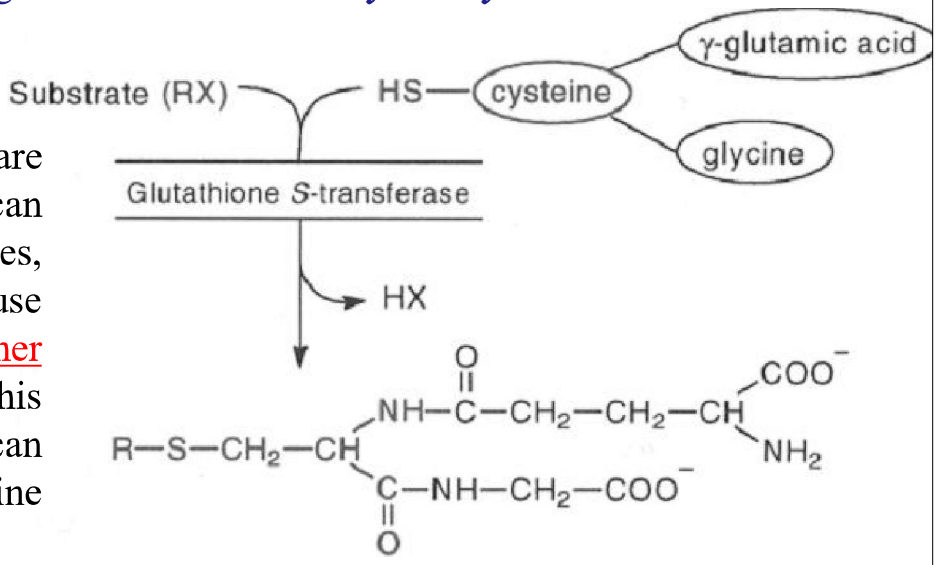
(3) Glutathione (GSH):

Glutathione (GSH) is a unique tripeptide (γ-glutamylcysteinylglycine) that has several important functions. Glutathione is found in most living organisms in its reduced form (GSH) in concentrations 500 times more than the oxidized form (GSSG).

Some Functions:

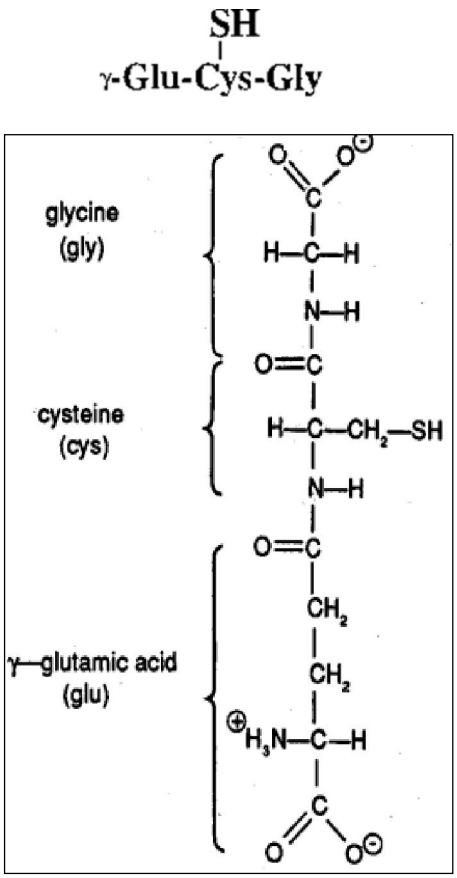
a. GSH is required in conjugation reactions catalyzed by Glutahione S-transferases (GSTs)



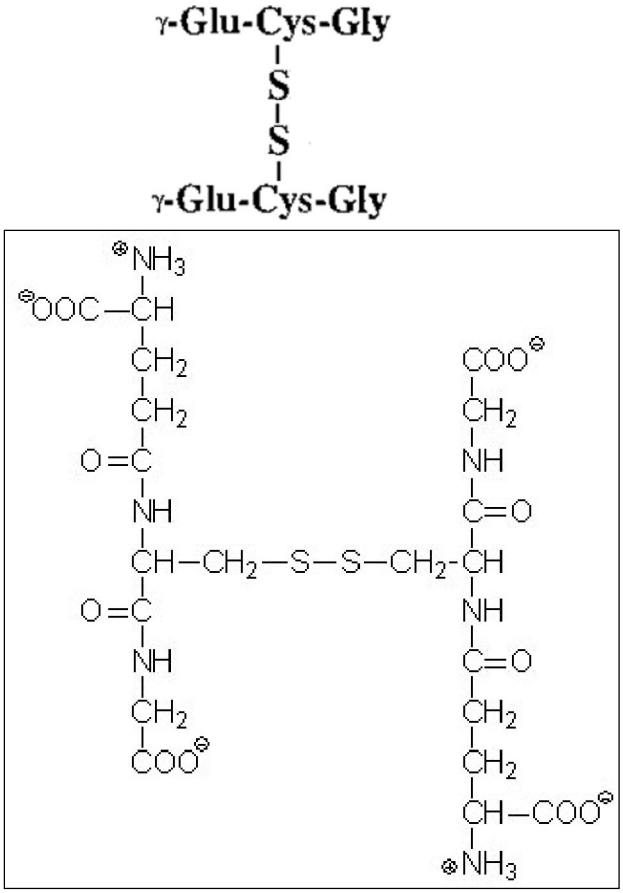
Hydrophobic substrates (RX) are potentially toxic species that can bind to critical nucleophiles, such as nucleic acids, and cause genetic mutations. Thioether conjugates formed in this reaction are hydrophilic so can be readily excreted in urine (detoxification)

Thioether conjugate

Reduced glutathione (GSH)



Oxidized glutathione (GSSG)

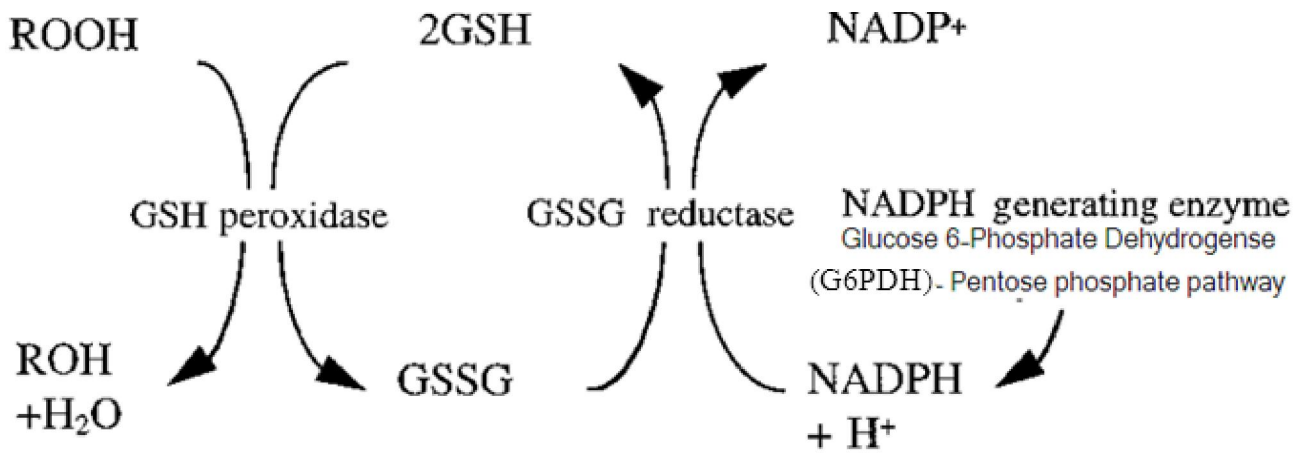


b. GSH is required as a reducing agent (antioxidant) to reduce organic and hydrogen peroxides (ROOH & H_2O_2) – detoxification. This reaction is catalyzed by the enzyme glutathione peroxidase (GSH is oxidized to GSSG)

c. GSH is required in high concentration in blood to keep a reducing environment. Glutathione reductase regenerates GSH from GSSG, where NADPH is required as a reducing agent to keep high [GSH]. The inability to maintain high [GSH] in RBC's leads to increased rates of oxidation of hemoglobin (Fe^{2+}) to methemoglobin (Fe^{3+}) leading to increased fragility of RBC's and consequently to hemolytic anemia.

The metabolic pathway called pentose phosphate pathway (PPP) in RBC's is essentially the only pathway for these cells to produce NADPH (the first reaction in this pathway is catalyzed by the enzyme glucose 6-phosphate dehydrogenase "G6PDH").

The most common inborn error in PPP is the result of mutation in G6PDH
Deficiency in the level of activity of G6PDH is the basis of favism and primaquine (an anti-malarial drug) sensitivity



Proteins

One or more polypeptide chains

One polypeptide chain - a monomeric protein

More than one - multimeric protein

Homomultimer - one kind of chain

Heteromultimer - two or more different chains

Hemoglobin, for example, is a heterotetramer It has two alpha chains and two beta chains

Size range is enormous

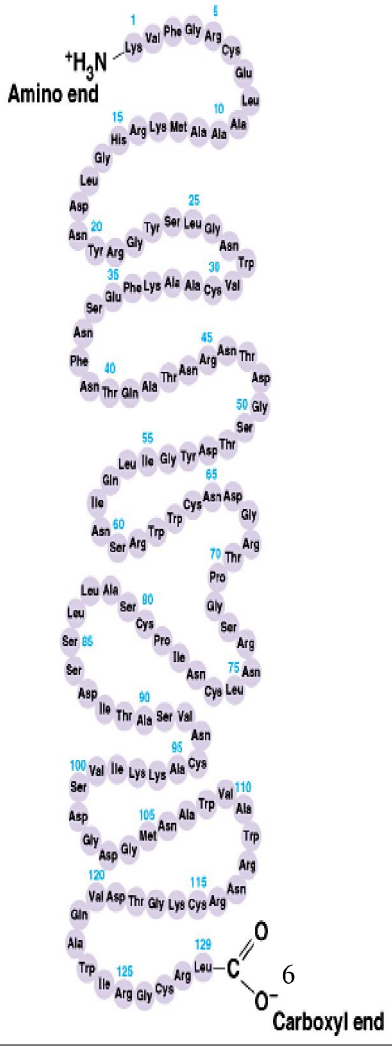
- Insulin - A chain of 21 residues, B chain of 30 residues -total MW of 5,733
- Glutamine synthetase - 12 subunits of 468 residues each - total MW of 600,000
- Connectin proteins - alpha - MW 2.8 million

Protein Structure

There are four levels of protein structure (primary, secondary, tertiary and quaternary)

Primary structure:

- The **primary structure** of a protein is its unique sequence of amino acids.
  - e.g. Lysozyme, an enzyme that attacks bacteria, consists of a polypeptide chain of 129 amino acids.
  - The precise primary structure of a protein is determined by inherited genetic information.
  - At one end is an amino acid with a free amino group the (the N-terminus) and at the other is an amino acid with a free carboxyl group the (the C-terminus).



High orders of Protein structure

- **A functional protein** is **not just** a polypeptide chain, but **one or more** polypeptides precisely **twisted, folded and coiled** into a molecule of unique shape (**conformation).** This conformation is essential for some protein function e.g. Enables a protein to recognize and bind specifically to another molecule e.g. hormone/receptor; enzyme/substrate and antibody/antigen.
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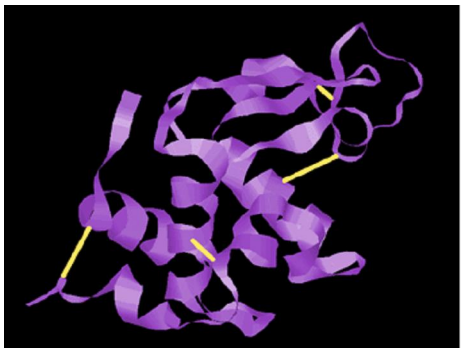
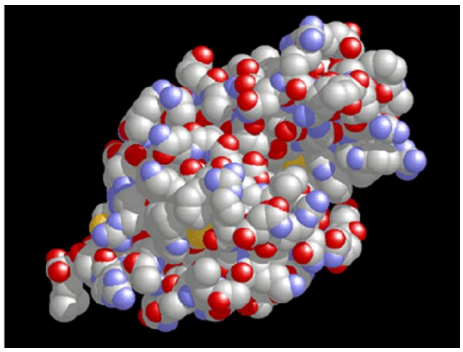




2- Secondary structure:

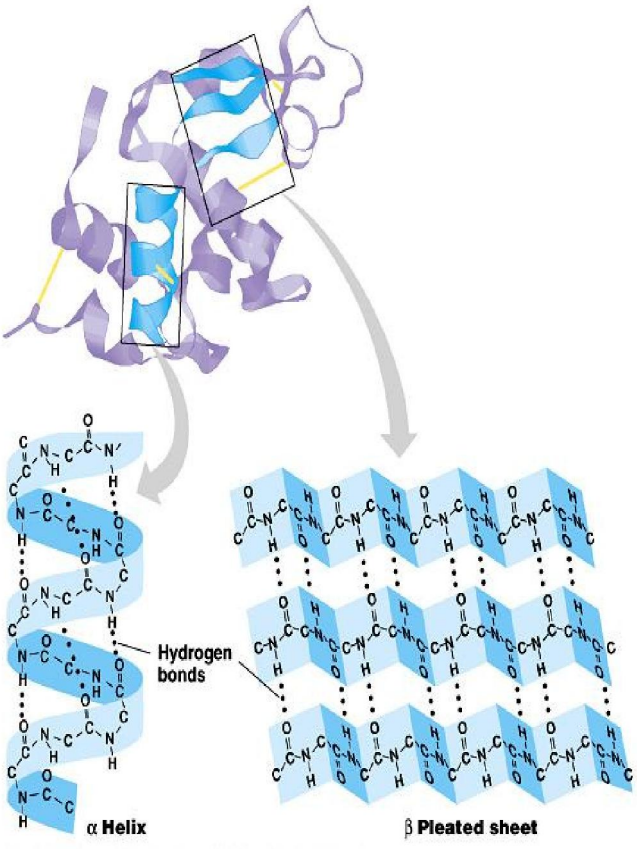
Local folding resulting from hydrogen bond formation between hydrogen of -NH group of peptide bond and the carbonyl oxygen of another peptide bond. According to H-bonding there are two main forms of secondary structure:

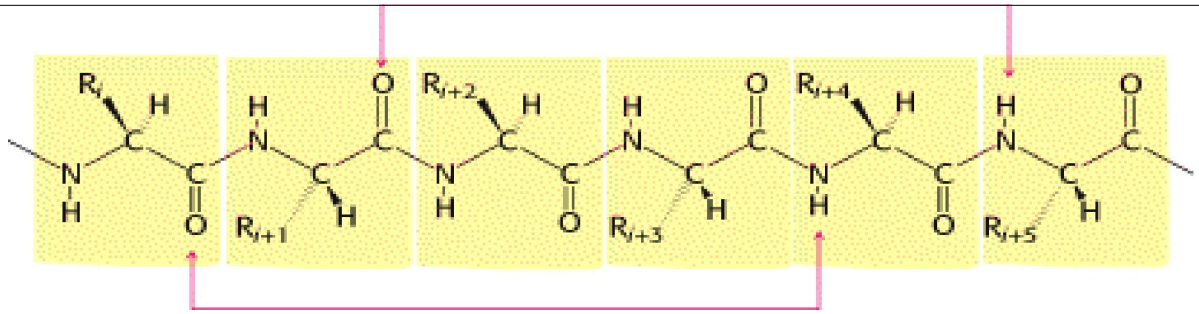
α-helix:

It is a spiral structure resulting from hydrogen bonding between **one peptide bond** and the **fourth one**

β-sheets:

is another form of secondary structure in which two or more polypeptides (or segments of the same peptide chain) are linked together by hydrogen bond between H- of NH- of one chain and carbonyl oxygen of adjacent chain (or segment).





Hydrogen bonding in α-helix: In the α-helix CO of the one amino acid residue (n) forms H-bond with NH of the fifth one (n+4).

Supersecondary structure or Motifs :

occurs by combining secondary structure.

The combination may be: α-helix-turn-α-helix-turn….

Or: β-sheet-turn-β-sheet-turn….

Or: α-helix-turn-β-sheet-turn-α-helix….

Turn (or bend): is short segment of polypeptides (3-4 amino acids) that connects successive secondary structures.

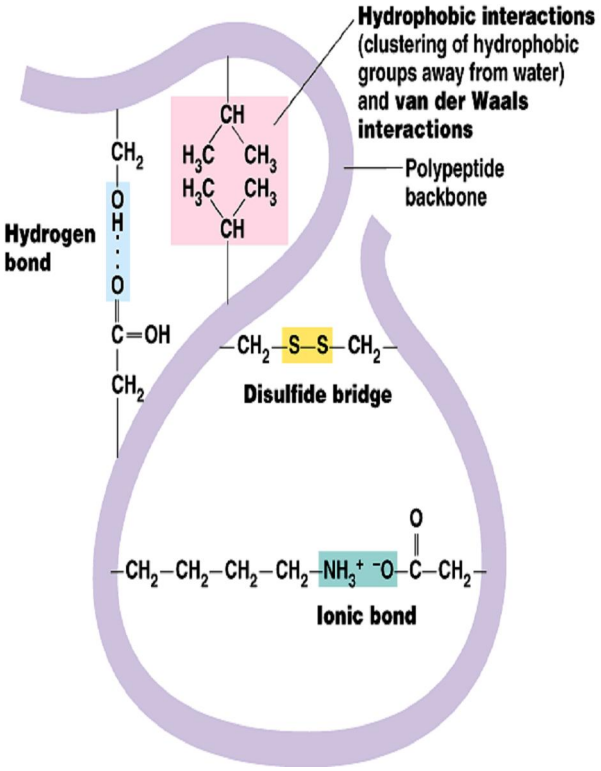
e.g. β-turn: is small polypeptide that connects successive strands of β-sheets.

- **Tertiary structure** is

  Additional folding determined by a variety of intrachain interactions between R groups of the polypeptide.

  a. **The weak interactions** include:
  - **Hydrogen bonds** among polar side chains
  - **Ionic bonds** between charged R groups (basic and acidic amino acids)
  - **Hydrophobic interactions** among hydrophobic (non polar) R groups.

  b. **Strong covalent bonds** include: **disulfide bridges**, that form between the sulfhydryl groups (SH) of cysteine monomers, stabilize the structure.



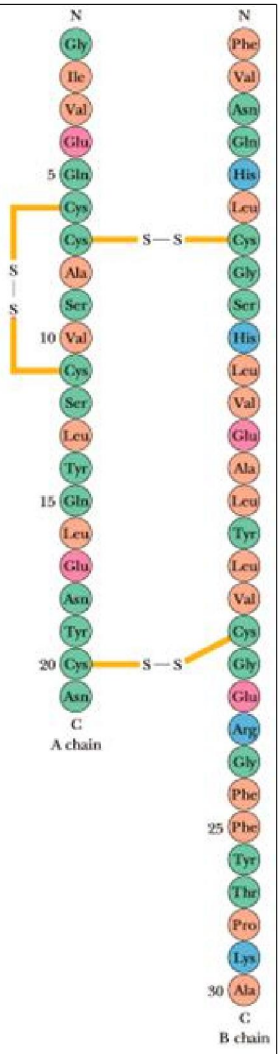
- **Quaternary structure:** results from the aggregation (combination) of two or more polypeptide subunits held together by interchain interactions.
- Examples on protein having quaternary structure:

– **Collagen:** is a fibrous protein of three polypeptides (trimeric) that are supercoiled like a rope.

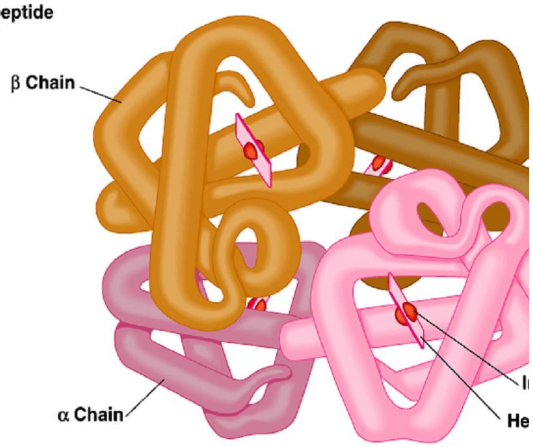
- This provides the structural strength for their role in connective tissue.

– **Hemoglobin:** is a globular protein with four polypeptide chains (tetrameric)

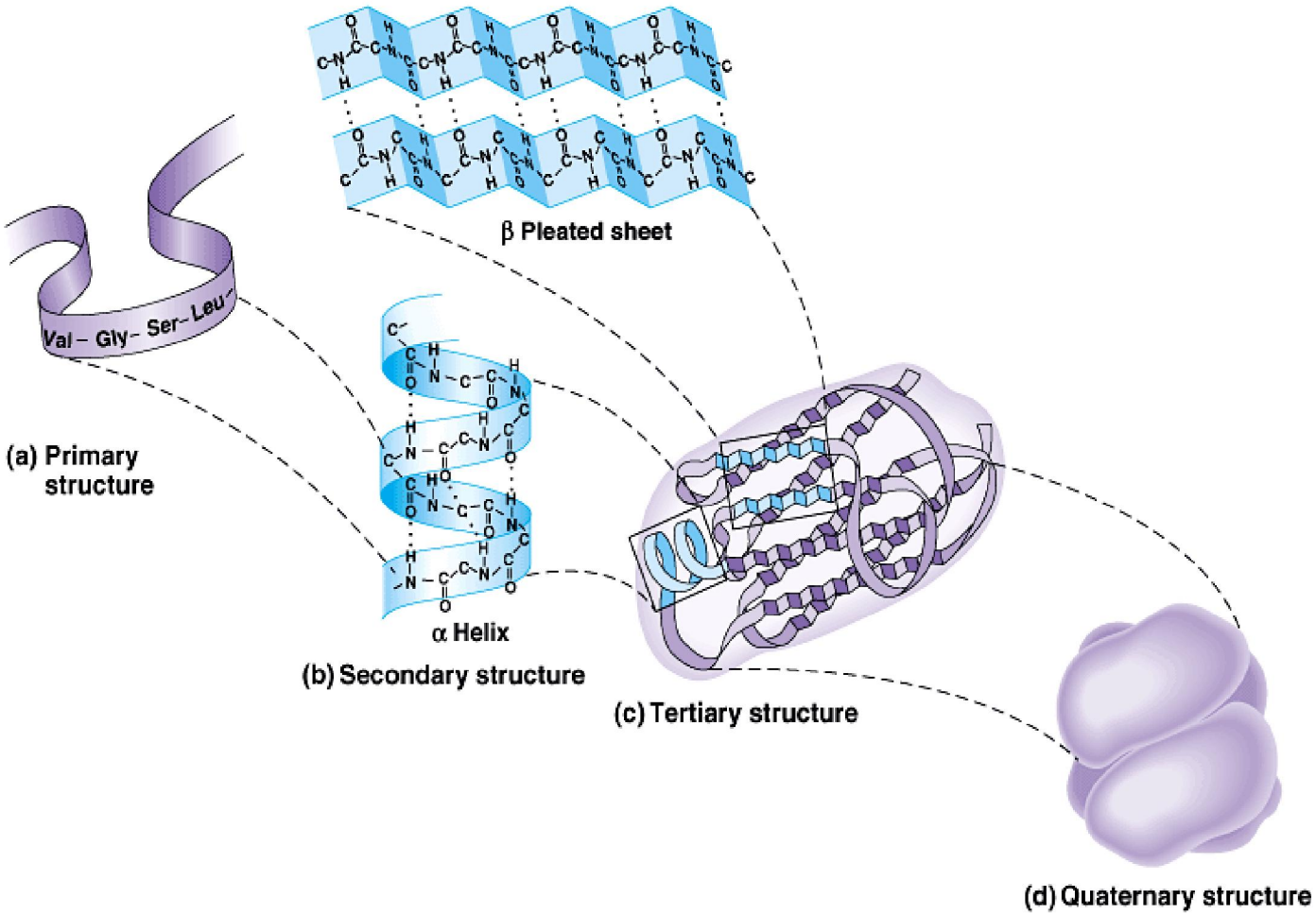
Insulin: two polypeptide chains (dimeric)



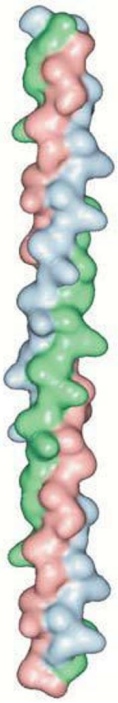
(a) Collagen



(b) Hemoglobin



* **Collagen:** protein of connective tissues found in bone, teeth, cartilage, tendons, skin and blood vessels.



- Collagen may be present as gel e.g. in extracellular matrix or in vitreous humor of the eye.
- Collagen are the most important protein in mammals. They form about 30% of total body proteins.
- There are more than 20 types of collagens, the most common type is **collagen I** (constitutes about 90% of cell collagens).
- **Structure of collagen:** three helical polypeptide chains (trimeric) twisted around each other forming triplet-helix molecule.
- ⅓ of structure is glycine, 10% proline, 10% hydroxyproline and 1% hydroxylysine. Glycine is found in every third position of the chain. The repeating sequence -Gly-X-Y-, where X is frequently proline and Y is often hydroxyproline and can be hydroxylysine.

Solubility: collagen is insoluble in all solvents and not digested.

- When collagen is heated with water or dil. HCl it will be converted into **gelatin** which is soluble , digestible and used as diet (as jelly). Gelatin is classified as derived protein.

Some collagen diseases:

1- Scurvy: disease due to deficiency of vitamin C (ascorbic acid) which is important coenzyme for conversion of proline into hydroxyproline and lysine into hydroxylysine. Thus, synthesis of collagen is decreased leading to abnormal bone development, bleeding, loosing of teeth and swollen gum.

2- Osteogenesis Imperfecta (OI): Inherited disease resulting from genetic deficiency or mutation in gene that synthesizes collagen type I leading to abnormal bone formation in babies and frequent bone fracture in children. It may be lethal.

* **Elastin:** present in walls of large blood vessels (such as aorta). It is very important in lungs, elastic ligaments, skin, cartilage, ...
It is elastic fiber that can be stretched to several times as its normal length.

Structure: composed of 4 polypeptide chains (tetramer), similar to collagen being having 33% glycine and rich in proline but in that it has low hydroxyproline and absence of hydroxy lysine.

Emphysema: is a chronic obstructive lung disease (obstruction of air ways) resulting from deficiency of α1-antitrypsin particularly in cigarette smokers.

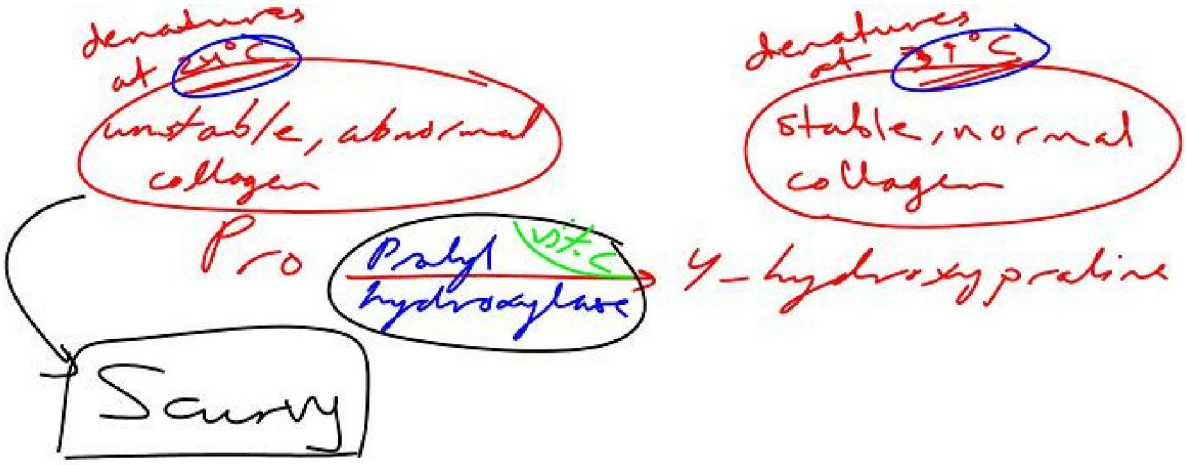
Role of α1-antitrypsin: Elastin is a lung protein. Smoke stimulate enzyme called elastase to be secreted form neutrophils (in lung). Elastase cause destruction of elastin of lung.

α1-antitrypsin is an enzyme (secreted from liver) and inhibit elastase and prevent destruction of elastin. So deficiency of α1-antitrypsin especially in smokers leads to degradation of lung and destruction of lung (loss of elasticity of lung), a disease called emphysema.

Derived proteins

Produced from hydrolysis of simple proteins.

e.g. - Gelatin: from hydrolysis of collagen
- Peptone: from hydrolysis of albumin



Classification of Proteins

Proteins can be classified according to their function (previously shown), shape (globular and fibrous) or chemical structure (simple and conjugated)

According to chemical structure

- Proteins containing only amino acids are called simple proteins
- Conjugated proteins have non amino acid constituents (chemical groups called prosthetic groups) covalently bound to proteins and essential for their activity
- Protein minus its prosthetic group is called an apoprotein
- Protein plus its prosthetic group is called a holoprotein

Classes of Conjugated Proteins

- Glycoproteins—carbohydrate residues
- Lipoproteins—associated with lipids
- Nucleoproteins—associated with nucleic acids
- Phosphoproteins—covalently modified by phosphate
- Metalloproteins—metal ion(s)
- Hemoproteins—heme (iron-porphyrin)
- Flavoproteins—flavin group, involved in oxidation-reduction

Globular and Fibrous Proteins

Fibrous Proteins

Little or no tertiary structure.
Long parallel polypeptide chains.
Cross linkages at intervals forming long fibres or sheets.
Usually insoluble.
Many have structural roles.
E.g. keratin in hair and the outer layer of skin, collagen (a connective tissue).

Globular Proteins

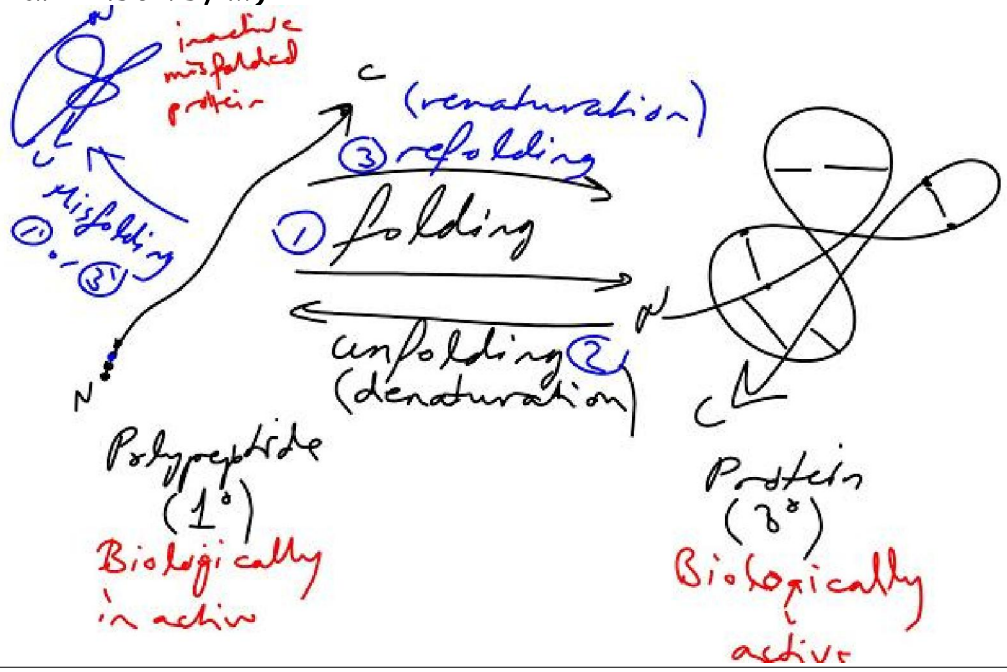
Have complex tertiary and sometimes quaternary structures.
Folded into spherical (globular) shapes.
Usually soluble as hydrophobic side chains in centre of structure.
Roles in metabolic reactions.
E.g. enzymes, hemoglobin in blood.

Protein Folding

- The process by which a protein goes from being an unfolded polymer with no activity to a uniquely structured and active protein.

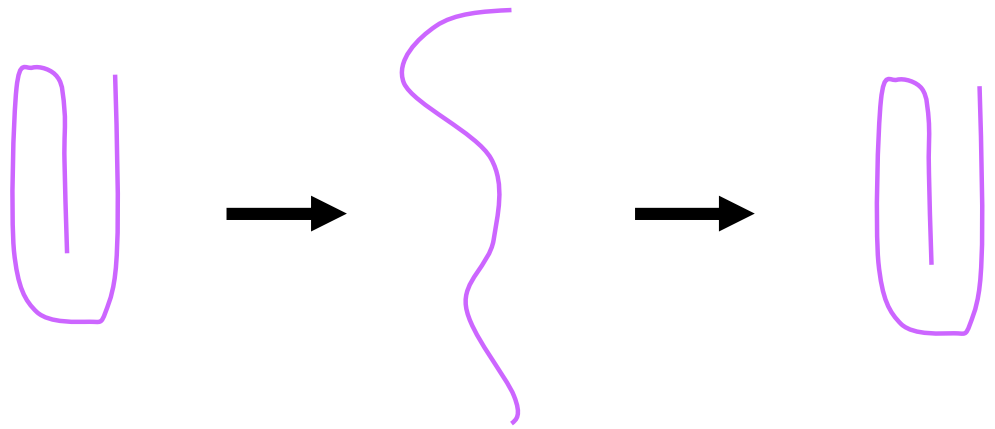
Why do we care about protein folding?

- If we understand how proteins fold, maybe it will help us predict their three-dimensional structure from sequence information alone.
- Protein misfolding has been implicated in many human diseases (Alzheimer's, Parkinson's, …)



Protein folding *in vitro* is often reversible

(indicating that the final folded structure is determined by its amino acid sequence)



37° C 70° C 37° C

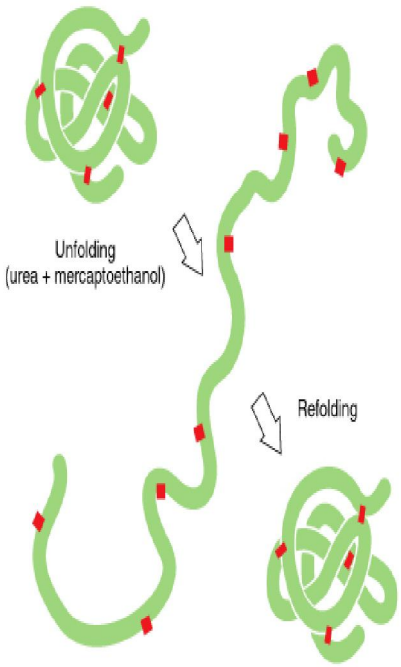
Molecular Chaperones

- Nature has a developed a diverse set of proteins (chaperones) to help other proteins fold.

- Over 20 different types of chaperones have been identified. Many of these are produced in greater numbers during times of cellular stress.

DENATURATION

- Under some conditions proteins will not fold into their biochemically functional forms. Temperatures above or below the range that cells tend to live in will cause thermally unstable proteins to unfold or "denature" (this is why boiling makes an egg white turn opaque). High concentrations of solutes, extremes of pH, mechanical forces, and the presence of chemical denaturants can do the same. Protein thermal stability is far from constant, however. For example, hyperthermophilic bacteria have been found that grow at temperatures as high as 122 °C, which of course requires that their full complement of vital proteins and protein assemblies be stable at that temperature or above.
- A fully denatured protein lacks both tertiary and secondary structure, and exists as a so-called random coil. Under certain conditions some proteins can refold; however, in many cases denaturation is irreversible. Cells sometimes protect their proteins against the denaturing influence of heat with enzymes known as chaperones or heat shock proteins, which assist other proteins both in folding and in remaining folded. Some proteins never fold in cells at all except with the assistance of chaperone molecules, which either isolate individual proteins so that their folding is not interrupted by interactions with other proteins or help to unfold misfolded proteins, giving them a second chance to refold properly. This function is crucial to prevent the risk of precipitation into insoluble amorphous aggregates.



From C. J. Epstein, R. F. Goldberger, and C. B. Anfinsen, *Cold Spring Harbor Symp. Quant. Biol.* 28:439, 1963.

Biochemistry 24311
Dr. Awni A. Abu-Hijleh