CHAPTER - I

Introduction and Review of Previous Work

A. General survey on DNA

The nucleic acids provide for some of the most essential functions in the living organism : the storage and transmission of its genetic make-up and the means by which this information is utilised in the synthesis of all cellular proteins, and hence of all cellular constituents. Nucleic acids, like proteins are macromolecules, consisting of a backbone of sugar (ribose in RNA and deoxyribose in DNA) and phosphate to which the various bases are attached. As a matter of fact, among them the former are the largest molecules known. As the name indicates, nucleic acid molecules are acidic and at physiological pH they carry a high density of negative charge. For this reason, they are found associated in the cell with various species of cations : frequently with basic proteins such as histones or histonelike entities; sometimes with oligoamines, such as cadaverine or spermine and very commonly with alkaline earth cations.

The bases found in nucleic acids are either pyrimidine or purines - the latter containing a fused pyrimidine-imidazole ring system. In deoxyribonucleic acid, the common bases are the pyrimidines, Thymine (T) and Cytosine (C),

and the purines, Adenine (A) and Guanine (G). Some 5-methyl cytosine occurs occasionally, especially in the DNA from higher plants and in certain bacteria and bacterial viruses. Uracil and 5-hydroxy methyl uracil have been reported as occurring in certain bacteriophage DNAs.

Nucleic acids, being polyanions, must be accompanied by an appropriate number of cations in their vicinity in order to maintain electrical neutrality. The extent of binding of these ions may vary greatly. On the one hand we have inorganic counter cations exemplified by the alkali metals, such as Na^{+}, Li^{+} etc., which serve only to neutralise the negative charges on the phosphate residues electrostatically; this allows the nucleic acid to assume its most compact conformation, as shown by increase in intrinsic viscosity, sedimentation co-efficient, hyperchromicity and specific rotation and a decrease in R_G, radius of gyration. Organic diamines such as putrescine and cadaverine, which usually accompany DNA in vivo, show a different behaviour : they are bound stoichiometrically, probably to the phosphate residues. Thus, within this stoichiometric range they are far more effective than the monovalent ions. In addition, aliphatic diamines and especially other relatively large organic molecules may exert other effects on DNA structure not duplicated by the inorganic ions.

Of great interest is the interaction of nucleic acids with certain polynuclear aromatic organic molecules and ions as well as inorganic ions. The interaction of nucleic acids with organic molecules and ions are important because such studies provide an opportunity for elucidating the molecular mechanism of the action of mutagens and carcinogens and also add to our knowledge of the detailed secondary and tertiary structure of DNA. It has been shown by Hinselwood and his collaborators that the quantitative aspects of inhibition of the growth of A.aerogenes by aminoacridines could be adequately accounted for in terms of a modification in the kinetic system of each cell in response to the presence of inhibitors (1). Albert and his co-workers in an extensive study of a large number of acridine dyes have shown that the flat ring system was essential to activity (2).

The theory of mutagenesis proposed by Freese (3) postulates that base analog mutagens act by altering base-pairing in DNA and that the so-called transversions resulting from mutagens with acridine type mutagens are caused by change of a purine into a pyrimidine. Brenner et al(4) criticized the above theory and proposed an alternative theory which states that acridines act as mutagens because they cause insertion or deletion of base pairs, forcing them 6.8 Å apart rather than the normal 3.4 Å distance

might lead to addition or subtraction of a base during replication of DNA. Such an alteration would be expected to result in the formation of a grossly altered protein or no protein at all rather than slightly modified protein. Experiments on acridine mutants of T_4 and T_{22} bacteriophage indicate that this does infact occur.

Before embarking upon the problem of interaction of organic and inorganic cations with DNA, it is worthwhile to make a survey of the binding of organic molecules as well as organic and inorganic ions with DNA.

B. <u>Interaction of DNA with dyes</u>:

Most of the studies concerning interaction of dyes by macromolecules involve cationic dyes. Although similar phenomenon may also occur for acid dyes, the underlying principle are not always the same.

Dyes of the acridine series, such as proflavine and acridine orange bind to DNA and when bound exhibit metachromasy and optical activity. With single chain polyanions, e.g., heparin and isotactic or atactic polystyrene sulphonate (5,6), the aminoacridines show a blue spectral shift. At low ratio of DNA to amino acridine (5) or to rosaniline (7) a blue spectral shift is observed, whereas at high ratios of DNA to aminoacridines a red spectral shift is observed (8). The blue spectral shift

has been interpreted as being caused by the interaction between adjacent dye ions in the aggregate (9) or stacked on the surface of the polyion (8). The red spectral shift, however, is usually attributed to the interaction of the heterocyclic ring system of the bound aminoacridine with the purine and pyrimidine bases of the DNA (8,10), which has been substantiated by a study of the fluorescence spectra of the complex formed between proflavine and DNA (11). It has been shown by Thomas et al.(12) that 2 to 3% of the base pairs form sites where the dye is strongly bound and fluoresces normally while in the other set of sites the binding constant is weaker and the fluorescence is completely quenched. Comparison with the complexes of proflavine with double stranded polynucleotides, poly (A+U), poly (I+C) and poly (G+C) confirm that strong binding sites correspond to A-T rich regions of DNA, while the quenched sites seem to require the presence of a neighbouring guanines.

Zama et al. (13) studied the C.D. spectra of DNA-acridine orange complex in the visible region at a DNA phosphorous to dye ratio (P/D) from 1 to 500. The C.D. spectrum of the DNA-AO complex in the P/D ratio between 1 and 40 consists of 4 components i.e., positive C.D. bands centered at 510 and 430 nm and negative C.D. bands

at 497 and 465 nm. The C.D. bands at 510 and 463 nm are optimum at P/D = 4 and the change of $\epsilon_l - \epsilon_r$ with P/D suggests that both of them are induced from the interaction between dye molecules bound to adjacent DNA binding sites, each of which is composed of 4 nucleotides. From studies by means of equilibrium dialysis and flow dichroism, Takaseda et al. (14) infers that acridine orange is oriented on DNA perpendicular to its main axis, like base pairs.

Interaction of DNA and RNA with Ethidium bromide, which inhibits the synthesis of nucleic acid in a variety of organisms (15,16,17,18) has been studied by M.J.Waring (19). Ethidium bromide bound to both DNA and RNA at sites, which appeared saturated when 1 drug molecule was bound for every 4 or 5 nucleotides. A secondary binding occurred, which caused precipitation of complex containing 1 drug molecule bound to 1 nucleotide. The primary binding to DNA was not influenced by the composition or by denaturation of DNA, but it was sensitive to salt concentration.

The binding of aminoacridines to DNA increases the thermal stability of DNA, the melting temperature Tm, of the amino-acridine - DNA complex being appreciably higher than that of native DNA (20). The shape of the thermal denaturation curve for the complex is also different from

that of the native DNA. The Tm usually increases on complexation with the dyes. Whether the dye released was originally bound by the primary or secondary mode has not been determined. Because of this effect, the melting temperature Tm has been defined as the temperature at which 50% hyperchromicity is attained from the onset of the rapid increase of the optical density. The value of Tm increases with 'r', the number of ligand molecules bound per atom of DNA phosphorus, reaching a maximum value of r corresponding to the plateau of the binding isotherms (21,20). This suggests that the increased stability is caused mainly by the primary binding process and that the secondary binding has little effect on the thermal stability of the DNA-dye complexes.

Direct evidence that the aminoacridine molecules bound by the primary binding process are intimately associated with the double helical structure of DNA arises from the binding studies of Chambron et al. (22), who studied the binding of proflavine to DNA at a range of temperatures above and below the thermal denaturation temperature Tm. There is a small but steady decrease in 'r' as the temperature is increased, the release of aminoacridine over the temperature range confirms the interpretation of the thermal denaturation curve. The main release of proflavine occurs, however, over a short range of

temperature corresponding to the melting of DNA-dye complex. The release of the dyes is clearly a cooperative phenomenon, and the double helical structure of DNA evidently is essential for the primary binding process to occur.

Binding of dyes with DNA also results in marked enhancement of viscosity (23,24) and diminution of the sedimentation co-efficient of the DNA (23). These changes are contrary to those expected on the basis of aggregation or simple electrostatic effects. Characteristic changes, which suggest considerable modification of the usual helical structure of DNA, are found in the X-ray diffraction patterns of fibres of the complex with proflavine (25,26). Measurement on dilute isotropic solution of DNA in the presence of proflavine showed that the dye (1) modified the structure of DNA but did not disrupt its rodlike configuration; (2) decreased its linear mass (3) decreased its radius of gyration. All these observations were satisfactorily explained by considering that the dye molecules were intercalated into the DNA helix between adjacent nucleotide pairs by extension and unwinding of the deoxyribose-phosphate backbone, rather than stacked on the outside of the helix. An apparent increase in the length of the DNA molecules as determined by autoradiography (27) and light scattering studies also accompany the binding of the dyes.

Models for the binding of dyes to DNA :

Peacocke and Skerrett (8) proposed a model for the binding of proflavine to DNA, where two sites were involved and was based mainly on the observation of a sudden change in the apparent binding constant at D/P = 0.22. They associated the strongly binding sites with monomers, and the weakly binding sites with the aggregates.

Bradly and Wolf (23) on the basis of spectral data proposed that the dyes bind to the outside of the DNA in such a way that neighbouring dye molecules could interact with each other. It was proposed that the dye molecules were bound principally by electrostatic forces and that they would tend to stack together in clusters on the DNA. They distinguished on the basis of absorption spectra between bound monomer and bound aggregate.

Lerman (23) proposed that the strong binding site of Peacocke and Skerrett be such that the dye is intercalated between two base pairs. In this model, the weaker binding site is on the exterior of the DNA molecule. The low angle X-ray diffraction data of Luzzati et al. (25) show a change in the mass per unit length of the proflavine DNA.

R.C. Armstrong et al. (29) studied the interaction of AO and Proflavine with DNA and made an addition to the intercalation binding model. They considered that an intercalated dye cation might associate with a non-intercalated dye cation to produce a spectroscopically distinct bound dimer.

C. Interaction with drugs:

The formation of non-covalently bound nucleic acid-drug complexes produces profound pharmalogical effects by interfering with biological processes in which nucleic acid participates (30,31). The therapeutic importance and the possibility for a detailed chemical understanding of the mechanism of action of these DNA-drug complexes provide sufficient stimulus to develop a rational methodology for optimising the thermodynamics, kinetics, sequence specificity, structural specificity and chemical specificity of these binding ligands.

Zimmer et al. (32) studied the interaction of the basic antibiotics, namely, Streptothricin, Streptomycin, Dihydrostreptomycin, Paromomycin, Kanamycin and Neomycin with DNA using spectrophotometric melting and titration and sedimentation measurements. On increasing the antibiotic concentration precipitation of insoluble DNA- antibiotic

complexes was observed. Solution of DNA-antibiotic complexes are formed at low ionic strength as follows from the stabilization of the DNA secondary structure against thermal denaturation by all antibiotics studied. It was also concluded that at low ionic strength basic antibiotics of amino-glycoside type were bound loosely to the negative charged phosphate groups of the DNA by electrostatic interaction.

Of the known antibiotics, actinomycin D has been the most extensively studied chemical in its reactivity toward DNA. The interest in actinomycin in this regard is attributable to its ability to interfere with the function of DNA, particularly as it relates to the transcription of RNA from a DNA template (33). At low concentrations, the antibiotic selectively turns off RNA synthesis both in vitro and in vivo without inhibiting the synthesis of either DNA or protein (34). At higher concentrations, synthesis of DNA may also be blocked. The binding of actinomycin to DNA is known to be dependent on the proportion of guanine present and upon helical structure (35). It is, however, specific for DNA as evidenced by the inability of actinomycin to bind either to natural or synthetic double helical polyribo nucleotides, even to those containing high proportions of guanine (35).

The increase in optical rotatory dispersion when actinomycin - DNA complex is formed showed that the molecular asymmetry of the actinomycin molecule was enhanced. This observation was in contrast to the observations made upon acridine - DNA complexes, where the optical activity was decreased with increasing DNA concentration. This showed that the optical activity of the actinomycin -DNA complex does not result from interactions between drug molecules specifically oriented by the DNA and the drug. The fixation of the actinomycin on helical DNA imposed a highly specific orientation of both the phenoxazine and the two lactone rings the result of which was the increase of the optical activity of the drug. The optical rotatory dispersion of the weak actinomycin -deoxyguanosine complex could be differentiated from the much firmer actinomycin - DNA complex, differing both in amplitude and shape. This was explained due to the greater steric freedom, which imposed much less constraint to the conformation on the bound actinomycin molecule (36).

Fielding et al. (37) while studying the binding of chloroquine to DNA proposed a model in which the drug stacks into the major groove of DNA by electrostatic interaction between the side chain amino and backbone phosphate groups, and by ring stacking both between adjacent

drug molecules and between the drug and the bases they protrude into the major groove. Although appreciable binding of pentaquine and primaquine with DNA occurred, the binding did not cause a significant change in the transition temperature or the viscosity of native DNA (30). The antimalarial 4-aminoquinoline, chloroquine induced marked changes in both the properties upon binding to native DNA. Interaction of the anti malarial aminoquinolines with nucleic acids and the consequent interference in nucleic acid synthesis and function may be one mode of antimalarial activity of these compounds.

D. Interaction with metal ions:

Nucleic acids and proteins have been known to form specific and /or non-specific complexes with many important metallic ions. These complexes have been demonstrated to play important roles in enzymatic or photosynthetic reaction in vivo. A metal can bind to the phosphate portion, or base portion or the hydroxyl portion of ribose and each base offers a number of sites, the total number of sites would be quite large.

Heavy metal interactions with nucleic acids are considered important because most of them selectively interact with bases. Yamane and Davidson (30) proposed separation of DNA of varying composition utilising the

specificity of Hg (II) for A - T rich and Ag (I) for G - C rich DNA. Gruenwedel and Davidson (40) have also found that Methyl Mercuric Hydroxide denatures DNA and binds selectively to Thymine rich or A and T rich strands depending upon its concentration. It could, therefore, be used for separation complementary strands of DNA. Separation and isolation of DNA strands permit study of the structure of the individual strands and their fate during DNA transfer or replication (41).

Nandi et al. (42) studied the effect of metal ions on the binding of cationic dyes to nucleic acids. Studies with different ions like Hg(II), Ag(I), Cu(II), Mg(II) and Na(I) in systems of nucleic acids of dyes like ethidium bromide, toludine blue, proflavine, acridine orange and methylene blue reveal that certain metals have preferential effect on certain dye-nucleic acid complexes and replace the intercalated dye molecules from their intercalating sites.

Attention has been paid to the specific changes of the DNA secondary structure induced by copper ion (43-52). The fact that copper decreases the melting temperature of the DNA molecule has been attributed to the formation of a complex between copper and the nitrogen bases during heating. Besides the phosphate sites, guanine as well as cytosine(53) have been regarded as preferred binding sites in the formation

of DNA – Cu complexes. The denaturation of DNA at low ionic strength in the presence of copper ions has been shown to be reversible with increasing ionic strength (43,44). These results led to the assumption that copper ion is complexed between guanine and cytosine (43). From NMR and IR studies it was concluded that the copper ion cross links involves N-7 of guanine and N-3 of cytosine as the most probable site of co-ordination (43).

The ability of Zn (II) to bring about the unwinding and rewinding of the DNA double helix under appropriate conditions has been demonstrated (54). The degree of DNA rewinding after heating can be correlated to the increasing order of base binding given as $Mg < Co, Ni < Mn < Zn < Cd < Cu$. Differential UV absorption spectra indicate that Zn^{2+} binding to base sites depends on the G-C content of DNA in contrast to Mg^{2+}, which interacts only with the phosphate groups. Acid titration of DNA in the presence of Zn^{2+} shows that protonation of G-C pairs is suppressed and destabilization toward acid denaturation occurs (55).

While it is possible to bring about unwinding and rewinding of DNA helix in the presence of some metal ions by controlling temperature, cadmium ions prevent the molecules from recombining. This is probably because cadmium ions assisted in breaking the H-bonds by displacing them through co-ordination on heating and preventing them from

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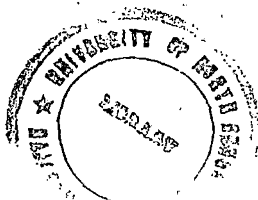

recombining during cooling (56).

Pt (II) selectively reacts with the bases in DNA. Zakharenko and Moshkovskii (57) have proposed that it could be used for studies on the sequence of bases in DNA. It is reported that cis Pt (II) compounds have antitumour activity (58) and their mode of action seems to be through forming interstrand links in a DNA chain (59).

Metal ion interactions are also potentially useful in physical techniques such as X-ray diffraction and electron microscopy (60) . The position of a heavy metal could easily be detected by X-ray in a macromolecule containing only relatively small atoms. For example, methyl mercuric hydroxide (61) which selectively interacts only with Thymine at low concentration could be made use of in the determination of the tertiary structures of t - RNA, which contain a lone thymine molecule.

Heavy atoms scatter electrons well and produce detectable contrasts in electron micrograph. Beer and co-workers and many others (62-65) have studied base selective staining of nucleic acids with heavy metals with a view to determine the base sequences of nucleic acids. Highton and Beer (66) have shown that when polyadenylic acid is stained with gold (III), it becomes faintly visible in electron microscope.

Many major alterations in nucleic acid and protein metabolism are the result of deficiency of metals present in RNA (67). For example, as a consequence of zinc deficiency, the RNA content of Euglena Gracilis is decreased, the amino acids are increased and the DNA content is doubled. The results are attributed to the changes in the conformation of nucleic acids brought about by their complexation with cations.

Metal ions and stability of nucleic acids:

There are many factors that contribute to the stability of ordered polynucleotide structure (68). There are internal forces arising in the case of polynucleotides from interactions of the charge distribution on the bases. This class of interaction can be thought as hydrogen bonding between the bases. A second class of electrostatic forces are those arising from the charges on the phosphate groups. Contribution from solvent also arise from interactions of the nucleic acid charges with the charge distribution of thesolvent molecules.

Metal ions which contribute to disturb the above factors that determine the stability of nucleic acids in solution may produce different effects depending on their role. Thus, a metal ion can act as a counter ion by interacting with the negatively charged phosphate groups and thus stabilize the structure, e.g. Mg (II) (69). It can

destabilize the Watson and Crick double helix by interacting with electron system of the bases i.e., by disturbing the hydrogen bonds e.g. Cu (II) (70,71). In the former case, the melting temperature of DNA increases and in the latter the melting temperature decreases.