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# General Techniques for Biomolecular Characterization

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**Abstract:** Nucleic acids and proteins are biomolecules which are made up of chains of building blocks called nucleotides and amino acids, respectively. The synthesis of proteins is itself catalyzed by proteins and enzymes, however this process is directed by genetic material in the cell, DNA. Two types of nucleic acids occur in cells: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Both these biomolecules, nucleic acids and proteins play very important and versatile roles in living systems. DNA acts as a carrier of genetic information whereas proteins which are translated from mRNA perform various functions in the biological processes. They are the important constituent of cell membrane, function as enzymes, antibodies and form different tissues in the body. Primary structure of a biomolecule is the linear arrangement in which monomers are joined together. Secondary and tertiary structure of these biomolecules are stabilized by a number of factors like hydrogen bonding, stacking interactions, ionic interactions, vander waal interactions etc. A detailed information about the formation of these structures, DNA/protein-ligand interaction, type of changes occurring during the enzymatic reactions can be studied by various biochemical and biophysical techniques. This review covers the basic principle of techniques like Gel-Electrophoresis, UV-Thermal Denaturation and Circular dichroism (CD) employed for the characterization of nucleic acids and proteins. Gel-Electrophoresis is an analytical tool, used by biochemists for the separation, purification and for information on structural status of biomolecules. UV-Thermal Denaturation and Circular dichroism (CD) are useful in structural studies of nucleic acids, proteins and nucleoproteins. UV-thermal denaturation is used for determining the stability of biomolecules. CD spectra of nucleic acids and proteins arise primarily from the spatial symmetry of the nucleotides and amino acids respectively, in the backbones of the macromolecules.

**Keywords:** Biomolecules, Gel-Electrophoresis, UV-Thermal Denaturation, Circular Dichroism,

## 1. Introduction

Nucleic acids and proteins are the most significant biomolecules present in the living system. They perform various essential functions in all biological processes. The term "Protein" was first proposed by J. J. Berzelius in 1838 and derived from the Greek word "proteios" meaning the first place. James Watson and Francis Crick discovered the double helical structure of DNA in 1953, using the X-ray diffraction pictures taken by Rosalind Franklin [1]. Deoxyribonucleic acid (DNA), a complex molecule is present in its supercoiled form in the nucleus of the eukaryotic organisms. This inscrutable biomolecule serves two central roles. First, it is responsible for the maintaining the genetic information and passing this information to each new cell through the process of replication. Second, it contains the genetic code which is transcribed into RNA (ribonucleic acid) which is then translated into proteins. According to the "central dogma of molecular biology" (Figure 1), a concept given by Francis Crick in 1957, the information encoded in the double stranded DNA is first transferred to single-stranded messenger RNA (mRNA) via the transcription process. After the process of splicing, mRNA moves to the cytoplasm where it is translated into proteins on the ribosome with the aid of tRNA (transfer RNA). The structure of DNA strongly demonstrates a central role common to all biological macromolecules: the important relation between structure and function. Structurally, DNA is a flexible molecule, and this flexibility is well pronounced in the polymorphic nature and the growing number of unusual DNA conformations. Apart from the double helical structure of DNA, it can also adopt unusual structures like triplexes, quadruplexes, parallel duplexes, i-motif etc. [2]. Alpha helix and beta-pleated sheets are the two main types of secondary structures of proteins. The molecular details of any biological phenomenon cannot be fully understood until the status of reacting molecules has been characterized. Therefore, it is necessary to know about the techniques/methods used for the separation and identification of biomolecules. In this review, we have made an attempt to discuss the physicochemical

techniques involved in the characterization of biomolecules.

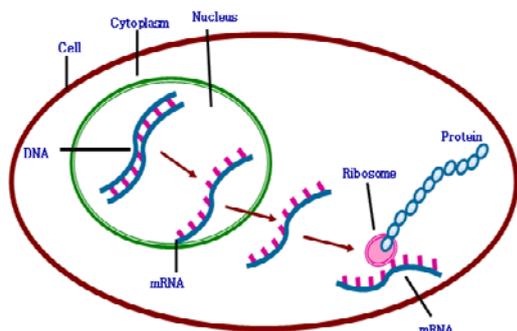


Figure 1: Schematic representation of Central Dogma of Molecular Biology

## 2. Techniques used to characterize Nucleic acid and proteins

### 2.1 Gel-Electrophoresis

Gel electrophoresis is a technique, used for the separation of macromolecules-especially nucleic acids, proteins and for protein-complex characterization. The relative rate at which an individual molecule move depends on several factor like net charge, molecular shape, charge to mass ratio, porosity and viscosity of the matrix through which the molecules migrates. The net charge depends on the number of positive and negative charges present in a molecule. Nucleic acids have a consistent negative charge due to the presence of phosphate backbone due to which they migrate towards the anode [3] whereas proteins can have either a net positive or net negative charge. These molecules will move towards the cathode if positively charged or towards the anode if negatively charged (Figure 2). Therefore, the different physical and chemical properties of a molecule are accountable for their movement through a gelatinous medium.

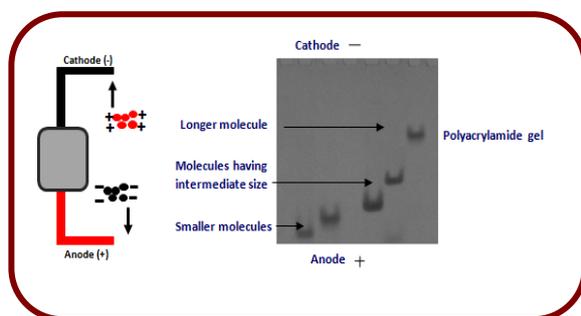


Figure 2: Migration of charged molecules in an electric field on the basis of (a) charge and (b) size

The basic principle involved in the process of electrophoresis is similar, but the major difference lies on the type of support medium like cellulose or thin gels. Polyacrylamide and agarose gels are used as support medium for larger molecules. For low

molecular weight biomolecules such as amino acids and carbohydrates, cellulose is used. However, denaturing gels are also performed to check the purity of the biomolecules.

### 2.1.1 Denaturing Gels

Denaturing gels have been used for the separation and purification of single stranded fragment of DNA and RNA. Denaturation is the destruction of the molecular conformation of the native protein and nucleic acids, and results in the loss of their biological activity. Denaturation can be carried by the addition of denaturants, strong acid or base (i.e. changing the pH of the solution), an organic solvent (e.g., alcohol or chloroform), or heat. Under non-denaturing conditions, such samples (nucleic acid or proteins) migrate in a manner determined largely by their intrinsic electrical charge.

#### (i) Urea-Polyacrylamide Gel Electrophoresis (Urea -PAGE)

Denaturing polyacrylamide gels are used to allow the separation of molecules by their relative size by keeping the molecules denatured. Formamide and urea is often used to denature DNA and RNA and allow their separation on the basis of size by keeping the molecules single stranded. Samples can also be heat treated for denaturation. The secondary structure of nucleic acid is stabilized by hydrogen bonding between the base pairs. Similar hydrogen bonds are involved in stabilization of secondary, tertiary and quaternary structures of proteins. High concentration of urea (7-8 M) interrupt the hydrogen bonding between two functional groups, leading to complete disruption of secondary, tertiary and quaternary structures.

#### (ii) Sodium Dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE)

SDS-PAGE is the technique used for the analysis of protein mixture qualitatively. It is mostly used for the purification of protein and their molecular mass determination. SDS ( $\text{CH}_3\text{-(CH}_2\text{)}_{10}\text{-CH}_2\text{OSO}_3^-\text{Na}^+$ ) is used to distort the secondary, tertiary and quaternary structure of proteins to produce linear polypeptide chains. Each protein in the mixture gets fully denatured by this technique. Consequently, SDS-polypeptide complexes will have net negative charge and migrate towards anode at rate based solely on size and molecular weight of the polypeptides. After electrophoresis and dye staining, mobilities are measured and molecular weight can be determined graphically.

### 2.1.2 Native Gels

#### (i) Polyacrylamide Gel

Polyacrylamide gel electrophoresis (PAGE) is a dynamic technique, used for the physical-chemical characterization of proteins and nucleic acid on the

basis of size, conformation, and net charge. These gels are formed by the free radical polymerization of acrylamide (monomer) into long chains and the cross linking agent is- N-N'-methylene-bisacrylamide. The initiation process is carried out by the addition of ammonium per sulfate and the base N, N, N', N'-tetramethylene diamine (TEMED). An alternative method that can be used to polymerize acrylamide gels is photopolymerisation. Photopolymerization can be instigated by riboflavin, which is used in place of ammonium persulfate and TEMED. The gel is kept in front of a bright light for 2-3 hours after being poured, and polymerization is initiated by the free radical generated by the photodecomposition of riboflavin. There are several advantages of using polyacrylamide gels such as, it is chemically inert, provides high resolution and gels can be prepared with wide range of pore sizes..

### (ii) Agarose Gel

PAGE is used for analyzing small fragments of nucleic acids (500bp) in molecular size; however, large nucleic acid fragments cannot be adequately analyzed due to the small pore sizes. These gels are used for both nucleic acid and proteins. These gels have a larger pore size in comparison to polyacrylamide gels and therefore can be used to analyze large biomolecules. Agarose is a polysaccharide made up of the agarobiose, which comprises alternating units of D-galactose and 3, 6-anhydro-L-galactose. The mobility of nucleic acids in agarose gels depends on the conformation of the nucleic acid, the molecular size and agarose concentration. For separation of nucleic acids, gels having 0.3 to 2.0 % agarose concentration are most effective. DNA molecules of the same size but with different conformations migrate at different rates. For example, the small, compact, supercoiled forms usually have the higher mobility, in comparison to the linear double stranded DNA. The extended, circular DNA migrates more slowly. However, agarose concentration and ionic strength are also considering factors while determining migration pattern of the various forms of DNA.

### 2.2 UV-thermal denaturation

Spectroscopy is a branch of science which deals with the study of interaction of electromagnetic radiation with matter and the measurements which are based on light and other forms of electromagnetic radiation are called spectrochemical methods/techniques. These techniques are widely used for the elucidation of molecular structure as well as the qualitative identification and quantitative estimations of both organic and inorganic compounds. The stability of the secondary and tertiary structure of biomolecules is important for many biological processes. The stability of nucleic acids and proteins can be evaluated by different spectroscopic techniques and

one of them is UV-Thermal Denaturation. Nucleic acids absorb strongly in the UV region (around 260 nm) whereas proteins absorb at 280 nm. As the temperature of the solution (containing DNA/protein sample) increases, the absorbance increases. This increase in absorbance (hyperchromicity) results from the unwinding of the two strands of DNA/dissociation of the secondary/tertiary structures to the primary structure. When a solution containing DNA is heated, a change in absorbance properties occurs, which reflect conformational changes in the molecule [4, 5]. The temperature at which 50% of the DNA sample is melted is called melting temperature ( $T_m$ ) and determines the thermal stability of the duplex or structure of interest. It not only provides the stability of any biomolecule of interest but is one of the possible methods to predict the stabilization or destabilization of a nucleic acid/protein-structure by a ligand.

The main factors affecting  $T_m$  values are biomolecule sequence and its length, salt concentration, oligomer concentration, the presence of denaturants (e.g. formamide) and various ligands etc. Presence of various ligands may increase or decrease the melting temperature depicting the stabilization or destabilization of present conformation respectively. Figure 3 shows the thermal denaturation profile of a double stranded DNA.

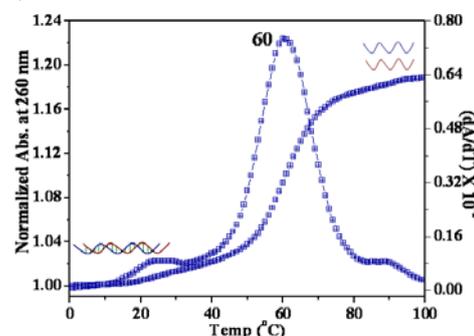


Figure 3: Thermal denaturation profile of DNA

### 2.3 Circular Dichroism (CD)

Circular Dichroism (CD) is powerful spectroscopic technique widely used in biochemistry to detect conformational alterations of nucleic acids, in particular DNA [6], proteins and DNA-protein interaction [7]. CD is a form of optical activity which arises from the differential absorption between the components of left- and right-circularly polarized light by a solution of chiral molecules. Optical activity is a characteristic of many organic and almost all biological molecules and, each optically active compound has a characteristic specific rotation.

A very large fraction of biological molecules contain optically active centers and when applied to molecules such as proteins, nucleic acids and carbohydrates, CD can provide their detailed

structural conformations. Non-chiral molecules exhibit no CD signal in solution, however a CD signal can be obtained when any achiral ligand binds to a chiral host [8].

The structures of proteins and DNA are examined in the wavelength range from 160 to 400 nm because in these regions electronic transitions occurs in the purine and pyrimidine bases, peptide backbone and side chains in proteins. Biopolymers such as nucleic acids and proteins are folded into secondary, tertiary and quaternary structures by ionic interactions, stacking interactions, hydrogen bonding and hydration. CD spectroscopy is quite informative in elucidating the conformations adopted by various nucleic acid and protein secondary structures, conformational changes due to interactions with ligands and environmental effects [9]. DNA exhibits structural polymorphism and can adopt either A-, B- and Z- conformation depending on the oligonucleotide sequence and solution conditions. The native conformation of DNA in solution under physiological condition is B- form. A typical B- form of DNA shows positive peak at 280 nm and a negative peak at 245 nm. Runs of homopurine•homopyrimidine DNA sequence [poly(dG)•poly(dC)], forms an A- like helix and give positive peak at 260 nm and negative peak at 240 nm. Z- DNA can form in alternating purine•pyrimidine tracts (dGCGCGCGC). The Z- form exhibits a negative peak at 290 and a positive peak at 260 nm. CD characteristics of different forms of DNA are shown in Figure 4.

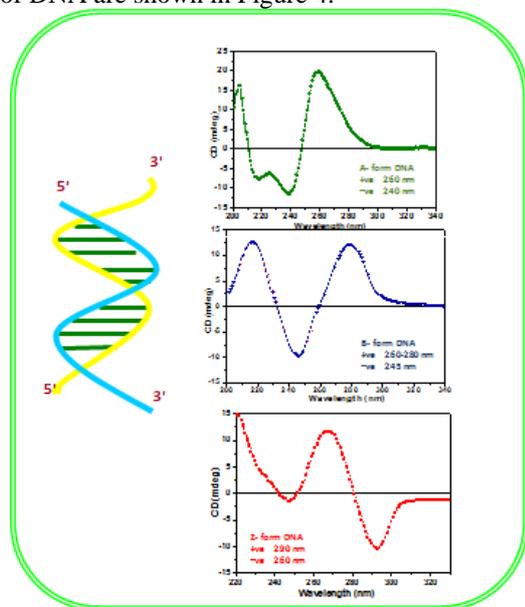


Figure 4: CD spectra of different forms of DNA

DNA triple helical structures can adopt A- or B- conformation depending on the oligonucleotide sequence, but appearance of an intense negative peak in the wavelength region 210-220 nm is characteristic of DNA triple- helical structures

(Figure 5a) [10]. CD spectra of antiparallel G-Quadruplex is characterized by a positive CD peak at 295 nm and a negative band at 260 nm (Figure 5b (1)). Parallel quadruplex folds have a positive signal at 260 nm and a negative signal peak at 240 nm (Figure 5b (2)) [11].

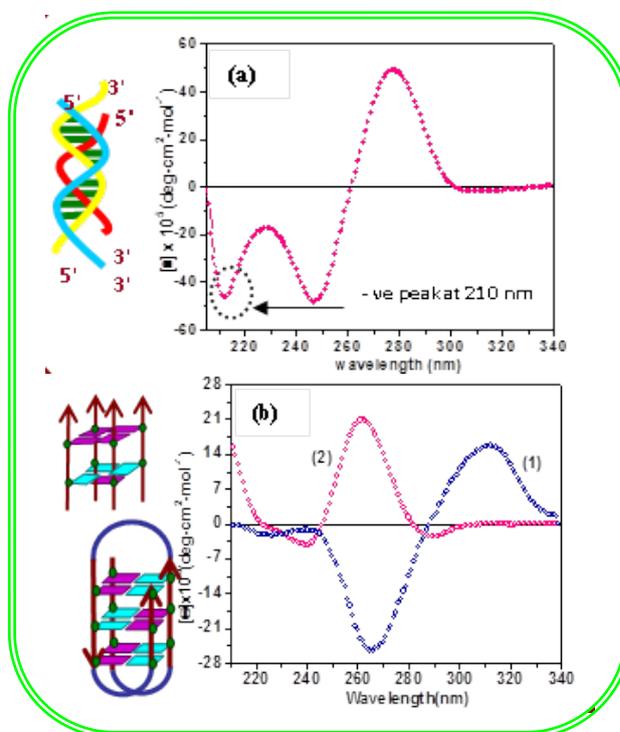


Figure 5: CD spectra of (a) DNA Triplexes and (b) G-Quadruplexes

Alpha-helix ( $\alpha$ ) and beta ( $\beta$ ) pleated sheets are two main type of secondary structures of proteins. CD signatures can be used to determine the approximate fraction of each secondary structure type present in any protein that is the beta-sheet conformation, alpha-helix conformation, or some other conformation. For determining any secondary structure in proteins, there are particular characteristics for CD spectra.  $\beta$ -pleated sheet is characterized by a negative peak at 218 nm and positive at 195 nm. Positive CD peak at 212 nm and negative at 195 nm is a characteristic peak of random coil conformation.  $\alpha$ -helix conformation shows positive peak at 192 nm and negative at 208 nm and 222 nm. Figure 6 displays the CD-spectra of BSA protein (Bovine Serum Albumin).

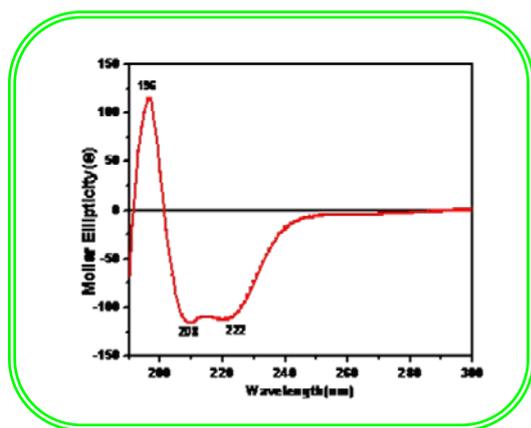


Figure 6: CD spectra of BSA Protein

CD is quite useful in determining conformational changes in polynucleotides and proteins. For example, these techniques can be used to study (i) the effect of temperature and pH on the biopolymers, (ii) transitions from single- to double-stranded polynucleotides and vice versa, (iii) structural changes in biomolecules on interaction with ligand, peptides and cations.

### 3. Conclusion

Nucleic acids and proteins are versatile macromolecules which can adopt various secondary and tertiary structures depending on the solution conditions. Detailed understanding of the structures formed by these biomolecules and their interactions is crucial for essentially all life processes. The fact cannot be denied that none of the biochemical or biophysical techniques can give a complete description about different structures adopted by these biomolecules or even of their complexes with various ligands. So, these techniques are used in combination with the other for structure elucidation. On one hand, gel-electrophoresis is employed to determine the molecularity of structures formed by any biomolecule, on the other, spectroscopic techniques like thermal denaturation and circular dichroism are used to check the stability and for detecting the conformation of these macromolecules, respectively. Nowadays, even more sophisticated techniques like nuclear magnetic resonance (NMR), surface plasma resonance (SPR), X-ray crystallography, and computational methods are utilized to have an in-depth knowledge at molecular level which might provide essential understanding about developing new drugs/medicine for the betterment of mankind.

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