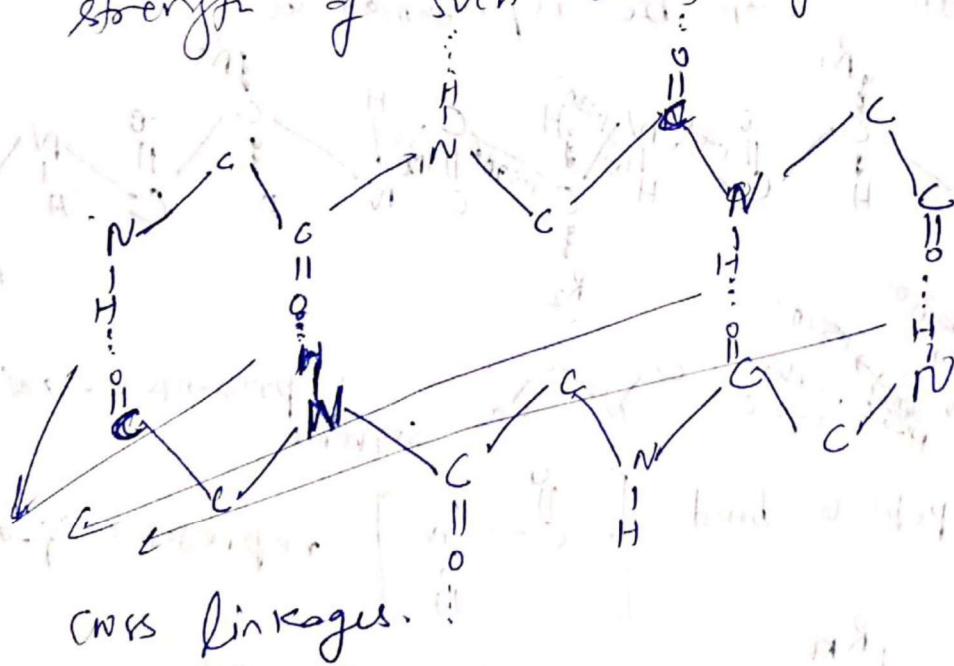
$\begin{array}{c} | \\ \text{R}_n \\ | \\ \text{C} \\ | \\ \text{H} \end{array}$ represents z-axis

So, we see that polypeptide chain has actually a 3-D structure. The H-atom and O-atom of the peptide (amide) bond form a 2-D plane with the main $-\text{N}-\text{C}-\text{C}-$ chain, and the side chain (R) and the H-atoms attached to α -C-atom form another 2-D plane at right angle to the former.

The polypeptide chains of protein molecules are oriented parallel to the fibre axis and are parallel to one another.

The individual planes again held tightly together by cross linkages which cross with the main $-N-C-C-$ chain.

The physical and chemical properties of protein are decided by the nature and strength of such cross linkages.

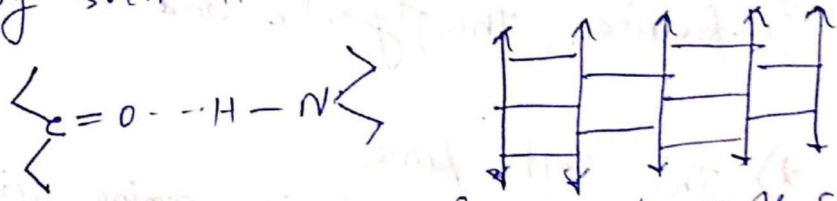


following are
 The cross linkages present in collagen are:-

- 1) The direct carbimino backbone link
- 2) The salt link
- 3) Amide link
- 4) Others.

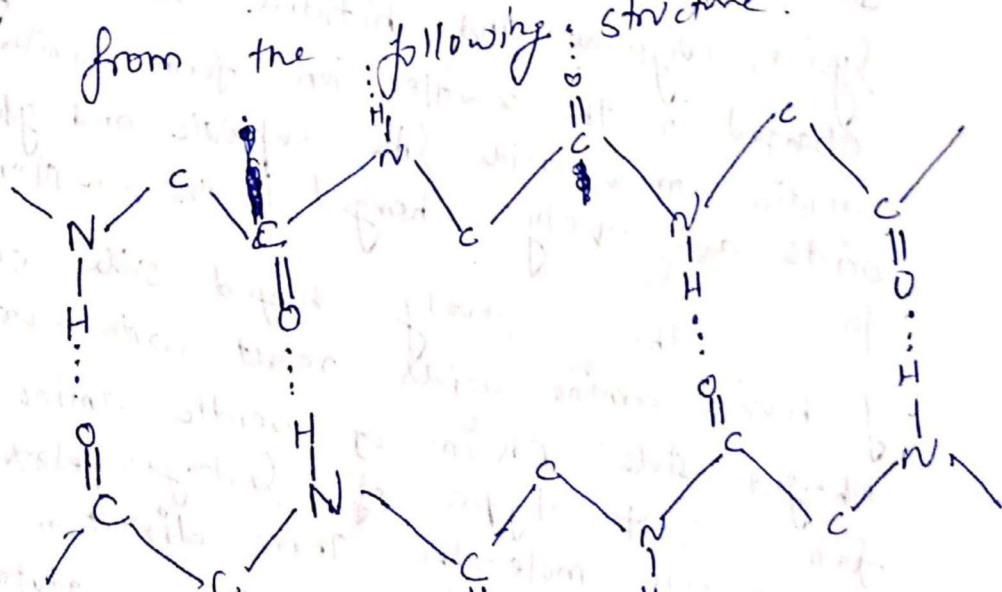
1) The direct carbimino link

This is a H-bond type of linkage formed between >C=O ~~carboxyl~~ carbonyl group and H-atom of >N-H imino group of polypeptide chains. The polypeptide chains of protein are grouped together by such H-bonds at several points.



This is much more clear

from the following structure.



on an average,
The H-bond energy is 8 Kcal/mole.
Even though the strength of individual
H-bond is very small, but the strength
of several such H-bonds in
polymers is very great and these
are actually responsible for the stability
of hide protein to action, heat, chemical,
enzyme and bacteria.

The H-bonds of collagen
are broken down and the latter is
converted into glue when digested with

- 1) water at $> 60^{\circ}\text{C}$
- 2) meta cresol $\geq 15^{\circ}\text{C}$
- 3) formic, thioglycolic and lactic acid $> 0^{\circ}\text{C}$

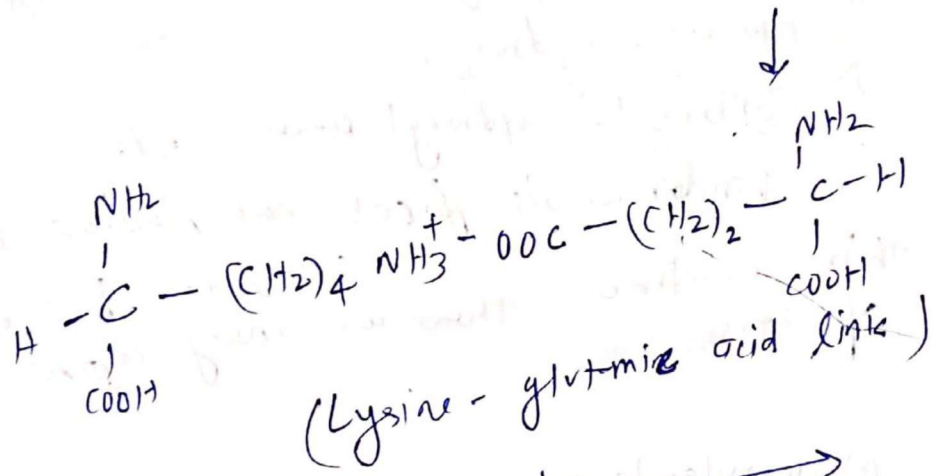
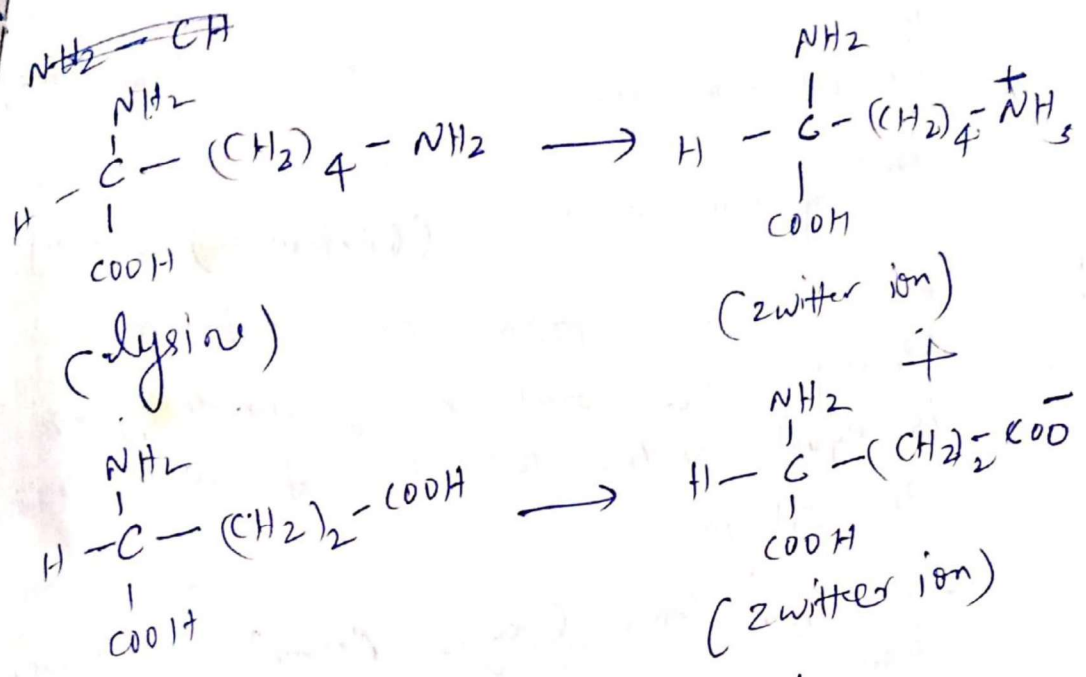
d) The salt link

In collagen, the basic amino acids like
lysine, arginine and histidine are +vely
charged in the zwitter ion form. Similarly the
acidic amino acids like aspartic and glutamic
acids are -vely charged in the zwitter ion
form.

These +vely charged side chains
of basic amino acids react with -vely
charged side chains of acidic amino acids
in protein molecules. Their direction and
form salt type of linkages (electrostatic
bonds) are not fixed. Ex: - Lysine - glutamic

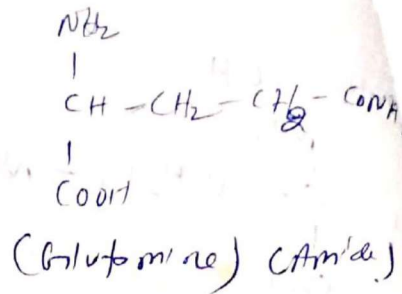
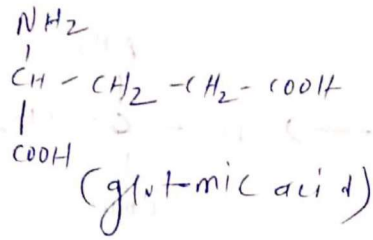
1 mole
1 mole
com
ben

(8)



10 to 11 A° in dry collagen
17 A° in swelled collagen

3) Amide link
 The other possible cross linkage possible cross linkage is amide cross linkage. A/c to most of the protein chemists, very small amount of acidic amino acids are found in collagen. most of them remains as asparagine linkage possible



From an experiment finding the total no. of amide groups is equal to the total no. of Serine residues in collagen.

4) Other cross linkages present in collagen

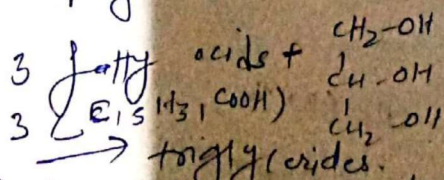
- a) Hydroxy - hydroxy
- b) Carboxyl - hydroxyl ester etc
- c) Vander waals forces are possible in their existence but about their existence there are many controversial opinions.

5) Macromolecule and Polymer

a) Macromolecule of high mol. wt.
 1) They are molecules made of smaller subunits.

2) All macromolecules are not polymers.

3) Ex:- Triglyceride is (Fat) a macromolecule not polymer.



4) Ex:- Lipids, some dyes

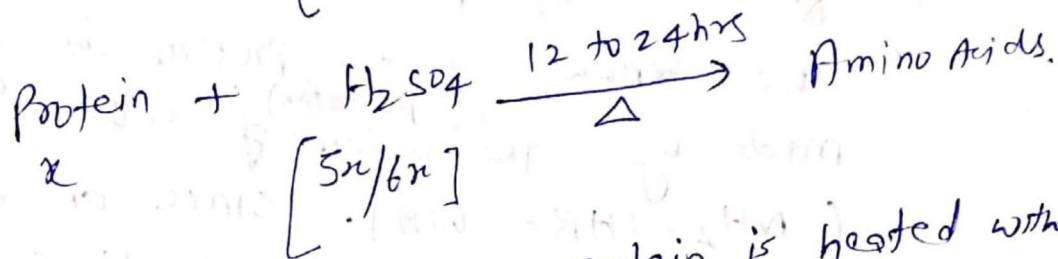
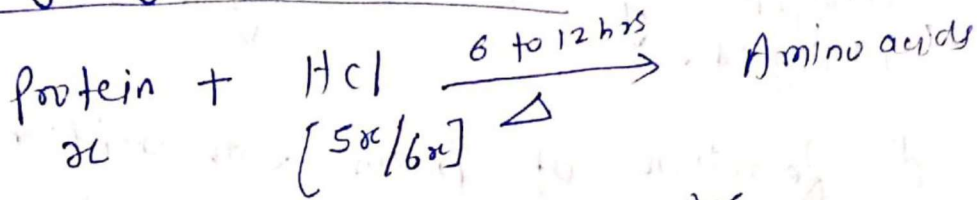
Polymer
 1) They are macromolecules made of repeating subunits.

2) All polymers are macromolecules.

3) Protein is both a polymer and a macromolecule. since protein is made by repeating units of amino acids.

4) Ex:- cellulose, polyethylene

b) Hydrolysis of protein : - (9)



When a protein is heated with 5 or 6 times its weight with strong HCl (for 6 to 12 hrs) / strong H_2SO_4 (for 12 to 24 hrs), it is hydrolysed / decomposed producing finally Amino acids. This is a direct proof that protein is composed of amino acids. Nearly 25 different amino acids (basic, acidic, aromatic, aliphatic etc.) have been isolated from proteins.

c) Amino acids present in collagen : -

Collagen consists of amino acids wound together to form triple-helices of elongated fibrils. It is mostly found in fibrous tissues such as tendons, ligament and skin.

Almost 21 amino acids approx. are found in collagen. which ranges from acidic (Aspartic, glutamic), basic (lysine, arginine, histidine, ornithine), aromatic (phenyl, alanine, tyrosine), aliphatic (Alanine, leucine, valine).

Tryptophan is an amino acid is absent in collagen by which collagen can be identified. sequence

d) Reaction of protein with acids & bases.

We know that protein is a polymer made by the union of several amino acids (NH₂-CHR-COOH). Since an amino acid molecule has both acidic (-COOH) and basic (-NH₂) groups and so it can react with both acid and base.

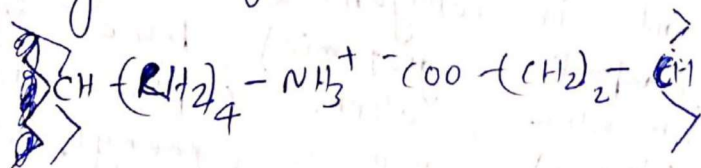
The acidic and basic groups in the side chains of some amino acids also increase the acidic and basic character of the respective amino acids.

Ex:- salt formation:- Amino acids react with each other

in a typical acid-base neutralisation reaction to form a salt. The reaction is simply the transfer of -H (H⁺) from the acid to the amine and the attraction of the +ve and -ve charges. Ex:- glycine and alanine may interact

in the zwitter ion form to make salt

b) Salt formation of side chains
Lysine - glutamic acid link



Qe / Sequence of amino acids present in (10)
Collagen.

Protein $\xrightarrow[\text{Strong HCl/H}_2\text{SO}_4]{\text{hydrolysis}}$ Amino acids $\xrightarrow[\text{suitable solvent}]{\text{Paper chromatography technique}}$ separated and identified by their respective Rf values

Quantitatively determined.

← Spectrophotometer / (mass spectroscopy)

The no. of gram molecules of glycine, proline and hydroxyproline in gelatine are nearly $\frac{1}{3}$, $\frac{1}{6}$ and $\frac{1}{9}$ of the total gram molecule of the amino acid residues respectively. The scientist therefore suggested in the polypeptide chain of collagen, glycine could be every 3rd residue, proline every 6th and hydroxyproline every 9th.

So, the following are the two possible amino acid sequences in polypeptide chain of collagen.

a) — G P X G P X G P X G P X — and

b) — G P X G P X G P X G P X —

where G represents glycine residue, P proline and X any other amino acid.

