



CSIR-NET

Council of Scientific & Industrial Research

LIFE SCIENCE

VOLUME – 2

BIO CHEMISTRY



CONTENT

BIOCHEMISTRY

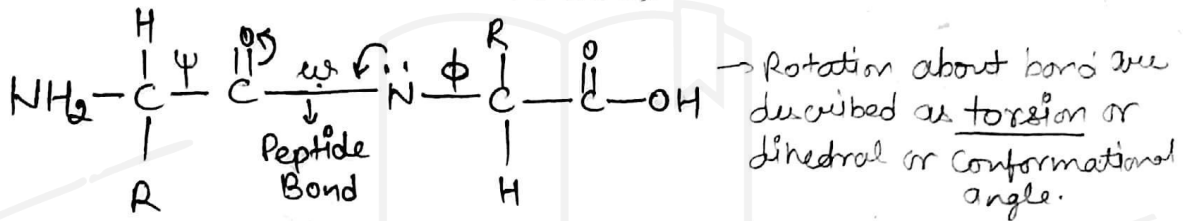
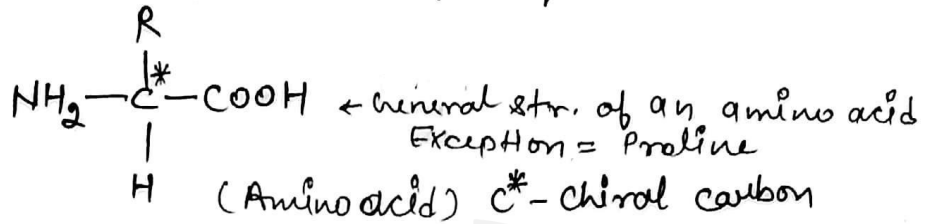
1. Protein	1
2. Enzyme Kinetics	22
3. Membrane Transport	52
4. Protein Folding	87
5. Protein Targeting/Sorting	95
6. Signal Transduction	136
7. Cell Division and Cancer	162
8. Apoptosis	185
9. Cytoskeleton	193

CELL - BIOLOGY (BIOCHEMISTRY)

PROTEIN

Amino acid - Building blocks of protein

→ Amino acid bind together and form protein.



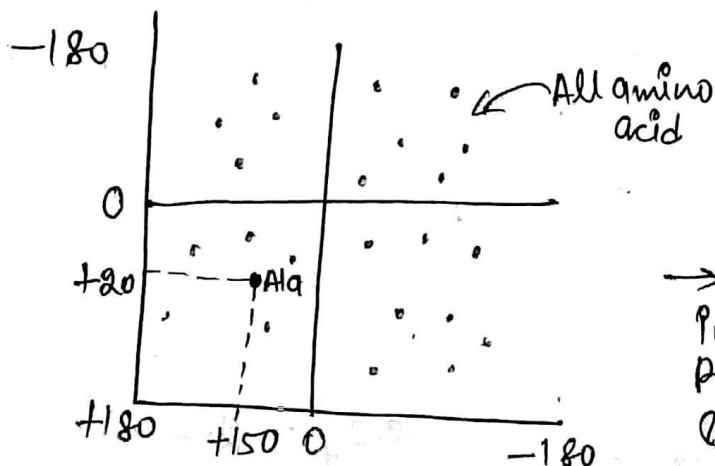
→ Peptide bond present in plane/planner (also c/d an amide bond)

→ CHON & two C_α is not in plane (2C_α)

→ $\phi = 0 \text{ to } \pm 180^\circ$
 $\psi = 0 \text{ to } \pm 180^\circ$

eg. Alanine ψ ϕ
+150 / +20
+120 / +30

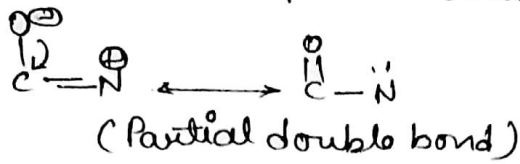
Ramchandran plot $^\circ$ -180° and +180°



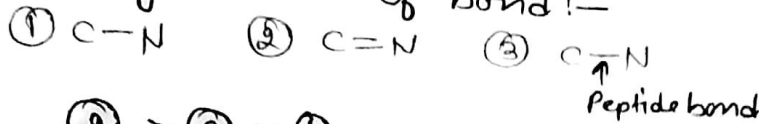
→ All amino acid ψ & ϕ value are noted from \hat{A}
Plot \hat{A} show ψ & ϕ

→ Ramcha. p. provide valuable information on the quality of protein, str & amino acid seq. & tertiary protein str.

→ Peptide bond present b/w C & N. It is show the resonance, so peptide bond is a partial double bond.



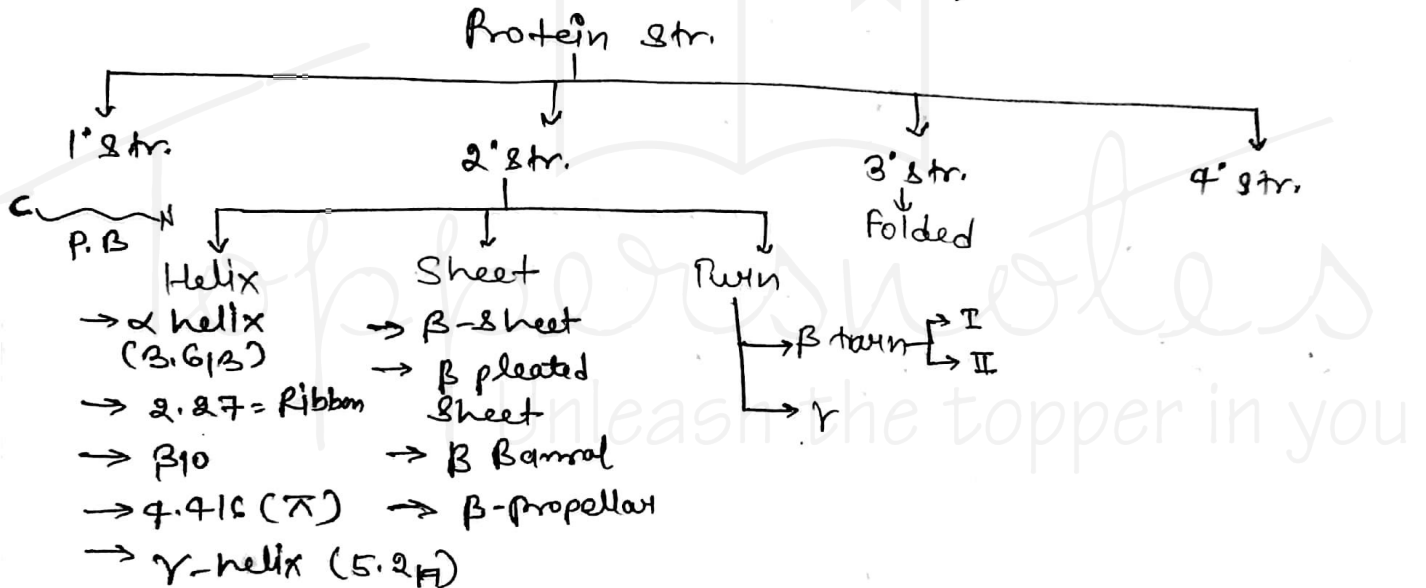
* strength order of bond:-



② > ③ > ① .

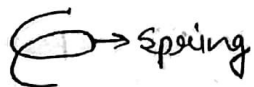
→ Ramchandran plot is show in 2° str. of protein.

* Protein structure :-

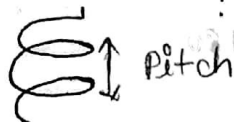


* Secondary str. (2° str.) :-

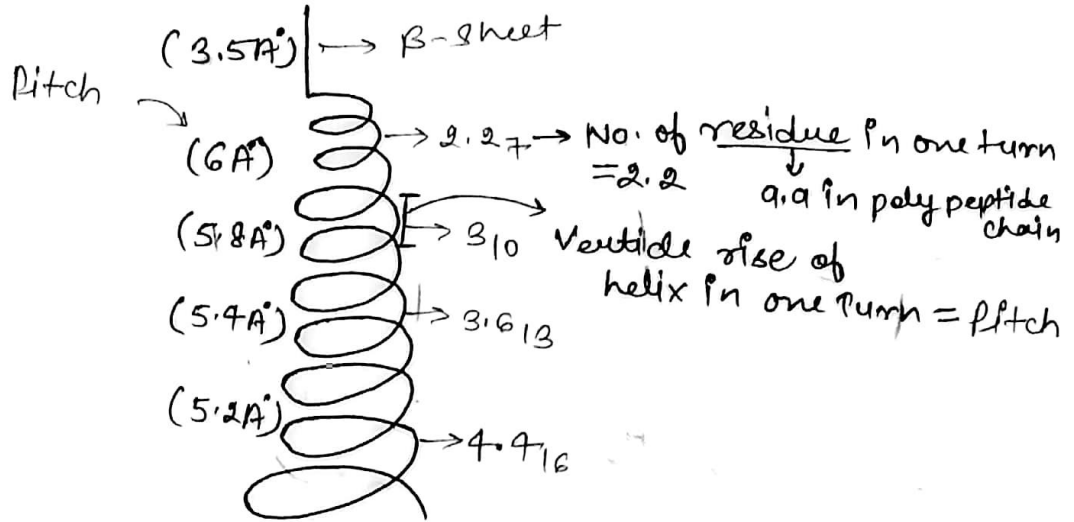
- Pauling & Corey - discover
- Diameter & No. of amino acid.
- Diameter ↑ then no. of amino acid is ↑
- Vertical rise present in spring



→ Maximum vertical rise present in β-sheet



2.27 = 2.2 amino acid



→ Vertical rise decrease according to spring size

* Pitch for all residue :-

$$\frac{\text{Pitch}}{\text{A.A (Residue)}} = \text{Distance b/w 2 amino acid}$$

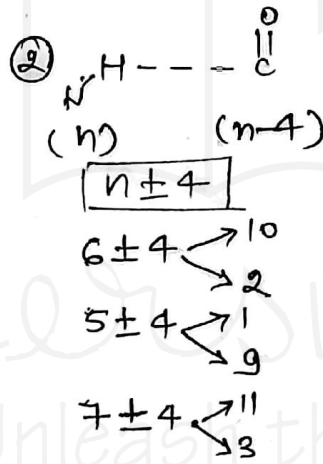
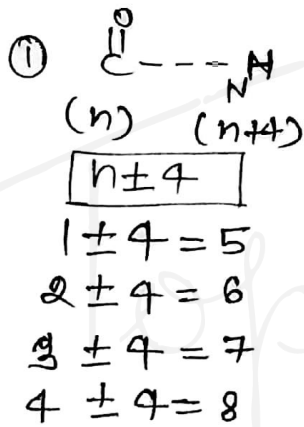
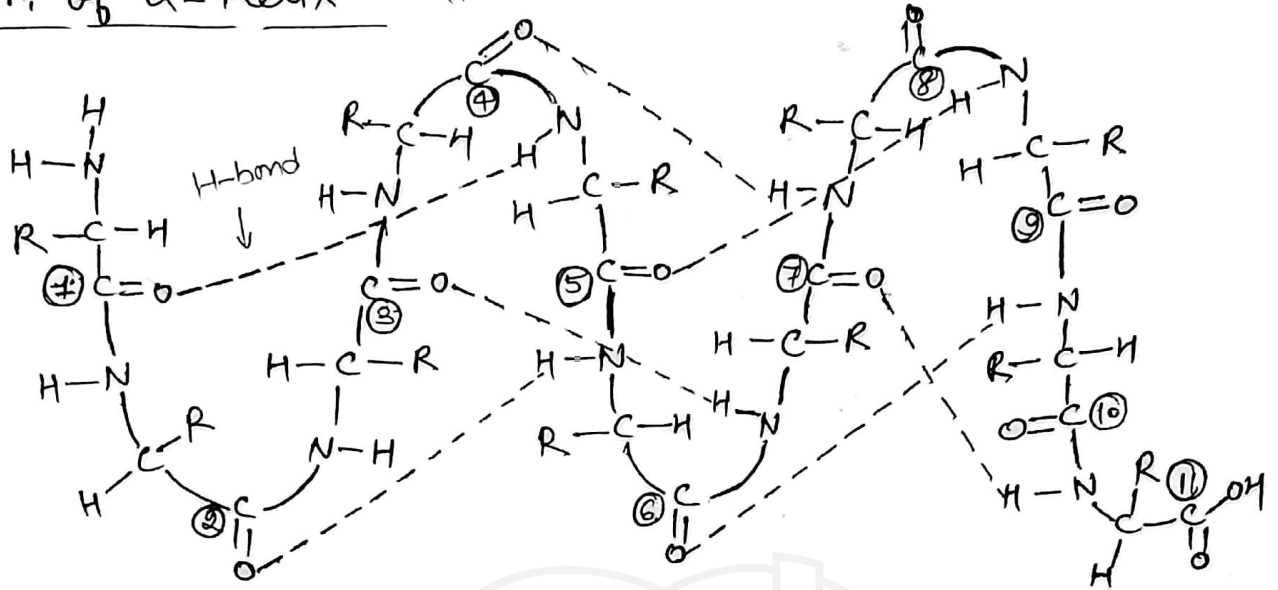
	Angle
$2.27 = \frac{6}{2.2} = 2.72^\circ \rightarrow \frac{360}{2.2} = 163.63$	
$3_{10} = \frac{5.8}{3} = 1.93^\circ \rightarrow \frac{360}{3} = 120$	
$3.6_{13} = \frac{5.4}{3.6} = 1.5^\circ \rightarrow \frac{360}{3.6} = 100$	
$4.4_{16} = \frac{5.2}{4.4} = 1.18^\circ \rightarrow \frac{360}{4.4} = 81$	
$5.2_{17} = \frac{5.2}{5.2} = 0.96^\circ = \frac{360}{4.4} = 69.23$	

Residue :- Protein is folded, three dimensional str. & amino acid in a polypeptide chain or protein is called as a residue.

- Proline is called as helix breaker & creates a sharp bend in helix.
- Silk fibroin (α -Keratin) - Antiparallel pleated sheet str
- Glycin provide High flexibility to the polypeptide chain

c ~~~~~ N terminal

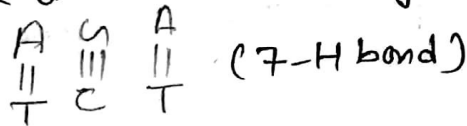
Str. of α -Helix - α Helix is called str.



$N_1, N_2, N_3, N_4 \rightarrow$ Capping

$C_1, C_2, C_3, C_4 \rightarrow$ Capping

\rightarrow 12 amino acid only 6 H-bond is int.



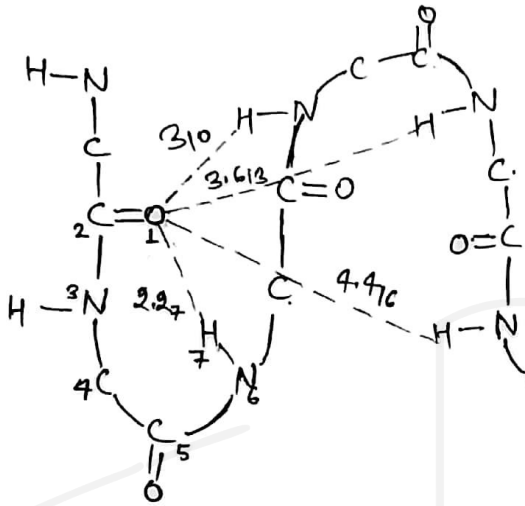
$\text{C} \equiv \text{C}$ melt on high temp.

\rightarrow First N_1, N_2, N_3 & N_4 H bond नहीं बनते हैं क्योंकि First NH group के नीचे 5th amino acid का R-C-H group आता है जो bond नहीं बनाता।

\rightarrow So first N_1, N_2, N_3 & N_4 & last C_1, C_2, C_3, C_4 group H bond नहीं बनते।

Ribbon- $2.2_7 = n \pm 2$
 $3.1_0 = n \pm 3$
 $3.6_{13} (\alpha \text{ Helix}) = n \pm 4$
 $4.4_{16} (\beta) = n + 5$

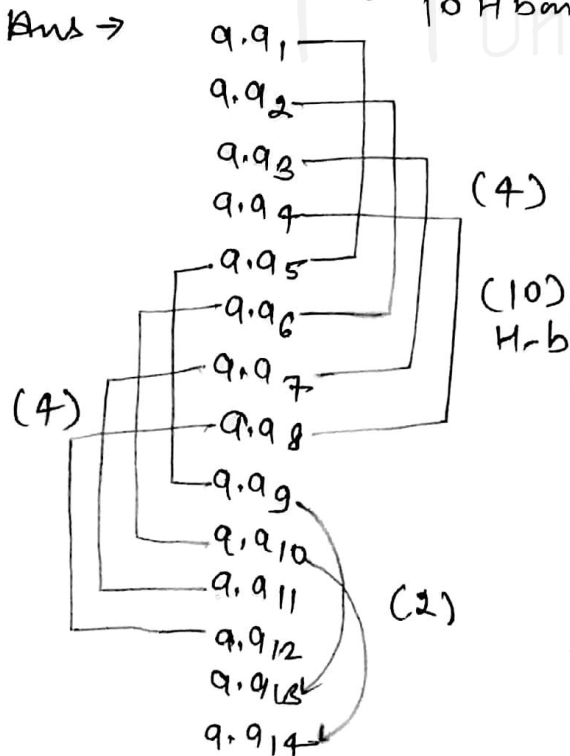
\rightarrow No of a.a in one turn
 $2.2_7 \rightarrow$ No of atoms participated in H-bond circle



\rightarrow Selenocysteine (Secor U) is the 21st standard amino acid. It has a str. similar to that of cysteine, but it contains Selenium rather than sulphur. It is incorporated in polypeptide during translation. It is specified by a triplet codon UGA (a stop codon) \rightarrow its own t-RNA containing the anticodon UCA \rightarrow Enzyme like glutathione Peroxidase & formate dehy.

$\rightarrow 2.2_7 \rightarrow 7$ atom is tut

Q. Calculate the total no. of H-bonding for by 14 a.a long peptide existing α -helical form.
 10 H bonding tut.



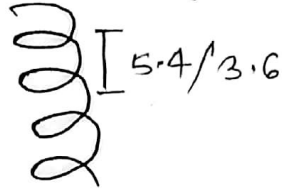
$10 \pm 4 = 14$

Chemical nature of R-group	Example
① Aliphatic	Gly, Ala, Val, Leu
② Aromatic	Phe, Tyr, Trp
③ Hydroxyl	Ser, Thr
④ Carboxylic	Asp, Glu
⑤ Sulphur containing	Cys, Met
⑥ Imino	Pro
⑦ Amino	Lys, Arg
⑧ Amide	Asn, Gln

\rightarrow 31% α Helix
 \rightarrow 28% β sheet

Q. Calculate the size of protein having 30 a.a present in the helix & π -Helix π , R, 3_{10} , β -sheet?

Ans \Rightarrow The size of protein having = 30 AA



$$\rightarrow 36 \text{ a.a} = \frac{36}{36} = 10 \text{ turn}$$

$$5.4 \times 10 = 54 \text{ \AA}$$

or

$$36 \text{ a.a ?}$$

$$\rightarrow \frac{5.4}{3.6} = 1.5$$

$$1.5 \times 36 = 54 \text{ \AA}$$

$$30 \text{ a.a ?}$$

$$1.5 \text{ \AA}$$

$$1.5 \times 30 \text{ a.a}$$

$$= 45 \text{ \AA}$$

$$\pi \text{ helix} = \text{Diagram of } \pi \text{ helix} \text{ with } 5.2$$

$$= \frac{5.2}{4.4} = 1.1$$

$$= 1.1 \times 30 = 35.45 \text{ \AA}$$

$$1.1 \times 36 = 39.6 \text{ \AA}$$

$$R \text{ helix} = \frac{6}{2.2} = 2.72 \text{ \AA}$$

$$= 2.72 \times 30$$

$$= 81.6 \text{ \AA}$$

$$2.72 \times 36 = 97 \text{ \AA}$$

$$3_{10} = \frac{5.8}{3} = 1.93 \text{ \AA}$$

$$= 1.93 \times 30 = 57.9 \text{ \AA}$$

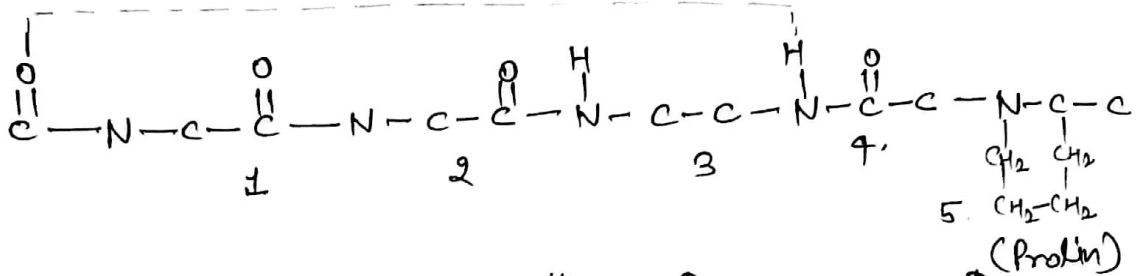
$$1.93 \times 36 = 69.48 \text{ \AA}$$

$$\beta \text{ sheet} - 3.5 \times 30 = 105 \text{ \AA}$$

→ Proline is helix breaker. It breaks the helix.

Q. Why proline is a helix breaker?

Ans -

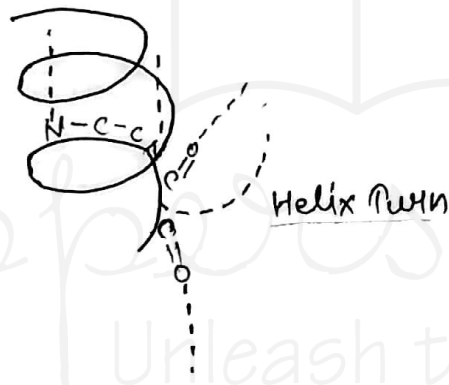


→ 4 amino acid का C=O 5th A.A के NH group से bind होता है but proline NH से bind होने पर H absent होता है व H-bonding नहीं कर पाता।

→ Helix Turn हो जाती है।

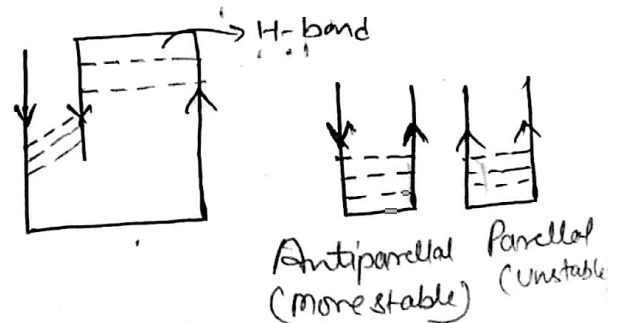
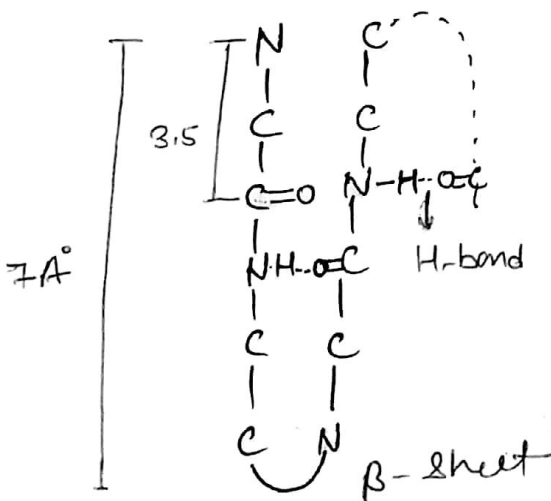
Proline - Amino acid

Amino acid - amine group
not.

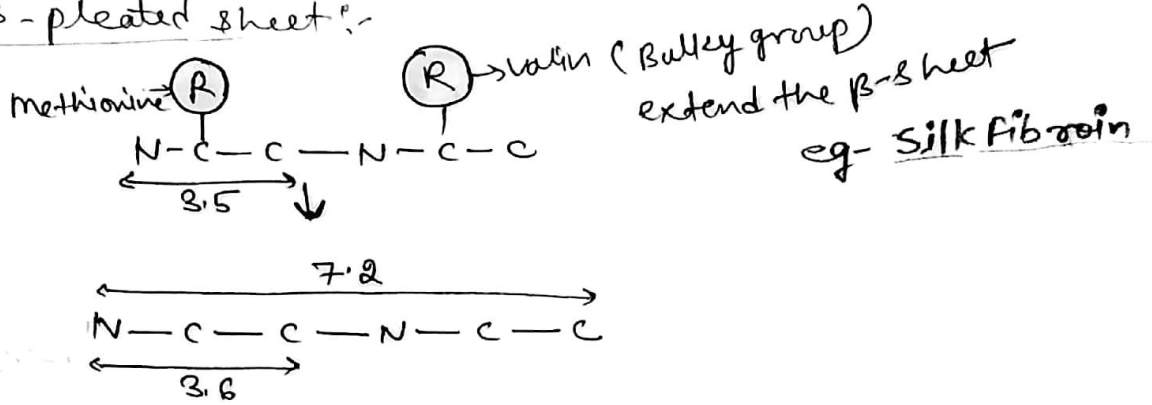


★ β-Sheet :-

→ β-Sheet is present in linear shape

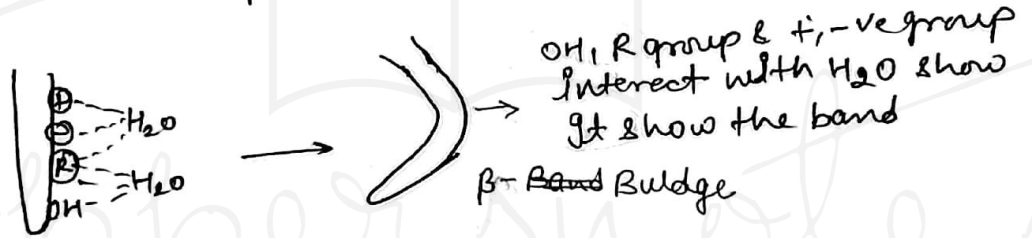
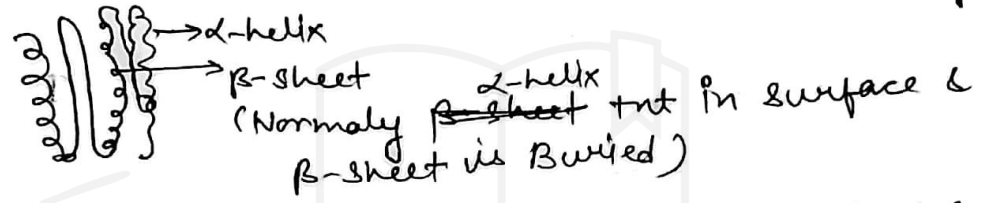


β-pleated sheet :-



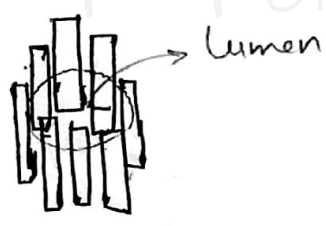
β-Bulge

(Buried - Placed under ground)

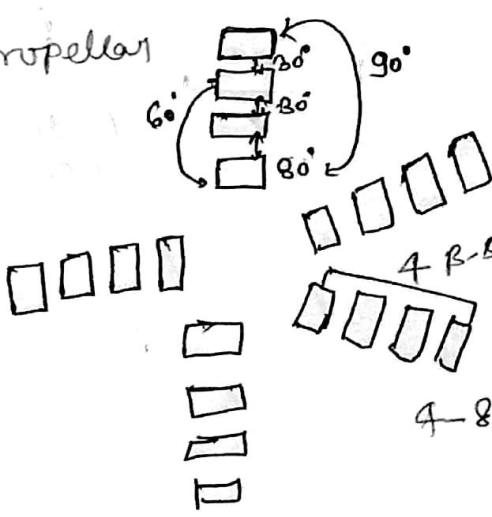


β-Barrel sheet

→ lumen is not & molecule move through it.



β-propeller

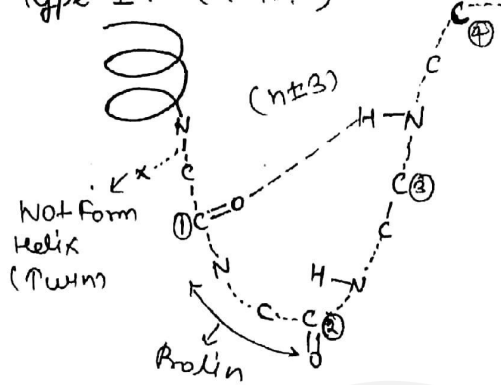


(Enzymes)
 → β-propeller not in hydrolases, Lyases & Transferase
 → β-propeller not in all enzymes.
 (W.D. Domain)
 → It also not in enzyme found & protein that bind with ATP, UTP, CTP, TTP etc

* Turn / β -Turn / β -Bend :- (E)

→ Ventchalam Turn (also known:

(i) Type I :- (α -turn)



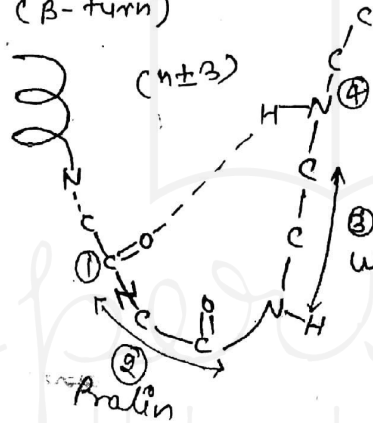
- 2nd position proline but
- C=O outside N-H inside
- (n±3)
- SO + bind with 4 NH

$$\phi_2 = -60^\circ / \psi_2 = -30^\circ$$

$$\phi_3 = -90^\circ / \psi_3 = 0^\circ$$

→ wycin & Proline commonly
but in turn

(ii) Type II (β -turn)

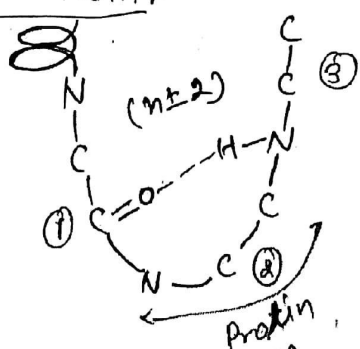


- C=O inside
- NH outside
- wycin & Proline group
is but

$$\phi_2 = -60^\circ / \psi_2 = 120^\circ$$

$$\phi_3 = -90^\circ / \psi_3 = 0^\circ$$

(iii) γ -Turn :-



- ① bind with ③rd
- 2 position proline is but

Disease caused by 3rd str.

- Cystic fibrosis
- Diabetes
- Hypercholesterolemia
- Osteogenesis

→ Aromatic a.a. = 60% in protein

→ Hydrophobic A.A. = P.A > Alanine > Glycine > Leucine

Strength of
H-bond 2-10 Kcal.

Gmp

★ Propensity of amino acid :-

↓

Tendency

* Propensity of a.a to form α helix / β -sheet / Turn :-

→ α -helix is unbranched

$$\text{Propensity} = \frac{F_{\alpha} \text{ in } \alpha \text{ helix}}{F_{\alpha}}$$



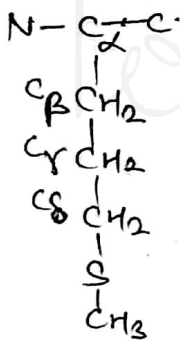
→ Only in Peptide backbone Channel primary Str is int.

→ After this change in α -Helix.

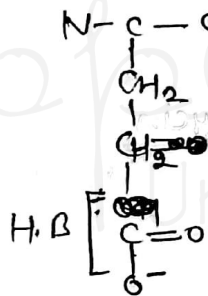
α helix [MELAK]

β -sheet

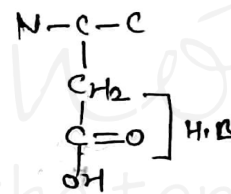
Methionine



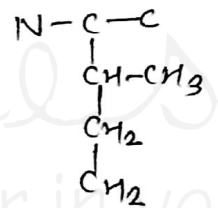
Glutamate



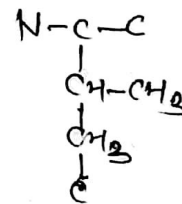
Aspartate



Isoleucine

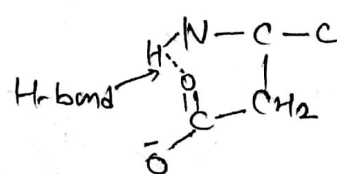
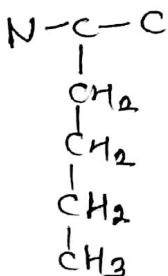


Valine



C_{β} is branch

Leucine



→ Histidine



→ Proline

β -sheet में C_{β} के साथ Bulky group bind करता है जो पाल के NH के साथ Bond बना लेता है।

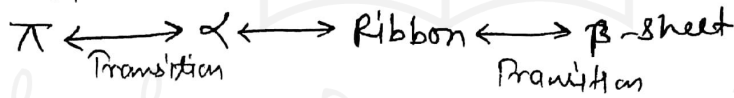
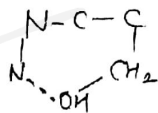
Two rule of β -sheet :-

- (1) Branched Rule :-
 → amino acid के 2nd carbon का β c branched होता है
 जिससे वह अपने पास के NH के साथ ही Bond बना लेता है
 → 100° angle होने के कारण all alkyl group
 outside होते हैं जिससे internal surface use नहीं आती



- (2) Based on H-bond :-

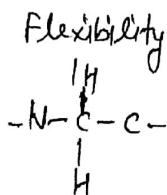
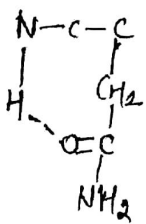
→ N-H rule को follow नहीं करता जिससे C=O अपने पास वाले NH group के साथ H bond बना लेता है



★ Turn Tendency :-

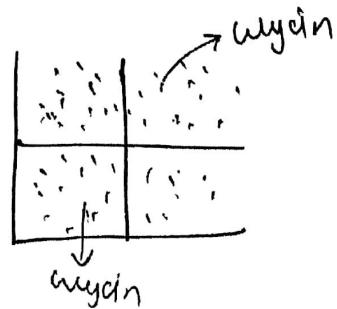
Turn tendency order

Asn, Asp
 (Asparagine) > (Aspartate) > Glycin > Prolin > Serine



Bulky alkyl group
 but H-bond is
 absent so Turn

Small group
 (0 to ±180)
 → Glycin plotted
 any where in R plot
 → ϕ & ψ all value +ve



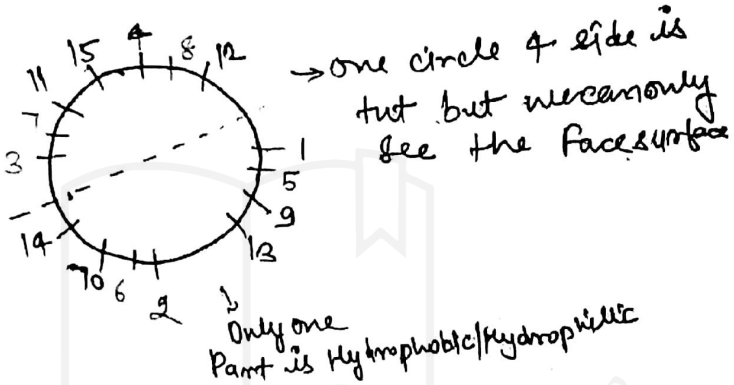
α Helix Order - Methionine - Highest tendency to form α -Helix

EMALK (Glutamic acid, Met, Ala, Leu, Lysin)

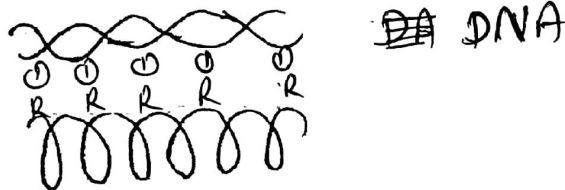
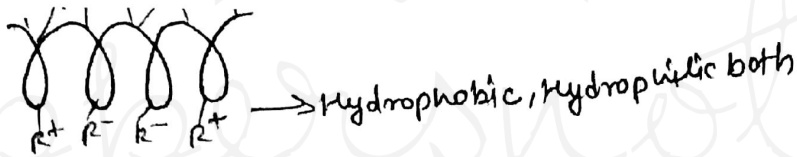
β -Sheet - (6 a.a require) \rightarrow Highest tendency

VIFAY Thr (Valin, Isoleucin, Phenyl Alanine, Tyrosine)

(Valin, Isoleucine, Phenyl Alanine, Tyr, Thr)



⑧

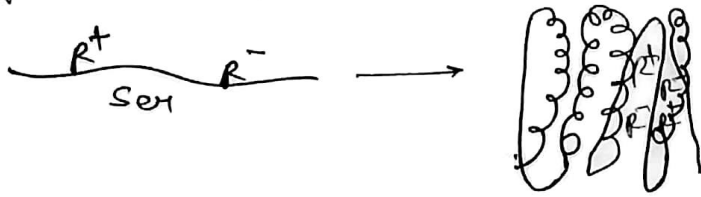


\Rightarrow Globular protein have a core of hydrophobic amino acid residue & water exposed, polar, charges hydrophilic residue on the surface.

\rightarrow This arrangement makes protein soluble in water & stable in conformation. The disulphide bond is the stability of a protein.

\rightarrow The protein folding process is achieved with help of chaperons.

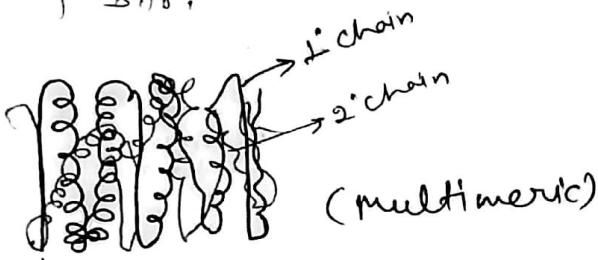
★ 3° str. :- Three dimensional str.



only monomeric

- In 3° str. follⁿ type bond tut
- ① Hydrophobic
 - ② electrostatic
 - ③ H-bond
 - ④ Vander walls
 - ⑤ Covalent → The covalent bond tut in a tertiary str. is the interchain disulphide bond. A disulphide bond (S-S bond) form b/w 2 cysteine residue due to oxidation of their thiol or sulfhydryl group.
- The oxidized dimer form of the amino acid cysteine is C/2 cystine. The other sulfur containing amino acid methionine cannot form disulphide bond.

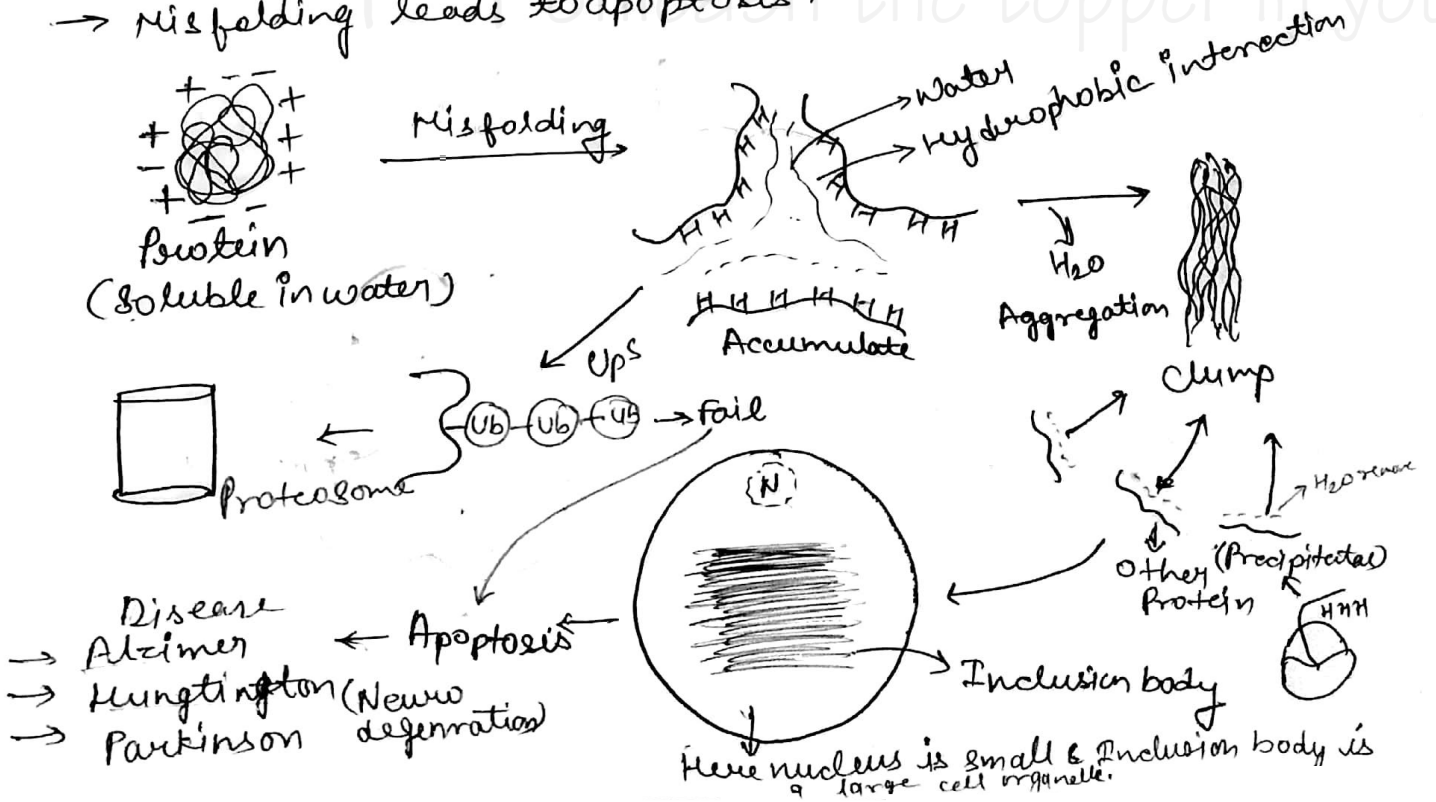
★ 4° str.

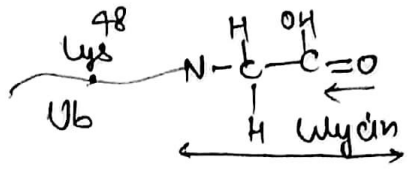
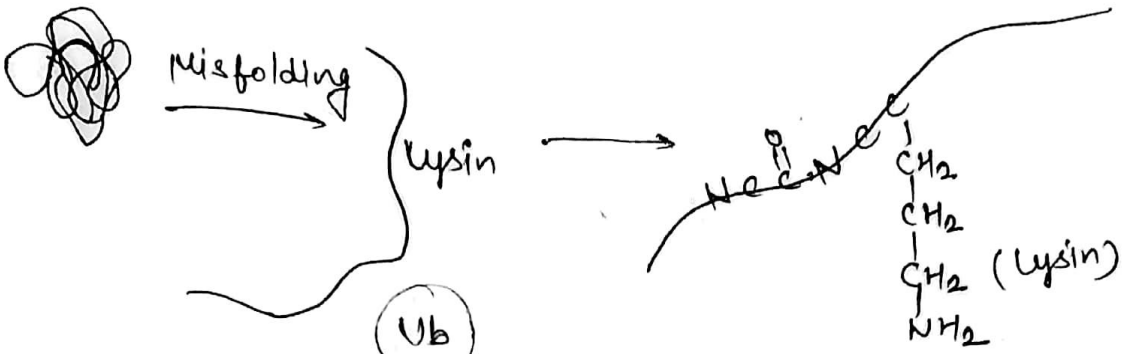


3° str.	4° str.
→ DNA poly I (1 unit) Monomeric	→ DNA poly II Multimeric (3 unit)
→ Myoglobin (1 unit) Protein-specific function depends on tertiary str.	→ Hemoglobin (α ₂ β ₂) 4° str. (multimeric protein)

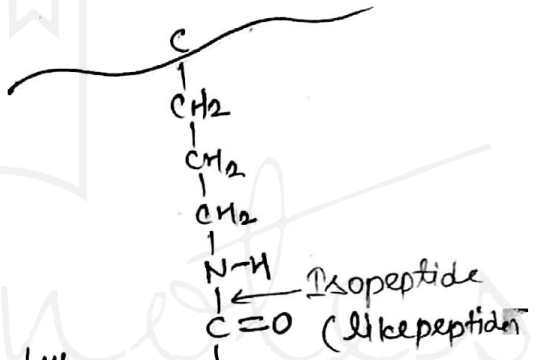
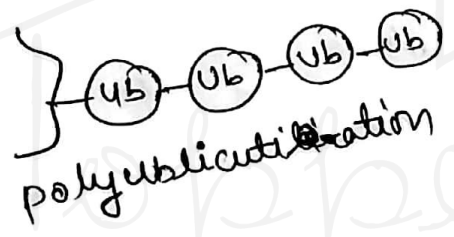
★ Protein degradation :-

→ Misfolding leads to apoptosis.

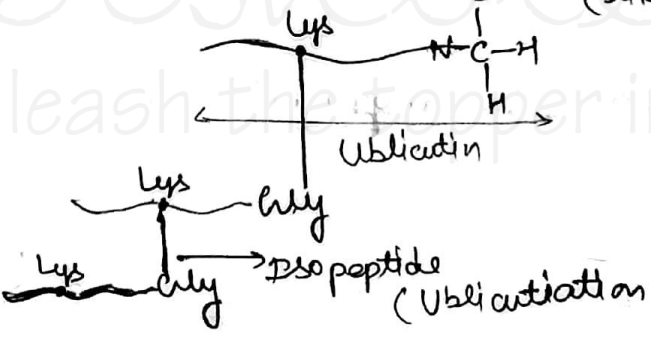




→ Ubi. is covalently attached to a lysine residue on a target protein is c/d Ubiquitination.



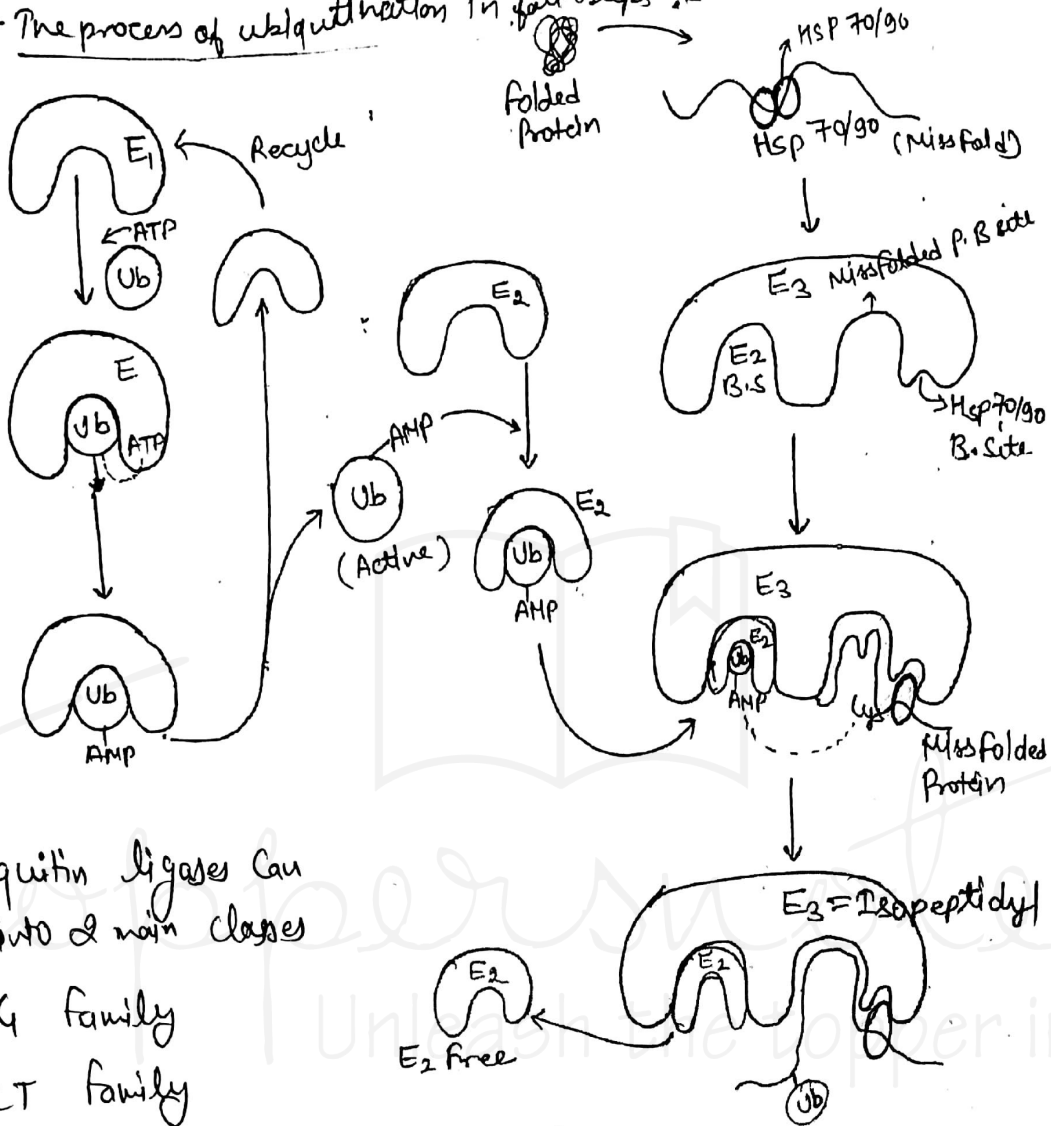
→ Poly ubiquitin is use
 → C & N & Carboxyl group involve of other chain C=O & N bond of other & Isopeptide bond



→ Reagents or conditions that can cause denaturation are called denaturing agents. Urea is most commonly used as denaturing agent.
 Detergent, β -mercaptoethanol & Heavy metal ions (mercury, lead) & Heat etc.



⇒ The process of ubiquitination in following steps :-



E₃ Ubiquitin ligases can be divided into 2 main classes

- ① RING family
- ② HECT family

E₁ = Ub Activating E₂
 E₂ = Ub Conjugating E₂
 E₃ = Ub Ligating E₂

45000 (protein)
 ↓
 1000 E₂
 Diversity
 E₃ > E₂ > E₁

E₃ ligase enzyme
 → PHD, HECT, RING, SCF, Mdm-2
 (RBX-1)
 → Skp-1, Cullin, F-box, APC
 → SCF (SKP-1 Cullin-F box)