CHAPTER - I

INTRODUCTION
In a living cell biological macromolecules such as nucleic acids and proteins or other organic molecules like lipids exist in an aqueous environment, the latter being different from one part of the cell to the other. For example, the aqueous environment in a cell nucleus is different from that of mitochondria and cell membrane, precisely because of the differences in structure and functional requirements. While water constitutes eighty per cent of the cellular materials, each cellular subsystem is enriched in all the necessary mono-valent and divalent inorganic ions, e.g. Na\(^+\), K\(^+\), Ca\(^{++}\), Mg\(^{++}\), Cl\(^-\), etc., maintained at a very specific level and controlling the ionic strength of the system. A continuous interaction of these ions with water molecules and other molecules (which most often develop electrical charges) during the various phases of life-process, gives rise to a dynamism in the system. As the biomacromolecules like proteins and DNA function as polyelectrolytes owing to the presence of polar groups and dipoles, one of the major interactions with small ions is electrical in nature.

Although the above mentioned interaction is similar to a certain extent to the ionic interaction of a simple system of electrolytic solution, it differs
in that a biomacromolecule is not free or mobile enough, instead, behaves as a semifixed site of electrical charges. As pointed out by Ling (1962) the forces of ionic interactions are both short-ranged and long-ranged ones, the latter always dominates and for all practical purposes the short-ranged ones can be neglected. The biomacromolecules, as mentioned in some cases, already provide charged sites and the electrostatic forces lead to the association of counter ions on those sites, to a degree much higher than ordinary electrolytic association. This happens because fixation of a site gives rise to a product, thermodynamically favoured (activation energy less) and the associated complex here leads to a more ordered state, hence, favoured entropically. Thus an array of charged particles (small molecules, water or inorganic ions) are bound on the macromolecular surface and this is extended to further counterion layers depending on the extent of interacting forces. However, the process of binding of ligands onto the large biomolecules is somewhat similar to association of ions at an interface where one of the layers provides fixed charged sites. Hence, the former process is viewed as one of adsorption (Ling, 1962).

Adsorption, which is known as the interaction of molecules of a subsystem with the molecular sites at an
interface of an adjacent subsystem, is henceforth, extended to a case where boundaries between subsystems are undefined. In a living system most often the boundaries between subsystems are not sharply defined. Nevertheless, the phenomenon of adsorption appears to play a great role in a number of biological processes.

In such adsorption interactions in a living system the electrostatic interaction amongst molecular species (free or semifixed) and water molecules, seem to be the real force of binding. If an inorganic ion is replaced by an organic molecule (aromatic and heterocyclic, which assumes electrical charge in solution), the process remains the same phenomenologically. And this corresponds to an important group of biochemical reactions like association of precursors with template DNA during transcription and translation, binding of drug molecules with proteins and DNA. Going a step further, the interaction or association of larger ligands with biomacromolecules demands a treatment similar to that of small ligand, but with appropriate modification and hence may fall within the purview of adsorption. It may be noted that in this case the ligand itself acts as a semi-fixed charged
surface. This category of adsorption includes protein-protein interaction antibody-antigen association, DNA-RNA association, DNA-protein interaction (nucleoprotein structure) and DNA-lipid interaction. Again, the biomacromolecule itself can behave as a ligand or adsorbate when appropriate surface is provided. For example, the association of external protein and DNA at a cell surface or tissue is primarily adsorption.

1.1 ADSORPTION OF SMALL MOLECULES (DRUG) ONTO BIOMACROMOLECULES (DNA)

As already mentioned the ligands undergoing adsorption (binding) onto biomacromolecules vary from small inorganic ions to organic molecules, aromatic and heterocyclic compounds, and all of these have important biological consequences. One important category of ligand is the drug molecule. Organic dyes have been used as tools to decipher biololecular structures. The study of dye-DNA interaction dates back to 1949 when Scatchard looked into the physical chemistry of the system in order to find the binding parameters followed by the work of Betesi and Hildebrand (1949). Starting from the simplest possible stoichiometry of ligand-macromolecule interaction, Scatchard arrived at a linear relation between fraction of
polymer sites bound (r) and macromolecule to drug ratio (P/D) which stands as a method to find out number of binding sites per drug molecule as well as total mode of binding (Fig.1.1.1).

Peacocke and Skerrett (1956) through some experimental measurements, e.g. spectrophotometry and equilibrium dialysis, reached the conclusion that in drug-DNA complex formation two types of binding are found: (i) a weak binding mode that takes place when the number (r) of drug molecules bound per nucleotide is equal or greater than 0.2 ($r > 0.2$) and (ii) a strong primary mode of binding that predominatest till $r \approx 0.2$.

The strong binding is, from chemical point of view, monomeric in nature and has essentially resulted from the charge transfer interaction of π electron cloud of purine and pyrimidine bases of the nucleotides. Obviously in such an interaction a specific geometry of the molecular system is demanded. Hydrophobic interactions between the base pairs and the dye rings stabilise the complex (Lochmann and Micheller, 1973). The free energy change in this case is about 6 to 9 Kcal/mole of the drug. Li and Crothers (1969) on the basis of kinetic measurements suggested that strong binding occurs in two steps:
Fig. 1.1.1. Scatchard plot: A typical example of plot of fraction of nucleotide bound (r) versus macromolecule to drug ratio in Drug-DNA (calf thymus) system.
first the drug (in cationic form) is electrostatically attracted towards the external phosphate group, followed by transfer of the drug molecules from a state of external binding into the grooves.

The latter mode of binding is known as intercalation (Lerman, 1961, Waring, 1965). According to Lerman (1961 and 1964), the aminoacridine cation lies centrally above and below the hydrogen bonds of the base pairs such that the positive nitrogen atom is near to the central axis of the molecule and equidistant from two polynucleotide chains (Fig. 1.1.2). The plane of the drug-rings are held within ± 36° of the plane of DNA bases (Lerman, 1963; Negata et al., 1966) forcing the bases to tilt slightly to accommodate the ligand. A modification to the above model (Pritchard, Blake and Peacocke, 1966) is that, while strongly bound, acridine lies between successive nucleotide bases on the same polynucleotide chain in a plane approximately parallel to the base planes but at an angle (looking down the polynucleotide chain) such that the positive ring nitrogen is close to the polynucleotide phosphate group (Fig. 1.1.3). It has been reported that intercalative binding results in a) increase in the distance between the bases from 3.4 Å to 6.8 Å (Waring, 1975) and the length of DNA
Fig.1.1.2. Lerman's model for intercalation of acridine into DNA bases.

Fig.1.1.3 A modification of Lerman's model for intercalation of acridine into DNA bases of the same strand.
Lerman's Model For Intercalation

Modified Model For Intercalation

The weak binding observed in low macromolecule to drug ratio, is an external attachment of the drug molecule to the DNA rod-like chain, where drug in its cationic state is electrostatically held by the negatively charged phosphates (Peacocke and Skerrett, 1956; Bradley and Wolf, 1959; Stone and Bradley, 1961). It should be noted that the binding can occur as an interaction with either ligand molecule which only contribute to this mode of binding or cationic part of the bound drug molecule (which has already formed a strong bond with DNA) projecting out as a branch on the DNA surface (Mukhopadhyay and Mookerjee, 1978). The activation energy is only a few (2 to 3) Kcal/mole of acridine. The tendency of the drug-molecules to bind externally is enhanced by (a) denaturation of the DNA, and (b) decrease in ionic strength (Peacocke and Skerrett, 1956; Gruenwedel et al. 1969; Record, 1975 and 1976).

Waring (1965), Crothers (1968), Fuller and Waring (1964), Krugh (1972), Sobell (1973), Orstein and Rein (1979) have contributed to the physical models of drug DNA-binding.

The binding of organic dyes has been found to depend on base composition and sequence. Since G.C base pair has polarizability greater than A.T base pair, the former is preferred by polar drugs (planar tricyclic dyes) (Muller & Crothers, 1975). This is further influenced by side-chains (Muller et al., 1975) and different ring systems (Le pecq et al., 1967). The binding of intercalating drugs to double stranded synthetic polynucleotides (Isenberg & Baird, 1967, Wells et al., 1970; Bontemps et al., 1974; Baguley et al., 1978; Wakelin et al., 1978) or complementary oligonucleo-
Fig. 1.1.4. Mechanistic scheme for frame-shift mutagenesis due to the interaction of a chemical mutagen with DNA.
DNA \textit{BACKBONE}

\begin{itemize}
\item \textbf{BASE SUBSTITUTION}
\item \textbf{BASE DELETION}
\item \textbf{SMALL DELETION "BULGE EFFECT"}
\end{itemize}
tides (Krugh et al. 1975; Patel et al. 1976 & 1980; Young et al. 1980) show specificity of base sequences in drug intercalation to DNA. Base pair specificity in drug binding has also been observed in external binding, e.g. proflavine has a preference for binding externally at G.C sequences (Ramstein et al 1972 and 1975).

The intercalative binding of drug to DNA might cause frameshift mutagenesis, which is the deletion or addition of base pairs to DNA, thus altering the codon readings. This process has been schematically demonstrated in Fig.1.1.4. However, mutagenicity has not yet been correlated to binding of drugs.

The macromolecule to dye ratio (denoted by P/D) reflects the molecular picture of ligand macromolecule interaction and determines the extent of binding (Bradley and Wolf, 1959) in their theoretical considerations, expressed the probability of binding as an appropriate function of P/D. It has been observed through experiments that with the rise of P/D ratio the extent of binding increases, but above certain value of P/D no more binding occurs (Mukhopadhyay and Mookerjee, 1976). This particular P/D value is referred to as saturation point and its value for a specific drug-DNA system, depends on ionic strength, pH etc. For inter-
calative binding the unwinding of the helices that takes place during the process, has a limit due to the stability of the overall macromolecular chain, leading to saturation in binding. As a whole the cause of this saturation phenomenon lies with the conformation of the macromolecule which keeps altering with increased binding of ligands (depending on the mode of binding) and finally attains a configuration which does not allow more ligand molecules to come in.

Bradley and Wolf (1959) first pointed out that the binding of ligands often follow cooperative mechanisms. In specific cases of binding of acridines with polynucleotides, they have shown that these drugs have a tendency to aggregate at the DNA surface, thus occupation of neighbouring binding sites is favoured. Similar cooperative effects are also observed in case of adsorption of mono and bivalent inorganic ions on proteins and tissues (Karreman, 1980). The question is whether the cooperative mechanism lies in inter ligand interaction (e.g. aggregation) or intersite interaction (adsorption sites) along the biomacromolecular chain or the tissue surface. Ling (1962) mentioned that because of site fixation the association of ligands at macromolecular sites is favoured. This association of ions results in redistribution of local charge densities and induces changes at the neighbouring sites.
Thus electrical signals are transmitted along the macromolecular chain. This is called I-effect (inductive) which can be positive or negative depending on the inducing charge (Ingold, 1953). Another electrostatic effect emanates from direct electrostatic interaction (Goulombic) of substituent groups and is transmitted through space along the axis of shortest separation of the interacting atoms, called a direct effect or D-effect. A combined effect of the above two is called F-effect. All these effects are parametrized with respect to different structural quantities. Ling (1962) pointed out that when a ligand is adsorbed on any site of a biomacromolecule, the neighbouring sites are influenced through F-effect. On the other hand, it has been pointed out that in case of acridine dyes, ligand-ligand interaction leads to cooperative binding (Bradley and Wolf, 1959). Hence, the cooperativity might arise at both adsorbate level and at adsorbent level. It was pointed out that although most systems probably show primarily either cooperative or anti-cooperative effects, mixed characteristics can be observed.

In the present studies, the drug proflavine (3,6 diaminoacridine) which has both carcinogenic and
Fig. 1.1.5. Structure of acridine group of drugs.
1. Acridine

2. 9-Aminoacridinium cation

3. Proflavine or 3,6-diaminoacridinium cation

4. Atebrin mepacrin or quinacrine cation, \( R = \text{CHMe} (\text{CH}_2)_3 \text{NET}_2 \)

5. Acranil cation \( R = \text{CH}_2\text{CH(OH)}\text{CH}_2\text{NET}_2 \)

6. Acridine orange, or 3,6-bis-dimethylaminoacridinium cation

7. Acriflavine or 3,6-diamino-10-methylacridinium cation

8. 9-Amino 1,2,3,4-tetrahydroacridinium

9. Ethidium cation

TH-1489
mutagenic activity has been used as a ligand for complexation with DNA (Fig. 1.1.5).

1.2 PROFLAVINE AS A LIGAND

Drugs found to inhibit or induce tumour growth, come into action via interaction with cellular macromolecules, e.g. especially DNA (Raven et al. 1960; Kersten and Kersten, 1965; Gottlieb, 1967; Newton, 1970; Horwitz, 1971; Hollstein, 1973). Investigations show that compounds derivable from the three ring anthracene like system either planar, aromatic and heterocyclic or partially saturated non-polar form, form stable complexes with DNA and inhibits DNA dependent RNA synthesis. This includes acridine group of drugs (Fig.1.1.5). However, the anthracycline group bears the strongest resemblance to the acridine dyes, e.g. Cinerubin, Nogalamycin, Daunomycin.

1.3 LIGAND-BINDING AND GENETIC EFFECTS

In a biological cell the genetic material DNA, which contains and propagates the entire information required by the cell, can be conceived of a specific arrangement of codons, each formed by three nucleotides with a specific sequence. The genetic code is read by enzymes as well as precursors during biochemical
processes like transcription, translation, replication. It is believed that the mechanism of deciphering the genetic code involves weak chemical interactions (which demand specific stereochemical conditions) between the template molecule and the precursors. Thus, any agent (radiation, ligand) perturbing the normal helical structure of DNA is likely to disturb the information content, hence, the biochemical processes. Carcinogens, anticancer agents (antibiotics) and mutagens, do suppress or alter genetic information as reported in several biological systems (Arcos and Argus, 1974). The question remains, how do these small molecules affect the genetic informations, whether by directly interacting with DNA or by some indirect means? Evidences are there to show that in a living cell most often the carcinogens (or mutagens) bind directly to DNA.

The diaminoacridines, i.e. proflavine (3,6-diaminoacridine), acriflavine (3,6-diamino-10-methylacridinium chloride), and acridine orange (bis-3,6-dimethylamino acridine) have been found to stain the nuclear and not the extranuclear nucleoproteins of living cells showing their direct interaction with nuclear DNA (de Bruyn et al, 1951). The action of acridines on viruses and bacteriophages,
already reported by DeMars (1953), is clearly the result of acridine-DNA binding. The mutagenic activity of some of the acridine drugs e.g. proflavine, is thought to take place via deletion and inversion of bases of DNA which is possible if only mutagen interacts at the DNA level. It has been observed (Peacocke, 1975; Tso and DiPaolo, 1979) that the cationic form and not the anionic or zwitterionic forms of acridines is bacteriostatic. For this activity the molecule should possess a minimum flat area of about $38 \AA^2$ demanding the corresponding binding site to be flat, so that attractive interactions are maximized. Since the nucleic acids are anionic, and possess flat purine or pyrimidine rings (as possible binding sites) it is the most likely binding site for bacteriostatic activity. The kinetic studies of inhibition of _A. serogenes_ by aminoacridines implicate nucleic acids as the site of acridine action on bacteria.

It may be questioned whether in a living cell the nuclear DNA or the extranuclear one is attacked by drug molecules. The examples given above demonstrate the involvement of nuclear DNA. However, there is some evidence showing the interaction of drug molecules with extranuclear DNA e.g. inhibition of multiplication of
episomes and kinetoplasts (a specialized portion of mitochondrion containing a large amount of DNA) (Peacocke, 1975). Hence one may conclude that drug molecules directly bind to DNA during certain biological events, although not always and affect the genetic information accordingly.
1.4 **ADSORPTION OF BIOPOLYMERS ONTO INORGANIC SURFACE**

The study of the behaviour of macromolecules at electrically charged interfaces becomes significant as a step leading towards the study of biological adsorptions. The surface activity of biopolymers like nucleic acids, proteins and enzymes has been a subject of much investigation (Bull, 1956, 1957; McLaren, 1954, 1958; Zittle, 1953; Chattoraj *et al.*, 1959, 1967, 1968; Upadhyay and Chattoraj, 1970, 1974, 1972) as an increasing number of biological phenomena involve adsorption of surface active molecules and ions at interfaces. Direct adsorption experiments with DNA onto alumina-water interface have also been carried out by different workers (Upadhyay and Chattoraj, 1968; Chari and Mookerjee, 1975; Upadhyay and Mookerjee, 1977). Although study on the adsorption of macromolecules from solution onto solid surfaces began about three decades ago (Adamson, 1967; Lipatov and Sergeeva, 1973) most of the work involved is of non-biological polymers. Biopolymers are usually more inflexible than the non-biological ones. In case of DNA, the inherent polyfunctionality of the polymer indicates that if one segment of the molecule gets adsorbed on a solid surface, the probability of adsorption of neighbouring segments will be greatly enhanced according to Ling's
concept. The unusual configurational behaviour of a macromolecule like DNA as evidenced by the properties of polymer solutes, suggests that the intramolecular configuration of the adsorbed molecules could be an important aspect of the adsorption process.

Frommer and Miller (1966, 1968) observed that denatured DNA adsorbs much faster than native DNA probably because of the higher diffusion coefficient of the denatured DNA. Chari and Mookerjee (1975) found that the quantity of gamma-irradiated DNA adsorbed on alumina is much greater than native and heat denatured DNA. Correspondingly, the adsorption of gamma-irradiated DNA is faster than the other forms of DNA studied. Miller (1961) studied the interaction of DNA with a charged mercury surface by measuring the effect of DNA on the differential capacity of the electrical double layer between the polarised mercury surface and 0.1 M sodium chloride solution containing varying concentrations of DNA. The lowering of the differential capacity varied with surface concentration at partially covered surfaces and reached a constant value when the surface was fully covered. Miller suggested that DNA preserves its double helix at a negatively charged surface but not at a positively charged one and also proposed a mechanism for the unfolding of the double
helix at a positively charged surface, which is presumed to be a very fast process. According to Miller, one can speculate that unfolding of the DNA double helix in biological system takes place at positively charged surfaces.

Miller (1965) followed up this work of adsorption studies of DNA on surfaces of copolymers of 4-vinylpyridine and styrene. He studied the electrophoretic mobility of the particles coated with either native or heat denatured DNA adsorbed from aqueous solution of different salt concentrations and observed that the electrophoretic mobility of the coated particles depends only on the salt concentration and the state of DNA molecules in solution but not on the composition of the surface of the copolymer particles before DNA adsorption. The shape of DNA molecules interacting with monolayers of copolymers with different surface characteristic was determined by electron microscopy (Gordon, 1970). The influence of surface charge on the extent of adsorption and structural changes of adsorbed DNA was studied. The amount of DNA adsorbed was found to depend both on copolymer concentration and surface treatment but not on the concentration of DNA remaining in solution. The interaction between DNA and electropositive or weakly electronegative copolymer surfaces were strong enough to break the structure of adsorbed DNA.
Chattoraj and Upadhyay (1968) have studied the adsorption of DNA and RNA at alumina-H$_2$O interface. They have used native, heat denatured, alkali and acid denatured DNA as adsorbates. The nature and shape of the 'adsorption versus concentration of DNA' curve for native DNA indicated Langmuir type adsorption. Frommer and Miller (1966) standardised a method for measuring the adsorption of tritium labelled compounds to study DNA adsorption on a polypeptide monolayer. Gordon (1970) studied the adsorption of DNA on mica after replacing the cation by Al$^{+3}$ through ion-exchange. The adsorption process monitored by electron microscopic observation showed that the amount of DNA adsorbed increased with ionic strength of solution. Of several multivalent cation tried only Al$^{+3}$ was effective in causing adsorption. Chattoraj et al (1969) studied the electrophoretic mobility of calf thymus DNA adsorbed on charged particles. Other studies on adsorption of nucleohistones by Upadhyay and Chattoraj (1972), Fasman and co-workers (1970), Upadhyay and Mookerjee (1977) and Akinrimisi et al. (1965) are also of much significance.

It may be mentioned that according to Ling's association-induction hypothesis the above process of adsorption involving biomacromolecules is cooperative specific (Ling, 1962; Karreman, 1980).
The Binding Scheme

The adsorption of DNA at a solid-liquid interface could be considered to take place via replacement of already adsorbed solvent molecules, \((H_2O)\) occupying the binding sites on the solid surface, by solute molecules. Therefore the following scheme is suggested:

\[ \nu(H_2O)_{\text{surface}} + P_{\text{soln.}} \xrightleftharpoons{} \nu(H_2O)_{\text{soln.}} + P_{\text{surface}} \]

\[ (H_2O)_{\text{surface}} + A^- \xrightleftharpoons{} (H_2O)_{\text{soln.}} + A^-_{\text{surface}} \]

where \(P\) denotes phosphates of DNA and \(A^-\) denotes anion of any other electrolyte present, say \(Cl^-\) of \(NaCl\), and \(\nu\) is the number of moles of solvent being replaced by a mole of phosphate.
1.5 ADSORPTION OF DNA ONTO LIPIDS

The surface activity of biopolymers finds a practical relevance in a situation with biosurfaces. In case of a living system while proteins and DNA interact with cell membrane (it is important in view of the biochemical behavior of the cell) the uptake or rejection of an external macromolecule depends on the behavior of the cell membrane towards the foreign molecule and if the macromolecule is uptaken, it is going to affect the structure and function of the cell.

The cell membrane, as it is known through series of experiments (Gorter and Grendel, 1925) and physical measurements (Fricke, 1925) is made up of lipid bilayer over all or almost all the surface of the cell and proteins. The Danielli and Davson membrane model (1935) which is the first model for cell membrane structure, is one of a rigid form. Later it was replaced, on the basis of more experimental findings, by alternative structural arrangements (flow diagram Fig.1.5.1). Observations that under appropriate conditions some lipids would adopt configurations other than a bilayer, appearance of granular structure at high magnification in electron micrographs and isolation of lipoprotein 'particles' led the membrane biologists to speculate that membranes might consist of
Fig. 1.5.1. Schematic diagram representing the chronology of different membrane models (from Membrane Structure, Ed. Finean and Michell, 1981).
laterally aggregated arrays of globular lipoprotein subunits. The identification of membrane proteins with exposed regions dominated by nonpolar amino acid side chains led to the realisation that such regions would be likely to associate with hydrocarbon region of the membrane lipid phase so that parts of these proteins might therefore be inserted deep into the membrane interior. This together with an emphasis on disordered or fluid packing of the lipid hydrocarbon chains and free lateral diffusion of membrane components, was then featured in a new membrane model, the 'Fluid-Mosaic' model proposed by Singer and Nicholson (1972). This has since been generally accepted as a more realistic expression of the general characteristics of membrane than any previous model. Experimental work on membranes has now advanced to the stage at which the structural patterns of individual membranes are being defined in some detail (Henderson and Unwin, 1975) so that individual membranes differ both in the spatial distributions of their molecular components and in the mobilities of these components.

The membrane proteins and glycoproteins are of two categories: (a) External or peripheral proteins, which are superficially attached to the hydrated surfaces of membranes and (b) Intrinsic or integral proteins
which are inserted to a significant extent into the non-polar interior of the membrane. On the other hand, the amount of lipid on a membrane is the amount needed to provide a continuous bilayer barrier around a cell or a segregated intracellular space, except that this quantity is diminished as the fraction of the membrane area that is occupied by the intrinsic proteins increases. The lipids that make up these bilayers are diverse, varying both from membrane to membrane and from organism to organism (Gurr and James, 1980).

In order to maintain the hydrophobic phase of the membrane as fluid rather than crystalline (which is a biological need for a living cell) at physiological temperatures, the hydrocarbon chain undergoes sufficient distortion by cis-double bonds, methyl branches or cyclopropane rings. And this seems to be a universal aspect of membrane lipid mixtures. In some membrane (e.g. plasma membranes of animal cells) this 'fluidity' is somewhat restricted by the presence of substantial quantities of cholesterol. However, such freedom of motion in the hydrocarbon phase does not require a complex lipid mixture, for example, the lipids of halobacteria seem to provide an appropriate environment for its membrane proteins even though all of the hydrocarbon chains are similar dihydrophytyle ethers (Heusar 1980);
also many enzymes isolated from membranes of complex lipid composition can function in much simpler lipid environments (Wiley and Skehel, 1977). From thermodynamic considerations the dominant structural theme in biological membranes will be a lipid bilayer, with each membrane protein either interacting with the polar surface of the bilayer or inserting some part of its bulk into the central hydrocarbon region, as indicated by its particular surface distribution of hydrophilic and lipophilic domains (Finean and Michell, 1981).

In a cell-membrane the organisation among water molecules is probably extensive but irregular, a state described as 'flickering clusters' of molecules (Frank, 1970) locally modified by solution, particularly macromolecules. The water structure with its dynamic form has an important role in biomembrane structure. Adequate knowledge of this combined with physicochemical studies on individual lipid and protein components help us to understand membrane structure (Benson, 1968; Fontana, 1971; Fuerstenan, 1971; Huang, 1972; Jones, 1975; Doniach, 1978; Gruenewald et al, 1979; Johnson and Garland, 1983; Ksai, 1983; Mathew and Balaram, 1983; Kosower, 1983).
1.6 **NUCLEIC ACIDS-MEMBRANE INTERACTION**

A living cell often encounters with an external macromolecule like nucleic acids and this is a basic necessity for various biochemical processes to go on. The process of uptake of DNA by a cell which is equivalent to an input of genetic information to a cellular system, begins with the interaction of nucleic acids with the outermost region of a cell i.e. membrane. It is known that replication of DNA in prokaryotic cells proceeds within a membrane complex of DNA (Sueoka, 1968; Harmon and Taber, 1977; Voikov et al. 1977). In eucaryotic cells too, association of chromosomes with the nuclear membrane appears to be necessary as evidenced by biochemical experiments (Infante et al 1976; Cabradilla and Toliver, 1975). Such interaction between polynucleotides and lipids has importance in transformation and viral infection. For instance, it has been shown by electron microscopy that during its infection, M13 bacteriophage DNA gets attached to cell membrane (Forsheit and Ray, 1971). Biochemical investigations show that in quite a few number of cases, interaction which matters is that with the membrane proteins and lipoproteins; hence the importance of nucleoprotein structures (Jack Griffith, 1972). However, over the whole range of interactions
between a cell membrane and nucleic acids, one is not certain whether the interaction is at the protein or the lipid level.

Transfer of polynucleotides across the membrane was demonstrated for prokaryotic (Spizizen et al., 1966) as well as for eukaryotic cells (Bhargava and Shanmugam, 1971). This process involves adsorption of polynucleotides on the cell surface; it penetrates and releases itself into the cell. The mechanism of crossing the hydrophobic barrier formed by proteinlipid membrane by large hydrophilic molecule is unknown. In particular, the nature of bonds which provide adsorption of polynucleotides on the membrane is yet to be understood. In quite a number of systems, one has thought that the adsorption mode is precisely DNA-protein interaction. However, there is a considerable bulk of information pointing to a strong effect of lipids upon the state of nucleic acids.

In studying DNA lipid interaction, Budker et al. (1978) used mitochondria as a convenient model object because their outside surface is a 'pure' protein-lipid membrane. It has been demonstrated by Budker et al. (1980) that polynucleotides are adsorbed by mitochondrial membranes as well as by liposomes formed either from total mitochondrial lipids or from phosphatidycholine.
The interaction has been reported to depend on the presence of bivalent cations like Mg\textsuperscript{++}. This leads to the idea that this interaction is due to action of bivalent cation as crosslinks between phosphate residues of polynucleotides and phosphate residues of the membrane bilayer. Budker et al, (1980) concluded that adsorption of DNA on membrane (which depend on the ratio of mono and bivalent cations) may regulate the rate of the synthesis of DNA and RNA.
1.7 COOPERATIVITY IN BIOMOLECULAR REACTIONS

A good number of biological processes show cooperative activities and this often originates from the molecular dynamics of the system. The phenomenon of cooperativity, which was suggested in a solid state system to account for spin-spin interaction, follows similar kinds of treatment both in non-living and living systems, known as Ising Models. For biomolecular systems, the phenomenon was first observed in the study of conformational changes in helix-coil transition in biopolymers and then to DNA to explain phase-transition or melting, on which an extensive study has been made. These are summarized below:

The problem of cooperative interaction is viewed as one in an ensemble of particles and sites, hence statistical mechanical methods are applied. The Algebra used are: (i) combinatorial method; (ii) the sequence generating function method; (iii) matrix method.

Schellman (1965) was first to deal with the theory of the helix-coil transition. The approach was developed for short-chain protein undergoing helix-coil transition. This was extended to an infinite chain by Peller (1959), Hill (1959) treated the problem in combinatorial method, known as Generalized Ising Model. Lifson and Zimm (1963),
Lifson (1963, 1964), Litan and Lifson (1965) applied the method of sequence generating functions in helix-coil transitions of protein and DNA to get results in proximity to experiments. The formalisms of Hill, Lifson and Litan and Lifson (1965) give identical results and treat any linear model where the only assumption made is that successive sequences are dependent on its length in any fashion. Lifson's method has the advantage over Hill's method in that the former treatment is easier, includes the possibility of more than two types of sequences and also gives results faster. However, these are applicable only to long-chains.

The matrix method seems to be the most convenient method to treat the problem of cooperativity in a chain of any length. Zimm and Bragg's model (1959) provides general method of treatment, so as the model of Miyake (1969), Lifson and Roig (1961), Nagai (1960). In all these models only nearest neighbour interactions have been considered for the sake of mathematical convenience. The theory of Gibbs and DiMarzio (1959) steps towards generalization of Ising Models. This was followed by application of these mathematical methods to different systems e.g. heteropolymer and the conformation of DNA (Poland et al. 1965, 1966; Vournakis et al. 1967) its melting (Zimm, 1960;

While extensive work has been done on cooperativity in conformational changes in biopolymers, Ising models find applications in other processes of biomolecular dynamics. An important problem is one of ligand-bipolymer binding (Silberberg and Simha, 1968; Zaredatelev et al., 1971; Gurskii, 1972; McGhee and Von Hippel, 1979; Kowalczykowski et al., 1980).

Schwarz (1970) applied Zimm-Bragg's method to dye-DNA binding. He also applied combinatorial method to this problem (1977). Later these methods have been modified for the cases of large ligands (1978), especially protein, by Epstein to approach nucleoprotein structures (1979). Dourlent (1975, 1976) has categorized ligand-DNA binding into finer sub-classes and dealt with respective binding constants.
1.8 EFFECT OF ULTRASOUND ON DNA

DNA has also been subjected to ultrasonication for investigating its adsorption characteristics at reduced chain length, hence reduced molecular weight. DNA may be degraded by many different processes when subjected to ultrasonic irradiation (e.g. local temperature up to \(10^4\)K, local pressures up to 10 atm, photoelectric effects, formation of free radicals, sonoluminescence and chemiluminescence). However, Hughes and Nyborg (1962) have convincingly demonstrated that the mechanical effect of stressing is the major cause of the initial degradation of polymers. Richards and Boyer (1965) have shown that 90% of DNA degradation occurs at C-O bonds of the DNA backbone and 10% at P-O sites (no C-O bond breaks were detected). The detailed mechanism by which DNA is degraded by ultrasonic irradiation, therefore, remains obscure. It has been observed that macro molecules do degrade to an apparently limiting molecular weight (Freifelder and Davison, 1962) after which time further ultrasonic irradiation has little or no effect. For DNA the limiting molecular weight distribution appears to be approximately the most probable Schultz distribution (Hall et al. 1981)
1.9 The Present Work

In the present work the following aspects have been covered -

1. The adsorption of DNA and drug bound DNA at aqueous alumina interface have been studied. The extent of adsorption and the adsorption isotherms were investigated. In case of drug-bound DNA the extent of drug binding were estimated, then the binding of drug-bound DNA onto the surface were evaluated; the latter values were compared with those of unbound DNA.

2. The above adsorption studies have been done for two different DNAs (a) calf thymus and (b) E.coli, and the results were compared to see the differences if any.

3. The adsorption studies at aqueous alumina interface were carried out with degraded (Ultrasonic exposed) and denatured (γ-irradiated) DNA and compared with that of normal DNA.

4. Adsorption of DNA onto lipids have been studied and similar points, namely the binding profiles, the extent of binding, saturation etc. have been investigated.

5. The mechanism of cooperative binding of ligands to DNA have been looked into. A new mode of binding has been proposed. In the light of the modified picture of
cooperative binding and the corresponding models, new solutions for binding parameters and degrees of co-operativity have been proposed.

6. The models for co-operativity have been considered in each of the above systems and the applicability was checked. The binding constants corresponding to various modes have been evaluated, simultaneously. This gave us indications of binding mechanism.

Although studies have been carried out by others on adsorption of free DNA at solid-liquid interface for various systems, the problem of adsorption of drug-bound DNA was not precisely looked into. In the present work, this has been dealt with for the first time. Moreover, an attempt has been also made to correlate the adsorption problem with ligand-DNA binding on which aspect some new ideas have been contributed through this work.