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Most Important★★★★★ (Learn Completely)
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Important★★★ (Selective Reading)
No Star Topics – Selective Reading Based on Check List Given after Every Subchapter

2.1 CHEMISTRY OF PROTEINS

Peptide Bond | Classification of Proteins

INTRODUCTION

Proteins are polymers of amino acid. Proteins contain Carbon, Hydrogen, Oxygen and Nitrogen as the major components. Nitrogen is characteristic of Protein. On an average, the **nitrogen content of ordinary proteins is 16% by weight**.

PEPTIDE BOND

All Proteins are linked by Peptide bond.

Alpha Carboxyl group of one amino acid reacts with alpha amino group of another amino acid to form a peptide bond or **CO-NH** bridge.

- N terminal end—The free NH_2 group of the terminal amino acid is called as N terminal end.
- C terminal end—The free COOH end is called C terminal end.
- The amino acids are sequenced from N-terminal end to C-terminal end.

Formation of Peptide Bond

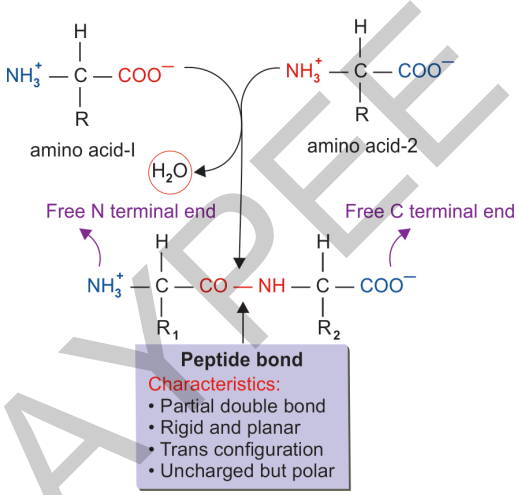


Fig. 2.1.1: Formation and characteristics of peptide bond

Atypical Peptide Bond (Pseudopeptide Bond) (Isopeptide Bond)

An amide bond formed between an amino group and a carboxyl group at least one of which is not an alpha group, seen in the side chains of proteins.

Characteristic Features of an Atypical Peptide Bond

- Occur **post-translationally**
- Can be formed **spontaneously or enzymatically**
- Can produce stably linked protein dimers, multimers or complexes
- Makes the **protein resistant** as proteases cannot hydrolyse isopeptide bond.

High Yield Points

Examples of Pseudopeptides

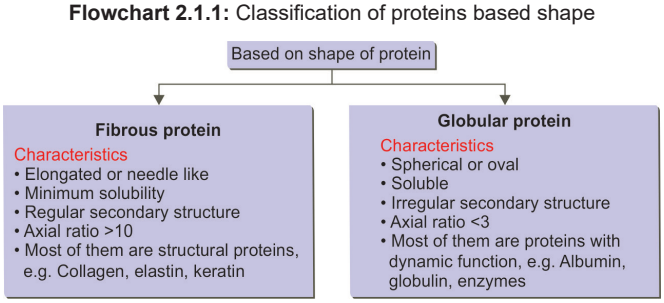
- Glutathione
- Thyrotropin releasing hormone
- Ubiquitin attached to protein
- Blood clots
- Cyclic peptide antibiotic like Tyrocidin and Gramicidin
- Heptapeptides like Dermorphin, Deltorphin.

Some Biologically Important Peptides

Peptide	Example
Tripeptide	Thyrotropin releasing hormone (TRH) Glutathione
Pentapeptide	Enkephalin
Octapeptide	Angiotensin II
Nonapeptide	Oxytocin vasopressin [ADH] bradykinin
Decapeptide	Angiotensin-I

CLASSIFICATION OF PROTEINS

Flowchart 2.1.1: Classification of proteins based shape



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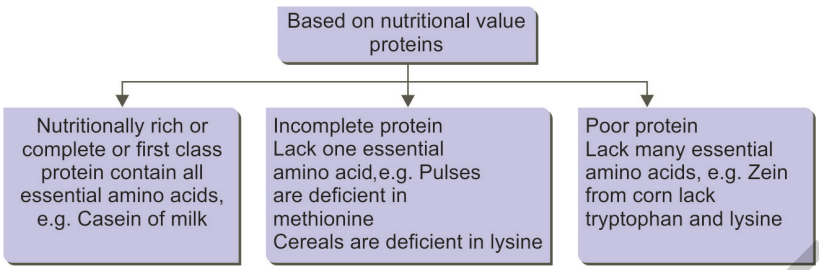
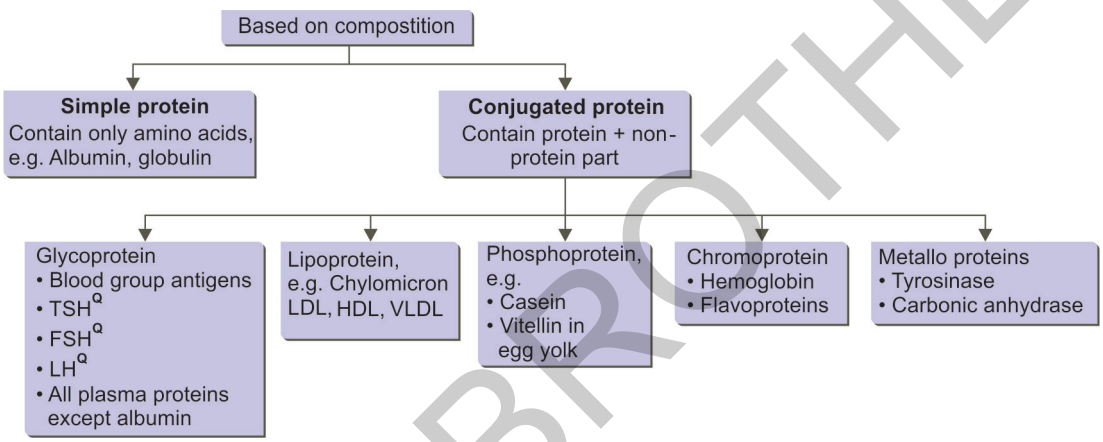
Flowchart 2.1.2: Classification of proteins based on nutritional value



Flowchart 2.1.3: Classification of proteins based on composition



Quick Review

- Nitrogen content in proteins is 16% by weight.
- TRH and Glutathione are atypical peptide.
- Proteins with structural function are fibrous protein.
- Fibrous protein has regular secondary structure.
- Most enzymes are globular proteins.
- TSH, FSH & LH are glycoproteins.
- Albumin is a simple protein.
- Casein of milk is a first class protein.
- Limiting amino acid in cereals is Lysine.
- Limiting amino acid in pulses is methionine.

MCQ Review Questions

1. True about Isopeptide bond is: *(PGI Nov 2011)*

a. It makes protein resistant.
b. Bond is formed between carboxy terminal of one protein and amino group of a lysine residue on another.
c. Involves in post-transcriptional modification of protein
d. Enzyme act as catalyst for bond formation

2. Which of the following is/are storage protein?

a. Myoglobin *(PGI May 2011)*
b. Ovalbumin
c. Ricin
d. Ferritin
e. Glutelin

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3. **Which one of the following can be homologous substitution for isoleucine in a protein in sequence?** *(AI 2006)*
   a. Methionine
   b. Aspartic acid
   c. Valine
   d. Arginine

4. **In a mutation if valine is replaced by which of the following would not result in any change in the function of protein?** *(AIIMS May 02)*
   a. Proline
   b. Leucine
   c. Glycine
   d. Aspartic acid

5. **At isoelectric pH protein:** *(PGI June 03)*
   a. Have no net charge
   b. Do not migrate
   c. Are positively charged
   d. Are negatively charged

6. **Isoelectric point is when:**
   a. Net charge of protein is zero
   b. Mass of protein is zero
   c. Protein
   d. Denaturation of protein occurs

7. **Biuret test is used for detection of:**
   a. Protein
   b. Cholesterol
   c. Steroid
   d. Sugar

8. **What type of protein is Casein?** *(CMC Ludhiana 2014)*
   a. Lipoprotein
   b. Phosphoprotein
   c. Glycoprotein
   d. Flavoprotein

9. **Which is/are not transport Protein?** *(PGI May 2012)*
   a. Transferrin
   b. Collagen
   c. Ceruloplasmin
   d. Hemoglobin
   e. Albumin

10. **Which of the amino acid is responsible for peptide bond?** *(PGI Nov 2014)*
    a. Amino group
    b. Carboxyl group
    c. Side chain
    d. Amide group
    e. Aldehyde group

Answers to Review Questions

1. **a, b, d. It makes protein resistant, Bond is formed between carboxy terminal of one protein and amino group of a lysine residue on another, Enzyme act as catalyst for bond formation**

Atypical Peptide Bond (Pseudopeptide Bond) (Isopeptide Bond)

A bond formed between an amino group and a carboxyl group at least one of which is not an alpha group. Seen in the side chains of proteins.

Characteristic features

- Occur post-translationally
- Can be formed spontaneously or enzymatically.
- Can produce stably linked protein dimers, multimers or complexes
- Makes the protein resistant as proteases cannot hydrolyse isopeptide bond.

Examples

- Glutathione
- Thyrotropin releasing hormone
- Ubiquitin attached to protein
- Blood clots

Application of spontaneous Isopeptide Bond formation

- Develop a new peptide tag called Isopeptag. Used in:
  - In vivo Protein targeting
  - Fluorescence Microscopy Imaging

2. **b, d, e. Ovalbumin, Ferritin, Glutelin**

(Ref: Chatterjee & Shinde 8/e page 82-85)

Storage protein

Proteins that act as store house of amino acids and metal ions that can be easily mobilised.

- Casein of milk
- Vitellin of egg yolk
- Ovalbumin of egg white
- Glutelin of wheat.
- Oryzenin of Rice
- Gliadin of Wheat
- Ferritin-stores iron

Other options

Myoglobin:

- It is a transport protein of Oxygen to Skeletal Muscle

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Ricin:
- Inhibitor of mammalian Protein Synthesis.
- From cast or be an in activates eukaryotic 28S ribosomal RNA

3. c. Valine

Conservative [Homologous] Substitution
- One amino acid replaced by another amino acid of similar characteristics
- Examples of homologous substitution is shown in the diagram given below.

Hydrophilic, Acidic	Asp	Glu				
Hydrophilic, Basic	His	Arg	Lys			
Polar, Uncharged	Ser	Thr	Gln	Asn		
Hydrophobic	Ala	Phe	Leu	Ile	Val	Pro

Nonconservative (Nonhomologous) Substitution
- One amino acid replaced by another amino acid of different characteristics

4. b. Leucine

5. a, b. Have no net charge, Do not migrate *(Ref: Harper 31/e page 20)*

Properties of proteins at isoelectric pH:
- They have no net charge.
- They do not have electrophoretic mobility
- Maximum precipitability
- Minimum solubility
- Minimum buffering action

6. a. Net charge of a protein is Zero *(Ref: Harper 31/e page 19)*

7. a. Protein

General test for Proteins

Cupric ions in alkaline medium forms violet colour with peptide bond nitrogen.

Ninhydrin Test

General test for all alpha Amino Acid

Amino acid + 2 mols of Ninhydrin ⟶ Aldehyde with 1 carbon atom less + CO_2 + Purple Complex (Ruhemann's Purple)

8. b. Phosphoprotein *(Ref: Chatterjea and Shinde 8/e page 85)*

Two important Phosphoproteins are
- Casein found in milk
- Ovovitellin found in egg yolk

9. b. Collagen *(Ref: Harper 31/e p 630 Table 52-3)*

10. a, b. Amino group, Carboxyl group
- Peptide bond is formed between amino group of one amino acid with carboxyl group of the next amino acid.

2.2 STRUCTURAL ORGANIZATION OF PROTEINS AND ITS STUDY

- Primary Structure
- Secondary Structure
- Tertiary Structure
- Quaternary Structure
- Study of Structure of Proteins

Proteins have different levels of structural organization; primary, secondary, tertiary and quaternary.

PRIMARY STRUCTURE

It is the linear sequence of amino acid held together by peptide bonds in its peptide chains.

Bond involved in primary structure is **Peptide Bond**, a type of covalent bond.

Amino acid sequence determines the 3D structure of the protein.Q

SECONDARY STRUCTURE

- The folding of short (3- to 30-residue), contiguous segments of polypeptide into geometrically ordered units.

High Yield Points
- Bonds involved in the secondary structure are primarily **noncovalent** bonds like
  - **Hydrogen bond (Most important Bond)**
  - **Hydrophobic bond**
  - **Electrostatic bond (Ionic bond, salt bridges)**
  - **Van der Waals forces.**

Secondary Structures of Proteins
- Alpha helix
- Beta pleated sheet
- Loops
- Bends
- Turns

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*Alpha Helix*Q

- Alpha helix is the **most common and stable** secondary structure.
- **Right-handed spiral structure**
- Structure stabilized primarily by **intra chain hydrogen bond** between carbonyl oxygen of 1st and amide nitrogen of 4th amino acid.
- Each turn formed by **3.6 Amino acyl** residues.
- Distance of 1 turn of alpha helix (called Pitch) is 0.54 nm.
- **Proline** can only be stably accommodated within the first turn of an α-helix
- Examples of proteins whose major secondary structure is α-helix
  - Hemoglobin
  - Myoglobin.

*Beta-Pleated Sheet*Q

- The **second most common** (hence "beta") recognizable regular secondary structure in proteins.
- Polypeptide chain is almost fully extended.
- They have a **zig-zag or pleated pattern**.
- In contrast to intrachain hydrogen bond in alpha helix, here it is **interchain hydrogen bond** between carbonyl oxygen and amide nitrogen of two adjacent chains.
- Adjacent strands in a sheet can run in the same direction (parallel) or opposite direction (antiparallel).

Example of proteins whose major secondary structure is

- Parallel β-sheet—Flavodoxin.
- Antiparallel β-sheet—Silk Fibroin.
- Both parallel and antiparallel β-sheet—Carbonic Anhydrase.

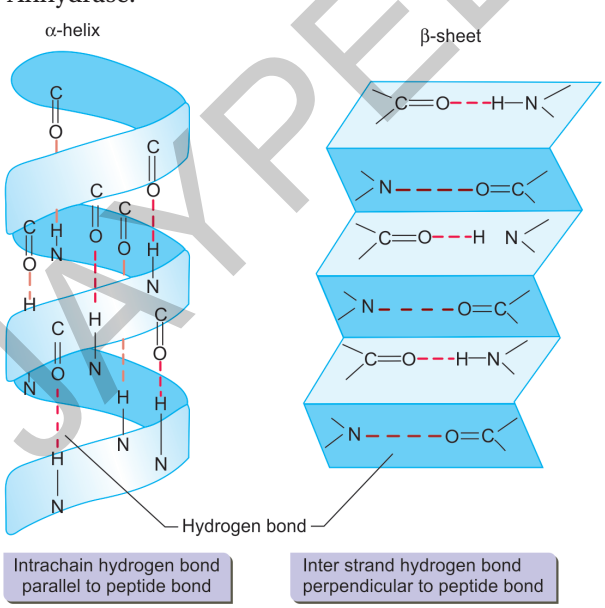

Fig. 2.2.1: Alpha helix

Turns and Bends

Short segments of amino acid that join two units of secondary structures.

Example: Beta turn

Loops

- Unlike bends and turns; loops are long segments of amino acid that joins two secondary structures.

IMAGE-BASED INFORMATION

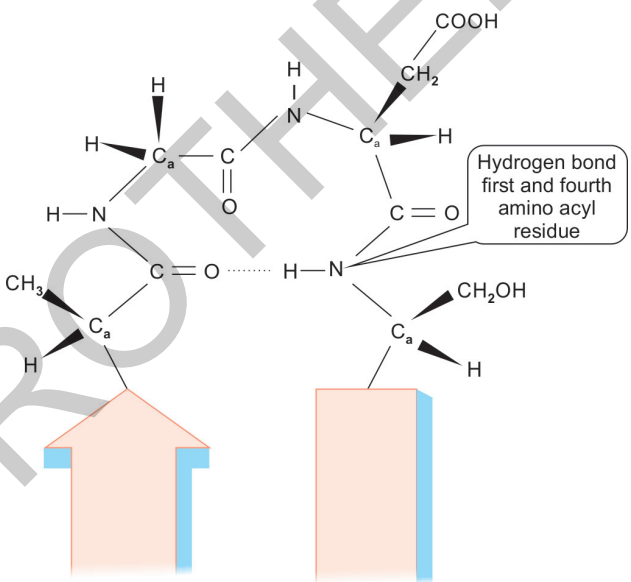

Fig. 2.2.2: Structure of beta turn

Beta Turn

- **Involves four amino acyl residues**
- **First amino acyl residue is hydrogen bonded to the fourth resulting in a tight 180° turn.**
- **Proline and Glycine are often present in β turn**

Amino Acids have Different Propensity for forming Alpha Helix, Beta Sheets and Beta Turns

The frequency of occurrence of certain amino acid residues determine the secondary structure formed.

Alpha helix

- Residues like Alanine, Glutamate, Methionine and Leucine tend to be present in the alpha helix.
- Most abundant amino acid is **Methionine** (relative frequency is 1.47) followed by Glutamate (1.44)
- Amino acid least present in alpha helix is **Proline**.

Amino acids that do not favor alpha helix areQ

- Amino acids with branches at β-carbon atom **valine, threonine and isoleucine** disrupt the stability.

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- Other amino acids that disrupt the stability are **serine, aspartate and asparagine.**
- **Proline** also disrupt the stability of alpha helix.
- **Glycine** induces bends in alpha helix.
- **Amino acids with bulky R group like Tryptophan**

Beta sheet

- Valine and isoleucine tend to be present in beta strands
- Most abundant amino acid in beta sheet is **Valine**
- Amino acid least present in beta sheet is **Proline**.

Turns

- Most abundant amino acid in turns is **Proline** (1.91) followed by Glycine (1.64).

Super Secondary Structure (Motifs)

Secondary Structural elements join to form **Super secondary structures**:

Examples are

- Beta-alpha-beta motif
- Greek key motif
- Beta meander motif
- Beta barrel:

DNA Binding motifs are examples of super secondary structure. They are:

- Helix-turn-helix motif
- Leucine zipper motif
- Zinc finger motif.



High Yield Points

Points to Ponder–DNA Binding Motifs

- The first motif described is the **Helix-turn-helix.**
- The second DNA binding motif is the **Zinc finger**.
- DNA binding motif which require Zinc for its activity Zinc Finger.
- DNA binding motif with leucine residues at every seventh position is **Leucine Zipper**.

Tertiary Structure

The entire three-dimensional conformation of a polypeptide is referred to as tertiary structure.

Domain

A domain is a section of protein structure sufficient to perform a particular chemical or physical task such as binding of a substrate.

Rossmann Fold

- It is a domain seen in the family of Oxidoreductases.
- They share a common N terminal **NAD(P)+ binding region called Rossmann fold**.

Oxidoreductase with Rossmann Fold[Q]

- Lactate dehydrogenase
- Alcohol dehydrogenase
- Glyceraldehyde-3-phosphate dehydrogenase
- Malate dehydrogenase
- 6-phosphogluconate dehydrogenase
- D-glycerate dehydrogenase

Quaternary Structure

If more than one polypeptides aggregate to form one functional protein, the spatial relationship between the polypeptide subunits is referred to as Quaternary structure.



High Yield Points

Bonds involved in Tertiary and Quaternary Structures are primarily non-covalent bonds.

- Hydrophobic Interaction
- Hydrogen Bond
- Electrostatic Bond
- Van der Waals Forces

Insulin has two polypeptide chains but it does not have Quaternary structure.

In Quaternary structure, the bond involved is primarily non-covalent bond. In insulin two polypeptide chains are connected by disulphide bond which is a Covalent Bond. Hence, even if Insulin has 2 polypeptide chain, it does not have quaternary structure.

Structure of Insulin

The first Hormone to be extracted in pure form

- This was done by **Banting and Best**
- **Banting** along with the director of the institute **John Macleod received Nobel Prize for the work**.
- The first protein in which complete sequencing was done
  - **Mr Frederick Sanger** was the man behind this work.
  - He used Sanger's Reagent for this.
  - He won Nobel Prize for his work
  - The first protein to be produced by Recombinant DNA Technology.

Primary Structure of Insulin

- Consist of two polypeptide chains
- Number of amino acids is 51
- A chain with 21 amino acids
- B chain-30 amino acids

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Disulfide Bonds in Insulin

Two interchain Disulphide Bonds:

- 7th Amino Acid in A chain to 7th Amino Acid in B chain.
- 20th Amino Acid in A chain to 19th Amino Acid in B chain.

One Intrachain Disulphide Bond

- 6th amino acid in A chain to 11th amino acid in A chain itself.

Species Variation in Insulin

- Restricted to 8, 9, 10 in A chain and C terminal amino Acid of B chain.
- Porcine and human insulin vary only in the terminal amino acid of B chain

Denaturation of Proteins

Nonspecific alteration in **secondary, tertiary and quaternary structures** of protein molecule when treated with a denaturing agent.

Two types of denaturation

1. **Reversible denaturation:** Denatured proteins are sometimes renatured when physical agent is removed.
2. **Irreversible Denaturation:** Denatured proteins are not renatured when physical agent is removed.
   - For example, Albumin heated is irreversibly denatured called **Heat Coagulation**.



High Yield Points

Characteristic features of denaturation are[Q]

- Loss of biological Activity
- Primary structure (i.e. the peptide bond) is not lost.
- Loss of secondary and tertiary and quaternary structures.
- Loss of Folding
- They assume a Random Coil Structure.

Concept Box

- Everything lost in denaturation except the primary structure (i.e. the peptide bond).
- Remember the peptide bond is a covalent bond which is the strongest bond.

STUDY OF PROTEIN STRUCTURE

Study of Primary Structure/Sequencing of Proteins

Methods of protein sequencing

1. End group analysis
2. Mass spectrometry
3. Molecular biology techniques

End Group Analysis

Identification of N-terminal and C-terminal amino acid in a polypeptide chain is called end group analysis.



High Yield Points

Identification of N Terminal Amino Acid:

- **Sanger's Technique** using Sanger's reagent **(1, Fluoro 2, 4 Dinitro Benzene, FDNB)**[Q]
- **Edman's Degradation Technique** using **Edman's reagent (Phenyl Isothiocyanate)**

Identification of C Terminal Amino Acid

- Using **Carboxypeptidase A and B**

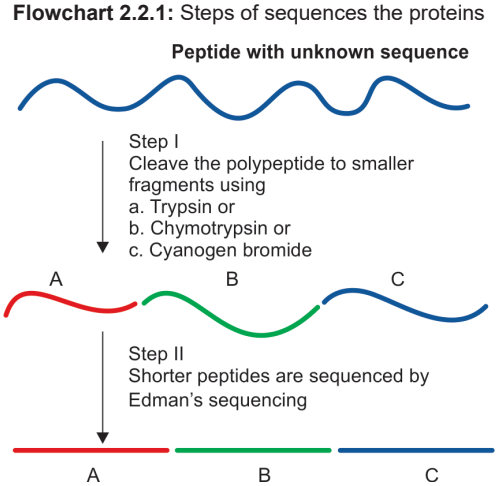
Sanger's Technique

- This was the first technique to determine the sequence of protein.
- Sanger's reagentis **1, Fluoro 2, 4 Dinitrobenzene**[Q]
- Sanger's Reagent derivatizes the amino terminal residues.
- The first protein to be sequenced by the method is **Insulin by Fredrick Sanger**. He got Nobel Prize in **1958**.
- Only dipeptides or tripeptides can be sequenced.

Edman's Technique

- By using Edman's reagent (Phenyl Isothiocyanate).
- Phenyl isothiocyanate derivatizes the amino terminal of polypeptide.
- Edman's Technique can sequence many residues (5–30 residues) of a single polypeptide sample unlike Sanger's sequencing.

Flowchart 2.2.1: Steps of sequences the proteins



Site of Cleavage of Polypeptide

- **Trypsin:** Carboxyl side of basic amino acids like **Lysine** and **Arginine**.

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- **Chymotrypsin:** Bulky nonpolar amino acids like **Phe, Tyr, Trp, Leu, Met**
- **Cyanogen bromide:** Carboxyl side of **methionine**.

Mass Spectrometers

Today mass spectrometers has emerged the **method of choice of protein identification**.

The principle used to identify protein based on mass (precisely saying on **mass/charge ratio**).

The molecular mass of each amino acid is unique, the sequence of the peptide can be reconstructed from the masses of its fragments.

The exceptions are molecular mass of (1) leucine and isoleucine and (2) glutamine and lysine.

Methods for Dispersion of Analyte into Vapor Phase

Analytes has to be converted to vapour phase by using various technique.

- Heating in a vacuum, but proteins and oligonucleotides are destroyed by heat.
- Electrospray Ionization
- Matrix assisted laser desorption and ionization (MALDI)[Q]
- Fast atom bombardment (FAB).

Types of Mass Spectrometers

Mass Spectrometers come in various configurations

1. Simple quadrupole mass spectrometers
2. Time of flight mass Spectrometers
3. Tandem mass spectrometry

Quadrupole Mass Spectrometers

- Quadrupole mass spectrometers generally are used to determine the masses of molecules of **4000 Da or less**

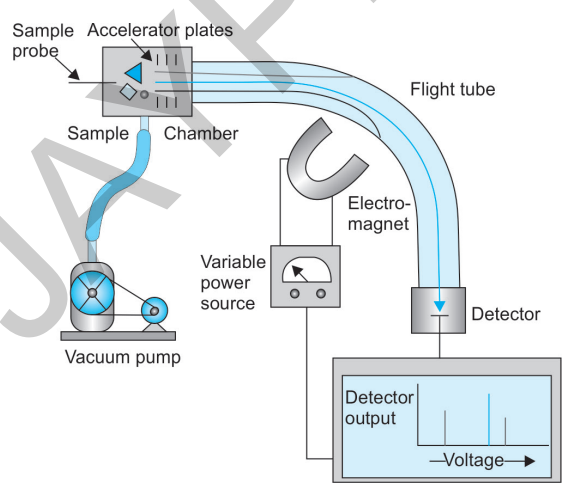

Fig. 2.2.3: Mass spectrometer

Time of Flight (TOF) Mass Spectrometer

Time-of-flight mass spectrometers are used to determine the large masses of complete proteins **(>4 KDa)**

Tandem Mass Spectrometry

Two mass spectrometers linked in series. For this reason, such tandem instruments are often referred to as MS–MS.

Advantage of Mass Spectrometers

- Method of choice Protein determination.
- Superior Sensitivity, speed and versatility.
- Detect **Post-translational modification** unlike Edman's reaction and DNA derived Protein sequence.
- Can be used for other biomolecules like oligonucleotide, carbohydrates as mass and charge are common properties of all this.

STUDY OF SECONDARY AND TERTIARY STRUCTURES

Study of Secondary Structure

- Circular dichroism
- Optical Rotatory dispersion chromatography
- Study of tertiary structures
- X-ray crystallography
- NMR spectroscopy
- UV light spectroscopy (most rapid method)
- Fluorescence spectroscopy
- Molecular modelling

Quick Review

- Bond of primary structure is peptide bond.
- Bonds of secondary structure is primarily noncovalent bonds like Hydrogen bond, hydrophobic bond, ionic bond and van der Waals forces.
- Proline inserts kinks in alpha helix hence it disrupt the stability of it.
- Glycine is also alpha helix breaker as it confer high flexibility.
- Most common secondary structure is alpha helix.
- Beta turn is an example of secondary structure.
- Beta barrel, Beta meander are super secondary structure.
- NAD(P) binding domain in certain oxidoreductase is called Rossmann fold.
- First protein to be sequenced completely is insulin by Frederick Sanger.
- Sanger's reagent is 1 Fluoro 2,4 DiNtrobenzene, (FDNB)
- Edman's reagent is phenyl isothiocyanate.
- Mass spectrometers identify proteins, oligonucleotides and carbohydrates
- Principle used to identify any compound by mass spectrometry is mass to charge ratio.
- Two mass spectrometers in series is called Tandem mass spectrometry.

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Check List for Revision

- Alpha helix
- Examples of secondary, tertiary and quaternary structures.
- Characteristics of denaturation of proteins.
- Structure of Insulin
- Sanger's and Edman's Technique.
- Bonds that stabilises different levels of organisation.
- Names of techniques to determine the structure of proteins.
- Mass spectrometers

MCQ
Review Questions

1. Amino acid sequence is not found by: *(NBE Pattern Question)*
a. Sanger's reagent
b. Benedict's reagent
c. Trypsin
d. Cyanogen bromide

2. Method used to study the structure of proteins include all, *except*:
a. UV Spectroscopy
b. NMR Spectroscopy
c. X-ray crystallography
d. Edman's technique

3. Sanger's reagent is chemically: *(NBE Pattern Question)*
a. 2, 4 Dinitrobenzene
b. 2, 4 Dinitrocresol
c. 1, Fluoro 2, 4 DinitroBenzene
d. 2, 4 FluorodinitroCresol

4. Which one of the following about protein structure is correct? *(PGI May 2015)*
a. Protein consisting of one polypeptide can have quaternary structure
b. The formation of disulphide bond in a protein requires that the two participating cysteine residues be adjacent to each other in the primary sequence of the protein
c. The stability of quaternary structure in protein is mainly the result of covalent bonds among the subunits.
d. The denaturation of proteins always leads to irreversible loss secondary and tertiary structure.
e. The information required for the correct folding of a protein is contained in the specific sequence of amino acid along the polypeptide chain.

5. An alpha helix of a protein is most likely to be disrupted if a missense mutation introduces the following amino acid within the alpha helical structure: *(AIIMS Nov 2002)*
a. Alanine
b. Aspartic acid
c. Tyrosine
d. Glycine

6. Proteins are linear polymers of amino acids. They fold into compact structures. Sometimes, these folded structures associate to form homo-or-heterodimers. Which one of the following refers to this associated form? *(AI 2006)*
a. Denatured state
b. Molecular aggregation
c. Precipitation
d. Quaternary structure

7. Which among the following is the structure of myoglobin?
a. Monomer
b. Homodimer
c. Heterodimer
d. Tetramer

8. Denaturation is resisted by which of the following bond? *(NBE Pattern Question)*
a. Peptide bond
b. Hydrogen bond
c. Disulphide bond
d. Electrostatic bond

9. Polypeptide formation in amino acid is by: *(NBE Pattern Question)*
a. Primary structure
b. Secondary structure
c. Tertiary structure
d. Quaternary structure

10. Rossman fold associated NADH domain is found in which enzyme? *(NBE Pattern Question)*
a. Pyruvate Dehydrogenase
b. Lactate Dehydrogenase
c. Alpha ketoglutarate Dehydrogenase
d. Isocitrate Dehydrogenase

11. In forming 3D structure following factors help: *(PGI May 2014)*
a. Peptide bond
b. Amino acid sequence
c. Interaction between polypeptide
d. Chaperone
e. Side chain

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Answers to Review Questions

1. b. Benedict's reagent *(Ref: Harper 31/e page 27, 32)*

Methods of protein sequencing Sanger's technique

- Sanger's reagent is 1, Fluoro 2, 4 Dinitrobenzene[Q]
- Sanger's reagent derivatizes the amino terminal residues.
- The first Protein to be sequenced by the methodis **Insulin by Fredrick Sanger. He got Nobel prize in 1958.**
- Only dipeptides or tripeptides can be sequenced.

Edman's Technique

- By using Edman's Reagent (Phenyl Iso-thiocyanate).
- Phenyl Isothiocyanate derivatizes the amino terminal of Polypeptide.

Edman's Technique can sequence many residues (5–30 residues) of a single polypeptide sample unlike Sanger's

2. d. Edman's Technique *(Ref: Harper 29/e Table 1.1)*

- Edman's reaction is used to detect sequence of amino acids in a polypeptide.

3. c. 1, Fluoro 2, 4 DinitroBenzene

- Sanger's reagent is 1 Fluoro 2, 4 Dinitrobenzene
- Edman's reagent is Phenyl Isothiocyanate

4. e. The information required for the correct folding of a protein is contained in the specific sequence of amino acid along the polypeptide chain

- Proteins with more than one polypeptide chain can only have quaternary structure.
- The cysteine residues need not be adjacent for the formation of disulphide bond

The stability of quaternary structure is by non covalent bonds.

- Denaturation can be reversible also.

5. d. Glycine *(Ref: Harper 31/e page 35)*

- Glycine induces bends in the alphahelix.
- Proline also disrupts the conformation of alpha helix.
- But proline is present in the first turn of alpha helix.

6. d. Quaternary structure *(Ref: Harper 31/e page 38)*

7. a. Monomer

- Myoglobin is a monomer
- Hemoglobin is a tetramer

8. a. Peptide bond

Denaturation of proteins

- Primary structure is not lost
- Hence peptide bond is not broken.
- Secondary and tertiary structure is lost.
- There is loss of folding

9. a. Primary structure *(Ref: Harper 31/e page 34)*

Primary structure

It is the linear sequence of amino acid held together by peptide bonds in its peptide chains. Bond involved in primary structure is Peptide Bond, a type of covalent bond.

Secondary Structure

- Configurational relationship between residues which are about 3-4 amino Acids apart in linear sequence.
- The folding of short (3- to 30-residue), contiguous segments of polypeptide into geometrically ordered units

Tertiary structure

The entire three-dimensional conformation of a polypeptide is referred to as tertiary structure.

Quaternary structure

If more than one polypeptides aggregate to form one functional protein, the spatial relationship between the polypeptide subunits is referred to as Quaternary structure.

10. b. Lactate dehydrogenase

Oxidoreductase with Rossmann Fold

- Lactate dehydrogenase
- Alcohol dehydrogenase
- Glyceraldehyde-3-phosphate dehydrogenase
- Malate dehydrogenase
- Quinoneoxidoreductase
- 6-phosphogluconate dehydrogenase
- D-glycerate dehydrogenase

11. b, c, d, e. Amino acid sequence, Interaction between polypeptide, Chaperone, Side chain
(Ref: Harper 31/e page 39, 574; Stryer 7/e page 60)

- Amino acid sequence determines the tertiary structure of proteins
- Side chains help in the formation of bonds involved in tertiary structure of proteins
- Interaction of polypeptide also helps the three dimensional structure of proteins.
- Chaperones helps in protein folding hence the three dimensional structure.
- But Peptide bond helps in the primary structure.

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2.3 BIOCHEMICAL TECHNIQUES

- Separatory Techniques of Proteins
- Precipitation of Proteins
- Electrophoresis
- Methods to Quantitate Proteins
- Chromatography

SEPARATORY TECHNIQUES OF PROTEINS

Different separatory techniques of Proteins

- Salt fractionation
- Ultra Centrifugation
- Electrophoresis
- Chromatography

Salt Fractionation (Salting Out)

Principle

The solubility of proteins is generally lowered at high salt concentrations, an effect called "**salting out**." The addition of a salt in the right amount can selectively precipitate some proteins, while others remain in solution.

Ammonium sulfate ($(NH_4)_2SO_4$) is often used for this purpose because of its high solubility in water.

Ultracentrifugation

Principle

Method to separate the protein based on **the mass, density**, to an extend to the shape also.

Electrophoresis

- **Migration of charged particle in an electric field is called Electrophoresis.**

Concept of Electrophoresis

- Sample is applied usually at the cathode end and the analyte moves towards the anode.
- So negatively charged particles moves faster.
- If a mixture of amino acids are separated then the negatively charged amino acid moves faster.

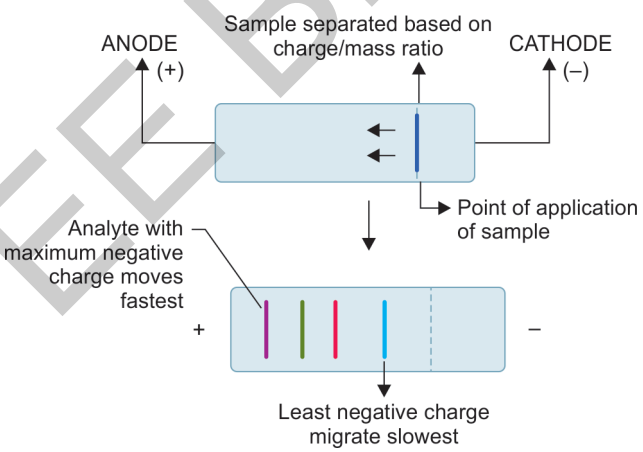

Fig. 2.3.1: Gel electrophoresis

Important Types of Electrophoresis

Type of electrophoresis	Supporting media used	Property used to separate the proteins
Agarose gel Electrophoresis	Agarose gel	Based on charge to mass ratio
Cellulose acetate Electrophoresis	Cellulose acetate membrane	
Poly acrylamide Gel Electrophoresis (PAGE)	Polymer of Acrylamide	Based on charge to mass ratio and molecular weight (Size)
Sodium Dodecyl Sulphate (SDS)-PAGE	Sodium dodecyl Sulphate and Polyacrylamide	Based on molecular weight (Size)
Capillary electrophoresis	Separation done in a capillary tube.	Based on the method of electrophoresis used
Isoelectric focusing	Supporting media with pH gradient	Based on isoelectric pH

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Two-Dimensional Electrophoresis

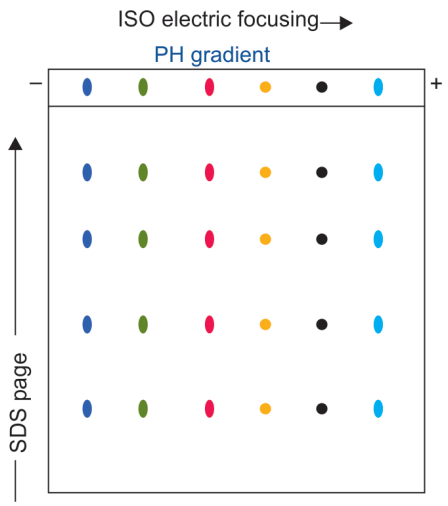


Fig. 2.3.2: Two-dimensional electrophoresis

In one direction SDS-PAGE and in the other direction isoelectric focusing. So separation based on both **molecular weight (Size) and isoelectric pH**.

Densitometry

- Method to quantitate the separated protein.

Compare the Different PAGES

PAGE

- Protein is separated based on molecular mass or molecular weight/size and charge.

SDS-PAGE

- SDS impart equal negative charge so that it masks the inherent charge of the Protein
- Now Proteins separate based on molecular weight (Size) only.

SDS-PAGE in conjunction with 2 Mercaptoethanol or dithiothreitol

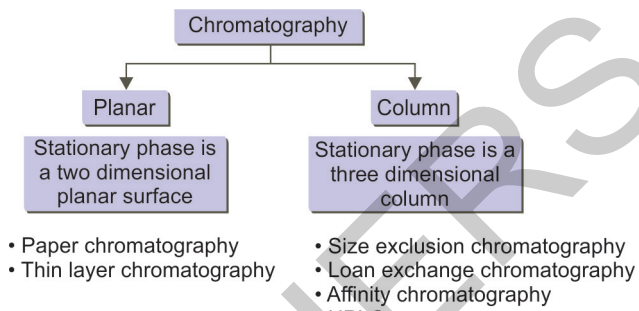
- Oxidatively cleave disulphide bond.
- So separate the components of multimeric proteins.

CHROMATOGRAPHY

The process by which components of a mixture are separated by differential distribution between a mobile phase and stationary phase. This technique invented by Mikhail Tsvet. Chroma = Color Graphein = to write. So Chromatography is color writing.

Classification of Chromatography

Flowchart 2.3.1: Classification of chromatography

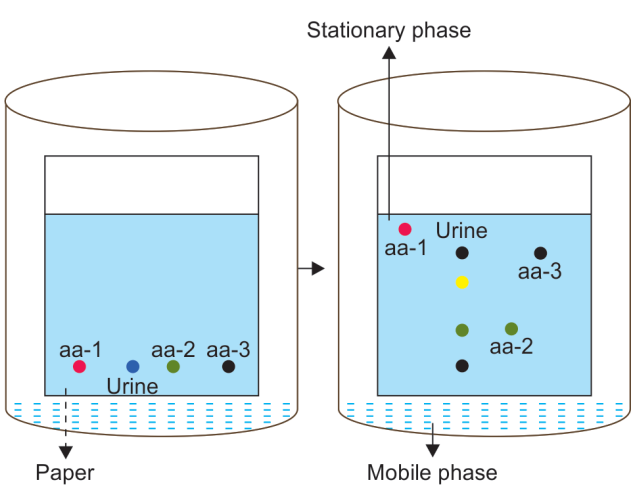


PLANAR CHROMATOGRAPHY

Thin Layer/Paper Chromatography

- Stationary phase is water or polar solvent coated on to paper fibres in paper chromatography.
- Mobile phase is a mixture of nonpolar solvents.
- Sample is applied on stationary phase.
- Sample is distributed on stationary phase by the mobile phase.
- The sample is applied on the support media.
- As the non polar solvent ascend on the stationary phase, the non polar component of the analyte move along the mobile phase, but polar component will remain in the stationary phase.

Paper Chromatography



- Presence of aa-2 and aa-3 inline urine sample
- Most nonpolar amino acid ascend faster than other polar amino acids

Fig. 2.3.3: Paper chromatography

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COLUMN CHROMATOGRAPHY

Size Exclusion Chromatography

- Stationary phase is a column of porous beads.
- Mobile phase is a mixture of analytes with different sizes.
- Smaller particles enter the porous beads and hence it take a labyrinthine path through the column.
- Larger particles cannot enter the pores hence will migrate faster in the column.
- So separation is based on molecular weight or size of the particle.

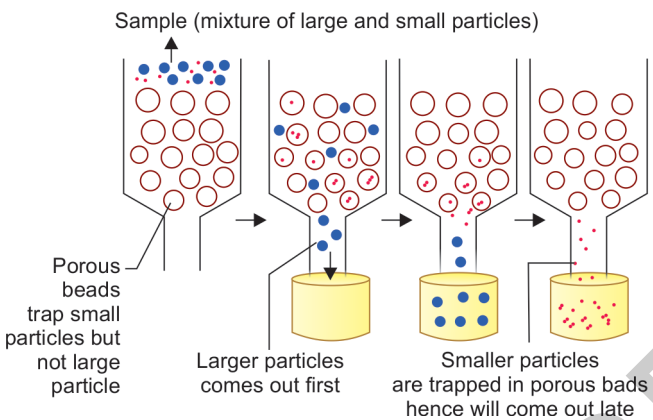

Fig. 2.3.4: Size exclusion chromatography

Affinity Chromatography

The beads in the column have a covalently attached chemical group. A protein with affinity for this particular chemical group will bind to the beads in the column, and its migration will be retarded as a result. So this is based on the biological activity.

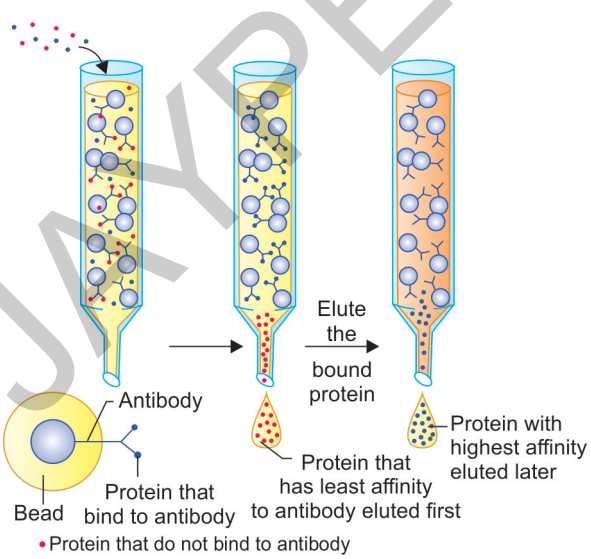

Fig. 2.3.5: Affinity chromatography

Ion Exchange Chromatography

This can be anion exchange or cation exchange chromatography. In cation exchange chromatography, the solid matrix has negatively charged groups. In the mobile phase, proteins with a net positive charge migrate through the matrix more slowly than those with a net negative charge, because the migration of the former is retarded more by interaction with the stationary phase. In anion exchange chromatography vice versa. So separation is based on the **charge of the analyte**.

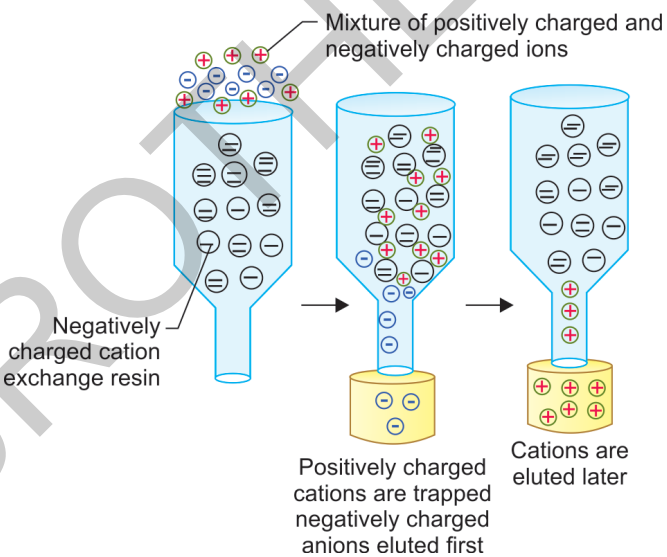

Fig. 2.3.6: Ion exchange chromatography

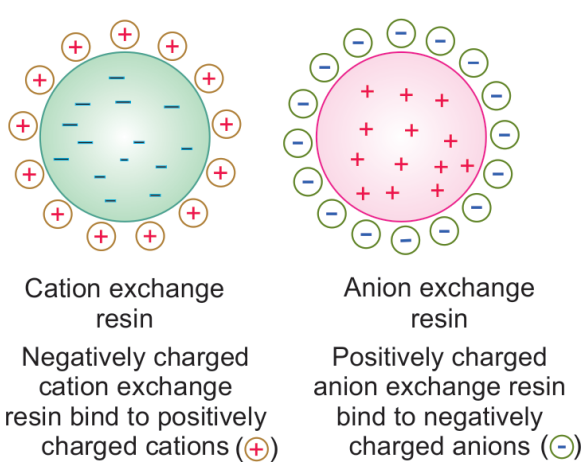

Fig. 2.3.7: Ion exchange resins

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Important Chromatographic Techniques

Chromatography	Stationary phase used	Property used for separation
Paper chromatography	Water held on a solid support of filter paper (or Cellulose)	Based on the polarity Least polar moves faster
Thin layer chromatography	Silica gel (Kieselguhr) spread on a glass plate or a plastic sheet or aluminum sheet.	Based on polarity Least Polar moves faster
Ion exchange chromatography	Column of Ion exchange resins Anion exchange or Cation exchange resins	Based on charge-charge interaction
• Size exclusion chromatography other names • Molecular sieve chromatography gel filtration chromatography • Gel Permeation Chromatography	Column of porous beads	Based on molecular weight (Size) Particles emerge in the descending order of Stokes Radius[Q]
Affinity chromatography[Q]	Column of resins bound to specific ligands used	Based on specific ligand binding behavior or biological activity
Hydrophobic interaction chromatography		Based on hydrophobic interaction
Adsorption chromatography		Adsorption and desorption of solute at a solid particle

High Pressure Liquid Chromatography (HPLC)

- Versatile technique among the Column methods of Chromatography

Concept Box

Concept of HPLC
- A modern refinement in chromatographic methods is **HPLC, or high-performance liquid chromatography**.
- Mobile phase solvent with pumped at a high pressure to the column.
- In the column any chromatographic methods like affinity column method or ion exchange resins can be used
- Using high pressure the transit time in the column is reduced hence less diffusional spreading of protein bands, so it greatly improve the resolution.

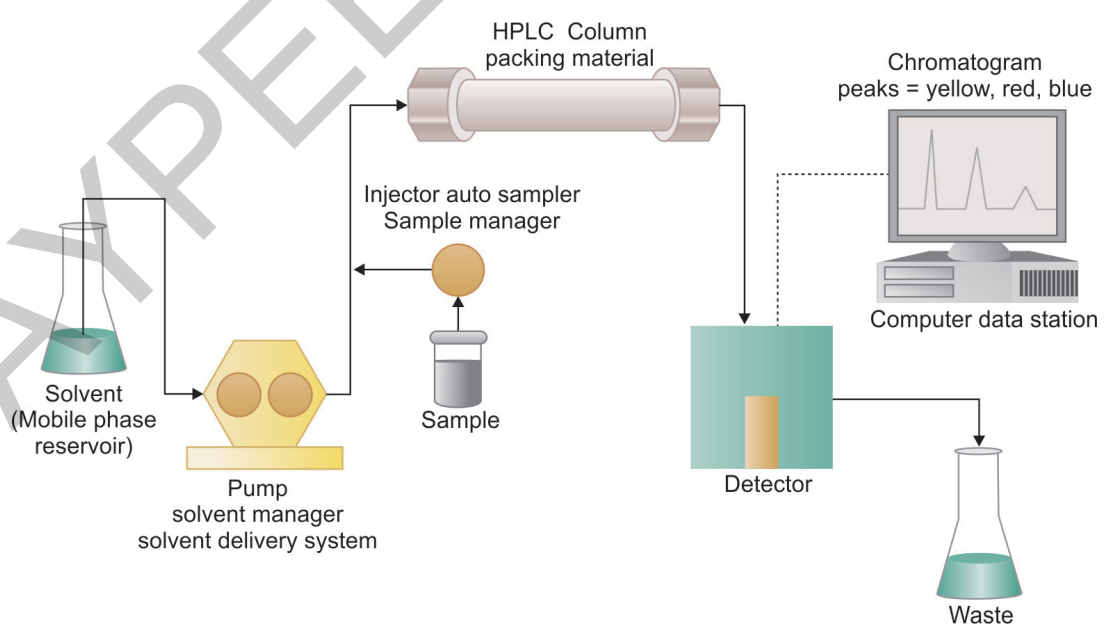

Fig. 2.3.8: Parts of HPLC

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Advantages

- Separation can be based on different property based on the column (affinity chromatography, ion exchange columns, Size exclusion, etc.) used.
- Better resolution and Less Transit Time
- Reproducible results
- Hence high performance (So also called high performance liquid chromatography)
- For separating hemoglobin fraction HPLC^{9} is used.
- Method of separation of proteins based on the differential distribution of analyte between stationary and mobile phase.

PRECIPITATION REACTIONS OF PROTEINS

Polar groups of proteins tend to attract water molecules around them to produce a shell of hydration. This makes the protein soluble in water. Any factor that neutralize the charge of protein or remove the shell of hydration will cause precipitation of proteins.

Methods to precipitate the protein by neutralizing the charge are:

- Precipitation by heavy metallic salt.
- Precipitation by acids.

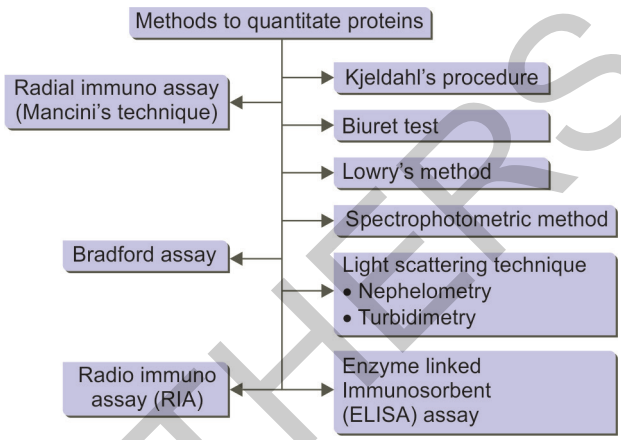
Methods to precipitate protein by removing the shell of hydration:

- Precipitation by neutral salts
- Precipitation by organic solvents

- Precipitation by heavy metallic salt
  - Heavy metals like Hg, Zn, Pb, etc. provide positively charged ions neutralize the negative charge of the protein.
- Precipitation by acids
  - Acids bring the pH of the medium to isoelectric pH, precipitability is maximum at isoelectric pH. This is because at isoelectric pH proteins carry no net charge, hence no shell of hydration.
  - The reagents used are **Phosphotungstic acid, Sulphosalicylic acid, Phosphomolybdic acid, Trichloroacetic acid**.
- Precipitation by neutral salts
  - Concentrated salt solution removes the shell of hydration.
  - Reagents used are **Ammonium sulphate**. This is called Salting out.
- Precipitation by organic solvents
  - Organic solvents reduce the dielectric constant of the water and decreases the water available for protein, hence it is precipitated.
  - The reagent used are ether, alcohol, acetone, etc.

Methods to Quantitate Proteins

Flowchart 2.3.2: Methods to quantitate proteins



Quick Review

- Most specific Chromatographic technique—affinity chromatography
- Most rapid method of separation of proteins is—capillary Electrophoresis
- Method to quantitate the separated protein is densitometry.
- Hydrophobic amino acid moves fastest along the stationary phase of thin layer chromatography or paper chromatography.
- The amino acid which moves fastest in a Thin layer Chromatography/Paper Chromatography—Isoleucine
- SDS-PAGE used in conjunction 2 Mercaptoethanol or dithiothreitol break component polypeptides of multimeric proteins.
- In electrophoresis, negatively charged amino acids and proteins move faster.
- In paper and thin layer chromatography, nonpolar amino acids move faster.
- Methods of separation in various separatory techniques
  - Electrophoresis: Charge to mass ratio
  - Isoelectric focusing: Isoelectric point
  - PAGE: Charge and Molecular weight/size
  - SDS-PAGE: Molecular weight/size
  - 2D Electrophoresis: Isoelectric point and molecular weight/Size
  - Size exclusion chromatography-molecular weight/size
  - Ion exchange chromatography-Charge
  - Affinity Chromatography: Ligand binding behavior

Check List for Revision

- Concept of electrophoresis
- Concept of different chromatographic techniques.
- Methods of separation in all chromatographic techniques.
- Basis of precipitation techniques.
- Names of methods to quantitate proteins

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