BIYANI GIRLS COLLEGE
First Internal Examination (2019-20)
M.Sc. Previous Year (BT)
Paper-Biological Macromolecules, Enzymology & Biotechnique

SET-A

Time: 1:30 Hours **MM-30**

Attempt all the questions

1. **Short Answers Questions (1 Marks Each; 1x8= 8 Marks)**

   i. What is optical isomerism?

Ans. Optical isomers are two compounds which contain the same number and kinds of atoms, and bonds (i.e., the connectivity between atoms is the same), and different spatial arrangements of the atoms, but which have non-superimposable mirror images. Each non-superimposable mirror image structure is called an enantiomer.

   ii. Define α helical structure of proteins?

Ans. The alpha helix (α-helix) is a common motif in the secondary structure of proteins and is a right hand-helix conformation in which every backbone N−H group hydrogen bonds to the backbone C=O group of the amino acid located three or four residues earlier along the protein sequence.

   iii. Give any 2 examples of sulphur containing amino acids?

Ans. The two amino acids with sulfur atoms in their side chains: cysteine and methionine.

   iv. What is hydrophilic-hydrophobic interactions?

Ans. Hydrophobic ("water hating") interactions are created because of the uncharged nature of the involved chemical groups. An example of such a chemical group is CH_3. All the bonds around the carbon atom are occupied.

Hydrophilic ("water loving) interactions are possible with polar chemical group. Water is polar because oxygen is far more electronegative than hydrogen and thus the electrons involved in an oxygen-hydrogen bond spend more time in proximity to the oxygen atom.

v. What are globular proteins, give 2 examples?

Ans. Globular proteins are spherical in shape and consist of long chains with numerous branches and offshoots which make them great as transport proteins. Examples of fibrous proteins are collagen, elastin, keratin, silk, etc. Examples of globular protein are myoglobin, hemoglobin, casein, insulin, etc

vi. Define pI?

Ans. The isoelectric point (pI, pH(I), IEP), is the pH at which a molecule carries no net electrical charge or is electrically neutral in the statistical mean. The standard nomenclature to represent the isoelectric point is pH(I).

vii. What is the effect of pH on protein solubility in water?

Ans. For all tested salts, the protein solubility increased when the pH increased. Maximum protein solubility was observed at pH8.0 because in this condition, the protein's positive and negative net charged molecules interact more with water. Protein solubility is lower in acidic pH than in alkaline pH.

viii. Define GS-GOGAT System?

Ans. Glutamine oxoglutarate aminotransferase (also known as Glutamate synthase) is an enzyme and frequently abbreviated as GOGAT. This enzyme manufactures glutamate from glutamine and α-ketoglutarate, and thus along with glutamine synthetase (abbreviated GS) plays a central role in the regulation of nitrogen assimilation in photosynthetic eukaryotes and prokaryotes.

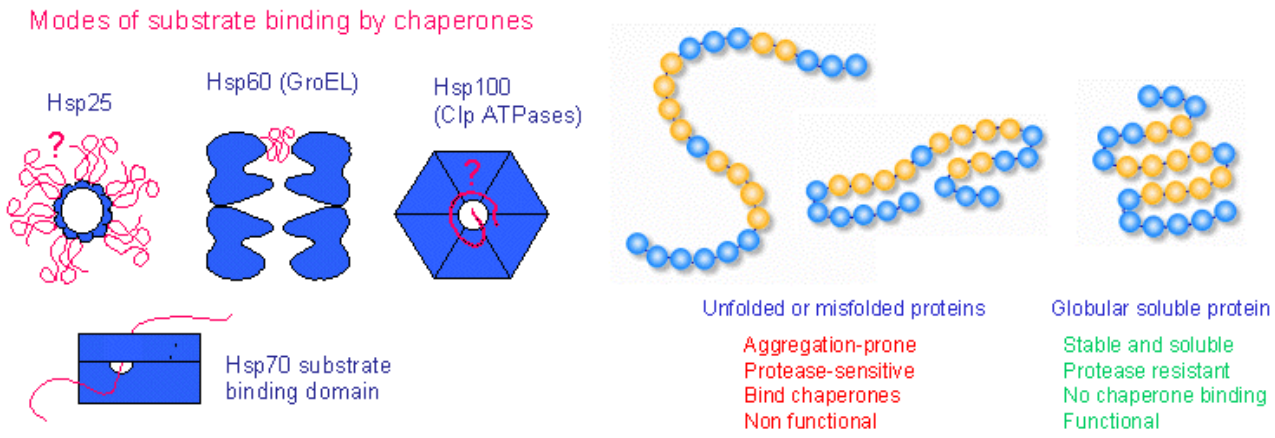
2. Medium Answer Questions (4 Marks each 2x4= 8 Marks)

i. What are molecular chaperones?

Ans. A large group of unrelated protein families whose role is to stabilize unfolded proteins, unfold them for translocation across membranes or for degradation, and/ or to assist in their correct folding and assembly. Molecular chaperones interact with unfolded or partially folded

protein subunits, e.g. nascent chains emerging from the ribosome, or extended chains being translocated across subcellular membranes. They stabilize non-native conformation and facilitate correct folding of protein subunits. They do not interact with native proteins, nor do they form part of the final folded structures. Some chaperones are non-specific, and interact with a wide variety of polypeptide chains, but others are restricted to specific targets. They often couple ATP binding/hydrolysis to the folding process. Essential for viability, their expression is often increased by cellular stress.

Main role: They prevent inappropriate association or aggregation of exposed hydrophobic surfaces and direct their substrates into productive folding, transport or degradation pathways.



ii. Describe about enantiomers of amino acids?

Ans. Amino acids (except for glycine) have a chiral carbon atom adjacent to the carboxyl group (CO_2^-). This chiral center allows for stereoisomerism. The amino acids form two stereoisomers that are mirror images of each other. The structures are not superimposable on each other, much like your left and right hands. These mirror images are termed enantiomers.

D/L and R/S Naming Conventions for Amino Acid Chirality

There are two important nomenclature systems for enantiomers. The D/L system is based on optical activity and refers to the Latin words *dexter* for right and *laevus* for left, reflecting left- and right-handedness of the chemical structures. An amino acid with the dexter configuration (dextrorotary) would be named with a (+) or D prefix, such as (+)-serine or D-

serine. An amino acid having the laevus configuration (levorotary) would be prefaced with a (-) or L, such as (-)-serine or L-serine.

Here are the steps to determine whether an amino acid is the D or L enantiomer:

1. Draw the molecule as a Fischer projection with the carboxylic acid group on top and side chain on the bottom. (The amine group will not be at the top or bottom.)
2. If the amine group is located on the right side of the carbon chain, the compound is D. If the amine group is on the left side, the molecule is L.
3. If you wish to draw the enantiomer of a given amino acid, simply draw its mirror image.

The R/S notation is similar, where R stands for Latin *rectus* (right, proper, or straight) and S stands for Latin *sinister* (left). R/S naming follows the Cahn-Ingold-Prelog rules:

1. Locate the chiral or stereogenic center.
2. Assign priority to each group based on the atomic number of the atom attached to the center, where 1 = high and 4 = low.
3. Determine the direction of priority for the other three groups, in order of high to low priority (1 to 3).
4. If the order is clockwise, then the center is R. If the order is counterclockwise, then the center is S.

3. Long Answer Questions (7 Marks each 2x7= 14 Marks)

i. Explain the working principal and instrumentation of SDS-PAGE?

Ans. SDS-PAGE (sodium dodecyl sulfate – polyacrylamide gel electrophoresis) is a technique used to separate the proteins according to their masses. Separation of macromolecules under the influence of the charge is called electrophoresis. The gel used in SDA-PAGE is polyacrylamide and agent which is used to linearize the proteins is SDS. Hence the name SDS-PAGE.

Principle of SDS-PAGE:

Protein samples and ladder are loaded into wells in the gel and electric voltage is applied. A reducing agent such as mercaptoethanol or dithiothreitol (DTT) (in the presence of a detergent i.e. SDS) breaks down the disulfide bridges that are responsible for protein folding; and a detergent such as SDS imparts negative charge to the proteins thereby linearizing them into polypeptides. Polyacrylamide provides a matrix for the polypeptides to run. Polypeptides run towards the positive electrode (anode) through the gel when electric field is applied. Electrophoretic mobility of the proteins depends upon 3 factors:

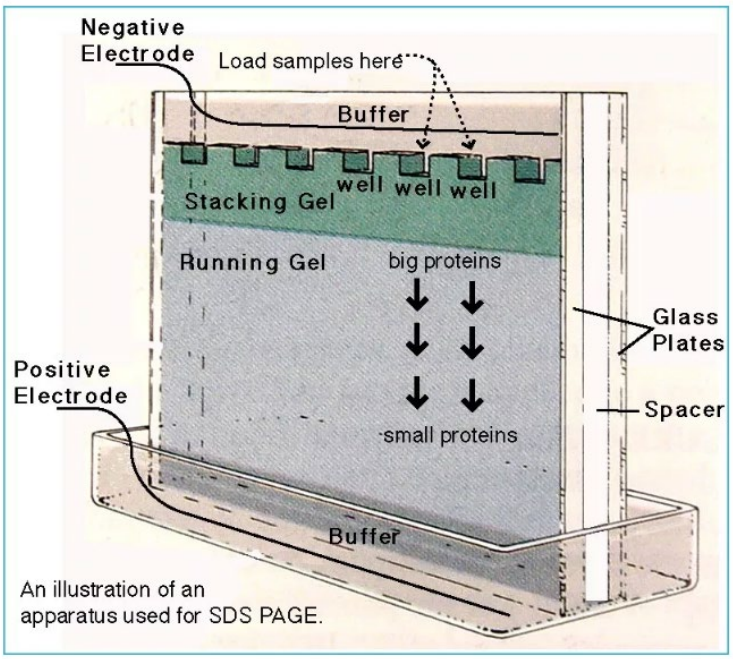
- Shape – All the proteins are in the primary structure after the treatment with a reducing agent. So, shape doesn't affect the protein separation.
- Charge – All the proteins are negatively charge proportional to their molecular weight after treatment with SDS. So charge doesn't affect the separation.
- Size– proteins get separated solely on the basis of their molecular weight.

Smaller polypeptides move faster because they have to face less hindrance, larger ones move slower because of greater hindrance. Hence proteins get separated ONLY on the basis of their mass.

Major steps of SDS-PAGE

Pouring of the resolving gel:

Resolving gel is poured between two glass plates (one is called short plate and the other one is tall plate), clipped together on a **casting frame**. Bubbles are removed by adding a layer of isopropanol on the top of the gel. (The level of the gel is predetermined by placing the comb on the glass-plates and leaving approximates 1cm space below the comb. Use a pen to mark the level. Now pour the gel up to this mark.) The gel is then allowed to solidify. When the gel is solidified, remove the isopropanol by using a filter paper.



An illustration of an apparatus used for SDS PAGE.

Pouring of the stacking gel:

When the resolving gel is solidified, stacking gel is loaded all the way to the top of the glass plates. Comb is placed just after loading. The gel is, then, allowed to polymerize (solidify). When stacking gel is solidified, comb is removed very carefully not damaging the well's shape.

Loading the ladder in wells

Add the ladder very carefully into the well which is on the extreme right using a micropipette. The samples are loaded into the other wells. Ladder is mostly pre-stained with the known molecular weight proteins.

Loading the samples in wells

Samples are loaded in each well with equal amount of the proteins mixture using micropipette. Be careful while loading the samples. Make sure not to damage the size of the wells or not to pour the sample out of the well instead of pouring inside it. At this stage, sample of the proteins appears to be blue because of a dye (bromophenol) used while preparing the sample.

Running the gel by applying voltage

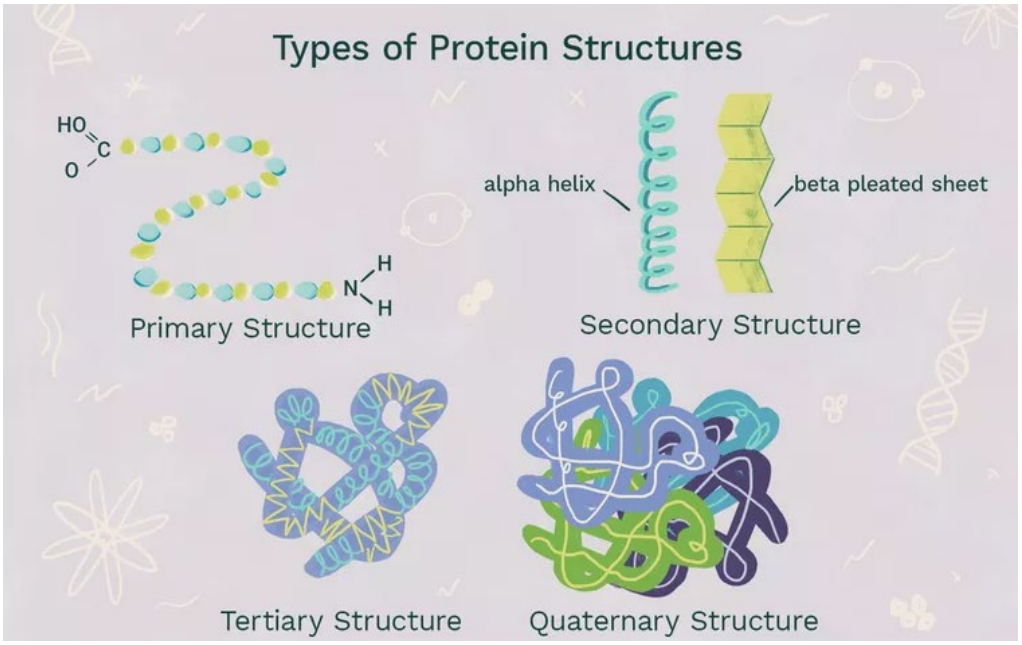
A voltage is applied after dipping the "sandwich of gel and glass plates" in running buffer. Turn of the voltage when the tracking dye has reached or crossed the gel. The gel is further proceeded for the subsequent analysis.

Subsequent analysis – Coomassie Blue Staining

The gel is rinsed with deionized water 3-5 times to remove SDS and buffer. It may create hindrance with the binding of the dye (0.1% Coomassie Blue) to the proteins. The gel is then dipped in Coomassie Blue stain (staining buffer) on a shaking incubator at room temperature. The invisible bands of the proteins beginning to appear within minutes but it takes approximately 1h for complete staining.

ii. Explain about different protein structures?

Ans.



Four Protein Structure Types

The four levels of protein structure are distinguished from one another by the degree of complexity in the polypeptide chain. A single protein molecule may contain one or more of the protein structure types: primary, secondary, tertiary, and quaternary structure.

1. Primary Structure

Primary Structure describes the unique order in which amino acids are linked together to form a protein. Proteins are constructed from a set of 20 amino acids. Generally, amino acids have the following structural properties:

- **A carbon (the alpha carbon) bonded to the four groups below:**
- **A hydrogen atom (H)**

- **A Carboxyl group (-COOH)**
- **An Amino group (-NH2)**
- **A "variable" group or "R" group**

All amino acids have the alpha carbon bonded to a hydrogen atom, carboxyl group, and an amino group. The **"R" group** varies among amino acids and determines the differences between these protein monomers. The amino acid sequence of a protein is determined by the information found in the cellular genetic code. The order of amino acids in a polypeptide chain is unique and specific to a particular protein. Altering a single amino acid causes a gene mutation, which most often results in a non-functioning protein.

2. Secondary Structure

Secondary Structure refers to the coiling or folding of a polypeptide chain that gives the protein its 3-D shape. There are two types of secondary structures observed in proteins. One type is the **alpha (α) helix** structure. This structure resembles a coiled spring and is secured by hydrogen bonding in the polypeptide chain. The second type of secondary structure in proteins is the **beta (β) pleated sheet**. This structure appears to be folded or pleated and is held together by hydrogen bonding between polypeptide units of the folded chain that lie adjacent to one another.

3. Tertiary Structure

Tertiary Structure refers to the comprehensive 3-D structure of the polypeptide chain of a protein. There are several types of bonds and forces that hold a protein in its tertiary structure.

- **Hydrophobic interactions** greatly contribute to the folding and shaping of a protein. The "R" group of the amino acid is either hydrophobic or hydrophilic. The amino acids with hydrophilic "R" groups will seek contact with their aqueous environment, while amino acids with hydrophobic "R" groups will seek to avoid water and position themselves towards the center of the protein.
- **Hydrogen bonding** in the polypeptide chain and between amino acid "R" groups helps to stabilize protein structure by holding the protein in the shape established by the hydrophobic interactions.

- Due to protein folding, **ionic bonding** can occur between the positively and negatively charged "R" groups that come in close contact with one another.
- Folding can also result in covalent bonding between the "R" groups of cysteine amino acids. This type of bonding forms what is called a **disulfide bridge**. Interactions called van der Waals forces also assist in the stabilization of protein structure. These interactions pertain to the attractive and repulsive forces that occur between molecules that become polarized. These forces contribute to the bonding that occurs between molecules.

4. Quaternary Structure

Quaternary Structure refers to the structure of a protein macromolecule formed by interactions between multiple polypeptide chains. Each polypeptide chain is referred to as a subunit. Proteins with quaternary structure may consist of more than one of the same type of protein subunit. They may also be composed of different subunits. Hemoglobin is an example of a protein with quaternary structure. Hemoglobin, found in the blood, is an iron-containing protein that binds oxygen molecules. It contains four subunits: two alpha subunits and two beta subunits.

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1. **Short Answers Questions (1 Marks Each; 1x8= 8 Marks)**

i. What is Zwitter ion?

Ans. Zwitterions (the word is derived from the German for "hybrid **ion**") are **ions** that are electrically neutral overall but contain nonadjacent regions of positive and negative charges; they are sometimes referred to as "dipolar **ions**." The best-known **examples** of zwitterions are the free amino acids found in cells.

ii. How amino acids work as natural buffer?

Ans. An amino acid can act as a buffer because it can react with added acids and bases to keep the pH nearly constant. In very acidic media, the NH_2 group is in the protonated form, and in very basic media, the COOH group is in the deprotonated form.

iii. What is optical activity?

Ans. Optical activity is the ability of a chiral molecule to rotate the plane of plane-polairsed light, measured using a polarimeter. A simple polarimeter consists of a light source, polarising lens, sample tube and analysing lens.

iv. What are polar amino acids, give any 2 examples with structure?

Ans. The polar group consist of 10 amino acids, two are negatively charged - aspartic acid and glutamic acid, 3 have a positive charge - arginine, lysine and histidine, and 5 are uncharged - asparagine, glutamine, serine, threonine and tyrosine.

v. What is isoelectric point?

Ans. The isoelectric point, is the pH at which a molecule carries no net electrical charge or is electrically neutral in the statistical mean.

vi. Define term SDS-PAGE?

Ans. SDS-PAGE is an electrophoresis method that allows protein separation by mass. The medium (also referred to as ′matrix′) is a polyacrylamide-based discontinuous gel. In addition, SDS (sodium dodecyl sulfate) is used. About 1.4 grams of SDS bind to a gram of protein, corresponding to one SDS molecule per two amino acids.

vii. What do you understand by tertiary structure of proteins?

Ans. Secondary structure hierarchically gives way to tertiary structure formation. Once the protein's tertiary structure is formed and stabilized by the hydrophobic interactions, there may also be covalent bonding in the form of disulfide bridges formed between two cysteine residues. Tertiary structure of a protein involves a single polypeptide chain; however, additional interactions of folded polypeptide chains give rise to quaternary structure formation.

viii. What is the effect of β-mercaptoethanol on protein structure?

Ans. 2-mercaptoethanol is used to reduce these disulfide bonds and irreversibly denature the proteins. This prevents them from digesting the RNA during its extraction procedure.

2. Medium Answer Questions (4 Marks each 2x4= 8 Marks)

i. What is secondary structure of proteins?

Ans. Formation of a secondary structure is the first step in the folding process that a protein takes to assume its native structure. Characteristic of secondary structure are the structures known as alpha helices and beta sheets that fold rapidly because they are stabilized by intramolecular hydrogen bonds, as was first characterized by Linus Pauling. Formation of intramolecular hydrogen bonds provides another important contribution to protein stability. α-helices are formed by hydrogen bonding of the backbone to form a spiral shape. The β pleated sheet is a structure that forms with the backbone bending over itself to form the hydrogen bonds. The hydrogen bonds are between the amide hydrogen and carbonyl oxygen of the peptide bond. There exists anti-parallel β pleated sheets and parallel β pleated sheets where the stability of the hydrogen bonds is stronger in the anti-parallel β sheet as it hydrogen bonds with the ideal 180 degree angle compared to the slanted hydrogen bonds formed by parallel sheets.

ii. What is protein folding, explain?

Ans. Protein folding is the physical process by which a protein chain acquires its native 3-dimensional structure, a conformation that is usually biologically functional, in an expeditious and reproducible manner. It is the physical process by which a polypeptide folds into its characteristic and functional three-dimensional structure from a random coil. Each protein exists as an unfolded polypeptide or random coil when translated from a sequence of mRNA to a linear chain of amino acids. This polypeptide lacks any stable (long-lasting) three-dimensional structure. As the polypeptide chain is being synthesized by a ribosome, the linear chain begins to fold into its three-dimensional structure. Folding begins to occur even during translation of the polypeptide chain. Amino acids interact with each other to produce a well-defined three-dimensional structure, the folded protein (the right hand side of the figure), known as the native state. The resulting three-dimensional structure is determined by the amino acid sequence or primary structure While these macromolecules may be regarded as "folding themselves", the process also depends on the solvent (water or lipid bilayer), the concentration of salts, the pH, the temperature, the possible presence of cofactors and of molecular chaperones. Proteins will have limitations on their folding abilities by the restricted bending angles or conformations that are possible. These allowable angles of protein folding are described with a two-dimensional plot known as the Ramachandran plot, depicted with psi and phi angles of allowable rotation.

3. **Long Answer Questions (7 Marks each 2x7= 14 Marks)**
   i. Explain the working principal and instrumentation of Diagonal electrophoresis?

Ans. Diagonal gel electrophoresis is a form of two-dimensional analysis useful for investigating the subunit composition of multisubunit proteins containing interchain disulfide bonds. Proteins are electrophoresed in the first dimension in a slab or tube gel under nonreducing conditions. The proteins are then reduced in the gel and this piece of gel is layered onto a second gel and electrophoresed. In the second gel, the proteins migrate at right angles to their original, first-dimension migration. The majority of cellular proteins are not disulfide-linked and will fall on the "diagonal" in this system; that is, they migrate approximately equal distances in both directions during electrophoresis and lie approximately on the diagonal line connecting opposite corners of the gel. Upon reduction, component subunits of proteins connected by interchain disulfide bonds will resolve below the diagonal because the individual subunits migrate faster than the disulfide-linked complex during the second electrophoresis.

A state of oxidative stress (OS) can occur when there is an imbalance between the rate of reactive oxygen species (ROS) production and their detoxification. Under OS conditions sulphur-containing residues are particularly susceptible to oxidation, and this can result in transient formation of intra- or inter-chain disulphide bridges. Diagonal electrophoresis is a relatively simple technique to analyse the formation of these bridges by sequential non-reducing/reducing electrophoresis. Proteins that do not form disulphides, electrophorese identically in both dimensions and form a diagonal after the second dimension, proteins that contained intra-chain disulphides lie above this diagonal, while those that formed inter-disulphides fall below the diagonal. These spots can be excised, tryptic digested, and identified by mass spectrometry. Identification of those proteins, which are reversibly modified, could play an important part in coupling redox status to protein function.

A method for identification of a particular kind of peptide in a mixture by identical electrophoretic steps, the second at a 908 angle to the first, with a chemical modification introduced between the steps. An example is the identification of tyrosine-bearing peptides in a mixture of peptides by treatment of the products of paper electrophoresis with iodine vapour to iodinate the tyrosine residues before the second electrophoresis Iodotyrosine peptides are then identified as those that deviate from the diagonal formed by all the other peptides when they are visualized by, for instance, the ninhydrin reaction.

ii. Explain about different steps involved in amino acid biosynthesis?

Ans. Synthesis of Amino Acids

Synthesis and/or collection of amino acids is critical for cell survival. They not only serve as the building blocks for proteins but also as starting points for the synthesis of many important cellular molecules including vitamins and nucleotides.

In most cases bacteria would rather use amino acids in their environment than make them from scratch. It takes a considerable amount of energy to make the enzymes for the pathway as well as the energy required to drive some of the reactions of amino acid biosynthesis. The genes that code for amino acid synthesis enzymes and the enzymes themselves are under tight control and are only turned on when they are needed.

The amino acids synthesis pathways can be grouped into several logical units. These units reflect either common mechanisms or the use of common enzymes that synthesize more than one amino acid. These categories are: simple reactions, branch chain amino acids, aromatic amino acids, threonine/lysine, serine/glycine, and unique pathways. The aromatic amino acids, threonine/lysine and serine/glycine pathways have a common beginning and then diverge to form the amino acid of interest.

Notice that each pathway begins with a central metabolite or something derived from "central metabolism". Using common compounds instead of synthesizing them from scratch saves energy and conserves genes since fewer enzymes are needed to code for the pathways.

A useful way to organize the amino acid biosynthetic pathways is to group them into families corresponding to the metabolic precursor of each amino acid (Table 21-1). This approach is used in the detailed descriptions of these pathways presented below.

Table 21–1 Amino acid biosynthetic families, grouped by metabolic precursor

α-Ketoglutarate	*Oxaloacetate*	*Phosphoenolpyruvate and erythrose-4-phosphate*
Glutamate	Aspartate	Tryptophan*
Glutamine	Asparagine	Phenylalanine*
Proline	Methionine*	Tyrosine
Arginine†	Threonine*	
	Lysine*	*Ribose-5-phosphate*
3-Phosphoglycerate	Isoleucine*	Histidine*
Serine		
Glycine	*Pyruvate*	
Cysteine	Alanine	
	Valine*	
	Leucine*	

* Essential amino acids.
† Essential in young animals.

In addition to these precursors, there is a notable intermediate that recurs in several pathways: **phosphoribosyl pyrophosphate** (PRPP). PRPP is synthesized from ribose-5-phosphate derived from the pentose phosphate pathway (see Fig. 14-22), in a reaction catalyzed by **ribose phosphate pyrophosphokinase**:

Ribose-5-phosphate + ATP $\longleftrightarrow$ 5-phosphoribosyl-1-pyrophosphate + AMP

Ribose phosphate pyrophosphokinase is allosterically regulated by many of the biomolecules for which PRPP is a precursor. PRPP is an intermediate in tryptophan and histidine biosynthesis, with the ribose ring contributing several of its carbons to the final structure of these amino acids. It is also of fundamental importance in the biosynthesis of nucleotides.

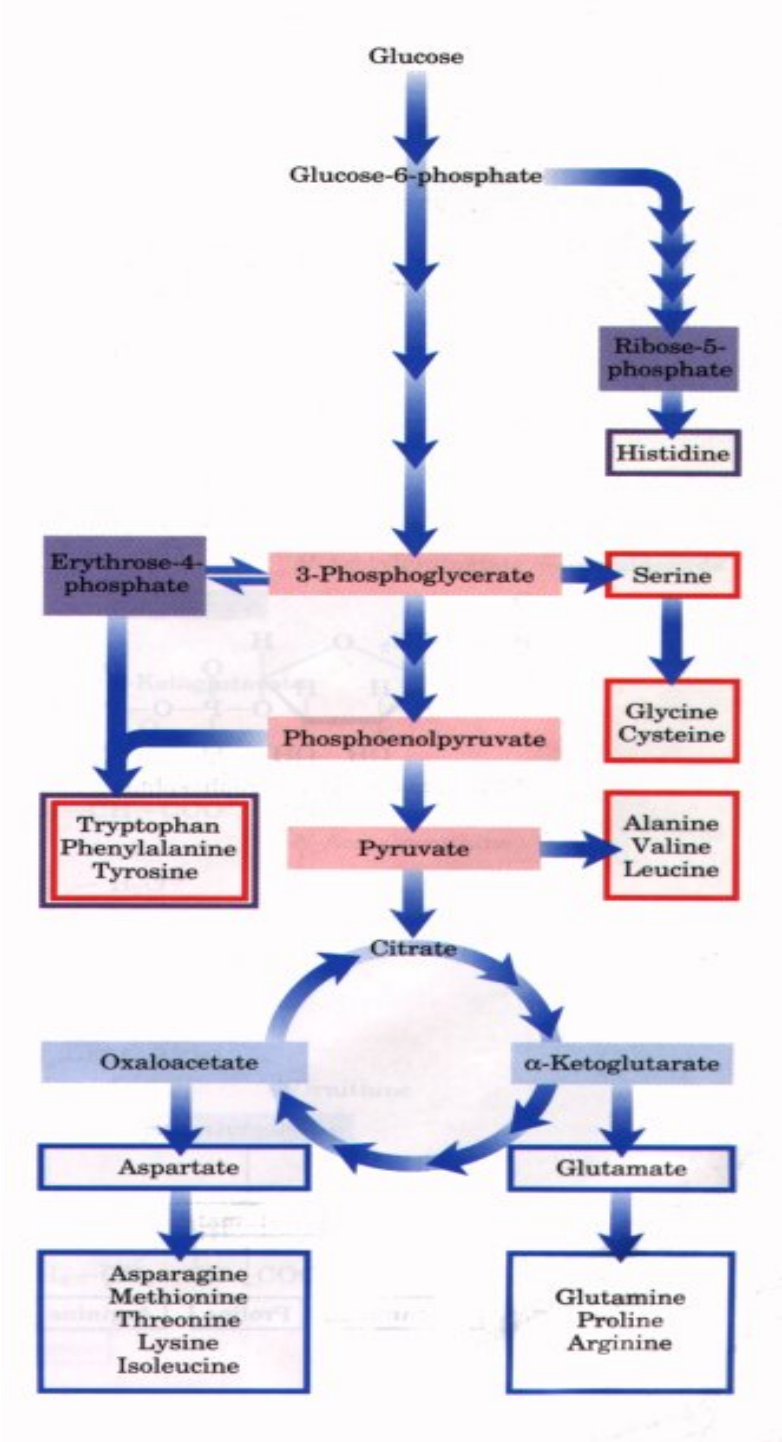

Figure - Overview of amino acid biosynthesis. Precursors from glycolysis (red), the citric acid cycle (blue), and the pentose phosphate pathway (purple) are shaded, and the amino acids derived from them are boxed in the corresponding colors. [The same device-color-matching precursors with pathway end products-will be used in illustrations of the individual pathways