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PG AND RESEARCH DEPARTMENT OF BIOCHEMISTRY

E-NOTES

SUBJECT NAME: CHEMISTYY OF BIOMOLECULES

SUBJECT CODE: GBC12

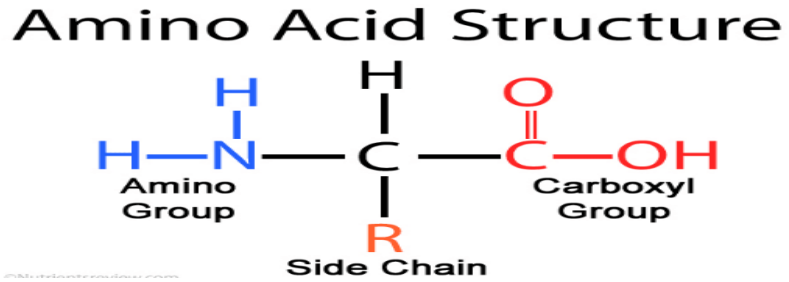
Unit-III: Amino acids and Proteins

Amino acids–classification, structure and physiochemical properties,chemical synthesis of peptides – solid phase peptide synthesis. Proteins – classification, purification, and criteria of homogeneity. Structural organization, sequence determination and characterization of Proteins. Conformation of proteins – Ramachandran plots. Denaturation of proteins.

Apoprotein and Prosthetic group- Porphyrins – Structure and properties of porphyrins – heme, Chlorophyll and Cytochromes.

Amino Acid

- It's a group of organic compounds containing two functional groups – **amino (-NH2) and carboxyl group (-COOH)**
- Its also called **ZwitterIon–both acidic and basic functional group (dipolar ion)**
- This property is known as **amphoteric and are often called ampholytes**
- Neither humans nor any other higher animals can synthesize 10 of the 20 common amino acids –**Essential Amino acids**



Classification

Amino acid has been classified under various ways

1.Structure

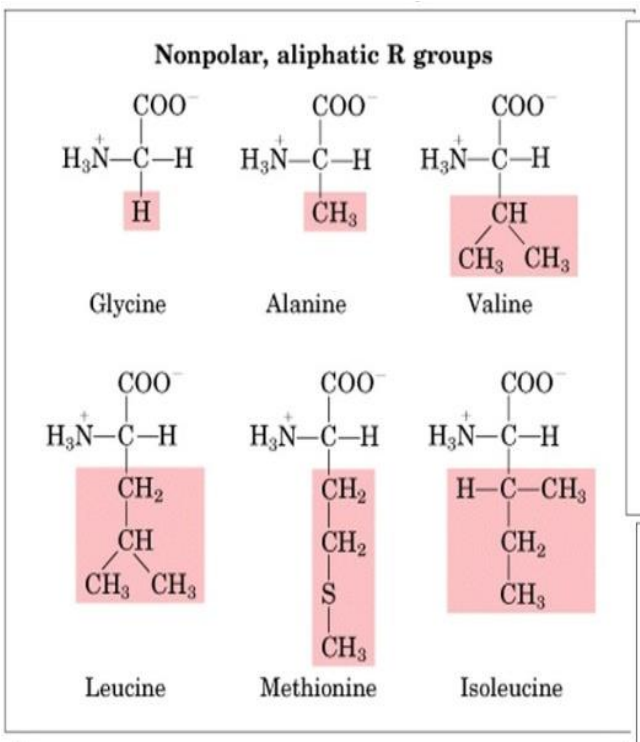
- With side chain containing **Aliphatic Side Chains**
- With Side Chains Containing **Hydroxylic(OH) Groups**
- With Side Chains Containing **Sulfur Atoms**
- With Side Chains Containing **Acidic Groups or Their Amides**
- With Side Chains Containing **Basic Groups**
- Containing **Aromatic Rings**
- **IminoAcid**

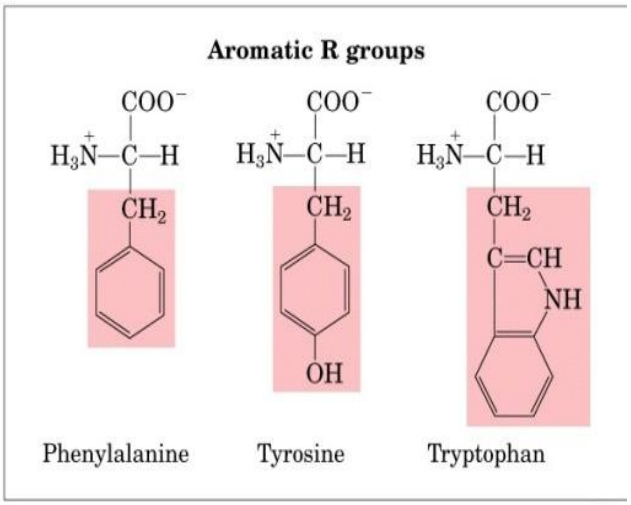
2. Polarity

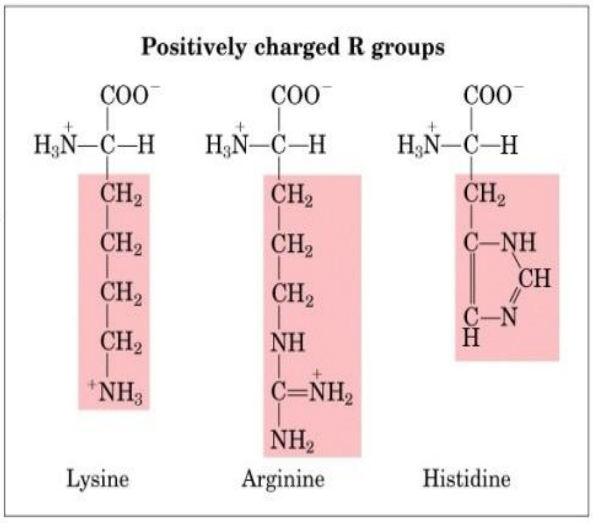
- Non Polar
- Polar

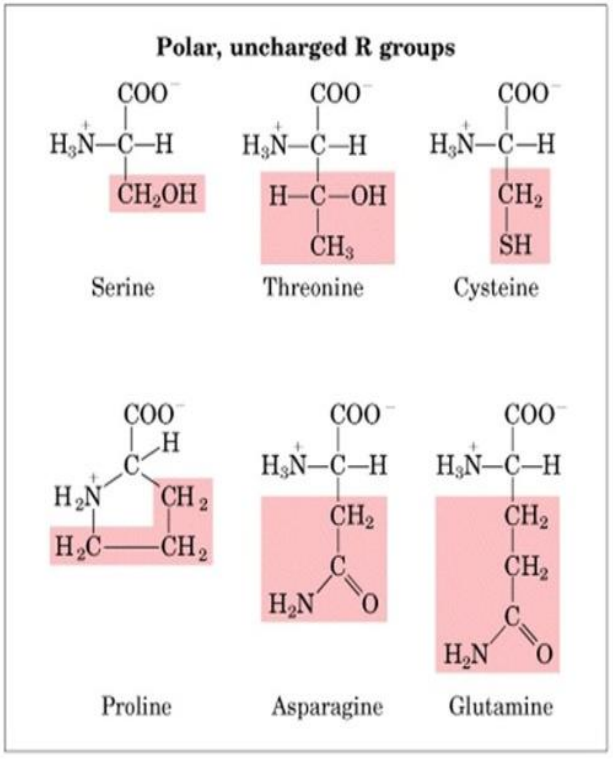
3. Nutritional

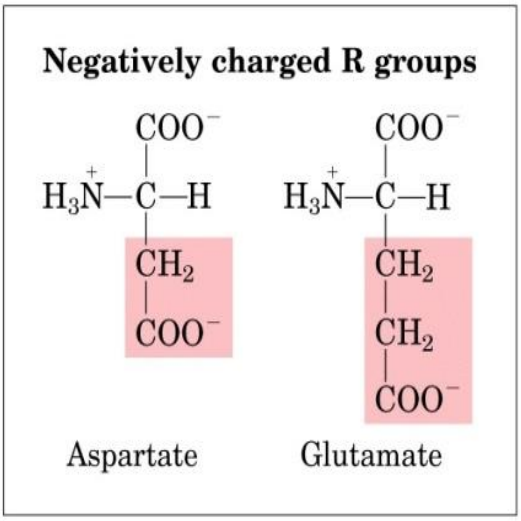
Essential and Non-essential





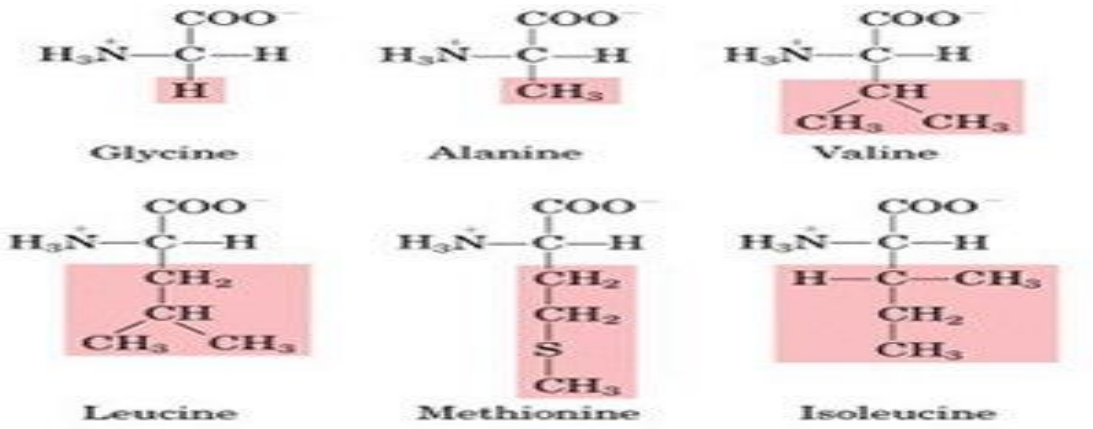






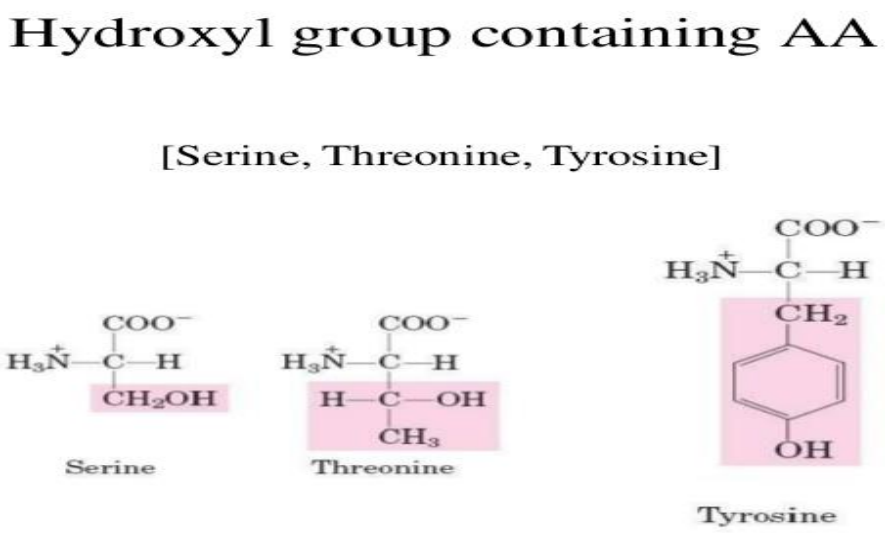
Amino acids with aliphatic side chains

- These are monoamino monocarboxylic acids.
- This group consists of the most simple amino acids glycine, alanine,valine, leucine and isoleucine.The last three amino acids (Leu, lle, Val) contain branched aliphatic side chains.



Hydroxyl group containing amino acids :

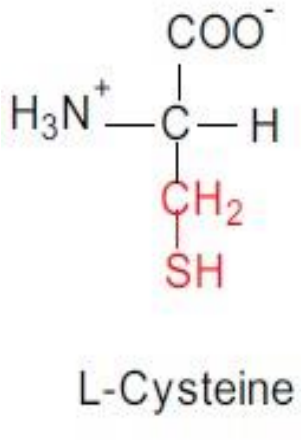
Serine, threonine and tyrosine are hydroxyl group containing amino acids.Tyrosine-being aromatic in nature is usually considered under aromatic amino acids.



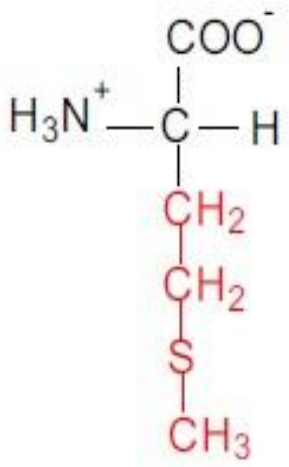
Sulfur containing amino acids

With sulfhydryl group and methionine with thioether group are the two

Amino acids incorporated during the course of protein synthesis. Cystine, another Important sulfur containing amino acid, is formed by condensation of two molecules of cysteine



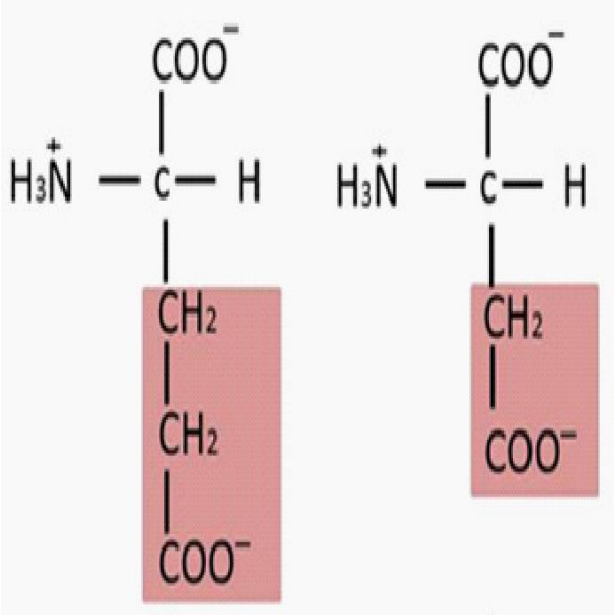
L-Cysteine



L-Methionine

Acidic amino acids and their amides

Aspartic acid and glutamic acids are dicarboxylic monoamino acids while asparagine and glutamine are their respective amide derivatives. All these four amino acids possess distinct codons for their incorporation into proteins

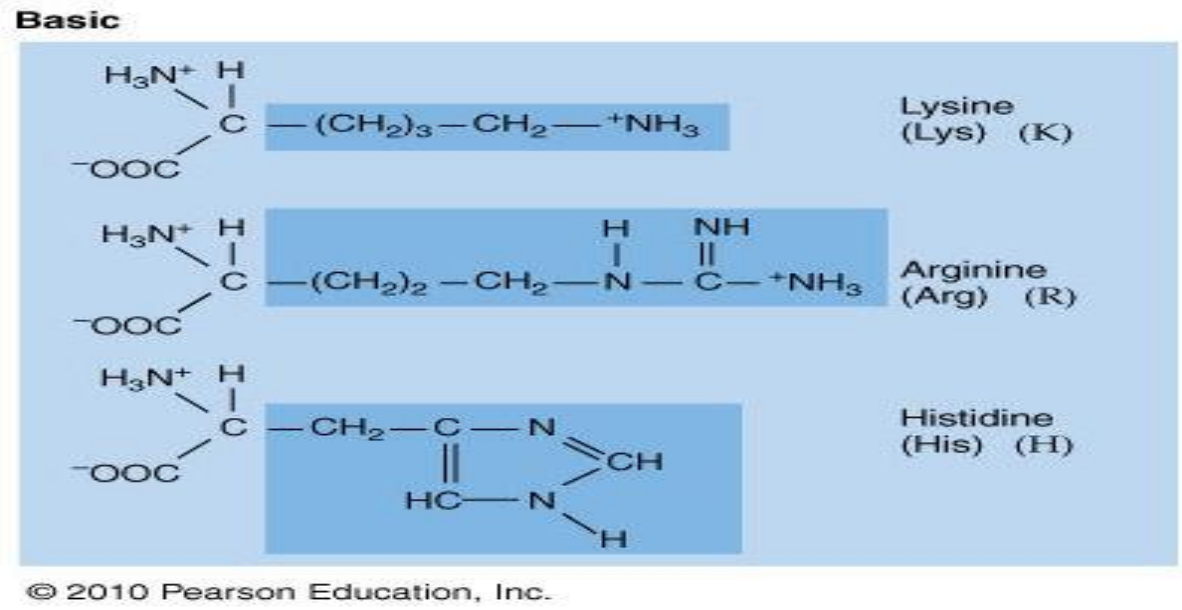


Basic amino acids

The three amino acids

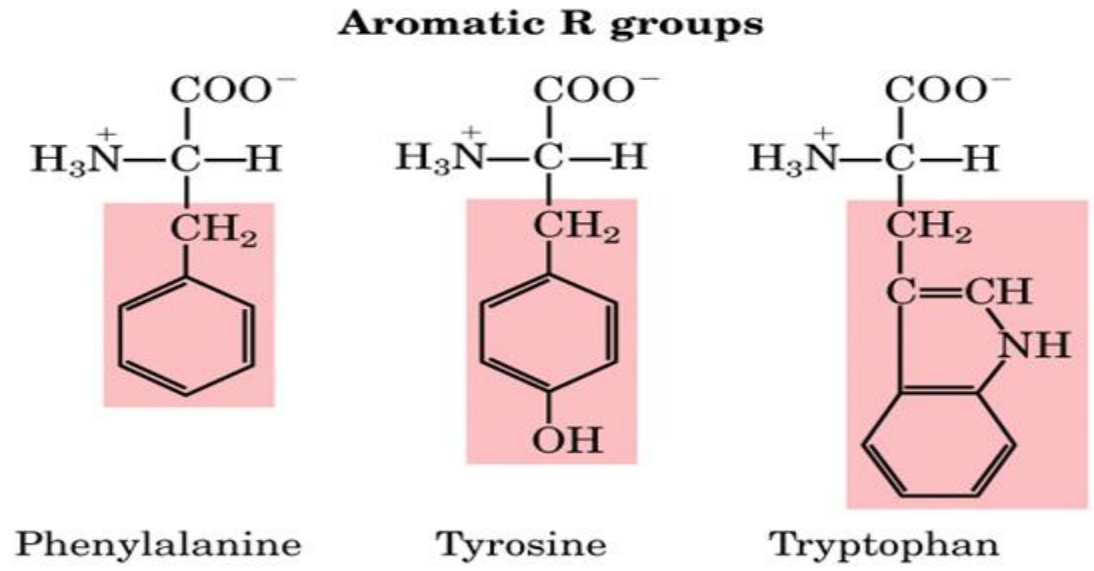
lysine, arginine (with guanidino group) and histidine (with imidazole ring) are dibasic mono carboxylic acids. They are

highly basic in character.



Aromatic amino acids

Pheny lalanine,tyrosine and tryptophan (with indole ring) are aromatic amino acids.

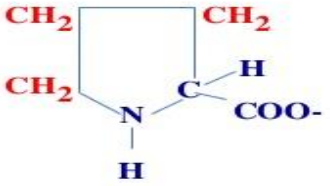


lmino acids

Proline containing pyrrolidine ring is a unique amino acid. lt has an imino group (=NH), instead of an amino group (NH2) found in other amino acids. Therefore proline is an a-imino acid.

7.Imino acids :

- **Proline containing pyrrolidine ring is a unique amino acid.**
- **It has an imino group (=NH). Threrefore, proline is an α-imino acid.**



Pyrroline

2 Classification according to polarity of side chain (R):

A. Non- polar Amino acids

e.g. Alanine, Leucine, Isoleucine, Valine, Methionine, Phenyl Alanine, Tryptophan and Proline

B. Polar Amino acids with no charge on "R" group

e.g. Glycine, Serine, Threonine, Cysteine, Glutamine, Asparagine and Tyrosine

C. Polar Amino acids with positive "R" group

e.g. Lysine, Arginine, Histidine

D. Polar Amino acids with negative "R" group

e.g. Aspartic acid and Glutamic acid

3 Nutritional classification:

1- Essential or Indispensable amino acids: Can't be synthesized in the body & have to be taken in the diet.

e.g. Valine, Isoleucine, leucine, Lysine, Methionine, Phenyl Alanine, Threonine and Tryptophan

2- Semiessential amino acids: These are growth promoters. Not synthesized in adequate amount in the body.

e.g. Arginine and Histidine.

3- Non essential or Dispensable amino acids: Can be synthesized in the body & may not be the component of diet.

e.g. Glycine, Alanine, Serine, Cysteine, Asparagine, Glutamine, Tyrosine, and Proline.

Physical Properties

1. Amino acids are colorless, crystalline solid.
2. All amino acids have a high melting point greater than 200^{o}
3. Solubility: They are soluble in water, slightly soluble in alcohol and dissolve with difficulty in methanol, ethanol, and propanol. R-group of amino acids and pH of the solvent play important role in solubility.
4. On heating to high temperatures, they decompose.
5. All amino acids (except glycine) are optically active.
6. Peptide bond formation: Amino acids can connect with a peptide bond involving their amino and carboxylate groups. A covalent bond formed between the alpha-amino group of one amino acid and an alpha-carboxyl group of other forming -CO-NH-linkage. Peptide bonds are planar and partially ionic.

Chemical Properties

1.Zwitterionic property

A zwitterion is a molecule with functional groups, of which at least one has a positive and one has a negative electrical charge. The net charge of the entire molecule is zero. Amino acids are the best-known examples of zwitterions. They contain an amine group (basic) and a carboxylic group (acidic). The -NH2 group is the stronger base, and so it picks up H+ from the -COOH group to leave a zwitterion. The (neutral) zwitterion is the usual form amino acids exist in solution.

2.Amphoteric property

Amino acids are amphoteric in nature that is they act as both acids and base since due to the two amine and carboxylic group present.

3.Ninhydrin test

When 1 ml of Ninhydrin solution is added to a 1 ml protein solution and heated, the formation of a violet color indicates the presence of α-amino acids.

4.Xanthoproteic test

The xanthoproteic test is performed for the detection of aromatic amino acids (tyrosine, tryptophan, and phenylalanine) in a protein solution. The nitration of benzoid radicals present in the amino acid chain occurs due to reaction with nitric acid, giving the solution yellow coloration.

5.Reaction with Sanger's reagent

Sanger's reagent (1-fluoro-2, 4-dinitrobenzene) reacts with a free amino group in the peptide chain in a mild alkaline medium under cold conditions.

6.Reaction with nitrous acid

Nitrous acid reacts with the amino group to liberate nitrogen and form the corresponding hydroxyl.

Solid Phase Peptide Synthesis (SPPS)

The control and regulatory mechanisms for many biological processes are dependent on **peptides** and proteins derived from α–amino acids. In addition, many modern medicines are now produced from peptides or derivatives of peptides. A few examples are anti-cancer agents, antibiotics or peptide based drugs that control blood pressure. For this reasons α–amino acids and peptide chemistry has become a central technology in organic chemistry, biochemistry, biotechnology and medicinal chemistry. Solid phase **peptide synthesis** has become the major automated synthesis method or technology used for the production of synthetic peptides.

The automation of solid phase peptide synthesis and the many improvements made in **peptide synthesis** instrumentation in recent years have made synthetic peptides and their derivatives more available to the scientific community and the biological industry as a whole. Synthetic peptides can be synthesized automatically using solid phase peptide synthesis and used to manufacture epitope-specific antibodies, map antibody epitopes and study enzyme binding sites or to design and synthesize novel peptide- or protein-mimetics or even whole enzymes.

To synthesize a peptide bond between two amino acids will need to be formed. The exact size of peptides is not well defined but it usually refers to flexible chains of up to 30 to 50 amino acids. However, peptides with up to 100 amino acids within the chain can now be synthesized.

The basic concept in solid phase peptide synthesis is the step-wise construction of a peptide chain attached to an insoluble polymeric support (see Figure 1. for general synthesis scheme).

Fmoc-Chemistry for Peptide Synthesis

Carboxyl group activation of the incoming amino acid utilizes one of the following methods:

1. BOP/HOBt/NMM:

The amino acid is mixed with the BOP (Castro's reagent or (benzotriazol-yloxy) tris (dimethylamino) phosphonium hexa-fluoro-phosphate), HOBt (1-hydro-xybenzotriazole) and sufficient NMM (N-methyl-morpholine) to ionize 50% of the HOBt.

2. DIPCDI:

DIPCDI (disopropylcarbo-diimide) can be used either with or without HOBt in a solution of DCM:DMF (di-chloromethane and dimethyl-formamide).

3. Active esters:

These are usually pentafluorophenyl esters (OPfp) of the amino acids. The pentafluorophenyl esters of serine and threonine are oils and therefore are employed in the more convenient form of the dihydrooxobenzotriazine ester (ODhbt).

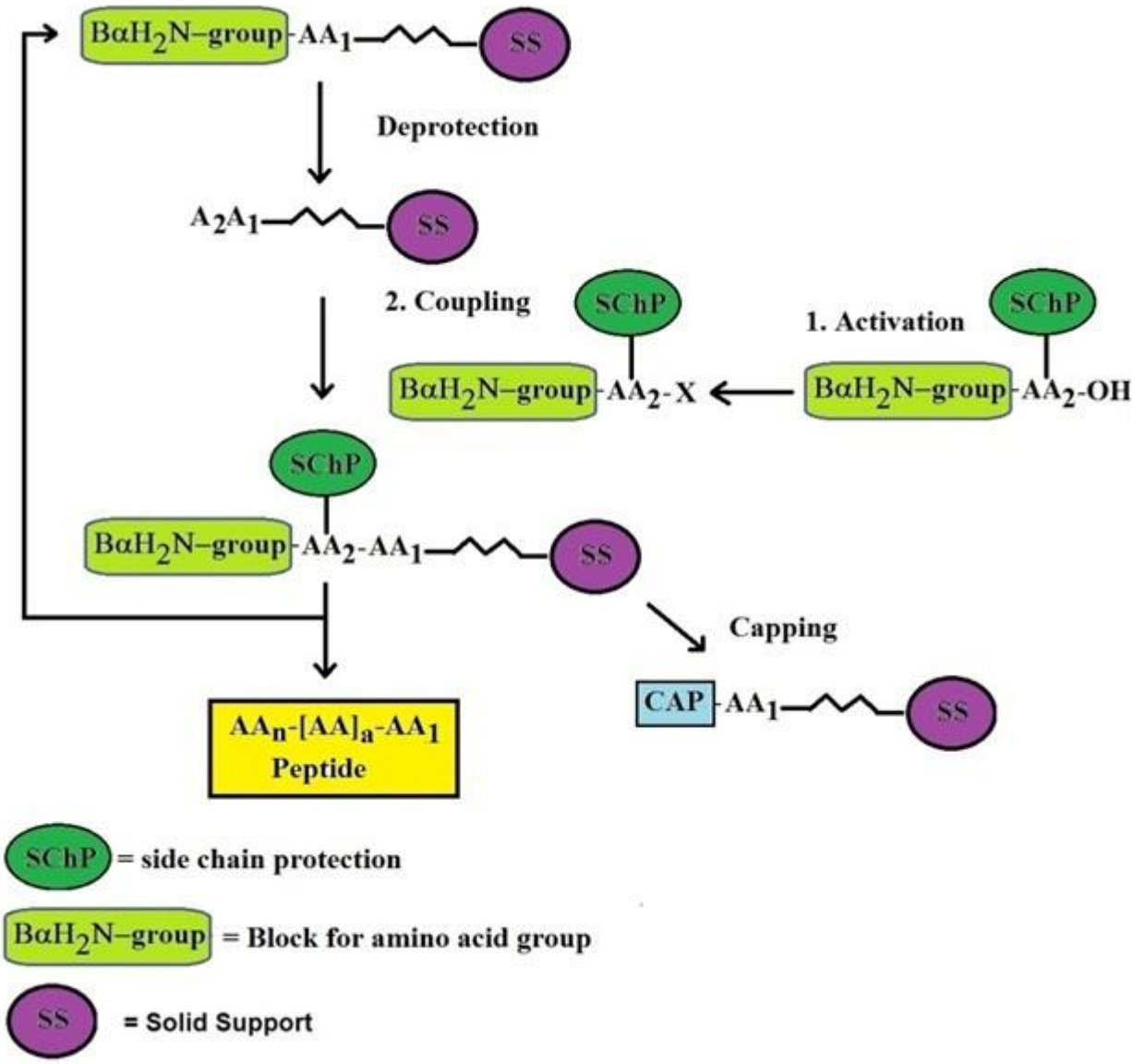


Figure 1: General Scheme for Solid Phase Peptide Synthesis

This approach permits unreacted reagents to be removed by washing without loss of product. Synthesis of the peptide chain proceeds from the carboxyl end of the amino terminus of the peptide.

The carboxyl moiety of each incoming amino acid is activated by one of several strategies and couples with the α-amino group of the preceding amino acid. The α-amino group of the incoming residue is temporarily blocked in order to prohibit peptide bond formation at this site. The residue is de-blocked at the beginning of the next synthesis cycle. In addition, reactive side chains on the amino acids are modified with appropriate protecting groups. The peptide chain is

extended by reiteration of the synthesis cycle. Excess reagents are used to drive reactions as close to completion as possible. This generates the maximum possible yield of the final product.

After fully assembling the peptide the side-chain protecting groups are removed, and the peptide is cleaved from the solid support, using conditions that inflict minimal damage on labile residues. The product is analyzed to verify the sequence thereafter. The synthetic peptide is usually purified by gel chromatography or HPLC. The blocking group used for blocking the α-amino group determines both the synthesis chemistry employed and the nature of the side-chain protecting groups. The two most commonly used α-amino protecting groups are Fmoc (9-fluorenyl-methoxy-carbonyl) and tBoc (tert.-butyloxycarbonyl). Fmoc side-chain protection is provided by ester, ether and urethane derivatives of tert.-butanol, while the corresponding tBoc protecting groups are ester, ether, and urethane derivatives of benzyl alcohol. The latter are usually modified by the introduction of electron-withdrawing halogens for greater acid-stability. Ether and ester derivatives of cyclopentyl or cyclohexyl alcohol are also employed.

The Fmoc protecting group is base-labile. It is usually removed with a dilute base such as piperidine. The side-chain protecting groups are removed by treatment with trifluoroacetic acid (TFA), which also cleaves the bond anchoring the peptide to the support. The tBoc protecting group is removed with a mild acid (usually dilute TFA). Hydrofluoric acid (HF) can be used both to deprotect the amino acid side chains and to cleave the peptide from the resin support. Fmoc is a gentler method than tBoc since the peptide chain is not subjected to acid at each cycle and has become the major method employed in commercial automated peptide synthesis.

Protein Classification

Proteins are complex high molecular weight nitrogenous molecule made up of amino acids.

Proteins are dehydration polymers of amino acids, with each amino acid residue joined to its neighbor by a specific type of covalent bond called Peptide bond.

All proteins are constructed from the same ubiquitous set of 20 amino acids.

Proteins are classified based on their composition/solubility, shapes, size and functions

1. Solubility or compositon
2. Simple proteins
3. Conjugated proteins
4. Derived proteins
5. **Simple proteins**
6. **— Contain only amino acid residues**

1.1

Simple proteins

1.1 Simple proteins

Classes of proteins	Characteristics	Example
i. Albumins	Soluble in water, Coagulate by heat Precipitate at high salt con.	Serum alb, egg alb, lactalb,
ii. Globulins	Insoluble in water, Soluble in dilute salt solutions, Precipitated by half saturated salt solutions.	Serum glo, tuberin(potato), Myosinogen(Muscle), Legumin(peas).
iii. Glutelins	Insoluble in water but soluble in dilute acids and alkalis. Mostly found in plants.	Glutenin(Wheat), oryzenin(rice).
iv. Prolamines	Insoluble in water and absolute alcohol but soluble in 70 to 80 percent alcohol.	Gliadin(wheat), zein(maize)
v. Protamines	soluble in water, dilute acids and alkalis. Not coagulable by heat. Basic proteins of low molecular weight,	Salmine (Salmon sperm)

vi. Histones	Soluble in water and insolulbe in very dilute ammonium hydroxide,	Globin of hemoglobin and thymus histones.
vii. Scleroproteins	Insoluble in water, dilute acids and alkalis.	Keratin(hair, horn, nail, hoof and feathers) collagen (bone and skin), elastin (ligament).

1.2 conjugated proteins

i. Nucleoproteins	Composed of simple basic proteins(protamines, or histones) with nucleic acids, found in nuclei. Soluble in water	Nucleoprotamines and nucleohistones.
ii. Lipoproteins	Combination of proteins with lipids, such as fatty acids, cholesterol, and phospholipids.	Lipoproteins of egg yolk, milk and cell membrane, blood.
iii. Glycoproteins	Combination of proteins with carbohydrate	Mucin(saliva), Ovomucoid, tendomucoid (tendon)
iv. Phosphoproteins	Contains phosporus radical as a prosthetic gp,	Caseinogen(milk), Ovovitellin(egg),
v. Metalloproteins	Contain metal ions as their prosthetic gp, the metal ions generally are Fe, Co, Mg, Mn, Zn, Cu etc. proteins as	Siderophilin (Fe), ceruloplasmin (Cu).
vi. Chromoproteins	Contain porphyrin (with a metal ion) as their prosthetic gp	Haemoglobin, myoglobin, catalase, peroxidase, cytochromes
vii Flavoproteins	Contain riboflavin as their prosthetic gp,	Flavoproteins of liver and kidney.

1.3 Derived Proteins

a. Primary derivatives		
i. Proteans	Derived in the early stage of protein hydrolysis by dilute acids and enzymes or alkalis.	Fibrin from fibrinogen
ii. Metaproteins	Derived in the later stage of protein hydrolysis by stronger acids and alkalis.	Acid and alkali metaproteins
iii. Coagulated proteins	They are denatured proteins formed by the action of heat, X-rays, UV rays	Cooked proteins, coagulated albumins.

2.Protein – shapes

Globular proteins - polypeptide folds tightly together on itself - most enzymes- lysozymes

Fibrous proteins - elongated structure - span great distances - hair - keratin

1. Many subunits attached together
2. Actin & collagen
3. Strong & rigid (Collagen - ligaments)
4. Strong & flexible (elastin - skin)

3. Based of function

Based on structure proteins are classified into

1. Structural proteins ; play structural role such as collagen, keratin etc
2. Functional proteins : antibodies, enzymes, etc.
3. Based on Size

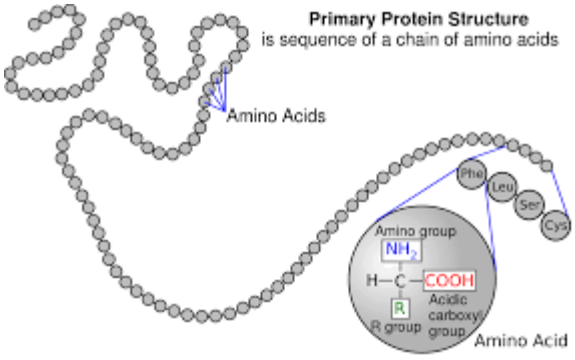
- A typical protein contains 200-300 amino acids, but some are much smaller and some are much larger
- Proteins range in molecular weight from 6,000 Daltons (insulin) to millions of Daltons (structural proteins)

- The linear sequence of amino acid residues in a polypeptide chain determines the three-dimensional configuration of a protein, and the structure of a protein determines its function.
- All proteins contain the elements carbon, hydrogen, oxygen, nitrogen and sulfur some of these may also contain phosphorus, iodine, and traces of metals like ion, copper, zinc and manganese.
- A protein may contain 20 different kinds of amino acids. Each amino acid has an amine group at one end and an acid group at the other and a distinctive side chain.
- The backbone is the same for all amino acids while the side chain differs from one amino acid to the next.

The structure of proteins can be divided into four levels of organization:

1. Primary Structure

- The primary structure of a protein consists of the amino acid sequence along the polypeptide chain.
- Amino acids are joined by peptide bonds.
- Because there are no dissociable protons in peptide bonds, the charges on a polypeptide chain are due only to the N-terminal amino group, the C-terminal carboxyl group, and the side chains on amino acid residues.
- The primary structure determines the further levels of organization of protein molecules.



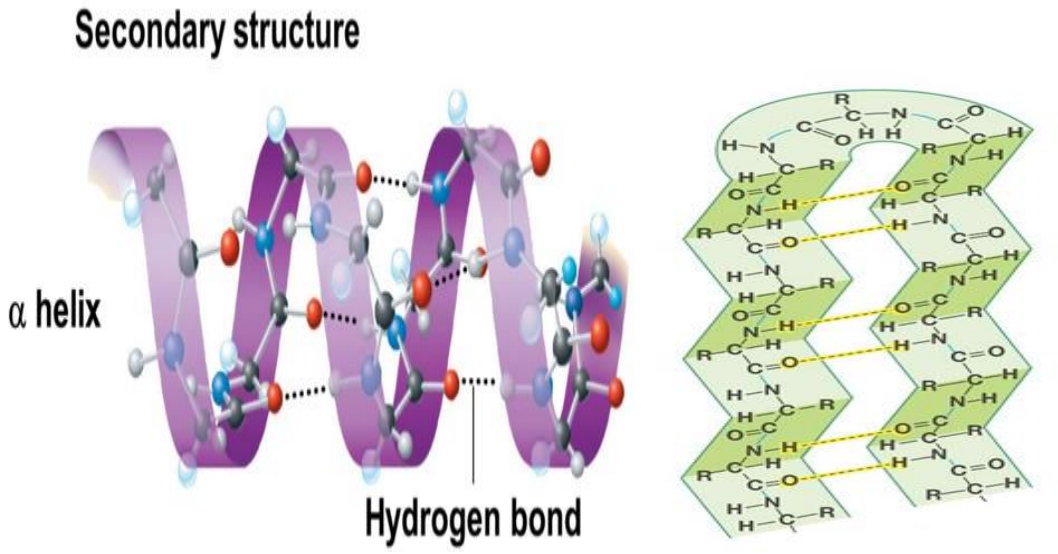
2. Secondary Structure

- The secondary structure includes various types of local conformations in which the atoms of the side chains are not involved.
- Secondary structures are formed by a regular repeating pattern of hydrogen bond formation between backbone atoms.
- The secondary structure involves α-helices, β-sheets, and other types of folding patterns that occur due to a regular repeating pattern of hydrogen bond formation.
- The secondary structure of protein could be :

1. **Alpha-helix**
2. **Beta-helix**

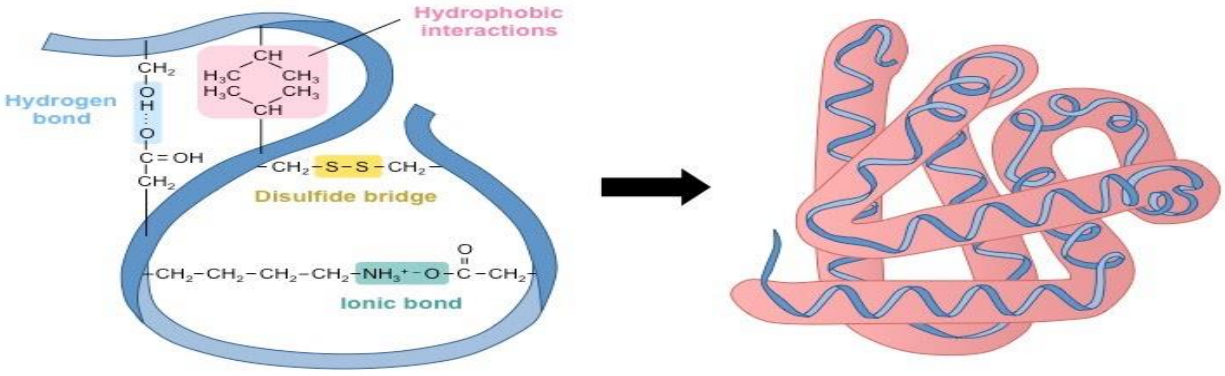
- The α-helix is a right-handed coiled strand.
- The side-chain substituents of the amino acid groups in an α-helix extend to the outside.
- Hydrogen bonds form between the oxygen of the C=O of each peptide bond in the strand and the hydrogen of the N-H group of the peptide bond four amino acids below it in the helix.
- The side-chain substituents of the amino acids fit in beside the N-H groups.
- The hydrogen bonding in a ß-sheet is between strands (inter-strand) rather than within strands (intra-strand).
- The sheet conformation consists of pairs of strands lying side-by-side.
- The carbonyl oxygens in one strand hydrogen bond with the amino hydrogens of the adjacent strand.

- The two strands can be either parallel or anti-parallel depending on whether the strand directions (N-terminus to C-terminus) are the same or opposite.
- The anti-parallel ß-sheet is more stable due to the more well-aligned hydrogen bonds.



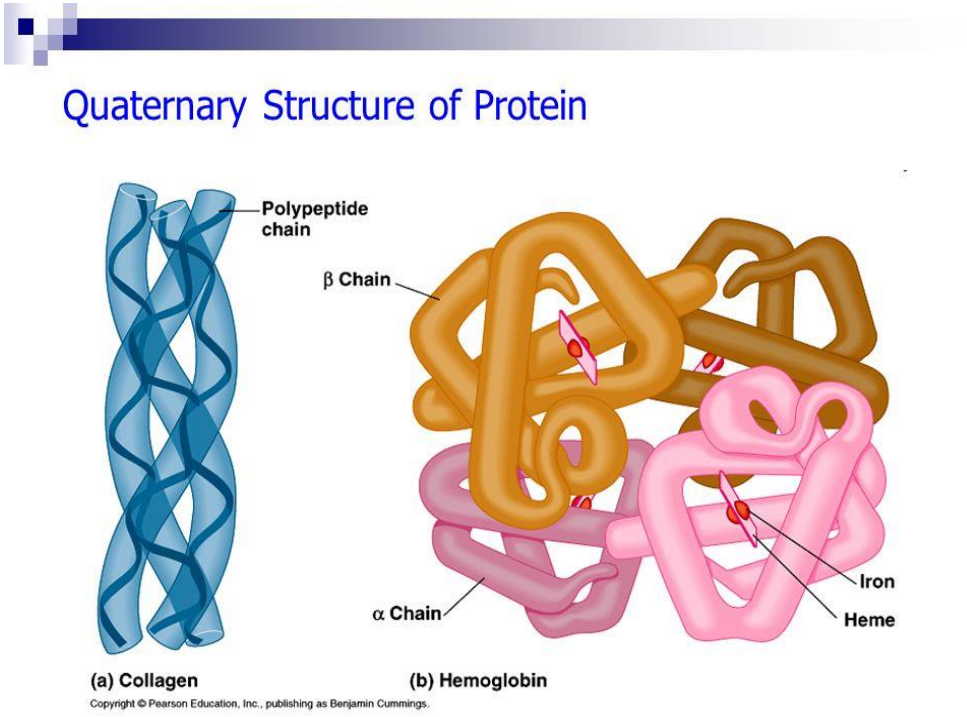
3. Tertiary Structure

- Tertiary structure of a protein refers to its overall three-dimensional conformation.
- The types of interactions between amino acid residues that produce the three-dimensional shape of a protein include hydrophobic interactions, electrostatic interactions, and hydrogen bonds, all of which are non-covalent.
- Covalent disulfide bonds also occur.
- It is produced by interactions between amino acid residues that may be located at a considerable distance from each other in the primary sequence of the polypeptide chain.
- Hydrophobic amino acid residues tend to collect in the interior of globular proteins, where they exclude water, whereas hydrophilic residues are usually found on the surface, where they interact with water.



4. Quaternary Structure

- Quaternary structure refers to the interaction of one or more subunits to form a functional protein, using the same forces that stabilize the tertiary structure.
- It is the spatial arrangement of subunits in a protein that consists of more than one polypeptide chain.



Purification, and criteria of homogeneity

Protein purification is a series of processes intended to isolate one or a few proteins from a complex mixture, usually cells, tissues or whole organisms. Protein purification is vital for the specification of the function, structure and interactions of the protein of interest. The purification process may separate the protein and non-protein parts of the mixture, and finally separate the desired protein from all other proteins. Separation of one protein from all others is typically the most laborious aspect of protein purification. Separation steps usually exploit differences in protein size, physico-chemical properties, binding affinity and biological activity. The pure result may be termed **protein isolate**.

Purpose

Protein purification is either preparative or analytical. **Preparative purifications** aim to produce a relatively large quantity of purified proteins for subsequent use. Examples include the preparation of commercial products such as enzymes (e.g. lactase), nutritional proteins (e.g. soy protein isolate), and certain biopharmaceuticals (e.g. insulin). Several preparative purifications

steps are often deployed to remove bi-products, such as host cell proteins, which poses as a potential threat to the patient's health. **Analytical purification** produces a relatively small amount of a protein for a variety of research or analytical purposes, including identification, quantification, and studies of the protein's structure, post-translational modifications and function. Pepsin and urease were the first proteins purified to the point that they could be crystallized.


Recombinant bacteria can be grown in a flask containing growth media.

Preliminary steps

Extraction

If the protein of interest is not secreted by the organism into the surrounding solution, the first step of each purification process is the disruption of the cells containing the protein. Depending on how fragile the protein is and how stable the cells are, one could, for instance, use one of the following methods: i) repeated freezing and thawing, ii) sonication, iii) homogenization by high pressure (French press), iv) homogenization by grinding (bead mill), and v) permeabilization by detergents (e.g. Triton X-100) and/or enzymes (e.g. lysozyme).[3] Finally, the cell debris can be removed by centrifugation so that the proteins and other soluble compounds remain in the supernatant.

Also proteases are released during cell lysis, which will start digesting the proteins in the solution. If the protein of interest is sensitive to proteolysis, it is recommended to proceed quickly, and to keep the extract cooled, to slow down the digestion. Alternatively, one or more protease inhibitors can be added to the lysis buffer immediately before cell disruption. Sometimes it is also necessary to add DNAse in order to reduce the viscosity of the cell lysate caused by a high DNA content.

Precipitation and differential solubilization

In bulk protein purification, a common first step to isolate proteins is precipitation with ammonium sulfate $(NH_4)_2SO_4$.[4] This is performed by adding increasing amounts of ammonium sulfate and collecting the different fractions of precipitated protein. Subsequently, ammonium sulfate can be removed using dialysis. During the ammonium sulfate precipitation step, hydrophobic groups present on the proteins are exposed to the atmosphere, attracting other hydrophobic groups; the result is formation of an aggregate of hydrophobic components. In this

case, the protein precipitate will typically be visible to the naked eye. One advantage of this method is that it can be performed inexpensively, even with very large volumes.

The first proteins to be purified are water-soluble proteins. Purification of integral membrane proteins requires disruption of the cell membrane in order to isolate any one particular protein from others that are in the same membrane compartment. Sometimes a particular membrane fraction can be isolated first, such as isolating mitochondria from cells before purifying a protein located in a mitochondrial membrane. A detergent such as sodium dodecyl sulfate (SDS) can be used to dissolve cell membranes and keep membrane proteins in solution during purification; however, because SDS causes denaturation, milder detergents such as Triton X-100 or CHAPS can be used to retain the protein's native conformation during complete purification.

Ultracentrifugation

Centrifugation is a process that uses centrifugal force to separate mixtures of particles of varying masses or densities suspended in a liquid. When a vessel (typically a tube or bottle) containing a mixture of proteins or other particulate matter, such as bacterial cells, is rotated at high speeds, the inertia of each particle yields a force in the direction of the particles velocity that is proportional to its mass. The tendency of a given particle to move through the liquid because of this force is offset by the resistance the liquid exerts on the particle. The net effect of "spinning" the sample in a centrifuge is that massive, small, and dense particles move outward faster than less massive particles or particles with more "drag" in the liquid. When suspensions of particles are "spun" in a centrifuge, a "pellet" may form at the bottom of the vessel that is enriched for the most massive particles with low drag in the liquid.

Non-compacted particles remain mostly in the liquid called "supernatant" and can be removed from the vessel thereby separating the supernatant from the pellet. The rate of centrifugation is determined by the angular acceleration applied to the sample, typically measured in comparison to the *g*. If samples are centrifuged long enough, the particles in the vessel will reach equilibrium wherein the particles accumulate specifically at a point in the vessel where their buoyant density is balanced with centrifugal force. Such an "equilibrium" centrifugation can allow extensive purification of a given particle.

Sucrose gradient centrifugation — a linear concentration gradient of sugar (typically sucrose, glycerol, or a silica based density gradient media, like Percoll) is generated in a tube such that the highest concentration is on the bottom and lowest on top. Percoll is a trademark owned by GE Healthcare companies. A protein sample is then layered on top of the gradient and spun at high speeds in an ultracentrifuge. This causes heavy macromolecules to migrate towards the bottom of the tube faster than lighter material. During centrifugation in the absence of sucrose, as particles move farther and farther from the center of rotation, they experience more and more centrifugal force (the further they move, the faster they move). The problem with this is that the useful separation range of within the vessel is restricted to a small observable window. Spinning a sample twice as long doesn't mean the particle of interest will go twice as far, in fact, it will go significantly further. However, when the proteins are moving through a sucrose gradient, they encounter liquid of increasing density and viscosity. A properly designed sucrose gradient will counteract the increasing centrifugal force so the particles move in close proportion to the time

they have been in the centrifugal field. Samples separated by these gradients are referred to as "rate zonal" centrifugations. After separating the protein/particles, the gradient is then fractionated and collected.

Size exclusion chromatography

Gel permeation chromatography

Chromatography can be used to separate protein in solution or denaturing conditions by using porous gels. This technique is known as size exclusion chromatography. The principle is that smaller molecules have to traverse a larger volume in a porous matrix. Consequentially, proteins of a certain range in size will require a variable volume of eluent (solvent) before being collected at the other end of the column of gel.

In the context of protein purification, the eluent is usually pooled in different test tubes. All test tubes containing no measurable trace of the protein to purify are discarded. The remaining solution is thus made of the protein to purify and any other similarly-sized proteins.

Separation based on charge or hydrophobicity

Hydrophobic interaction chromatography

HIC media is amphiphilic, with both hydrophobic and hydrophilic regions, allowing for separation of proteins based on their surface hydrophobicity. Target proteins and their product aggregate species tend to have different hydrophobic properties and removing them via HIC further purifies the protein of interest.Additionally, the environment used typically employs less harsh denaturing conditions than other chromatography techniques, thus helping to preserve the protein of interest in its native and functional state. In pure water, the interactions between the resin and the hydrophobic regions of protein would be very weak, but this interaction is enhanced by applying a protein sample to HIC resin in high ionic strength buffer. The ionic strength of the buffer is then reduced to elute proteins in order of decreasing hydrophobicity

Ion exchange chromatography

Ion exchange chromatography separates compounds according to the nature and degree of their ionic charge. The column to be used is selected according to its type and strength of charge. Anion exchange resins have a positive charge and are used to retain and separate negatively charged compounds (anions), while cation exchange resins have a negative charge and are used to separate positively charged molecules (cations).

Before the separation begins a buffer is pumped through the column to equilibrate the opposing charged ions. Upon injection of the sample, solute molecules will exchange with the buffer ions as each competes for the binding sites on the resin. The length of retention for each solute depends upon the strength of its charge. The most weakly charged compounds will elute first, followed by those with successively stronger charges. Because of the nature of the separating

mechanism, pH, buffer type, buffer concentration, and temperature all play important roles in controlling the separation.

Ion exchange chromatography is a very powerful tool for use in protein purification and is frequently used in both analytical and preparative separations.

Nickel-affinity column. The resin is blue since it has bound nickel.

Lyophilization

If the solution doesn't contain any other soluble component than the protein in question the protein can be lyophilized (dried). This is commonly done after an HPLC run. This simply removes all volatile components, leaving the proteins behind.

Ultrafiltration

Ultrafiltration concentrates a protein solution using selective permeable membranes. The function of the membrane is to let the water and small molecules pass through while retaining the protein. The solution is forced against the membrane by mechanical pump, gas pressure, or centrifugation.

Evaluating purification yield

The most general method to monitor the purification process is by running a SDS-PAGE of the different steps. This method only gives a rough measure of the amounts of different proteins in the mixture, and it is not able to distinguish between proteins with similar apparent molecular weight.

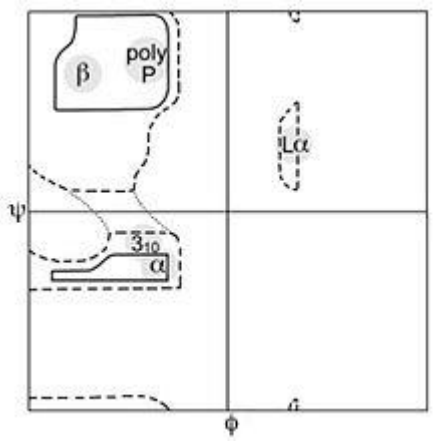
If the protein has a distinguishing spectroscopic feature or an enzymatic activity, this property can be used to detect and quantify the specific protein, and thus to select the fractions of the separation, that contains the protein. If antibodies against the protein are available then western blotting and ELISA can specifically detect and quantify the amount of desired protein. Some proteins function as receptors and can be detected during purification steps by a ligand binding assay, often using a radioactive ligand.

In order to evaluate the process of multistep purification, the amount of the specific protein has to be compared to the amount of total protein. The latter can be determined by the Bradford total protein assay or by absorbance of light at 280 nm, however some reagents used during the purification process may interfere with the quantification. For example, imidazole (commonly used for purification of polyhistidine-tagged recombinant proteins) is an amino acid analogue and at low concentrations will interfere with the bicinchoninic acid (BCA) assay for total protein

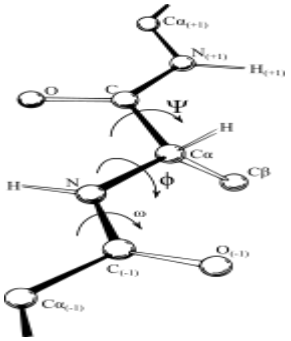
quantification. Impurities in low-grade imidazole will also absorb at 280 nm, resulting in an inaccurate reading of protein concentration from UV absorbance.

Another method to be considered is Surface Plasmon Resonance (SPR). SPR can detect binding of label free molecules on the surface of a chip. If the desired protein is an antibody, binding can be translated directly to the activity of the protein. One can express the active concentration of the protein as the percent of the total protein. SPR can be a powerful method for quickly determining protein activity and overall yield. It is a powerful technology that requires an instrument to perform.

Ramachandran plot

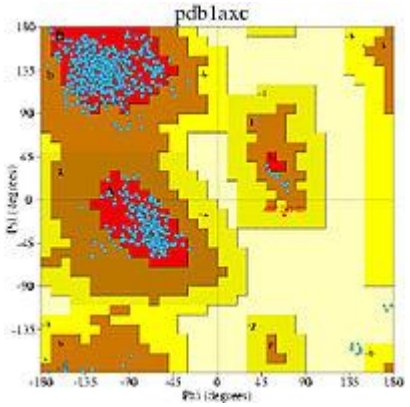


Original hard-sphere, reduced-radius, and relaxed-tau φ,ψ regions from Ramachandran, with updated labels and axes



Backbone dihedral angles φ and ψ (and ω). All three angles are at 180° in the conformation shown

In biochemistry, a **Ramachandran plot** (also known as a **Rama plot**, a **Ramachandran diagram** or a **[φ,ψ] plot**), originally developed in 1963 by G. N. Ramachandran, C. Ramakrishnan, and V. Sasisekharan,[1] is a way to visualize energetically allowed regions for backbone dihedral angles ψ against φ of amino acid residues in protein structure. The figure on the left illustrates the definition of the φ and ψ backbone dihedral angles[2] (called φ and φ' by Ramachandran). The ω angle at the peptide bond is normally 180°, since the partial-double-bond character keeps the peptide planar.[3] The figure in the top right shows the allowed φ,ψ backbone conformational regions from the Ramachandran et al. 1963 and 1968 hard-sphere calculations: full radius in solid outline, reduced radius in dashed, and relaxed tau (N-Cα-C) angle in dotted lines.[4] Because dihedral angle values are circular and 0° is the same as 360°, the edges of the Ramachandran plot "wrap" right-to-left and bottom-to-top. For instance, the small strip of allowed values along the lower-left edge of the plot are a continuation of the large, extended-chain region at upper left.



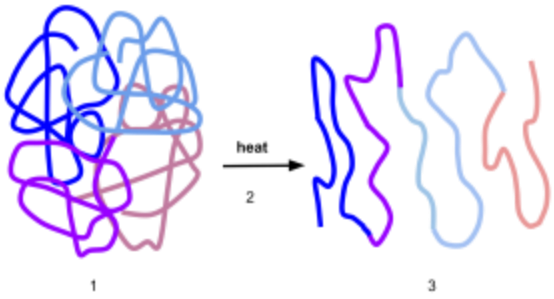
A Ramachandran plot generated from human PCNA, a trimeric DNA clamp protein that contains both β-sheet and α-helix (PDB ID 1AXC). The red, brown, and yellow regions represent the favored, allowed, and "generously allowed" regions as defined by ProCheck

Uses

A Ramachandran plot can be used in two somewhat different ways. One is to show in theory which values, or conformations, of the ψ and φ angles are possible for an amino-acid residue in a protein (as at top right). A second is to show the empirical distribution of datapoints observed in a single structure (as at right, here) in usage for structure validation, or else in a database of many structures (as in the lower 3 plots at left). Either case is usually shown against outlines for the theoretically favored regions.

Denaturation of Proteins

These biomolecules are also required for the proper maintenance of our bodies. We have studied about the different structures of proteins; it has a unique three-dimensional structure.



Denaturation of Proteins

The stability of protein and its structure depends on physical and chemical conditions.

- Temperature and pH affect their stability to a great extent.
- Denaturation of the proteins is a condition when the unique three-dimensional structure of a protein is exposed to changes.
- Due to changes in temperature, pH or other chemical activities, the hydrogen bonds present in the proteins get disturbed. This results in the unfolding of globular proteins and uncoiling of the helix structure.
- The uncoiling of helix structure affects the chemistry of proteins and they lose their biological activity. This phenomenon of losing their activity and uncoiling of helix structure due to physical or chemical changes is called the denaturation of proteins.
- During denaturation of proteins, the secondary and tertiary structures get destroyed and only the primary structure is retained.
- Covalent bonds are broken and interaction between amino-acid chains gets disrupted. This results in the loss of biological activity of the proteins.

Process of Denaturation of Proteins

- Secondary, tertiary and quaternary protein structure is easily changed by a process called denaturation. These changes can be quite damaging.
- Heating, exposure to acids or bases and even violent physical action can cause denaturation to occur.
- The albumin protein in egg white is denatured by heating so that it forms a semisolid mass. Almost the same thing is accomplished by the violent physical action of an egg beater in the preparation of meringue.
- Heavy metal poisons such as lead and cadmium change the structure of proteins by binding to functional groups on the protein surface.
- Denaturation of proteins can be done by bringing in physical changes as well as the introduction of chemicals.

- Most of the denaturation processes are irreversible, but it has been seen (in very few cases) that some of the denaturation processes can be reversed; it is then called as renaturation of protein.
- Some of the common cases of denaturation of proteins are coagulation of egg white when an egg is subjected to boiling. Here the denaturation occurs due to change in temperature.
- Curdling of milk is another example of denaturation of proteins where the formation of lactic acid by microbial action results in denaturation.

Protein is a vast subject of research in science. The development of technology has rendered us the ability to extract so much information about these biomolecules. Learning is a continuous process and even the scientists keep learning new things about these biomolecules.

Prosthetic group

A **prosthetic group** is the non-amino acid component that is part of the structure of the heteroproteins or conjugated proteins, being covalently linked to the apoprotein.

Not to be confused with the cofactor that binds to the enzyme apoenzyme (either a holoprotein or heteroprotein) by non-covalent binding a non-protein (non-amino acid)

This is a component of a conjugated protein that is required for the protein's biological activity. The prosthetic group may be organic (such as a vitamin, sugar, RNA, phosphate or lipid) or inorganic (such as a metal ion). Prosthetic groups are bound tightly to proteins and may even be attached through a covalent bond. They often play an important role in enzyme catalysis. A protein without its prosthetic group is called an apoprotein, while a protein combined with its prosthetic group is called a holoprotein. A non-covalently bound prosthetic group cannot generally be removed from the holoprotein without denaturating the protein. Thus, the term "prosthetic group" is a very general one and its main emphasis is on the tight character of its binding to the apoprotein. It defines a structural property, with oppostion of the term "coenzyme" that defines a functional property.

Prosthetic groups are a subset of cofactors. Loosely bound metal ions and coenzymes are still cofactors, but are generally not called prosthetic groups. In enzymes, prosthetic groups are involved in the catalytic mechanism and required for activity. Other prosthetic groups have structural properties. This is the case for the sugar and lipid moieties in glycoproteins and lipoproteins or RNA in ribosomes. They can be very large, representing the major part of the protein in proteoglycans for instance.

The heme group in hemoglobin is a prosthetic group. Further examples of organic prosthetic groups are vitamin derivatives: thiamine pyrophosphate, pyridoxal-phosphate and biotin. Since prosthetic groups are often vitamins or made from vitamins, this is one of the reasons why vitamins are required in the human diet. Inorganic prosthetic groups are usually transition metal ions such as iron (in heme groups, for example in cytochrome c oxidase and hemoglobin), zinc (for example in carbonic anhydrase), copper (for example in complex IV of the respiratory chain) and molybdenum (for example in nitrate reductase).

List of prosthetic groups

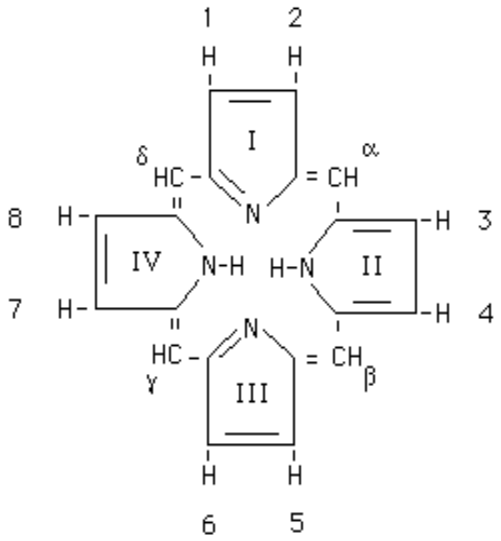
Prosthetic group	Function	Distribution
Flavin mononucleotide	Redox reactions	Bacteria, archaea and eukaryotes
Flavin adenine dinucleotide	Redox reactions	Bacteria, archaea and eukaryotes
Pyrroloquinoline quinone	Redox reactions	Bacteria
Pyridoxal phosphate	Transamination, decarboxylation and deamination	Bacteria, archaea and eukaryotes
Biotin	Carboxylation	Bacteria, archaea and eukaryotes
Methylcobalamin	Methylation and isomerisation	Bacteria, archaea and eukaryotes
Thiamine pyrophosphate	Transfer of 2-carbon groups, α cleavage	Bacteria, archaea and eukaryotes
Heme	Oxygen binding and redox reactions	Bacteria, archaea and eukaryotes
Molybdopterin	Oxygenation reactions	Bacteria, archaea and eukaryotes
Lipoic acid	Redox reactions	Bacteria, archaea and eukaryotes

Porphyrin

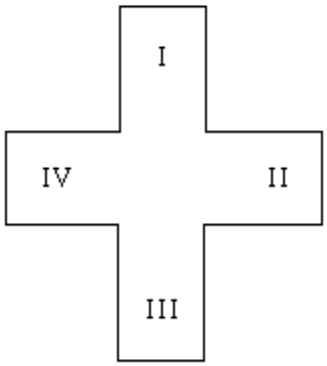
Porphyrin, any of a class of water-soluble, nitrogenous biological pigments (biochromes), derivatives of which include the hemoproteins (porphyrins combined with metals and protein). Examples of hemoproteins are the green, photosynthetic chlorophylls of higher plants; the hemoglobins in the blood of many animals; the cytochromes, enzymes that occur in minute

quantities in most cells and are involved in oxidative processes; and catalase, also a widely distributed enzyme that accelerates the breakdown of hydrogen peroxide.

Porphyrins have complex cyclic structures. All porphyrin compounds absorb light intensely at or close to 410 nanometres. Structurally, porphyrin consists of four pyrrole rings (five-membered closed structures containing one nitrogen and four carbon atoms) linked to each other by methine groups (—CH=). The iron atom is kept in the centre of the porphyrin ring by interaction with the four nitrogen atoms. The iron atom can combine with two other substituents; in oxyhemoglobin, one substituent is a histidine of the protein carrier, and the other is an oxygen molecule. In some heme proteins, the protein is also bound covalently to the side chains of porphyrin.



Porphin, showing the four pyrrole rings and the Roman numerals which designate them. Arabic numbers indicate positions at which substituents may be attached. Greek letters denote the methene bridges.



Schematic representation of porphin.

Properties

Porphyrins are strongly colored compounds. The variety of available colors is apparently unlimitedalthough there is an observable inclination towards red and purple. The color is a consequence of the complicated electronic spectra of porphyrins, which contain intense absorptions in the visible region (called **Q bands**). Even more intense (ten times and more) is the **Soret band** found in the near UV, so named after its discoverer (Another conspicuous characteristic of porphyrins, directly associated with their optical properties,is the **aromaticity** of the macrocycle. It is most evidently manifested in the ^{1}H NMR spectra by the downfield shifts of peripheral protons and the strongly upfield shifts of the inner NH's(usually negative on the scale).

Porphyrin aromaticity is most frequently described in terms of the **[18]annulene model**, proposed by E. Vogel. According to that model, a delocalization pathway is distinguished in the macrocycle, as shown in Figure 4, which is aromatic in the traditional Hückel sense. The porphyrin is thus viewed as a bridged diaza[18]annulene.

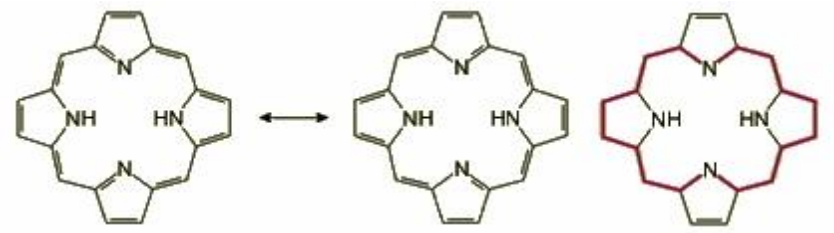


Fig. 4. Delocalization pathway in porphyrin according to Vogel's [18]annulene model.

Porphyrins are weak bases and can be protonated to form dications. In the unprotonated porphyrin (called *free base*) the two inner protons are mobile and jump freely among the four nitrogens. The *trans* (21,23-H) tautomer, shown in all schemes, is energetically preferred to the *cis* form (21,22-H).

Porphyrins form a great number of complexes with metal ions and some nonmetals (Figure 5).The coordinating environment provided by porphyrins is very flexible and can be fine-tuned to particular oxidation and spin states by varying peripheral substitution and axial ligands. This tunability was probably Nature's reason to choose porphyrin as its "workhorse macrocycle."

Of the naturally ocurring metalloporphyrins the iron complexes, called *hemes*, are by far the most important. They make the reactive centers of numerous heme proteins, responsible for oxygen transport and storage (hemo- and myoglobin), electron transfer (cytochromes), and oxidation of organic substrates (oxygenases of the P450 family).