CBCS 3RD SEM (MAJOR)
Credits: 4

Unit 3 (II): Proteins

BONDS STABILIZING PROTEIN STRUCTURE, LEVELS OF ORGANIZATION IN PROTEINS, DENATURATION, INTRODUCTION TO SIMPLE AND CONJUGATE PROTEINS

By-
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Definition

Proteins are large biomolecules, or macromolecules, consisting of one or more long chains of amino acid residues. Proteins perform a vast array of functions within organisms, including catalyzing metabolic reactions, DNA replication, responding to stimuli, providing structure to cells, and organisms, and transporting molecules from one location to another. Proteins differ from one another primarily in their sequence of amino acids, which is dictated by the nucleotide sequence of their genes, and which usually results in protein folding into a specific 3D structure that determines its activity.

Protein Bonds

Proteins are the polymers of amino acids. Amino acids are joined together by a special type of covalent bond (peptide bond) to form linear structures called polypeptides. The polypeptides are then folded into specific structures to form the functional conformation of the protein. The folding of proteins into specific shapes and conformations are assisted and stabilized by many types of bonds in them. Some of these bonds are strong bonds whereas others are weak interactions. Important types of bonds involved in protein structure and conformation are Peptide bonds, Ionic bonds, Disulfide bonds, Hydrogen bonds and Hydrophobic Interactions.

Bonds Stabilizing Protein structure

Types of bonds

There are 5 types of chemical bonds that play important roles in determining and stabilizing 3-D protein structure.

They are:

1) Peptide bonds

2) Ionic Bonds

3) Disulfide Bonds

4) Hydrogen bonds

5) Hydrophobic bonds

Peptide Bonds

- Peptide bond definition: a covalent bond formed between the carboxylic group of one amino acid and the group of another amino acid
- Peptide bond is a strong covalent bond with high bond dissociation energy.
- It is formed by the joining of two amino acid residues during protein synthesis.
- The carboxylic group (-COOH) of one amino acid combine with the amino group ($-NH_2$) of another amino acid to form the peptide bond.
- Peptide bond formation is an example for a condensation or elimination reaction.
- One molecule of water is eliminated during the formation of peptide bond by the condensation reaction of two amino acids.
- The resulting compound after the peptide bond formation is called a dipeptide.

- A dipeptide has a free amino group at one end and a carboxylic group at the other end.
- The free amino group or carboxyl group of a dipeptide can form another peptide bond with another peptide bond with a third amino acid and so on.
- Many amino acids join together in this manner to form a polypeptide.
- Peptide bonds formation is facilitated by the enzyme **Peptidyl transferase** during the translation process of protein synthesis.
- Peptidyl transferase enzyme is a ribozyme; it is a part of the ribosomal RNA (rRNA) of large subunit of Ribosome.
- In prokaryotes the 23S rRNA and in eukaryotes the 28S rRNA acts as the Peptidyl transferase enzyme.

- The primary structure of the protein is stabilized by the peptide bond.

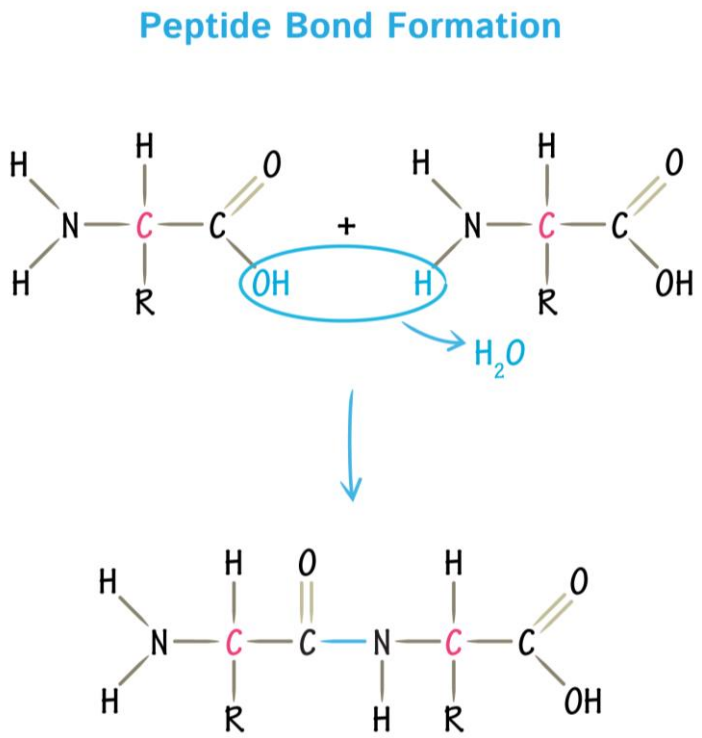


FIG: Primary structure and peptide bond.

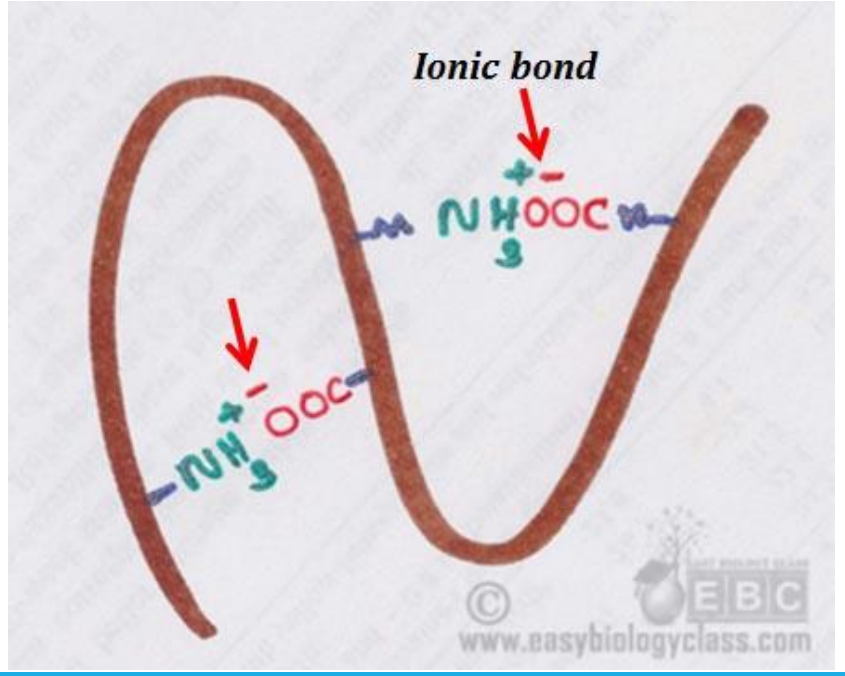
Ionic Bond

- Ionic Bond definition: a chemical bond formed between the two ions of opposite charges.
- In proteins, the ionic bonds are formed between the ionized acidic or basic groups of amino acids.
- The R groups (side chains) of certain amino acids contain additional acidic ($-COO^-$) or basic ($-NH_3^+$) groups.
- These R groups can ionize to produce charged groups at certain pH.
- Acidic R groups will be negatively charged since they release the H^+ ions

$$-COOH \longrightarrow COO^- + H^+$$

- Basic R groups will be positively charged since they accept H^+ ions from the medium.

$$NH_2 + H^+ \longrightarrow NH_3^+$$

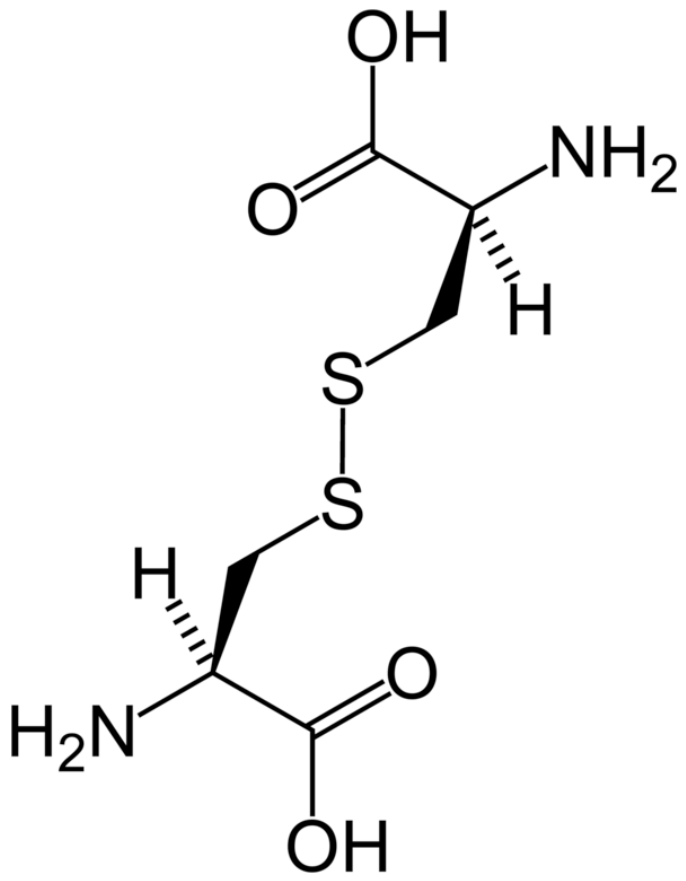


- After the ionization of side chain as mentioned above, the amino acids in the protein chain can attract/repel each other based on their charges. {This is also called “Electrostatic Interactions”}
- The attraction of oppositely charged R groups results in the formation of ionic bonds.
- Ionic bonds are weak bonds and they are very fragile in an aqueous medium.
- Even a change in pH may break down the ionic bonds.
- This is the reason for the denaturation of proteins in acidic or basic medium.
- Tertiary and Quaternary structures of proteins are stabilized by ionic bonds.

Disulfide bonds

- Disulfide bond definition: A covalent bond formed from two thiol groups of two cysteine residues in a protein.
- The cysteine (Cys or C, a sulfur containing amino acid) contain a highly reactive sulfhydryl group (-SH) in its side chain (R group).
- The sulfhydryl is highly polar and highly reactive.
- If two molecules of a cysteine line up alongside each other, the neighboring sulfhydryl groups can be oxidized.
- This reaction results in the formation of a permanent covalent connection between two cysteine residues called disulfide bond.
- Disulfide bond in protein chemistry is better known as the disulfide bridge or S-S bond.

- They are very strong bonds that are not easy to break.
- A disulfide bond may be formed between the cysteine residues of same polypeptide chain or different polypeptide chain of a functional protein.
- Disulfide bonds stabilize the tertiary structures of the protein.



Hydrogen bonds

- Hydrogen bond definition: Hydrogen bond is an electrostatic attraction between a hydrogen atom, which is covalently bound to a high electronegative atom (such as Oxygen and Nitrogen), to another electronegative atom of same or different molecules of their close neighborhood.
- Hydrogen present in the-OH group of $-NH_2$ of amino acids become slightly electropositive.
- This is due to the high electronegativity of O and N when compared to hydrogen.
- Due to the high electronegativity, Oxygen and Nitrogen attract the shared electron of hydrogen more towards them.
- Thus hydrogen attached to these high electronegative atoms will get a partial positive charge called **δ** positive whereas the electronegative atoms will get a partial negative charge called **δ** negative.

- Consequently, the slightly positive H is then attracted towards the neighboring electronegative oxygen of –C=O or nitrogen atom –NH_2 group.
- These –C=O and NH_2 groups occur along the length of the polypeptide chain in regular sequence.
- Thus the formation of hydrogen bonds gives a regular shape to the polypeptide chain such as alpha helix and beta plates.
- Hydrogen bonds are very weak bonds.
- Occurrence of hydrogen bonds in high frequency makes a considerable contribution towards the molecular stability of proteins.
- Hydrogen bonds are involved in stabilizing the secondary, tertiary and quarternary structure of proteins.

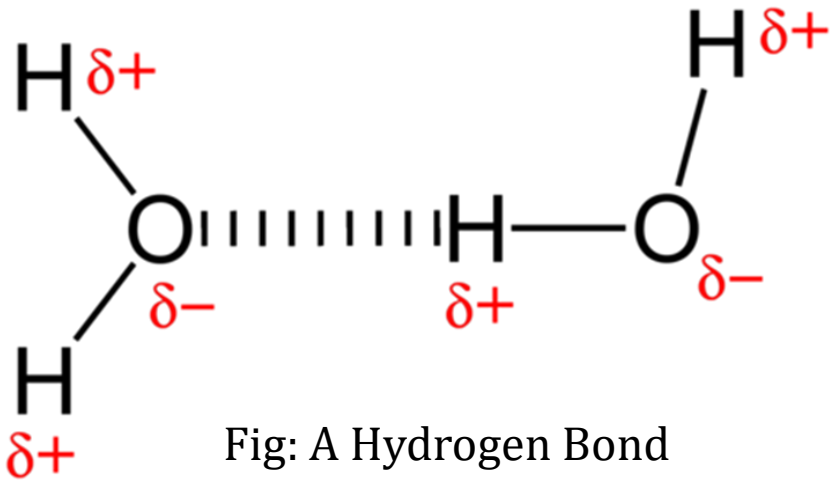
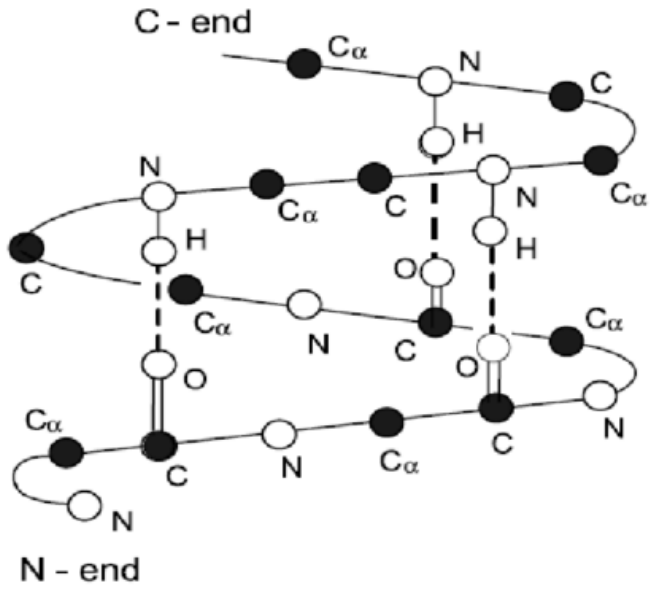


Fig: A Hydrogen Bond



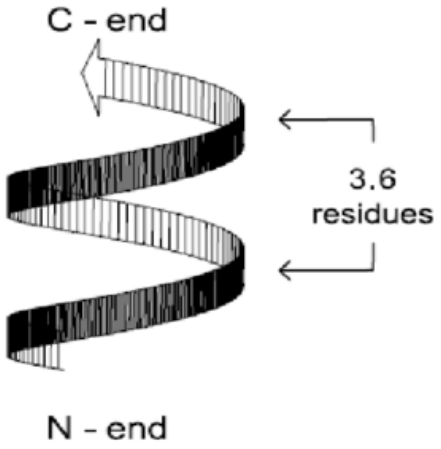


Fig: **α** -Helix

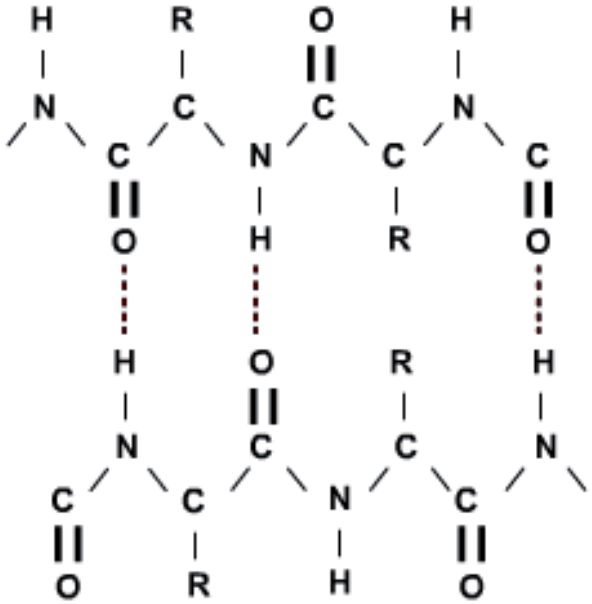


Fig: **β**-plated sheet

Hydrophobic Interactions

- Some R groups in the amino acids are non-polar.
- Example: Alanine, valine, isoleucine, leucine and methionine.
- The non-polar R groups are hydrophobic and they try stay away from water.
- In a long polypeptide chain there may be many such non-polar amino acids which may be adjacent to each other or separated by polar R groups.
- In an aqueous environment (inside the cell) the linear polypeptide will fold into such a shape that the hydrophobic amino acids come in close contact with each other and they try to exclude the water due to its hydrophobicity.
- By this method, the peptide chain of a globular protein will fold into a spherical shape in the aqueous environment.

- In a folded protein the hydrophobic groups tend to orient towards the inner side of the protein.
- The hydrophilic residues will form a shell over the hydrophobic residues.
- The hydrophilic shell makes the protein soluble in the aqueous environment.
- Similarly, in the unit membrane, the orientations of membrane proteins are also affected by the hydrophilic and hydrophobic interactions.
- The hydrophobic domain of membrane protein orient towards the exterior of the membrane whereas the hydrophobic domain will orient towards the interior (to the lipid portion).

Protein Structure/ Levels of organization of proteins.

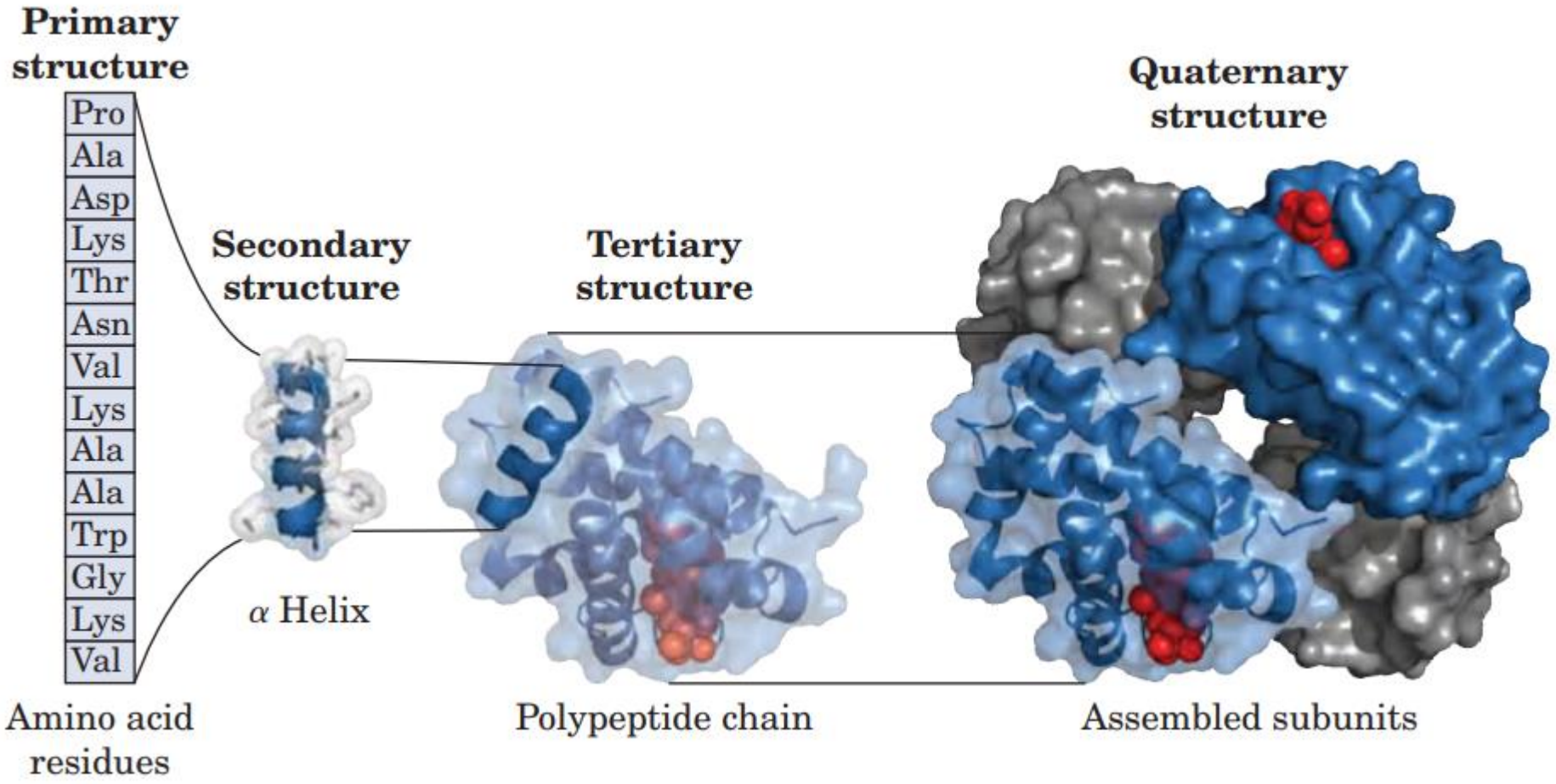


Fig: Levels of structure in proteins. The primary structure consists of a sequence of amino acids linked together by peptide bonds and includes any disulfide bonds. The resulting polypeptide can be arranged into units of secondary structure, such as an helix. The helix is a part of the tertiary structure of the folded polypeptide, which is itself one of the subunits that make up the quaternary structure of the multi-subunit protein, in this case hemoglobin.

Introduction

Most proteins fold into unique 3D structures. The shape into which a protein naturally folds is known as its native conformation. Although many proteins can fold unassisted, simply through the chemical properties of their amino acids, others require the aid of molecular chaperones to fold into their native states. Biochemists often refer to four distinct aspects of a protein's structure:

1. Primary Structure
2. Secondary Structure
3. Tertiary Structure
4. Quaternary Structure

Protein primary structure

Definition

Protein primary structure is the linear sequence of amino acids in a peptide or protein. By convention, the primary structure of a protein is reported starting from the amino-terminal (N) end to the carboxyl-terminal (C) end. Protein biosynthesis is most commonly performed by ribosomes in cells. Peptides can also be synthesized in the laboratory. Protein primary structures can be directly sequenced, or inferred from DNA sequences.

Formation

- **Biological:**

Amino acids are polymerized via peptide bonds to form a long backbone, with the different amino acid side chains protruding along it. In biological systems, proteins are produced during translation by a cell's ribosomes. Some organisms can also make short peptides by non-ribosomal peptide synthesis, which often use amino-acids other than the standard 20, and may be cyclized, modified and cross-linked.

- **Chemical:**

Peptides can be synthesized chemically via a range of laboratory methods. Chemical methods typically synthesize peptides in the opposite order (starting at the C-terminus) to biological protein synthesis (starting at the N-terminus).

Notation

Protein sequence is typically notated as a string of letters, listing the amino acids starting at the amino-terminal end through to the carboxyl-terminal end. Either a three letter code or single letter code can be used to represent the 20 naturally occurring amino acids, as well as mixtures or ambiguous amino acids (similar to nucleic acid notation).

Peptides can be directly sequenced, or inferred from DNA sequences. Large sequence databases now exist that collate known protein sequences.

20 Natural amino acid notation

Ambiguous amino acid notation

Amino Acid	3-Letter	1-Letter	Symbol	Description	Residues represented
Alanine	Ala	A	X	Any amino acid, or unknown	All
Arginine	Arg	R	B	Aspartate or Asparagine	D, N
Asparagine	Asn	N	Z	Glutamate or Glutamine	E, Q
Aspartic acid	Asp	D	J	Leucine or Isoleucine	I, L
Cysteine	Cys	C	Φ	Hydrophobic	V, I, L, F, W, M
Glutamic acid	Glu	E	Ω	Aromatic	F, W, Y, H
Glutamine	Gln	Q	Ψ	Aliphatic	V, I, L, M
Glycine	Gly	G	π	Small	P, G, A, S
Histidine	His	H	ζ	Hydrophilic	S, T, H, N, Q, E, D, K, R, Y
Isoleucine	Ile	I	+	Positively charged	K, R, H
Leucine	Leu	L	-	Negatively charged	D, E
Lysine	Lys	K			
Methionine	Met	M			
Phenylalanine	Phe	F			
Proline	Pro	P			
Serine	Ser	S			
Threonine	Thr	T			
Tryptophan	Trp	W			
Tyrosine	Tyr	Y			
Valine	Val	V			

Modification

In general, polypeptides are unbranched polymers, so their primary structure can often be specified by the sequence of amino acids along their backbone. However, proteins can become cross-linked, most commonly by disulfide bonds, and the primary structure also requires specifying the cross-linking atoms, e.g., specifying the cysteines involved in the protein's disulfide bonds. Other crosslinks include desmosine.

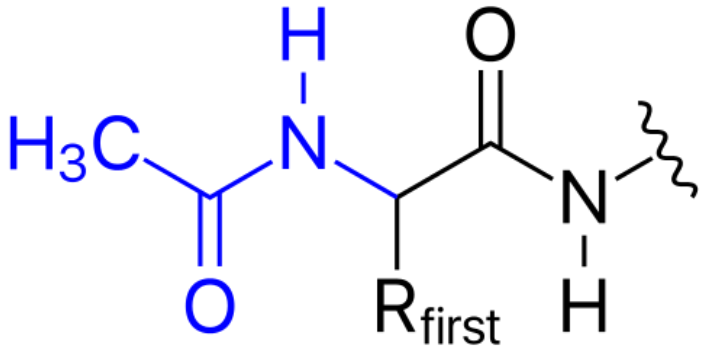
Isomerization

The chiral (asymmetric) centers of a polypeptide chain can undergo racemization. Although it does not change the sequence, it does affect the chemical properties of the sequence. In particular, the L-amino acids normally found in proteins can spontaneously isomerize at the $\mathbf{C^{\alpha}}$ atom to form D-amino acids, which cannot be cleaved by most proteases. Additionally, proline can form stable trans-isomers at the peptide bond.

Post-translational modification

Finally, the protein can undergo a variety of posttranslational modifications, which are briefly summarized here.

The N-terminal amino group of a polypeptide can be modified covalently, e.g.,



1. **Acetylation –C(=O)-CH_3 :**
The positive charge on the N-terminal amino group may be eliminated by changing it to an acetyl group (N-terminal blocking)

2. Formylation -C(=O)H :
The N-terminal methionine usually found after translation has an N-terminus blocked with a formyl group. This formyl group (and sometimes the methionine residue itself, if followed by Gly or Ser) is removed by the enzyme deformylase.

3. Pyroglutamate:
An N-terminal glutamine can attack itself, forming a cyclic pyroglutamate group.

4. Myristoylation: -C(=O)-$(CH_2)_{12}$-CH_3
Similar to acetylation. Instead of a simple methyl group, the myristoyl group has a tail of 14 hydrophobic carbons, which make it ideal for anchoring proteins to cellular membranes.

The C-terminal carboxylate group of a polypeptide can also be modified, e.g.,

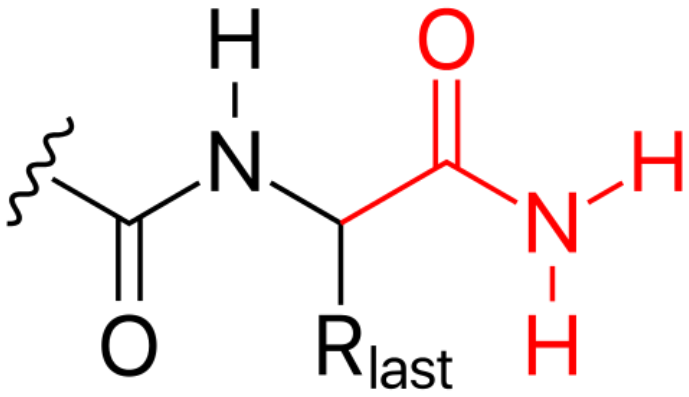


Fig: C-terminal amination

5. Amination:
The C-terminus can also be blocked (thus, neutralizing its negative charge) by amination.

6. Glycosyl phosphatidylinositol (GPI) attachment:
Glycosyl phosphatidylinositol(GPI) is a large, hydrophobic phospholipid prosthetic group that anchors proteins to cellular membranes. It is attached to the polypeptide C-terminus through an amide linkage that then connects to ethanolamine, thence to sundry sugars and finally to the phosphatidylinositol lipid moiety.

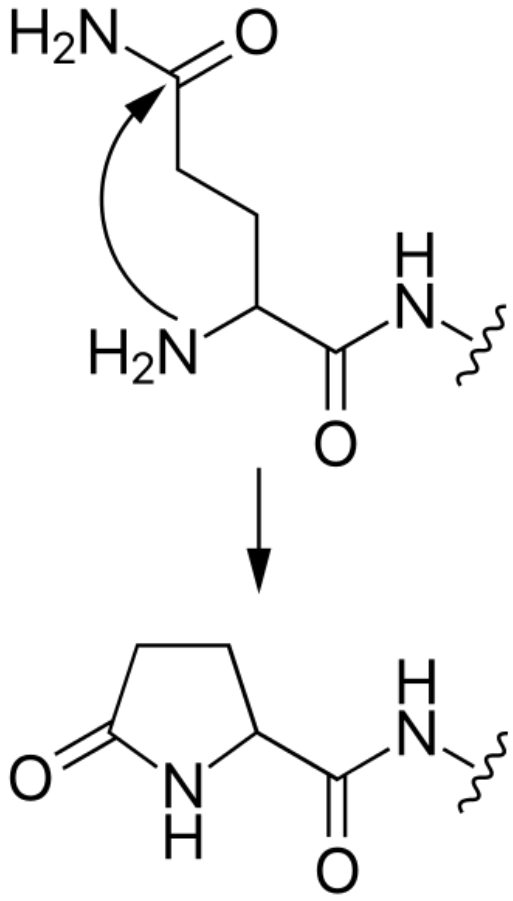


Fig. Formation of pyroglutamate from an Nterminal glutamine

7. Phosphrylation

Aside from cleavage, phosphorylation is perhaps the most important chemical modification of proteins. A phosphate group can be attached to the sidechain hydroxyl group of serine, threonine and tyrosine residues, adding a negative charge at that site and producing an unnatural amino acid. Such reactions are catalyzed by kinases and the reverse reaction is catalyzed by phosphatases. The phosphorylated tyrosines are often used as "handles" by which proteins can bind to one another, whereas phosphorylation of Ser/Thr often induces conformational changes, presumably because of the introduced negative charge. The effects of phosphorylating Ser/Thr can sometimes be simulated by mutating the Ser/Thr residue to glutamate.

8. Glycosylation

A catch-all name for a set of very common and very heterogeneous chemical modifications. Sugar moieties can be attached to the sidechain hydroxyl groups of Ser/Thr or to the sidechain amide groups of Asn. Such attachments can serve many functions, ranging from increasing solubility to complex recognition. All glycosylation can be blocked with certain inhibitors, such as tunicamycin.

9. Deamidation

In this modification, an asparagine or aspartate side chain attacks the following peptide bond, forming a symmetrical succinimide intermediate. Hydrolysis of the intermediate produces either aspartate or the β-amino acid, iso(Asp). For asparagine, either product results in the loss of the amide group, hence "deamidation".

10. Hydroxylation

Proline residues may be hydroxylates at either of two atoms, as can lysine (at one atom).

Hydroxyproline is a critical component of collagen, which becomes unstable upon its loss.

The hydroxylation reaction is catalyzed by an enzyme that requires ascorbic acid (vitamin C), deficiencies in which lead to many connective-tissue diseases such as scurvy.

11. Methylation

Several protein residues can be methylated, most notably the positive groups of lysine and

arginine. Arginine residues interact with the nucleic acid phosphate backbone and commonly form hydrogen bonds with the base residues, particularly guanine, in protein–DNA complexes. Lysine residues can be singly, doubly and even triply methylated. Methylation does not alter the positive charge on the side chain, however.

12. Acetylation

Acetylation of the lysine amino groups is chemically analogous to the acetylation of the Nterminus. Functionally, however, the acetylation of lysine residues is used to regulate the

binding of proteins to nucleic acids. The cancellation of the positive charge on the lysine

weakens the electrostatic attraction for the (negatively charged) nucleic acids.

13. Sulfation

Tyrosines may become sulfated on their atom. Somewhat unusually, this modificationoccurs in the Golgi apparatus, not in the endoplasmic reticulum. Similar to phosphorylatedtyrosines, sulfated tyrosines are used for specific recognition, e.g., in chemokine receptors on the cell surface. As with phosphorylation, sulfation adds a negative charge to a previously neutral site

14. Prenylation and Palmitoylation $–C(=O)-(CH_2)_{14}-CH_3$

The hydrophobic isoprene (e.g., farnesyl, geranyl, and geranylgeranyl groups) and palmitoyl groups may be added to the atom of cysteine residues to anchor proteins to cellular membranes. Unlike the GPI and myritoyl anchors, these groups are not necessarily added at the termini.

15. Carboxylation

A relatively rare modification that adds an extra carboxylate group (and, hence, a double negative charge) to a glutamate side chain, producing a Gla residue. This is used to strengthen the binding to "hard" metal ions such as calcium.

16. ADP-Ribosylation

The large ADP-ribosyl group can be transferred to several types of side chains within proteins, with heterogeneous effects. This modification is a target for the powerful toxins of disparate bacteria, e.g., Vibrio cholerae, Corynebacterium diphtheriae and Bordetella pertussis.

17. Ubiquitination and SUMYOlation

Various full-length, folded proteins can be attached at their C-termini to the sidechain ammonium groups of lysines of other proteins. Ubiquitin is the most common of these, and usually signals that the ubiquitin-tagged protein should be degraded.

Most of the polypeptide modifications listed above occur post-translationally, i.e., after the protein has been synthesized on the ribosome, typically occurring in the endoplasmic reticulum, a subcellular organelle of the eukaryotic cell.

Many other chemical reactions (e.g., cyanylation) have been applied to proteins by chemists, although they are not found in biological synthesis.

Cleavage and ligation

In addition to those listed above, the most important modification of primary structure is peptide cleavage (by chemical hydrolysis or by proteases). Proteins are often synthesized in an inactive precursor form; typically, an N-terminal or C-terminal segment blocks the active site of the protein, inhibiting its function. The protein is activated by cleaving off the inhibitory peptide. Some proteins even have the power to cleave themselves. Typically, the hydroxyl group of a serine (rarely, threonine) or the thiol group of a cysteine residue will attack the carbonyl carbon of the preceding peptide bond, forming a tetrahedrally bonded intermediate [classified as a hydroxyoxazolidine (Ser/Thr) or hydroxythiazolidine (Cys) intermediate].

This intermediate tends to revert to the amide form, expelling the attacking group, since the amide form is usually favored by free energy, (presumably due to the strong resonance stabilization of the peptide group). However, additional molecular interactions may render the amide form less stable; the amino group is expelled instead, resulting in an ester (Ser/Thr) or thioester (Cys) bond in place of the peptide bond. This chemical reaction is called an N-O acyl shift.

The ester/thioester bond can be resolved in several ways:

- Simple hydrolysis will split the polypeptide chain, where the displaced amino group becomes the new N-terminus. This is seen in the maturation of glycosylasparaginase.
- A β-elimination reaction also splits the chain, but results in a pyruvoyl group at the new Nterminus. This pyruvoyl group may be used as a covalently attached catalytic cofactor in some enzymes, especially decarboxylases such as S-adenosylmethionine decarboxylase (SAMDC) that exploit the electron-withdrawing power of the pyruvoyl group.

- Intramolecular transesterification, resulting in a branched polypeptide. In inteins, the new ester bond is broken by an intramolecular attack by the soon-to-be C-terminal asparagine.
- Intermolecular transesterification can transfer a whole segment from one polypeptide to another, as is seen in the Hedgehog protein autoprocessing.

Protein secondary structure

Introduction

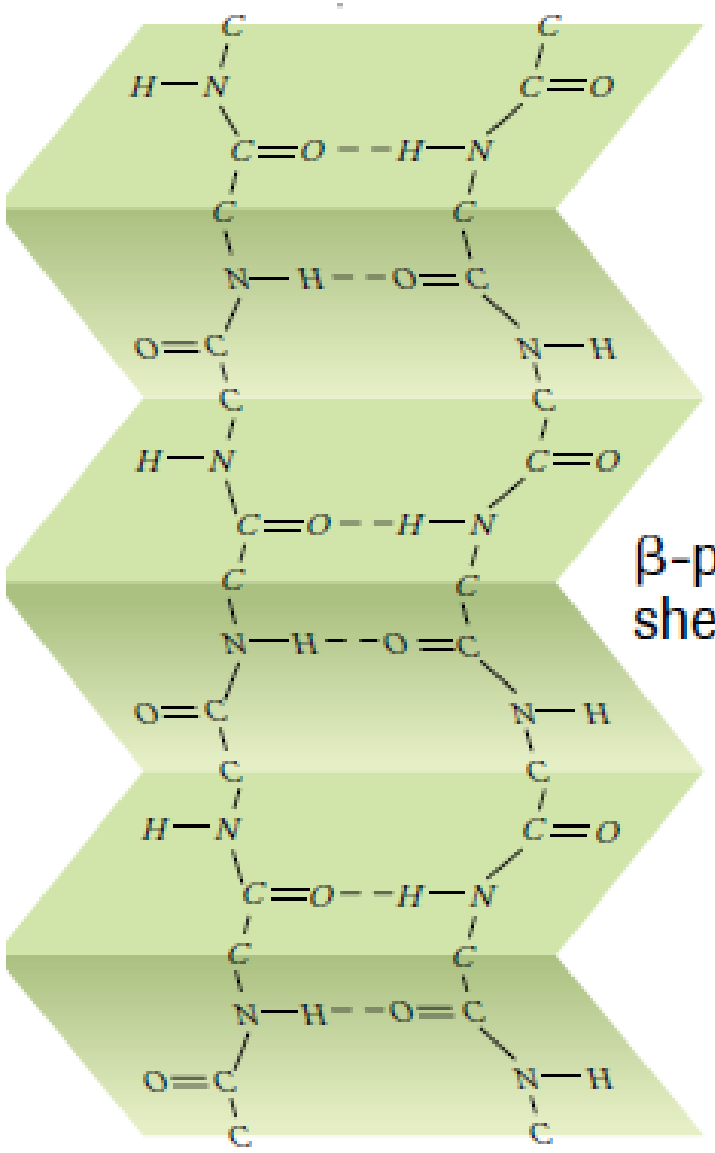
Protein secondary structure is the three dimensional form of local segments of proteins. The two most common secondary structural elements are alpha helices and beta sheets, though beta turns and omega loops occur as well. Secondary structure elements typically spontaneously form as an intermediate before the protein folds into its three dimensional tertiary structure.

Secondary structure is formally defined by the pattern of hydrogen bonds between the amino hydrogen and carboxyl oxygen atoms in the peptide backbone. Secondary structure may alternatively be defined based on the regular pattern of backbone dihedral angles in a particular region of the Ramachandran plot regardless of whether it has the correct hydrogen bonds.

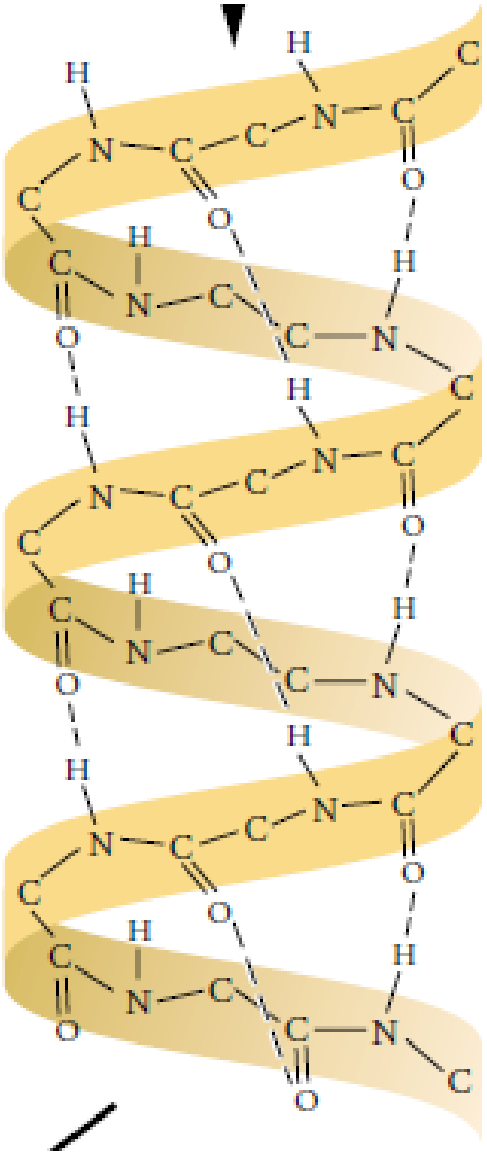
The concept of secondary structure was first introduced by Kaj Ulrik Linderstrøm-Lang at Stanford in 1952. Other types of biopolymers such as nucleic acids also possess characteristic secondary structures.

α helices and β sheets

The most common secondary structures are alpha helices and beta sheets. Other helices, such as the 310 helix and π helix, are calculated to have energetically favorable hydrogen-bonding patterns but are rarely observed in natural proteins except at the ends of α helices due to unfavorable backbone packing in the center of the helix. Other extended structures such as the polyproline helix and alpha sheet are rare in native state proteins but are often hypothesized as important protein folding intermediates. Tight turns and loose, flexible loops link the more "regular" secondary structure elements. The random coil is not a true secondary structure, but is the class of conformations that indicate an absence of regular secondary structure.



β-pleated sheet



α helix

Protein tertiary structure

Introduction

Protein tertiary structure is the three dimensional shape of a protein. The tertiary structure will have a single polypeptide chain "backbone" with one or more protein secondary structures, the protein domains. Amino acid side chains may interact and bond in a number of ways. The interactions and bonds of side chains within a particular protein determine its tertiary structure. The protein tertiary structure is defined by its atomic coordinates. These coordinates may refer either to a protein domain or to the entire tertiary structure. A number of tertiary structures may fold into a quaternary structure.

Determinants & Protein folding

Protein folding is the physical process by which a protein chain acquires its native 3-dimensional structure, a conformation that is usually biologically functional, in an expeditious and reproducible manner. It is the physical process by which a polypeptide folds into its characteristic and functional three-dimensional structure from a random coil.

Each protein exists as an unfolded polypeptide or random coil when translated from a sequence of mRNA to a linear chain of amino acids. This polypeptide lacks any stable (long-lasting) three-dimensional structure (the left hand side of the first figure). As the polypeptide chain is being synthesized by a ribosome, the linear chain begins to fold into its three-dimensional structure. Folding begins to occur even during translation of the polypeptide chain. Amino acids interact with each other to produce a well-defined three-dimensional structure, the folded protein (the right hand side of the figure), known as the native state. The resulting three-dimensional structure is determined by the amino acid sequence or primary structure (Anfinsen's dogma).

Thermostability

A protein folded into its native state or native conformation typically has a lower Gibbs free energy (a combination of enthalpy and entropy) than the unfolded conformation. A protein will tend towards low-energy conformations, which will determine the protein's fold in the cellular environment. Because many similar conformations will have similar energies, protein structures are dynamic, fluctuating between a large these similar structures.

Globular proteins have a core of hydrophobic amino acid residues and a surface region of water-exposed, charged, hydrophilic residues. This arrangement may stabilise interactions within the tertiary structure. For example, in secreted proteins, which are not bathed in cytoplasm, disulfide bonds between cysteine residues help to maintain the tertiary structure. There is a commonality of stable tertiary structures seen in proteins of diverse function and diverse evolution. For example, the TIM barrel, named for the enzyme triosephosphateisomerase, is a common tertiary structure as is the highly stable, dimeric, coiled coil structure. Hence, proteins may be classified by the structures they hold. Databases of proteins which use such a classification include SCOP and CATH.

Kinetic traps

Folding kinetics may trap a protein in a high-energy conformation, i.e. a high-energy intermediate conformation blocks access to the lowest-energy conformation. The high-energy conformation may contribute to the function of the protein. For example, the influenza hemagglutinin protein is a single polypeptide chain which when activated, is proteolytically cleaved to form two polypeptide chains. The two chains are held in a high-energy conformation. When the local pH drops, the protein undergoes an energetically favorable conformational rearrangement that enables it to penetrate the host cell membrane.

Metastability

Some tertiary protein structures may exist in long-lived states that are not the expected most stable state. For example, many serpins (serine protease inhibitors) show this metastability. They undergo a conformational change when a loop of the protein is cut by a protease.

Chaperone proteins

It is commonly assumed that the native state of a protein is also the most thermodynamically stable and that a protein will reach its native state, given its chemical kinetics, before it is translated. Protein chaperones within the cytoplasm of a cell assist a newly synthesised polypeptide to attain its native state. Some chaperone proteins are highly specific in their function, for example, protein disulfide isomerase; others are general in their function and may assist most globular proteins, for example, the prokaryotic GroEL/GroES system of proteins and the homologous eukaryotic heat shock proteins (the Hsp60/Hsp10 system).

Cytoplasmic environment

Prediction of protein tertiary structure relies on knowing the protein's primary structure and comparing the possible predicted tertiary structure with known tertiary structures in protein data banks. This only takes into account the cytoplasmic environment present at the time of protein synthesis to the extent that a similar cytoplasmic environment may also have influenced the structure of the proteins recorded in the protein data bank.

Ligand binding

The structure of a protein, for example an enzyme, may change upon binding of its natural ligands, for example a cofactor. In this case, the structure of the protein bound to the ligand is known as holo structure, of the unbound protein as apo structure.

Tertiary structure is held by:

- Hydrogen Bonds - formed between amino acids at different points in the chain.
- Disulphide Bonds - a strong double bond (S=S) formed between the Sulphur atoms within the Cysteine monomers.
- Ionic Bonds - formed between 2 oppositely charged 'R' groups (+ and -) found close to each other.
- Hydrophobic and Hydrophilic Interactions: amino acids may be hydrophobic or hydrophilic; in a water based environment, the hydrophobic parts of globular protein are orientated towards centre and the hydrophilic parts are towards edges.

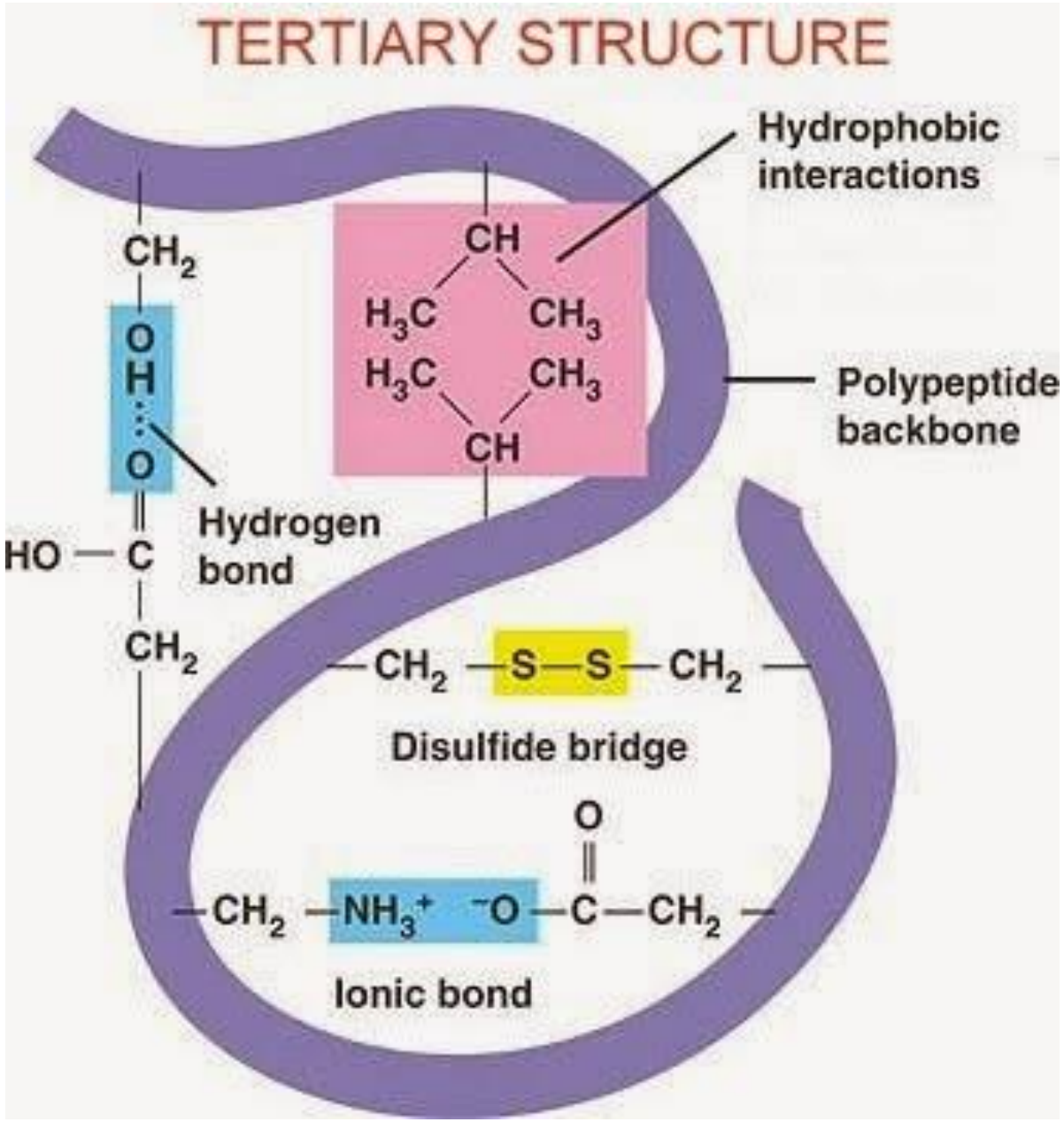


Fig: Protein tertiary Structure

Protein Quaternary Structure

Introduction

Protein quaternary structure is the number and arrangement of multiple folded protein subunits in a multisubunit complex. It includes organizations from simple dimers to large homooligomers and complexes with defined or variable numbers of subunits. It can also refer to biomolecular complexes of proteins with nucleic acids and other cofactors.

Description and examples

Many proteins are actually assemblies of multiple polypeptide chains. The quaternary structure refers to the number and arrangement of the protein subunits with respect to one another.

Examples of proteins with quaternary structure include hemoglobin, DNA polymerase, and ion channels. Enzymes composed of subunits with diverse functions are sometimes called holoenzymes, in which some parts may be known as regulatory subunits and the functional core is known as the catalytic subunit. Other assemblies referred to instead as multi-protein complexes also possess quaternary structure.

Examples include nucleosomes and microtubules. Changes in quaternary structure can occur through conformational changes within individual subunits or through reorientation of the subunits relative to each other. It is through such changes, which underlie cooperativity and allostery in "multi-meric" enzymes, that many proteins undergo regulation and perform their physiological function.

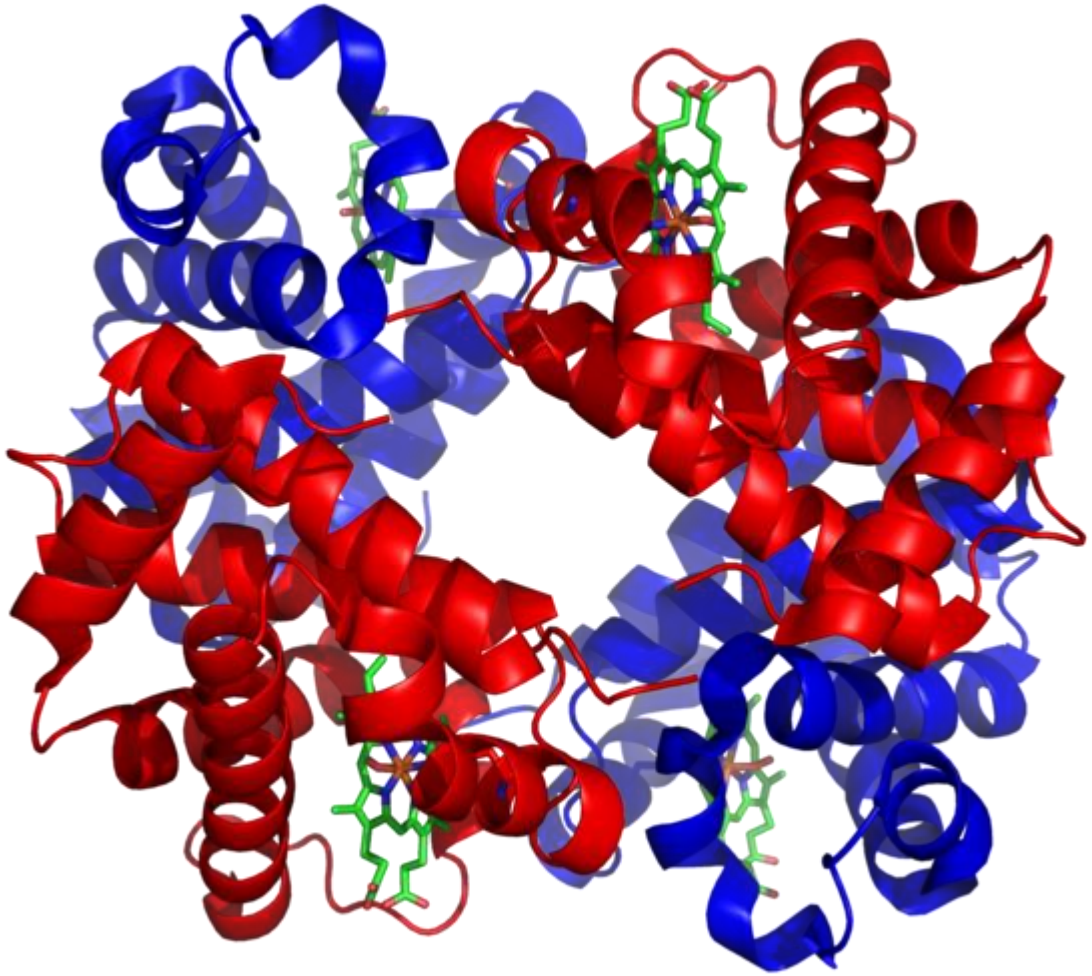


Fig: Protein Quaternary Structure

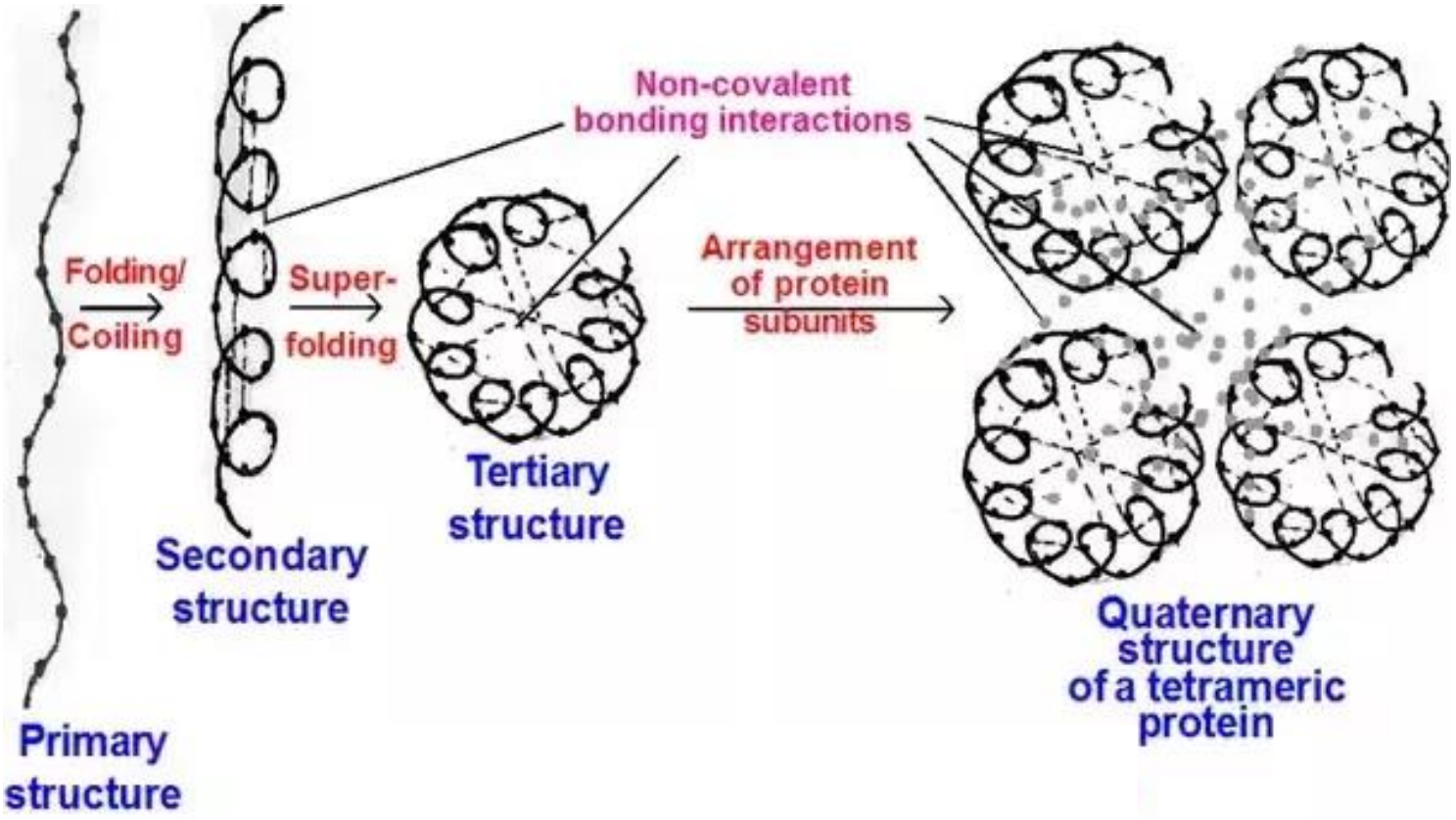
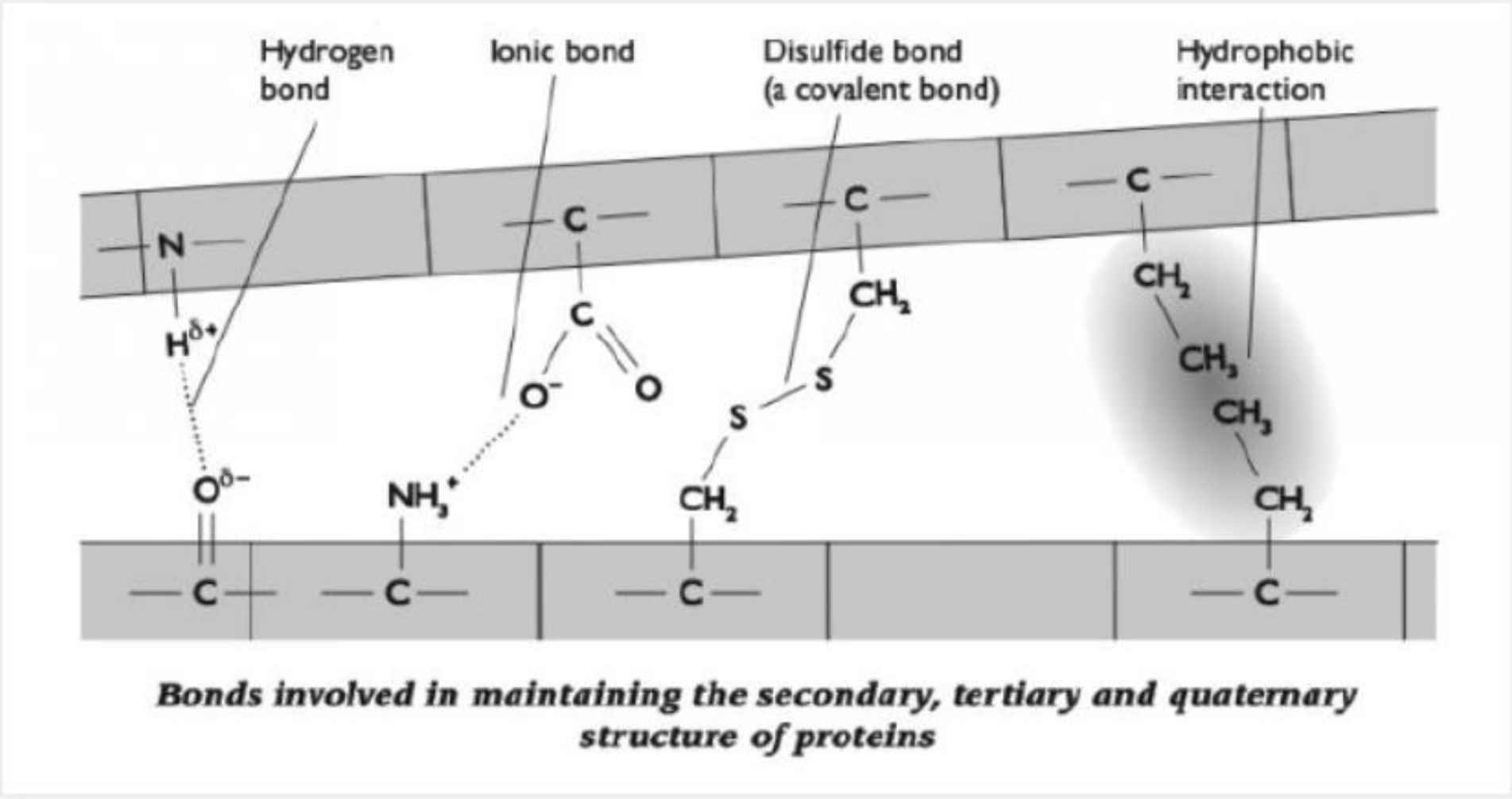


Fig: Structural Organization of porteins



Bonds involved in maintaining the secondary, tertiary and quaternary structure of proteins

Quaternary structure: ≥ 2 polypeptide chains join together to form a protein.

Some proteins are made up of multiple polypeptide chains, sometimes with an inorganic component (e.g. a haem group in haemoglogin) called a Prosthetic Group. These proteins will only be able to function if all subunits are present.

The polypeptide chains are held by the same type of bonds as in the tertiary structure.

Denaturation of protein

Introduction

Denaturation is a process in which proteins or nucleic acids lose the quaternary structure, tertiary structure, and secondary structure which is present in their native state, by application of some external stress or compound such as a strong acid or base, a concentrated inorganic salt, an organic solvent (e.g., alcohol or chloroform), radiation or heat. If proteins in a living cell are denatured, this results in disruption of cell activity and possibly cell death. Protein denaturation is also a consequence of cell death. Denatured proteins can exhibit a wide range of characteristics, from conformational change and loss of solubility to aggregation due to the exposure of hydrophobic groups. Denatured proteins lose their 3D structure and therefore cannot function.

Protein folding is key to whether a globular or membrane protein can do its job correctly; it must be folded into the right shape to function. However, hydrogen bonds, which play a big part in folding, are rather weak and thus easily affected by heat, acidity, varying salt concentrations, and other stressors which can denature the protein. This is one reason why homeostasis is physiologically necessary in many life forms.

This concept is unrelated to denatured alcohol, which is alcohol that has been mixed with additives to make it unsuitable for human consumption.

Proteins or Polypeptides are polymers of amino acids. A protein is created by ribosomes that "read" RNA that is encoded by codons in the gene and assemble the requisite amino acid combination from the genetic instruction, in a process known as translation. The newly created protein strand then undergoes posttranslational modification, in which additional atoms or molecules are added, for example copper, zinc, or iron. Once this post-translational modification process has been completed, the protein begins to fold (sometimes spontaneously and sometimes with enzymatic assistance), curling up on itself so that hydrophobic elements of the protein are buried deep inside the structure and hydrophilic elements end up on the outside. The final shape of a protein determines how it interacts with its environment.

Protein folding consists of a balance between a substantial amount of weak intra-molecular interactions within a protein (Hydrophobic, electrostatic, and Van Der Waals Interactions) and protein-solvent interactions

As a result, this process is heavily reliant on environmental state that the protein resides in. These environmental conditions include, and are not limited to, temperature, salinity, pressure, and the solvents that happen to be involved. Consequently, any exposure to extreme stresses (e.g. heat or radiation, high inorganic salt concentrations, strong acids and bases) can disrupt a protein's interaction and inevitably lead to denaturation.

When a protein is denatured, secondary and tertiary structures are altered but the peptide bonds of the primary structure between the amino acids are left intact. Since all structural levels of the protein determine its function, the protein can no longer perform its function once it has been denatured. This is in contrast to intrinsically unstructured proteins, which are unfolded in their native state, but still functionally active and tend to fold upon binding to their biological target.

How denaturation occurs at levels of protein structure?

- In quaternary structure denaturation, protein sub-units are dissociated and/or the spatial arrangement of protein subunits is disrupted.
- Tertiary structure denaturation involves the disruption of:
  - Covalent interactions between amino acid side-chains (such as disulfide bridges between cysteine groups).
  - Non-covalent dipole-dipole interactions between polar amino acid side-chains (and the surrounding solvent).
  - Van der Waals (induced dipole) interactions between nonpolar amino acid side-chains.
- In secondary structure denaturation, proteins lose all regular repeating patterns such as alpha-helices and beta-pleated sheets, and adopt a random coil configuration.
- Primary structure, such as the sequence of amino acids held together by covalent peptide bonds, is not disrupted by denaturation.

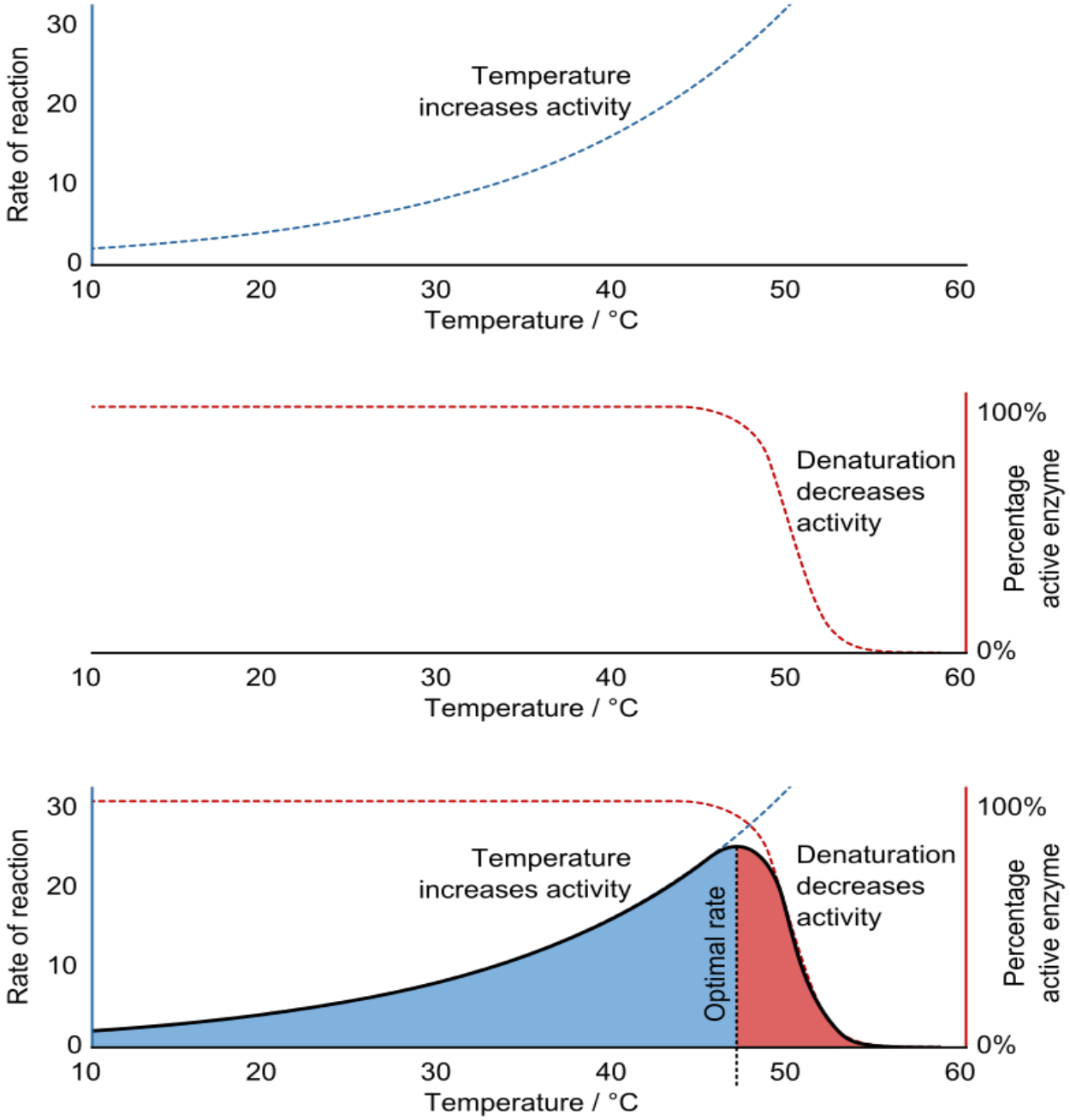


Fig: The effects of temperature on enzyme activity. Top - increasing temperature increases the rate of reaction (Q10 coefficient). Middle - the fraction of folded and functional enzyme decreases above its denaturation temperature. Bottom - consequently, an enzyme's optimal rate of reaction is at an intermediate temperature.

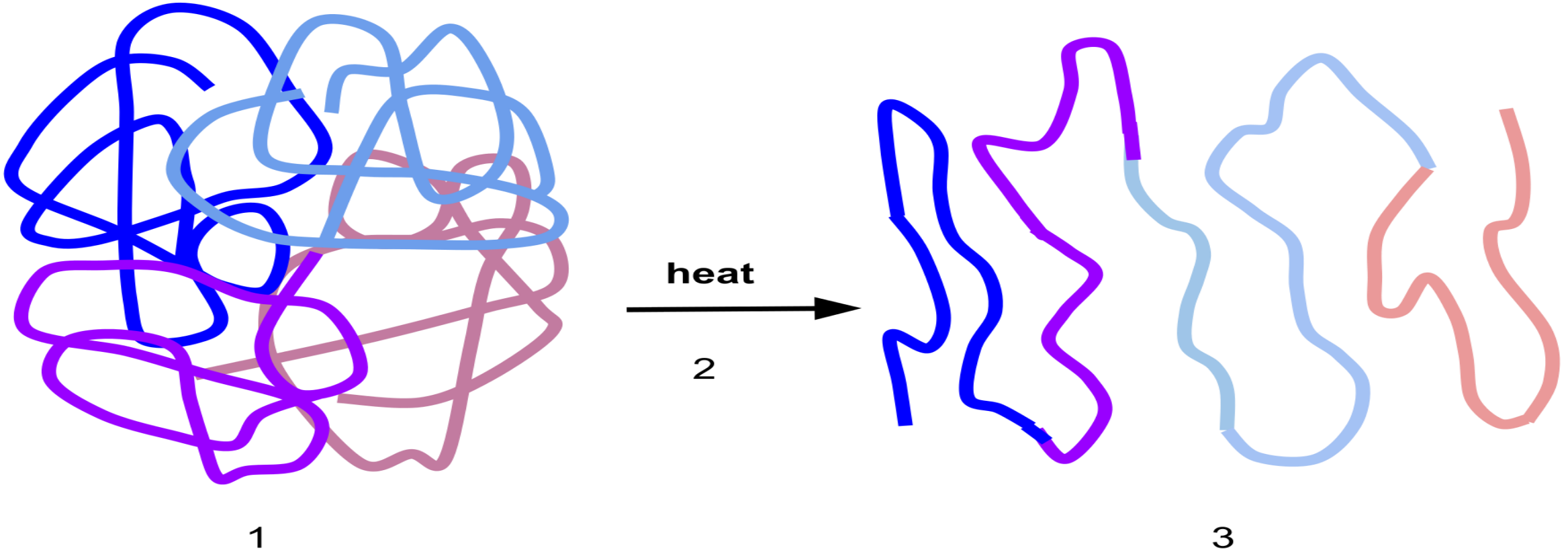


Fig: Process of denaturation:
1) Functional protein showing a quaternary structure
2) When heat is applied it alters the intramolecular bonds of the protein
3) Unfolding of the polypeptides (amino acids)

Loss of function

Most biological substrates lose their biological function when denatured. For example, enzymes lose their activity, because the substrates can no longer bind to the active site, and because amino acid residues involved in stabilizing substrates' transition states are no longer positioned to be able to do so. The denaturing process and the associated loss of activity can be measured using techniques such as dual-polarization interferometry, CD, QCM-D and MP-SPR.

Loss of activity due to heavy metals and metalloids

By targeting proteins, heavy metals have been known to disrupt the function and activity carried out by proteins It is important to note that heavy metals fall into categories consisting of transition metals as well as a select amount of metalloid. These metals, when interacting with native, folded proteins, tend to play a role in obstructing their biological activity. This interference can be carried out in a different number of ways. These heavy metals can form a complex with the functional side chain groups present in a protein or form bonds to free thiols. Heavy metals also play a role in oxidizing amino acid side chains present in protein. Along with this, when interacting with metalloproteins, heavy metals can dislocate and replace key metal ions. As a result, heavy metals can interfere with folded proteins, which can strongly deter protein stability and activity.

Reversibility and irreversibility

In many cases, denaturation is reversible (the proteins can regain their native state when the denaturing influence is removed). This process can be called renaturation.
This understanding has led to the notion that all the information needed for proteins to assume their native state was encoded in the primary structure of the protein, and hence in the DNA that codes for the protein, the so-called "Anfinsen's thermodynamic hypothesis".

Denaturation can also be irreversible. This irreversibility is typically a kinetic, not thermodynamic irreversibility, as generally when a protein is folded it has lower free energy. Through kinetic irreversibility, the fact that the protein is stuck in a local minimum can stop it from ever refolding after it has been irreversibly denatured.

Protein denaturation due to pH

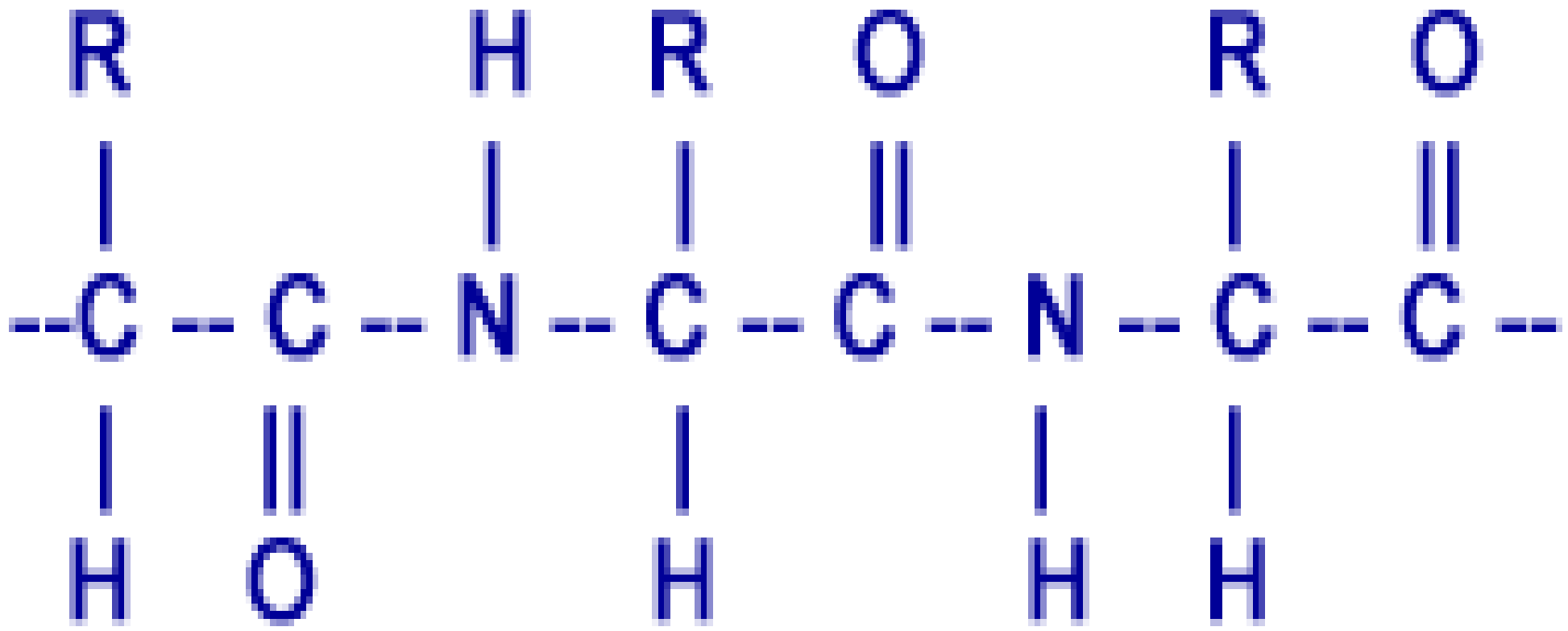
Denaturation can also be caused by changes in the pH which can affect the chemistry of the amino acids and their residues. The ionizable groups in amino acids are able to become ionized when changes in pH occur. A pH change to more acidic or more basic conditions can induce unfolding.
Acid-induced unfolding often occurs between pH 2 and 5, base-induced unfolding usually requires pH 10 or higher.

Introduction to Simple and Conjugate protein

Simple Protein: A Glossary of Terms

Simple proteins are made up of amino acid subunits joined together by peptide bonds. When hydrolyzed by enzymes, simple proteins yield only the amino acids from which they are comprised of. Examples of simple proteins include albumins, globulins, glutelins and albuminoids.

- **simple protein** - a protein that yields only amino acids when hydrolyzed
- **protein** - any of a large group of nitrogenous organic compounds that are essential constituents of living cells; consist of polymers of amino acids; essential in the diet of animals for growth and for repair of tissues; can be obtained from meat and eggs and milk and legumes; "a diet high in protein"
- **actin** - one of the proteins into which actomyosin can be split; can exist in either a globular or a fibrous form
- **albumen, albumin** - a simple water-soluble protein found in many animal tissues and liquids



A PROTEIN FRAGEMENT

- **globin, haematohiston, hematohiston** - a colorless protein obtained by removing heme from hemoglobin; the oxygen carrying compound in red blood cells
- **glutelin** - a simple protein found in the seeds of cereals
- **histone** - a simple protein containing mainly basic amino acids; present in cell nuclei in association with nucleic acids
- **prolamine** - a simple protein found in plants
- **protamine** - a simple protein found in fish sperm; rich in arginine; simpler in composition than globulin or albumin; counteracts the anticoagulant effect of heparin
- **albuminoid, scleroprotein** - a simple protein found in horny and cartilaginous tissues and in the lens of the eye

Conjugate Protein

A conjugated protein is a protein that functions in interaction with other (non-polypeptide) chemical groups attached by covalent bonding or weak interactions.

Many proteins contain only amino acids and no other chemical groups, and they are called simple proteins. However, other kind of proteins yield, on hydrolysis, some other chemical component in addition to amino acids and they are called conjugated proteins. The non-amino part of a conjugated protein is usually called its prosthetic group. Most prosthetic groups are formed from vitamins. Conjugated proteins are classified on the basis of the chemical nature of their prosthetic groups.

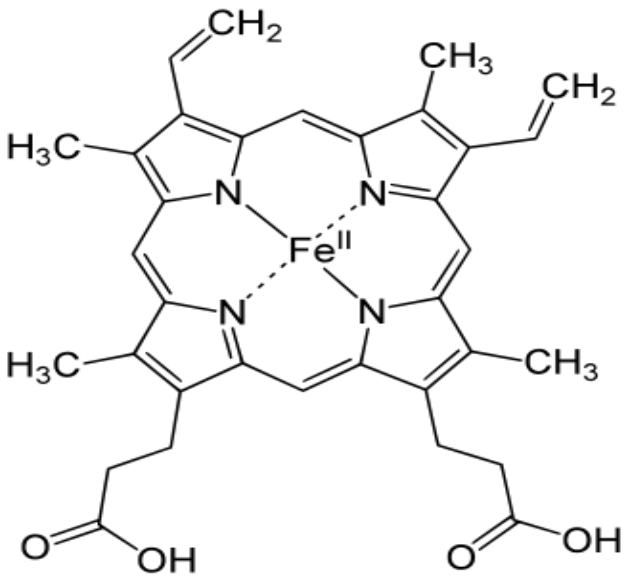


Fig: Heme – prosthetic group of hemoglobin

Some examples of conjugated proteins are lipoproteins, glycoproteins, phosphoproteins, hemoproteins, flavoproteins, metalloproteins, phytochromes, cytochromes, opsins and chromoproteins.

Hemoglobin contains the prosthetic group known as heme. Each heme group contains an iron ion (Fe^{2+}) which forms a co-ordinate bond with an oxygen molecule (O_2), allowing hemoglobin to transport oxygen through the bloodstream. As each of the four protein subunits of hemoglobin possesses its own prosthetic heme group, each hemoglobin can transport four molecules of oxygen.

Glycoproteins are generally the largest and most abundant group of conjugated proteins.

Thank You