CHAPTER 8

CHROMATIN AS A TARGET FOR THE DNA-BINDING ANTICANCER DRUGS

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Abstract: Chemotherapy has been a major approach to treat cancer. Both constituents of chromatin, chromosomal DNA and the associated chromosomal histone proteins are the molecular targets of the anticancer drugs. Small DNA binding ligands, which inhibit enzymatic processes with DNA substrate, are well known in cancer chemotherapy. These drugs inhibit the polymerase and topoisomerase activity. With the advent in the knowledge of chromatin chemistry and biology, attempts have shifted from studies of the structural basis of the association of these drugs or small ligands (with the potential of drugs) with DNA to their association with chromatin and nucleosome. These drugs often inhibit the expression of specific genes leading to a series of biochemical events. An overview will be given about the latest understanding of the molecular basis of their action. We shall restrict to those drugs, synthetic or natural, whose prime cellular targets are so far known to be chromosomal DNA

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The history of modern chemotherapy of cancer dates back to as early as 1946 when [Goodman et al. 1946] produced the first report of clinical results from 67 patients treated with nitrogen mustards for Hodgkin’s disease, lymphosarcoma, and leukemia. From then onwards, the search for newer and better anticancer agents has continued with an ever-increasing pace. Drugs designed vary not only in their sources, chemical compositions and modes of action, but also in their targets. Whereas certain anticancer drugs target nucleic acids, others target specific

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metabolic and signaling enzymes and even microtubules. Unfortunately, the major problem of anticancer drugs is their cytotoxicity. Lack of specificity of these compounds along with their chemically reactive centers augments their cytotoxic effects.

Chromatin is well recognized as a target for anticancer agents, in that both constituents of chromatin – chromosomal DNA and the associated chromosomal proteins have been utilized as drug targets for the past few decades. From the biochemical perspective, drugs have been designed that (1) may block the template property of DNA at the chromatin level, thereby inhibiting the action of replication or transcription machinery [Straney and Crothers, 1987; Gniazdowski and Czyz, 1999]; (2) may inhibit the function of DNA associated proteins such as topoisomerase, DNA methyltransferase, high mobility group of proteins (HMGs), TATA binding proteins (TBPs), histone acetyltransferases (HATs), histone deacetylases (HDACs) [Schäfer and Jung, 2005]; (3) may block the telomere function. All of the above processes lead to cell death.

In this review article we shall mostly confine ourselves to drugs that target the DNA, thereby falling into the first category mentioned above. The following figure depicts the target sites of the DNA-interactive anticancer drugs. Since the discovery of the double helical nature of DNA and the associated central dogma of molecular biology, there has been a plethora of studies to understand the structural basis of recognition of these drugs and the biological consequence of this recognition upon the nucleic acid template under in vitro condition. Interestingly, though many DNA-binding drugs, synthetic or from natural sources, are used to treat various types of cancer after extensive clinical trials, their mode of action inside the normal and neoplastic cells are still not very well known. In many cases there has been no extensive studies aimed at the target validation for these drugs. This is almost certainly due to the imperative social necessity to employ them to alleviate the sufferings and prolong the life expectancy of cancer-afflicted patients before a comprehensive understanding of their mode of action. In the last two or three decades efforts have been made to attain an incisive understanding of their interaction at the cellular level along with the validation of their targets at the level of cellular organelle.

More recently the concept of ADMET profile (Absorption Distribution Metabolism Excretion Toxicity profile) has further streamlined the molecular pharmacology aspects of these drugs with the ultimate objective of providing efficient target directed drugs with least toxicity.

Since chromatin targeted DNA-binding anticancer drugs take advantage of the dynamic nature of chromatin, so for the appreciation of the molecular and structural aspects of chromatin as target for these drugs, a brief introduction to the chromatin structure is essential. The nucleus houses over two meters of DNA, compacted to nearly one hundred thousandth of its dimension by a hierarchical scheme of folding, with an equal mass of proteins. This nucleoprotein complex is called ‘chromatin’ [Widom, 1998; Richmond and Widom, 2000]. The term chromatin structure is open in the sense that it covers a wide range of phenomena and levels
of organization, encompassing the atomic details of nucleosome architecture to the large-scale arrangements of interphase chromosomes that make up the nucleus.

It is now well established that the packaging of DNA in the eukaryotic nucleus, the principal target site of the DNA-binding antibiotics, involves several distinct hierarchical events. The first level of compaction occurs when DNA is wrapped around an octamer of core histones to form the repeating subunit nucleosome. An additional stretch of linker DNA connects adjacent nucleosomes. The linker DNA and nucleosome core are associated with linker histone H1 (Kornberg and Lorch, 1999). Each of the four core histones comprises a structured central domain, an amino-terminal tail, and in some cases a carboxyterminal tail (H2A and H3) (Van Holde et al., 1995). N and C terminal tail sequences contribute nearly 28% of their mass. The high-resolution structure of the nucleosome core particle (Luger et al., 1997, 2006; Davey et al., 2002) has shown that two turns of the DNA superhelix in the nucleosome core are arranged in such a way that they create sufficient gaps for the amino-terminal tails of both H2B and H3 histones to pass through to the outside of the core particle. H2A and H4 tails pass across the superhelix on the flat faces of the particle to the outside as well. Histone tails are exposed. They are posttranslationally modified during eukaryotic transcription. The modifications alter the charge distribution pattern on the N-terminal tails. It is an essential
requirement for the eukaryotic gene expression (Workman and Kingston, 1998). Along with sliding of the core particles along the linker (chromatin remodeling) the tail domains also play an important role in the access of transcription factors and other DNA-binding proteins to the nucleosomal templates. These tails have extreme biological significance as they play a vital role in regulating the nucleosomal spacing (i.e. in chromatin remodeling) and the state of condensation of chromatin. The inter-conversion of fluid interphase chromatin (Gasser, 2002) from transcriptionally blocked to transcriptionally active states is possibly tightly regulated by reversible modifications of selective amino acid residues in the tail parts of the histones, of other associated proteins, and of DNA (Jenuwein and Allis, 2001; Berger, 2002; Urnov, 2002). Trypsinized nucleosomes leading to chopped N-terminal tails have been found to be more accessible to transcription factors such as TFIIIA, DNase cleavage, and other sequence-specific DNA binding proteins (Ausio et al., 1989; Juan et al., 1994; Garcia-Ramirez et al., 1992). Thus the tails could impede access of proteins and DNA-binding drugs to nucleosomal DNA (Workman and Kingston, 1998).

In fact, a nucleosome is a dynamic system that can take up many subtly different conformations and substructures at all organizational levels and thereby allow chromosomal DNA to be accessed while simultaneously being packaged into highly condensed chromosomal structures. The resulting accessibility of DNA has fundamental implications in all physiological processes that use DNA as substrate, such as transcription, replication, DNA repair and recombination. Nucleosome dynamics may be intrinsic or may be due to the action of protein-mediated pathways. Using stopped-flow fluorescence resonance energy transfer, (Li and Widom, 2004), have shown that the ends of nucleosomal DNA unwrap and rewrap rapidly (within 50–250 ms) from the histone surface. This timescale is sufficient to permit transcription factor and anti-cancer drug binding to nucleosomal DNA during its partially unwrapped stage and to allow access of the transcription machinery. Like the nucleosomes, the higher level of organization, the chromatin fiber is also capable of assembling and disassembling of superstructures by both intrinsic and protein mediated mechanisms. A recent report (Bucceri et al., 2006) using yeast DNA repair by photolyase as a model system to monitor the rapid accessibility of nucleosomal DNA in yeast on a second time scale suggests that spontaneous unwrapping of nucleosomes rather than histone dissociation or chromatin remodeling provides DNA access to transcription factors, enzymes and probably small DNA-binding ligands. Thus, study of the kinetics might be relevant to understand the molecular approach of the anticancer drugs towards chromosomal DNA (Sischka et al., 2005).

1. DNA AS TARGET FOR ANTICANCER DRUGS

The structure of the DNA molecule makes it an extremely versatile target for anticancer drugs. It has a negatively charged phosphate backbone, hydrogen accepting and donating functional groups from the bases in the major and minor grooves, phosphate oxygen atoms and aromatic hydrophobic components of the
bases able to promote van der Waals interactions. DNA is polymorphic, and has been observed in several different conformations (e.g. A, B, Z etc.) that differ in the geometry of the double helix, including the depth and width of major and minor grooves [Saenger, 1984]. The groove shapes and hydration patterns in a particular conformation are also sequence dependent to certain extent. Along with these, the molecule of DNA also has several levels of structural organization. The most basic level is the primary base sequence. The roll, tilt and twist from one base pair to the next in the double strand depend on the base, thereby giving rise to sequence dependent microheterogeneity in the DNA backbone. The bending of the backbone arising from stretches of A-bases is probably the extreme example of such feature. The small and large DNA binding ligands recognizes this structural variability along the double helix. It leads to sequence specific recognition. Higher levels of organization include the DNA secondary structures, such as hairpins, and Holliday junctions, triple helices and DNA quadruplexes. All of the above features have been exhaustively exploited in designing anticancer drugs.

2. MOLECULAR AND STRUCTURAL BASIS OF DNA-BINDING ANTICANCER DRUG ACTION

Anticancer drugs targeted to DNA bind to the same by either non-covalent forces or covalent interactions. The primary and most important step in the functioning of a DNA targeting drug is the base-specific recognition of DNA by the drug. This initial process of recognition is driven by size and shape complementarities between the drug and its DNA – binding site. Non-covalent forces such as the coulombic force, van der Waals interactions, hydrogen bonding and stacking interaction stabilize the primary complex formed. For drugs that interact with DNA via non-covalent forces, the active primary complex is responsible for its intracellular function; whereas, for drugs that interact with DNA via covalent forces, the covalent bond formation is preceded by the non-covalent reversible drug-DNA association that determines the specificity, because it positions the reactive part of the drug along the target site in the DNA. The general mechanism of action of DNA targeting anti-cancer drugs is shown below:
3. GENERAL APPROACHES TO STUDY ASSOCIATION OF ANTICANCER DRUGS WITH DNA AT THE CHROMATIN LEVEL

There have been two basic approaches. First one involves isolation of the chromatin and nucleosome from the healthy and diseased cell line. The second approach is the reconstitution of the model target such as nucleosome followed by the association with the drug(s). The second approach has been extensively employed to identify the binding site in the protein-nucleic acid complex. A pre-knowledge about the components and their arrangements in the reconstituted system sometime makes it the preferred approach. Different biophysical, biochemical and genetic techniques have been employed to understand the mode of association and the effect of the drugs upon chromatin/nucleosome structure and function.

3.1. Non-covalently Interacting Anticancer Drugs

There are two principal modes by which drugs can bind non-covalently to DNA: intercalation and external groove binding.

3.1.1. Intercalators

Intercalating drugs consist of planar heteroaromatic ring systems, which have the potential to be inserted between two adjacent base pairs in a helix. The complex is stabilized by $\pi$-$\pi$ stacking interactions between the drug molecule and the DNA base pair accommodating the ring. In general, intercalating drugs are characterized by the presence of one or more planar aromatic rings, parallely oriented and/or separated by linker regions of varying lengths. Structure of a typical intercalator, Actinomycin, is given below:

![Actinomycin D](image)

Intercalation results in structural perturbations in DNA so as to lengthen it by $\sim 1$ bp spacing (i.e. $\sim 3.4$ Å) and there is also some unwinding of the DNA.
helix. As a result, the DNA adopts a C3′-endo –(5′, 3′)-C2′-endo mixed puckering conformation around the intercalation site. Intercalators are known to bind the DNA via both the minor and major grooves.

Mono-alkylators (e.g. daunomycin and related anthracycline drugs) contain a planar chromophore group of 4 fused six-membered rings, substituted at different loci and containing an amino sugar residue. The chromophores are inserted in a ‘head-on’ fashion with the long axis of the aglycone moiety oriented at right angles to the long axis of adjacent base pairs. One of the cyclohexane rings protrudes into the minor groove leaving the amino sugar in the minor groove.

The anthracycline antibiotic daunomycin and its derivatives are antitumor drugs widely used in cancer chemotherapy to treat myelogenous leukemia and solid tumors (Gianni et al., 1983, Myers et al., 1988, Carrion et al., 2004). Cellular DNA is the primary target for these drugs. Daunomycin acts by intercalation of its planar aglycon chromophore between DNA base pairs, and its amino sugar ring lies in the minor groove of the double helix (Barcello et al., 1988, Chaires et al., 1982, 1990, Chaires, 1996) leading to inhibition of replication and transcription.

These drugs were few of those whose association with chromatin and nucleosome was studied (Mizuno et al., 1975, Zunino et al., 1980, Chaires et al., 1982, 1983, 1990, Ganguli et al., 1983, Terasaki et al., 1984, Fritzsche et al., 1987, Simpkins et al., 1984, Chaires, 1996, Chakrabarti et al., 1996) when the molecular nature of the chromatin was established. The first detailed structural study (Chaires et al., 1982) employed equilibrium, hydrodynamic, and electric dichroism studies of the complex of daunomycin with H1-depleted 175 bp nucleosomes, along with some comparative data for ethidium. The results showed that in contrast to ethidium, daunomycin binding to nucleosomes is strongly reduced relative to the affinity for free DNA. The salt concentration dependence of the binding constant suggested that approximately one Na⁺ ion is released from both nucleosomes and free DNA upon daunomycin binding. The early melting transition of nucleosomes is preferentially stabilized by low levels of both drugs, but more markedly by ethidium.
Ethidium also stabilizes the second nucleosome melting transition, but daunomycin does not. Dichroism and rotational relaxation time measurements indicated that daunomycin unfolds nucleosomes. The data favored an unfolded structure in which the nucleosome elongates along the DNA superhelical axis. Higher concentration of the drug at a ratio more than 0.15 per DNA base pair promotes nucleosome aggregation. The authors suggested that the activity of daunomycin as an antitumor agent arises out of its special intercalation geometry that strongly prefers free DNA regions to the bent helices found in nucleosomes and chromatin. As a result of this preference there is an increased local concentration of the drug in the genetically active regions of nuclear DNA in which nucleosomal structure is less prevalent. Presumably the abundance of such regions in tumor cells makes them especially sensitive to daunomycin. There have been many other reports, which aimed to quantitatively define the affinity of daunomycin and related drugs with chromatin and nucleosome (van Helden and Wild, 1982). Fluorescent probes were used to examine the effect of adriamycin on supercoiled DNA and calf thymus nucleosomes.

Equilibrium dialysis and sedimentation velocity analysis (Rabbani et al., 1999) were also employed to characterize the binding of the antitumor drug daunomycin to chicken erythrocyte chromatin before and after depletion of linker histones and to its constitutive DNA under several ionic strengths (5, 25, and 75 mM NaCl). The equilibrium dialysis experiments showed that the drug binds cooperatively to both the chromatin fractions and to the DNA counterpart within the range of ionic strength used in this study. A significant decrease in the binding affinity was reported at 75 mM NaCl. Binding of daunomycin to DNA does not significantly affect the sedimentation coefficient of the molecule in contrast to the effect noticed when the drug binds to chromatin and to its linker histone-depleted counterpart. In these instances, preferential binding of the drug to the linker DNA regions induces an unfolding of the chromatin fiber that is followed by aggregation. Transient electrical dichroism studies also supported the condensation of the chromatin fiber.

Earlier reports showed that treatment of adriamycin to Novikoff hepatoma nuclei enhanced the DNA fragmentation by micrococcal nuclease (Ross et al., 1978; Gyapay et al., 1985). Exposure of murine thymocytes to doxorubicin triggered rapid DNA degradation, as indicated by the appearance of a major subdiploid population demonstrated by DNA flow cytometry (Zaleskis et al., 1994). Electron microscopic comparison of samples with large subdiploid populations versus those with little or no such subset revealed significantly more cells with the characteristic features of apoptosis. Daunorubicin – induced internucleosomal DNA fragmentation was also reported in acute myeloid cell lines. A plausible mechanism of this DNA-degradation may be the chemical activation of the drug by an intracellular redox system, which leads to the production of active oxygen species (Akman et al., 1992; Quillet-Mary et al., 1996). There are reports of DNA base modifications induced in isolated human chromatin by NADH dehydrogenase- catalyzed reduction of doxorubicin. Induction of apoptosis via DNA cleavage has now been accepted as one of the actions of the drug and its related compounds.
In a recent study [Nair et al., 2005] the transcription inhibitory effect upon a particular gene by the drug and the related compound WP361 was demonstrated with the urokinase receptor (uPAR). It is transcriptionally activated in several cancers and contributes to tumor progression by promoting cell migration and proteolysis. The bisanthracycline (WP631) represses uPAR gene expression and cell migration of RKO colon cancer cells by interfering with transcription factor binding to a chromatin-accessible -148/-124 promoter region. It was suggested from DNaseI hypersensitivity, genomic footprinting, and chromatin immunoprecipitation experiments that chromatinized -148/-124 regulatory region of the uPAR promoter is accessible to small molecules and that WP631, which disrupts the interaction of DNA binding proteins with this region, diminishes uPAR expression and function.

Recently, a novel observation has raised question about the DNA as the only target of daunomycin at the chromatin level inside the cell. It was shown using a compositionally defined chicken erythrocyte chromatin fraction that the drug is also able to interact with chromatin-bound linker histones without any noticeable binding to core histones [Rabbani et al., 2004]. The drug can interact in an equal fashion with both histone H1 and H5 and to a greater extent with core histones H3/H4 and H2A/H2B as free proteins in solution. Binding of daunomycin to linker histones appears to primarily involve the trypsin-resistant (winged-helix) domain of these proteins.

Since anthracycline antibiotics play an important role in cancer chemotherapy, the necessity for an improvement of their therapeutic index has enthused an ongoing search for anthracycline analogues with improved pharmacological properties. Cardiac toxicity is a negative feature in their use as drugs. Along with DNA, the DNA topoisomerase II was recognised to be another prime cellular target. Several anthracyclines interfere with topoisomerase II functions by stabilizing a reaction intermediate in which DNA strands are cut and covalently linked to tyrosine residues of the enzyme [Binaschi et al., 2001]. Investigations on the sequence specificity of doxorubicin in vitro and in nuclear chromatin of living cell have led to a molecular model of drug receptor on the topoisomerase II-DNA complex. Anthracyclines are likely placed at the interface between the DNA cleavage site and the active site of the enzyme, forming a DNA-drug-enzyme ternary complex. Moreover, a quite detailed structure-function relationship has been established for anthracyclines. These studies have revealed that (a) drug intercalation is necessary but not sufficient for topoisomerase II poisoning; (b) the deletion of the 4-methoxy and 3′-amino substituents results in an enhancement of the drug activity; and (c) the 3′ substituent of the sugar moiety markedly influences the sequence selectivity of anthracycline-stimulated DNA cleavage. These relationships have been exploited during the last decade by several groups in the search for new anthracycline drugs with lower side effects and higher activity against resistant cancer cells. A scheme utilizing the DNA replication in Xenopus egg extract system to simultaneously evaluate DNA-interacting drugs as potential anti-cancer agents and gain insight into the mechanism of drug action has been proposed [Kumar et al., 2004].
the authors this system might be useful for large scale screening of DNA-interacting chemotherapeutic compounds in cellular milieu.

3.1.2. **External groove binders**

The grooves (minor and major) of the DNA molecule are of immense structural and chemical significance. The difference in the pattern of hydrogen bond donors and acceptors in the minor and major grooves allow for sequence readout by these group of ligands. Thus, the external groove binders search for the shape and size of DNA grooves, which are specifically recognized by them. The molecules may even induce structural changes in the DNA duplex for better structural complementarity between drug and DNA. The drug can also alter its own structure by ‘induced fit’ type of mechanism (Spolar et al. 1994, Chaires 1997).

3.1.2.1. **Minor groove binders.** Minor groove binders are typically composed of several aromatic rings such as pyrrole, furan, or benzene that are connected by bonds with torsional freedom. In all complexes of minor groove binders with DNA, the drug displaces the ‘spine of hydration’ and fits snugly into the minor groove. These drugs generally adopt a characteristic curved shape isohelical with the target groove. Van der Waals interactions, hydrophobic forces, and hydrogen bonds stabilize the resulting DNA–drug complex.

Minor groove binders have a binding preference for A•T base pairs, probably because there are favorable hydrophobic contacts between the adenine C2 hydrogen atoms and the aromatic rings in the drug. Besides, the A•T base pairs possess hydrogen bond acceptors such as C2 carbonyl oxygen of thymine or N3 nitrogen of adenine that can readily interact with any hydrogen bond donors. Although similar hydrogen bonding opportunities exist at G•C base pairs, the amino group of guanine may be a steric block to hydrogen bonds involving either guanine N3 or cytosine C2 atoms. The favorable curvature of some tailor made ligands can overcome this and can bind to G•C base pair as well.

However, with the discovery of a new motif – the side-by-side pyrrole-imidazole amino acid pairing, or 2:1 (ligand: DNA) complex, it is possible to distinguish all four Watson-Crick base pairs (G•C, C•G, A•T and T•A) (Dervan et al. 1999). Using the 2:1 motif for recognition of the minor groove, a 6-bp sequence can be read even at subnanomolar concentrations (Tranger et al. 1996). Minor groove binding drugs are comprehensively reviewed in Zimmer (1986).

**Aureolic acid group of antibiotics:** We have given below an extended summary of the work done with this group of antibiotics, because we have been actively involved in understanding the mode of action of these DNA-binding antibiotics. The summary also illustrates the model example of how different biophysical and biochemical approaches were undertaken to characterize the association of these drugs with chromosomal DNA and the sequential effect of this binding upon the chromatin structure.
Aureolic acid group of antitumor antibiotics, mithramycin (MTR) and chromomycin A₃ (CHR), from *Streptomyces plicatus* and *Streptomyces griseus*, respectively, are clinically employed for testicular carcinoma and Paget’s disease [Calabresi and Chabner, 1991]. With a gross structural similarity, MTR and CHR have difference in the nature of sugar rings connected to aglycone ring via O-glycosidic bond. Antitumor properties of MTR and CHR in experimental tumors have been ascribed to their inhibitory roles in replication and transcription processes during macro molecular biosyntheses [Wohltet al., 1999]. They inhibit the expression of proto-oncogenes like *c-myc*, that have an important role in the regulation of cell proliferation and differentiation [Synder et al., 1991]. A potential use of these antibiotics is as neurological therapeutics for the treatment of diseases associated with aberrant activation of apoptosis [Chatterjee et al., 2001].

Prime cellular target of these antibiotics is DNA. A bivalent cation such as Mg²⁺ is an essential requirement for their association with DNA at and above physiological pH [Dimaraco et al., 1975]. We have shown that in absence of DNA these antibiotics
bind to Mg\(^{2+}\) and form two different types of complexes, complex I (1:1 in terms of antibiotic : Mg\(^{2+}\)) and complex II (2:1 in terms of antibiotic : Mg\(^{2+}\)) (Dimaraco et al., 1975; Aich and Dasgupta, 1990; Aich et al., 1992a, b). The equilibria associated with the reversible association along with the affinity constants are given below:

\[
\text{Drug} + \text{Mg}^{2+} \rightleftharpoons \text{Drug : Mg}^{2+} \quad (\text{Complex I})
\]

\[
\text{Drug : Mg}^{2+} + \text{Drug} \rightleftharpoons (\text{Drug})_2 : \text{Mg}^{2+} \quad (\text{Complex II})
\]

| Antibiotic     | Type of complex | Apparent association constant (M\(^{-1}\)) | Stoichiometry |
|----------------|----------------|------------------------------------------|---------------|
| Mithramycin    | I              | \(1.8 \times 10^4\)                      | 1.1           |
|                | II             | \(1.6 \times 10^3\)                      | 2:1           |
| Chromomycin    | I              | \(1.9 \times 10^4\)                      | 1:1           |
|                | II             | \(5.8 \times 10^2\)                      | 2:1           |

Since the second complex contains two molecules of drug, therefore, we refer to it as dimer complex. Keeping in view the milli molar concentration of the metal ion present in the cell, possibility of the formation of dimer complex is more under \textit{in vivo} conditions. However, in certain cases of cancer the metal ion concentration goes down to micro molar range. Under these unusual conditions, complex I is formed. Recently we have shown that mithramycin forms only dimer complex with Zn\(^{2+}\), another metal ion playing an important role as cofactor in many enzymes and DNA binding proteins like transcription factors.

These complexes are DNA binding ligands at and above physiological pH and bind to DNA via minor groove (Dimaraco et al., 1975; Cons and Fox, 1989; Aich and Dasgupta, 1990; Aich et al., 1992a, b; Aich and Dasgupta, 1995). It was established in our laboratory from spectroscopic and thermodynamic studies that the modes of binding of the two ligands with natural DNA, polynucleotides and oligomeric duplexes are different (Dimaraco et al., 1975; Aich and Dasgupta, 1990; Aich et al., 1992a, b; Aich and Dasgupta, 1995). We also illustrated the role of DNA minor groove size and the accessibility of the 2-amino group in the minor groove of guanosine in drug–DNA interaction using designed nucleotide sequences (Aich and Dasgupta, 1995; Majee et al., 1997; Chakrabarti et al., 2000–2001, 2002). Detailed NMR studies from other laboratories have helped to understand how the bulky complex II is accommodated at the cost of a considerable widening of the minor groove in B-DNA type structure (Keniry et al., 1993; Sastry et al., 1995). In our laboratory we have shown from a detailed thermodynamic analysis of the association of the dimer complex with different DNAs, natural and oligonucleotides, with defined sequences, that B to A type transition in the groove leads to a positive
change in enthalpy. This is compensated by a positive change in entropy arising from
the release of bound water in the minor groove (Aich and Dasgupta, 1995; Majee et al., 1997; Chakrabarti et al., 2000–2001, 2002). Sugars present in the antibiotics
play a significant role during the association with nucleic acids (Majee et al., 1997; Chakrabarti et al., 2000–2001, 2002). Absence of substituents like acetoxy group
in the sugar moieties of mithramycin imparts conformational flexibility of greater
degree than chromomycin. Therefore, the drug dimer of mithramycin has been
found to have a better conformational plasticity than chromomycin when it binds
to the minor groove of DNA.

Two approaches are usually taken to study the effect of the association of DNA
binding anticancer drugs upon the structure of chromatin and nucleosome. The
first one is reconstitution of the model nucleosome in the presence of the drugs.
This has been reported earlier in the case of mithramycin (Fox and Cons, 1993;
Carpenter et al., 1993). In our laboratory, so far we have taken the second approach
of comparing the association of the anticancer drugs with isolated chromatin at
various levels.

Spectroscopic studies such as absorbance, fluorescence and CD have demon-
strated directly the association of the above complexes with chromatin and its
components under different conditions. We made a comparison of the affinity
parameters, apparent dissociation constant and binding stoichiometry, in order to
throw light on the nature of the association. The reduced binding affinity of the
antibiotic: Mg\(^{2+}\) complexes to nucleosome or chromatin might be a consequence of
bending of double helix or, additionally, of unusual DNA conformations induced
by the histone binding. Alternatively, one can say that histone-DNA contacts and
N-terminal tail domains of individual core proteins in nucleosome core particle
reduce the accessibility of nucleosomal DNA to antibiotic: Mg\(^{2+}\) complexes. In the
chromatin, presence of linker H1 further reduces the binding potential of the ligand.
Structural and thermodynamic studies are mutually complementary and both are
necessary for understanding molecular basis of the binding process. Therefore we
made a comparison of the associated energetics. Linear van’t Hoff plot characteristic
of the association processes reported here implies the small value of heat capacity
changes. It can be attributed to the absence of any major conformational alterations
involving histones and DNA. In developing correlation between thermodynamic and
structural data, it is essential to consider that enthalpy-entropy compensation leads
to the observed free energy change. We resolved the enthalpy contributions from
three plausible sources: (a) the molecular interactions between bound ligand and
polymer binding site, (b) conformational changes in either DNA or drug molecule
or the complex.

Antibiotic:Mg\(^{2+}\) complex induced alteration in the ultrastructural changes in the
native and H1 depleted chromatin were monitored by thermal melting analysis,
polyacrylamide gel mobility assay, dynamic light scattering experiments and
transmission electron microscopic studies. Micrococcal nuclease digestion is the
biochemical probe to assess the accessibility of the antibiotic: Mg\(^{2+}\) complexes to
nucleosomal DNA.
The antibiotic: Mg$$^{2+}$$ complexes bind to nucleosomal DNA in presence of histones none of which bind to them. Role of the histones is probably limited to steric hindrance for the access of these complexes to the minor groove of DNA, though the possibility of noncovalent interactions between the DNA bound ligand and the potential hydrogen bonding site(s) in the histones can not be overlooked. In general we have noticed that the presence of histones leads to an increase in the dissociation constant and binding site size compared to naked DNA. Further increase in these parameters for nucleosome core particle provides indirect support that they bind to both core and linker DNA. Nuclease digestion pattern of the chromatin in presence of the antibiotics also favors the above proposition. Presence of the antibiotics reduces the accessibility of the nuclease to the potential cleavage sites in the linker region. This observation implies that presence of the anticancer drugs bound to the chromatin is a potential obstruction for the entry of the transcription factor(s) at the target promoter sites in the gene. Stabilization of the chromatin structure as indicated from the increase in transition temperatures as a sequel to the binding of the ligands would make the RNA polymerase induced opening of the duplex
energetically costly during transcription. Furthermore, during nucleosome tracking, another key step in eukaryotic transcription, histone-DNA contacts in the nucleosome region need to be ruptured.

Depletion of histone H1 after covalent modification from chromatin is a key step in eukaryotic transcription (Lee et al., 1993; Juan et al., 1994; Rice and Allis, 2001). A comparison of the association of the antibiotic: Mg\(^{2+}\) complexes with the normal and H1 depleted chromatin suggests that smaller ligands, like anticancer drugs, have better accessibility for H1 depleted chromatin compared to native chromatin. H1 depleted chromatin is also more prone to aggregation upon association with the complex I of the antibiotic: Mg\(^{2+}\) complexes. It is also less accessible to micrococcal nuclease. We propose that H1 depleted chromatin is a better target of these antibiotics compared to native chromatin. This observation is particularly significant in case of neoplastic cells where most of the cell nuclei are transcriptionally active, and, therefore, contain H1 depleted chromatin.

We modified the nucleosome by chopping the N-terminal tails sticking out of the core particle. From a scrutiny of the spectroscopic features of two bound complexes (for mithramycin and chromomycin) and comparison of the binding and associated thermodynamic parameters, we noticed the following features. N-terminal and intact and chopped core particles interact differentially with the same antibiotic: Mg\(^{2+}\) complex. Tryptic removal of the N-terminals tail domains of core histones enhances the binding potential and access of the antibiotic: Mg\(^{2+}\) complexes to the nucleosomal DNA. The association of the DNA-binding anticancer drugs with nucleosome core particle leads to a slow release of the nucleosomal DNA. Such disruption of histone-DNA interaction might be one of the mechanisms of the transcription inhibitory potential of these drugs. Compared to the N-terminal intact nucleosome, the N-terminal chopped nucleosome is more susceptible to disruption (shown in a carton below). Release of free DNA has also been reported when another groove binder DAPI interacts with reconstituted nucleosome (Fitzgerald and Anderson, 1999).

We extended this study to include daunomycin, which is widely used as an anticancer drug, in order to examine whether mode of binding to DNA influences the association to normal and N-terminal chopped nucleosome. The extent of the above effects is more pronounced in case of the intercalator, daunomycin. N-terminal tail domains protect the eukaryotic genome from external agents, such as anticancer drugs and the degree of protection is dependent upon the mode of binding to DNA. Although N-terminal tails are structureless entities of the nucleosome (Van Holde et al., 1995), our studies indicate that they have a part in maintaining the structural integrity of the nucleosome. The DNA release has been noted for other drugs also (unpublished observations from our laboratory) and may be a general feature at least with mononucleosome.

Notwithstanding the importance of in-depth analysis of the structural features of the association of the anti-gene transcription inhibitors with DNA, it is clear that structural studies on the association of these compounds with chromatin and its components like nucleosome and H1 histone under different conditions are essential
to get a molecular picture of how they function in vivo. To the best of our knowledge very few extensive structural studies at the level of chromatin as presented above have yet been done with any other anticancer drugs (or synthetic ligand) working via the inhibition of transcription \cite{Gottesfeld et al. 2002}. It is now well known that chromatin of the cell under neoplastic conditions is highly transcriptionally active. Even in a normal cell the process of transcription is dynamic in terms of the chromatin structure. Remodeling of the chromatin is a key factor in this process \cite{Tsukiyama and Wu 1997, Kadam and Emerson 2002}. Our future goal is to ascertain the molecular role of these small transcription inhibitors in this dynamic process.

Distamycin and other minor groove binders of the same class: In order to detect the binding loci of this class of groove binders in the nucleosomal DNA, hydroxyl radical and DNase I footprinting studies were carried out on the complexes of four AT-selective minor groove binding ligands (Hoechst, distamycin, netropsin and berenil) with DNA fragments which have been reconstituted with nucleosome core particles \cite{Brown and Fox 1996}. Hydroxyl radical footprints of reconstituted tyrT DNA show that all four ligands induce changes in the phased cleavage pattern, consistent with the suggestion that they cause the DNA to rotate by 180° on the nucleosome surface. Regions to which the ligands are bound are turned away from the protein surface, thereby minimising electrostatic repulsion between the cationic charges on the ligand and protein.

In one of the earlier reports hydroxyl radical footprinting was employed to analyze the interaction of distamycin and actinomycin with the 5s ribosomal RNA genes of Xenopus \cite{Churchill et al. 1990}. The two drugs showed different hydroxyl radical footprints. Distamycin gives a conventional (albeit high-resolution) footprint, while actinomycin does not protect DNA from hydroxyl radical attack, but instead induces
discrete sites of hyper reactivity. The results suggest that the shape of the DNA rather than the specific sequences leads to the recognition by distamycin. Similar trends were noticed from the footprinting studies of the reconstitution of two fragments of Xenopus borealis DNA 135 and 189 base-pairs long with chicken erythrocyte histones, after incubation with echinomycin (a bisintercalating antitumour antibiotic) or distamycin [Low et al., 1986, Portugal and Waring, 1987]. Controlled digestion of these defined sequence core particles using DNAase I revealed new cleavage products, indicative of a change in orientation of the DNA molecule on the surface of the nucleosome. This new rotational setting of DNA within the core particle appears to be practically independent of DNA sequence [Brown and Fox, 1996]. This study has shown that minor groove binding ligands like distamycin, netropsin and berenil alter the rotational positioning of DNA fragments on nucleosome core particles.
Combilexins are a group of synthetic DNA ligands having a sequence-specific minor groove-binding element combined with an intercalating chromophore, which stabilizes the DNA complex and can interfere with topoisomerases (Pindur et al., 2005 and related references therein). Collectively, the structural and kinetic data concur that the conjugate threads through the DNA double helix so that its acridine chromophore could intercalate, leaving the netropsin moiety and the methanesulfonanilino group positioned within the minor and major grooves of the double helix, respectively. The hybrid maintains the AT selectivity conferred by the netropsin moiety. The threading-type intercalation process, evidenced by stopped-flow measurements, is affected when the DNA is wrapped around histones. The composite drug can bind to both the DNA linker segments and the nucleosomal cores in chromatin. In contrast to its constituents, it antagonizes the salt-induced condensation of chromatin.

The (A•T)-selective recognition of these group of DNA binding ligands have been used to selectively block these regions during the experiments to understand the activation or repression of a gene by transcription factor. One such typical example is the following report (Kas et al., 1989, 1993). Scaffold-associated regions (SARs) are A+T-rich sequences defined by their specific interaction with the nuclear scaffold. The interaction of distamycin with SAR sequences leads to a complete suppression of binding to either scaffolds or histone H1, implying that (dA.dT)$_n$ tracts play a direct role in mediating these specific interactions and that histone H1 and nuclear scaffold proteins may recognize a characteristic minor groove width or conformation. The effect of distamycin on these specific DNA–protein interactions in vitro also proposes that binding of SARs to the nuclear scaffold and SAR-dependent nucleation of H1 assembly might be important targets of the drug in vivo (Sumer et al., 2004).

In fact much progress has been made in recent years in developing small molecules that target the minor groove of DNA. Synthesis of molecules that recognize specific DNA sequences with affinities comparable to those of eukaryotic transcription factors is a major stride in this direction. This makes it feasible to modulate or inhibit DNA/protein interactions in vivo, a major step towards the development of general strategies of anti-gene therapy. Examples from anti-parasitic drugs also suggest that synthetic molecules can affect a variety of cellular fractions crucial to cell viability by more generally targeting vast portions of genomes based on their biased base composition. Approaches based on selective interactions with broad genomic targets such as satellite repeats, essential for cellular proliferation employ synthetic polynucleotides or diamidines that bind the DNA minor groove. These highly selective agents are capable of interfering with specific protein/DNA interactions that occur in A+T-rich repeated sequences that constitute a significant portion of eukaryotic genomes. The satellite localization of cellular proteins that bind the minor groove of DNA via domains such as the AT hook motif is highly sensitive to these molecules (Susbielle et al., 2005).
3.1.2.2. **Major groove binders.** Mainly proteins recognize the major grooves of DNA. Non-peptidyl compounds have a tendency to bind to the minor groove, potentially allowing simultaneous major groove recognition by proteins. However, oligomers called triplex forming oligonucleotides (TFOs) (Thuong and Hélène, 1993) can bind to polypurine–polypyrimidine duplex sequences in the major groove to form hydrogen bond with bases on the purine strand. TFOs bind within the existing major groove of DNA and the orientation of the third strand relative to the duplex is dependent on the sequence.

Peptide nucleic acids (PNAs with a peptide like backbone) also bind via the major groove (Nielsen, 1999). PNAs form a triplex (Lohse et al., 1999), which then result in the displacement of the non-complementary oligopyrimidine DNA strand. This has been extensively reviewed by Hurley (2002).

3.2. **Covalently Interacting Anticancer Drugs**

Anticancer drugs that interact with DNA covalently do so by a primary non-covalent interaction, which is immediately followed by covalent interaction with either the phosphate backbone, or DNA bases, or deoxyribose sugar moieties. Covalent interactions lead to any of the following effects: cleavage of DNA strand, cross-linking, alkylation and adduct formation and base release. These events are mutually interconnected. Mono-alkylating agents alkylate the nucleophilic sites within the double helix. Bifunctional alkylating agents cross-link the two strands of DNA and as a result, the integrity of DNA, as a template is lost.

3.2.1. **DNA adduct forming or alkylating agents**

Alkylating agents were the first drugs to be used to treat highly proliferating cancers. They are mainly of two types: the relatively non-specific nitrogen mustards and the even less selective nitrosoureas.

Nitrogen mustards form strong electrophiles through the formation of carbonium ion intermediates. Most common sites of attack are the N7 atoms of adenine and guanine bases in the major groove. N2 and N3 atoms are the other potential sites. Chloroethyl side chains are the main structural features of nitrogen mustards such as Chlorambucil, Melphalan, and Cyclophosphamide. Chlorine is a good leaving group, therefore, it facilitates nucleophilic attack of nitrogen to form an imminium ion in a strained ring system. This readily undergoes alkylation at N7 of guanine in the major groove to form a monoalkylation product. Bifunctional alkylating agents can undergo a second cyclization of the second side chain and form a covalent bond with another nucleophilic group, possibly an N7 of another guanine or some other nucleophilic moiety. This results in the cross-linking of the two complementary strands of DNA, primarily at 5’GpUC sequences (Hansson et al., 1987). Cyclophosphamide requires activation by cellular mixed function oxidases. It is a non-specific pro-drug of the active metabolite phosphoramidate mustard and the most widely used alkylating agent.
Nitrosoureas encompass a class of compounds that breakdown to very unstable intermediates leading to indiscriminate reactions. Among this class are included Streptozotocin, which is used in the treatment of Hodgkin’s disease (Schein et al., 1974), triazenes such as Decarbazine, widely used for malignant melanoma (Cohen et al., 1998), and the more recent Temozolomide, used for gliomas (Friedman et al., 2000). Temozolomide is a monoalkylating drug that methylates guanine residues in DNA following a DNA facilitated rearrangement. Figure shows the chemical structures of some DNA alkylation agents.

3.3. DNA Cleaving Anticancer Drugs

This class consists of potent antitumor agents that mainly exhibit a radical mechanism. Among the members of this class, esperamicin, calicheamicin, dynemicin and neocarzinostatin function by an endiynne mechanism. They undergo an inducible chloroaromatization to an aryl or indenyl diradical, which abstracts hydrogens from proximate deoxyribosyl sites, leading to DNA scission (Smith et al., 1994). Bleomycin, on the contrary, has a different mechanism of action. The amino terminal tripeptide of Bleomycin molecule seemingly intercalates between guanine-cytosine base pairs of DNA. The opposite end of the Bleomycin peptide binds Fe(II) and serves as a ferrous oxidase, able to catalyze the reduction of molecular oxygen to superoxide or hydroxyl radicals responsible for DNA strand scission (Takeshita et al., 1978; Giloni et al., 1981). Recently, a new class of drugs is being developed – pyrazolo-triazoles that are DNA cleaving agents upon
photoactivation. The reaction proceeds from the lowest excited singlet state to an azoimine that converts thermally or photochemically to an intermediate. The intermediate in the singlet state reacts directly or undergoes intersystem crossing to the triplet. It is capable of hydrogen abstraction followed by DNA cleavage.

Constraints on the structure of nucleosomal and the linker DNA induced by the associated histones and the non-histone chromosomal proteins will play an important role in the loci of DNA-damage in the nucleus by this group of antibiotics. One of the initial reports employed Chinese Hamster cell nuclei (Kuo and Samy, 1978). They were reacted with neocarzinostatin and its DNA was analyzed on non-denaturing agarose gel. A series of bands with a multiplicity of 175 bp was obtained. A similar result was also obtained when the DNA samples were electrophoresed under denaturing gels. Later, a detailed study reported the DNA damage in HeLa nuclei and isolated nucleosome core particles with several more members of the enediyne family of antitumor antibiotics such as calicheamicin γ1 (CAL), esperamicin A1 (ESP A1), esperamicin C (ESP C), and neocarzinostatin (NCS). All three enediyne antitumor antibiotics produce DNA damage in HeLa nuclei that was modulated at the level of the nucleosome (Yu et al., 1994; Smith et al., 1996). DNA damage induced by ESPA1 and NCS was limited to the linker DNA. On the other hand, the damage produced by CAL and ESP C also occurred in the nucleosome core with a 10-nucleotide periodicity. The differences in the site of the damage have been ascribed to the structural differences between the enediyenes. Distinctive features of drug structure that may limit damage to the nucleosome core include the presence of substituents on both sides of the CAL/ESP-type core, and the presence of an intercalating moiety, such as the naphthoate of NCS and possibly the anthranilate of ESP A1. These observations further emphasize the point...
that DNA-damage by the covalently interacting drugs depend on the geometry of the drug–DNA complex resulting from the first step of the non-covalent association between them. Keeping in view the fact that these antigenic compounds are potent cytotoxic agents, there is scope to understand how these drugs might switch off the active genes for transcription. As proposed earlier it is possible that the conversion of nucleosomes to nuclease hypersensitive sites is a mechanism of transcription inhibition for the active genes. However, the chemically active nature of these drugs also make them possible players in any one or more steps in the chromatin remodeling, an essential pre-requisite for transcriptional activity of the active chromatin.

3.3.1. Bleomycin

The antitumor antibiotic bleomycin (structure shown below) is a glycopeptide that binds to the DNA minor groove and induces sequence specific single and double strand breaks in DNA by a free radical mechanism. It forms a coordination complex with Fe (II) and in the complexed state it combines with oxygen to produce a highly reactive species, which specifically abstracts hydrogen from the C4’ of deoxyribose sugar moiety. This leads to strand breakage or production of abasic sites. In the process of formation of double strand breaks, bleomycin primarily induces single strand breaks at pyrimidines of G-C and G-T sequences which is followed by secondary cleavage on the opposite strand (Povirk and Goldberg, 1987; Steighner and Povirk, 1990).

Biochemical and cytological studies of bleomycin actions on chromatin and chromosomes have revealed that bleomycin interacts with nuclei isolated from a variety of mammalian cells to release nucleosomes. i.e. bleomycin cleaves chromatin at linker regions (Kuo and Samy, 1978). Moreover, the ability of bleomycin to induce DNA lesions depends on nucleosome repeat length (Lonn et al., 1990), the number of DNA lesions created being lower for salt-incubated nuclei with short average nucleosome repeat length (140–145 bp) compared to nuclei with longer (190–195 bp) repeat length. bleomycin induced DNA cleavage is also asymmetric towards the periphery of nucleosome bound DNA (Smith et al., 1994), where marked inhibition of cleavage is observed toward the upstream side, but negligible inhibition occurs towards the downstream side for chromatin, reconstituted with *Xenopus laevis* 5S rRNA gene.

Another factor, which is of prime importance in the context of bleomycin activity on chromatin, is the degree of chromatin compaction. Restriction enzyme digestion of DNA from drug-treated nuclei along with Southern blotting procedures has shown that bleomycin preferentially cleaves the chromatin at actively transcribing regions (Kuo, 1981). In fact, the DNA sensitivity to bleomycin is inversely correlated with the degree of chromatin coiling (Lopez-Larraza and Bianchi, 1993). CHO cells with decondensed chromatin show higher DNA sensitivity to bleomycin than CHO cells with maximal chromatin compactness. Furthermore, a comparative study of the response of mosquito (ATC-15) and mammalian (CHO) cells to bleomycin reveals that ATC-15 cells, which have higher chromatin condensation compared to
CHO cells, also show a lower sensitivity to bleomycin \cite{Lopez-Larraza2006}. The ATC-15 cells exhibit satisfactory growth at bleomycin doses that produce a permanent growth arrest of CHO cells, thereby suggesting that mosquito cells might have linker DNA shorter than that of mammalian cells.

Bleomycin induced chromosomal damage in Chinese hamster bone marrow gives rise to micronuclei by means of lagging chromatin; main and micronuclei eventually become asynchronous in consecutive cell cycles and mitosing main nuclei induce premature chromosome condensation in the micronuclei \cite{Kurten1975}.

The cells respond to bleomycin induced chromatin damage by activating nuclear poly (ADP-ribose) polymerase (PARP), which regulates chromatin structure and DNA repair. Closely associated with PARP, is the activity of cellular integrins. A fluorescence microscopy based study using wild type and PARP knockout mouse lung endothelial cells and the PARP inhibitor, 3-aminobenzamide showed that integrin clustering protect wild type cells from DNA breakage and 3-aminobenzamide and PARP knockout inhibit this protection. Hence, the antigenotoxic effect of integrin activation requires PARP, but at the same time, integrins alter chromatin structure by both PARP-dependent and independent mechanisms \cite{Sidik1990}.

Ultimately the DNA lesion is repaired by a ‘short patch’ repair mechanism, which, in linker regions of nucleosomes or open regions of chromatin (where lesions are generally concentrated) is associated with minimal nucleosome rearrangement \cite{Jones2001}.
3.4. DNA Cross-linkers: Cisplatin and Mitomycin C

The most widely used drug in this category is cisplatin. Therefore, we have summarized below its action at the chromatin level. The anti-tumour drug cis-diamminedichloroplatinum(II) (cisplatin) is employed for the treatment of ovarian and testicular carcinomas, as well as solid tumours (Loehr and Einhorn, 1984; Zamble and Lippard, 1995).

Cisplatin

The covalent binding of cisplatin to cellular DNA mediates the cytotoxicity of the anti-cancer agent (Roberts and Thomson, 1979; Bruhn et al., 1992; reviewed in Jamieson and Lippard, 1999). The reaction of cisplatin with DNA results in covalent cisplatin-DNA adducts that can inhibit DNA replication. The most prevalent covalent adduct is an intra-strand cross-link formed between the N-7 of two adjacent guanine residues (Dabrowiak and Bradner, 1987; Bruhn et al., 1992). Inter-strand DNA crosslinks, as well as some DNA–protein cross-links, also occur. An interesting observation is that linker histone H1 binds preferentially to cisplatin damaged DNA (Yaneva et al., 1997). The sequence specificity of cisplatin DNA damage using a polymerase stop assay (Temple et al., 2000) has shown that runs of consecutive guanines is the most prevalent with lesser damage at AG, GA and GC dinucleotides. A similar DNA sequence selectivity has also been found in intact human cells (Murray et al., 1998; Davies et al., 2000). Footprinting techniques, utilising bleomycin and DNase I as the damaging agents, were employed to establish the precise location of positioned nucleosomes with respect to the DNA sequence in reconstituted chromatin (Galea and Murray, 2002). Reconstituted nucleosomal DNA was treated with cisplatin and drug-induced DNA adduct formation was quantitatively analysed with a polymerase stop assay using Taq DNA polymerase. The results from the studies show that the preferred site of cisplatin DNA binding was in the linker region of the nucleosome. The effect of chromatin structure upon cisplatin damage has also been studied in the intact human cells using epsilon-globin promoter as the DNA target. The study had shown that chromatin structure has a large impact upon the degree of damage, particularly, the binding of a transcription factor resulted in an enhancement of the DNA damage. Protein induced distortion of the DNA could lead to the formation of novel adduct that could evade the normal repair pathway thereby leading to the anti-tumor activity of the drug. In the cell, exposure of the damaged
DNA duplex to the exterior of the nucleosome is necessary for the damage repair of the platinum lesion by the appropriate machinery. The rotational setting of the nucleosomal DNA on the surface of the histone octamer decides the solvent accessibility of the nucleotides (Danford et al., 2005 and related references therein). Enzymatic digestion by exonuclease III of the nucleosome substrates suggested that the platinum cross-link affects the translational positioning of the DNA, forcing it into an asymmetric arrangement with respect to the core histone proteins. These phasing phenomena might play an important role in the recognition and processing of platinum-DNA adducts in cancer cells treated with these drugs.

Mitomycin C (structure shown below) is a naturally occurring antitumor antibiotic, used in cancer chemotherapy, particularly for the treatment of bladder cancer (Bradner, 2001). The cytotoxicity of this drug arises due to inter and intra-strand DNA crosslinking, following adduct formation. Mitomycin C induces the formation of monoadduct at guanine N2. Similarly, two guanine residues in proximity in the DNA minor groove may cross-link with each other through their respective 2-amino groups (Iyer and Szybalski, 1963; Dorr et al., 1985; Keyes et al., 1991). Crosslinking renders the DNA unsuitable as a template for replication or transcription to occur, and if left unrepaired, are highly cytotoxic.

It has been found that mitomycin C treatment initiates certain active cellular processes, which result in non-random chromatid interchanges. The frequency of exchange between homologous chromosomes by far exceeds what is expected by chance (Shaw and Cohen, 1965; Morad et al., 1973). Quadriradical formation predominantly occurs with human chromosomes 1, 9, and 16 and exchange breakpoints appear within the C bands of these chromosomes. Furthermore, the frequency of exchange events in these chromosomes tends to be directly correlated with the size of their paracentromeric heterochromatic bands (C-bands). Abdel-Halim et al., (2005) have reported that majority of exchange breakpoints of chromosome 9 are located within the paracentromeric heterochromatin and that over 70% of the exchanges occur between its homologues in G0/G1 and S-phase cells. It therefore implies that mitomycin C treatment induces heterochromatin-specific pairing and formation of exchanges.
Mitomycin C treatment is often followed by the induction of cross-link repair in vivo. The initiation of cross-link repair possibly occurs when the DNA replication or transcription machinery are stalled at the damaged site. Single strand DNA foci appear as an outcome of cross-link repair and these single strand DNA foci may occur in S-phase cells (Rothfuss and Grompe, 2004). A detailed in vivo analysis of mitomycin C induced DNA damage and repair (Lee et al., 2006) identifies the involvement of Xeroderma pigmentosum (XP) proteins. Induction of single strand DNA patches by mitomycin C is completely dependent on the presence of XPG and XPE proteins, as human mutant cells, defective in XPG and XPE fail to form single strand DNA foci on treatment with mitomycin C. Moreover, mitomycin C induced cross-link causes XPG to localize exclusively in the nucleus and to associate with chromatin. However, treatment of XPF deficient cells with mitomycin C results in a strong reduction of chromatid interchange frequency (Abdel-Halim et al., 2005). Lack of XPF also significantly delays the formation of mitomycin C induced single strand DNA foci in vivo. Thus, XPG, XPE and XPF, all play unique roles in the repair of mitomycin C induced DNA damage.

3.5. Anticancer Drugs Targeting the Structural Organization of DNA

Different levels of structural organization of DNA are targets for anticancer drugs. The first, most basic level is the chemical interaction of the drug with the DNA double helix. The DNA primary base sequence may be targeted in a non-sequence-specific (global) manner or the same may be targeted at repetitive DNA sequences such as AT rich regions (ORIs and MARs) or specific DNA sequences (e.g. oncogenes). Similarly, the secondary DNA structures such as DNA quadruplexes (telomeres), hairpins, Holliday junctions and triple helices also serve as potential targets for anticancer drugs.

3.6. Targeting the DNA Primary Base Sequence

Non-sequence-specific or global DNA damage is generally caused by random DNA alkylation, cross-linking or strand scission, the mechanisms and consequences of which have been previously mentioned.

3.7. Targeting the Repetitive DNA Sequences

Clusters of repetitive DNA sequences are present over vast areas of the human genome. In some cases, these sequences may provide important regulatory functions. Repetitive DNA often has the ability to take on non-B-form DNA conformations, which might recruit certain regulatory proteins that participate in control of gene expression. AT-islands, containing nearly 85–100% AT sequences may function as matrix attachment regions (MARs) that organize DNA loops on the nuclear
matrix and coordinate nuclear activities such as DNA replication, transcription and mitosis (Woynarowski, 2002). Moreover, origins of replication and certain promoters contain AT rich sequences. Unfortunately, these repetitive sequences are unstable. Polymerase slippage or unequal recombination (Debrauwere et al., 1997) may cause expansion or deletion of the repetitive elements and this is often associated with disease state. Therefore, selective damage to AT-rich DNA might be an important mechanism of drug action since binding to these sequences affects specific gene expression by preventing transcription factor binding, increasing the affinity of a transcription factor for its sequence, or creating unnatural binding sites (Gniazdowski et al., 2005).

Efforts are being made to design anticancer drugs that will specifically interact with AT-rich sequences and interfere directly with the metabolic processes therein. In fact, certain cyclopropyindoline compounds such as CC-1065, adozelesin, and bizelesin have been studied that alkylate the N3 of adenine in the minor groove of AT-rich DNA sequences. These compounds have been shown to inhibit DNA replication in cell free and cell based yeast and mammalian systems (McHugh et al., 1994, 1999; Cobuzzi et al., 1996; Woynarowski and Beerman, 1997; Weinberg et al., 1999; Wang et al., 2001). In addition, certain aminoindoline compounds have been found to target AT-rich sites located within the c-MYC gene in vitro (Nelson et al., 2005).

3.8. Targeting Specific DNA Sequences or Oncogenes

Certain drugs have been designed that span DNA and recognize a limited number of specific sequences. The pyrrole-imidazole polyamides (Dickinson et al., 2004) are the most discriminatory sequence selective DNA binding agents that inhibit transcription factor binding in vitro. However, these hairpin polyamides have not been found effective in vivo (Dudouet et al., 2003). Chlorambucil conjugated polyamides have been designed that cause cell cycle arrest in G2/M. Molecules such as polyamide 1-CBI (1-chloromethyl)-5-hydroxyl-1,2-dihydro-3H-(benz[e]indole) conjugate exhibit sequence specific DNA alkylation in mammalian cells. 1-CBI is able to damage encapsidated SV40 DNA by penetrating the virions (Philips et al., 2005). Moreover, the differences in sequence specificities of DNA alkylation of these conjugated polyamides lead to marked differences in biological activities (Shinohara et al., 2006).

Ecteinascidin (Et-743) is a minor groove-alkylating agent, currently in clinical development. The drug alkylates N2 of the central guanine of the DNA binding triplet and causes a conformational change in DNA, with the minor groove widening and the double helix bending towards the major groove (Pommier et al., 1996; Garcia-Nieto et al., 2001; Hurley et al., 2001; Zewail-Foote and Hurley, 1999, 2001). This compound demonstrates a unique potential to alter gene expression of discrete loci based on the presence of GC boxes in the promoter regions.
4. DNA SECONDARY STRUCTURES AS TARGETS FOR ANTICANCER DRUGS

DNA secondary structures containing stretches of single stranded DNA are present in the human genome and are involved in the regulation of crucial processes such as transcription. Hairpins or cruciforms are the potential recognition sites for binding of transcription factors. Of equal importance are telomeres, the DNA-protein complexes marking the ends of chromosomes. Facile interconversion between double and single stranded DNA and G-quadruplex at physiological conditions renders these secondary DNA structures attractive candidates for biological signaling molecules and consequently, potential targets for anticancer agents.

4.1. Targeting DNA Quadruplexes

Telomeres are DNA protein complexes at the ends of eukaryotic chromosomes that protect the linear DNA ends from erosion over multiple replication cycles and also from being recognized as double strand breaks and subsequent repair by exonucleolytic trimming and end-to-end fusion. Chromosomal telomeres contain 3’ G-rich overhang of 150—200 bp that forms a G-quartet structure. It is in the form of stacked tetrads of guanines in a cyclic Hoogsteen hydrogen bonding arrangement. G-quartets can be stabilized by sodium and potassium ions and this stabilization can inhibit telomerase activity. A number of small molecules have been identified that interact with G-quartets. For instance, 2,6-diamidoanthraquinone BSU1051 has been found to interact with and stabilize the G-quartet, and thereby inhibit telomerase activity ([Sun et al.] 1997). A 3,6,9-trisubstituted acridine is also a potent inhibitor of telomerase. Apart from these, certain cationic porphyrins such as TMPyP₄ are another class of agents that bind to G-tetrads by interactive stacking. TMPyP₄ has selectivity for intermolecular G-quadruplex structures ([Liu et al.] 2005). Telomes-
Chromatin is a natural G-quadruplex intercalating agent, which holds greater promise compared to previously studied G-quadruplex targeted molecules. On treatment of multiple myeloma cells with Telomestatin, inhibition of telomerase activity occurs along with reduction in telomere length followed by cell growth inhibition (Shammas et al., 2004). A third class of G-tetrad interacting compounds comprises of perylenetetracarboxylic diimide PIPER which shows binding characteristics, similar to the porphyrins. These compounds not only bind to G-quadruplexes, but also induce their formation in cells (Han et al., 1999).

Apart from telomeres, G-quadruplexes are also present in the upstream promoter regions of certain oncogenes. G-quadruplex targeted molecules may interact at these sites as well. In fact, the cationic prophyrin, TMPyP4 and the core modified expanded prophyrin analogue 5,10,15,20-[tetra(N-methyl-3-pyridyl)]-26,28-diselenasapphyrin chloride (Se2SAP) have been found to cause repression of transcriptional activation of c-MYC in cells by G-quadruplex stabilization (Seenisamy et al., 2005).

4.2. Targeting Hairpins and Holliday Junctions

Hairpins, cruciforms or single strand DNA containing secondary structures play an important role in the regulation of transcription. A molecule that can selectively bind to hairpins has the potential to block transcription by interfering with protein recognition of that specific site. The transcription inhibitor Actinomycin D and its analogues bind nearly 10 fold more tightly to the hairpin conformation formed from the single stranded DNA 5′-A7TAGT4A3TAT7-3′ than to the same strand in fully duplexed form.

4.3. Targeting DNA Triplexes

Specific targeting of triplex DNA structures is one of the most important strategies of ‘antigene’ based chemotherapy (Jenkins, 2000). The main aim is to target individual gene sequences at the DNA duplex level to modulate their expression or interactions with DNA binding proteins or to interfere with the vital template processes. A genomic DNA duplex is targeted using either a triplex forming oligonucleotide (TFO) or peptide nucleic acid analogue (PNA) to produce local DNA triplex structures that can inhibit the transcription of specific genes. The specificity of these triple helical structures stems from the Z—X·Y base triplets formed by Hoogsteen or reverse Hoogsteen hydrogen bonding arrangements involving pyrimidine or purine bases (Z) in the third strand and the purine strand (X) of the host DNA duplex. The triple helical structures formed have low thermal and thermodynamic stability and this poses a problem for effective targeting. So DNA triplex-targeted drugs mainly aim at stabilization of DNA triplexes.

Significant increase in triplex stability can be achieved by using intercalating agents, either by conjugate attachment to the third strand TFO or as a separate adjunct ligand, if binding of this residue can preferentially stabilize a
triple stranded DNA. Various fused and unfused heterocycles have been studied. They include naphthylquinoline, BePI (benzo[e]pyridoindole), imidazothioxanthone, acridine derivatives, coralyne, and the phenothiazinium dye, methylene blue. These molecules have (i) an extended planar aromatic system to maximize π-overlap with successive base triplet planes, (ii) a cationic charge on the intercalated chromophore and (iii) a pendant side-chain terminating in an amine residue that can be protonated. Due to these characteristics, the compound is easily delivered to the target DNA and it anchors the bound ligand through interaction with the grooves or the anionic phosphodiester backbone.

Efforts have also been made to design groove directed drugs for triplex stabilization. Ideally, these agents should have little or no inherent affinity for the underlying duplex in order to prevent intergroove cross talk and consequent binding induced displacement of the third strand. However, most of the compounds studied are mainly A/T specific with high affinity for DNA duplexes rather than DNA triplexes.

5. PROTEIN–DNA COMPLEXES AS MOLECULAR TARGETS FOR ANTICANCER DRUGS

Chromosomal DNA is the scaffold on which DNA binding proteins assemble and regulate vital cellular processes such as DNA packaging, transcription, replication, recombination and repair. Since these proteins use DNA as template for carrying out their respective functions, they are found associated with DNA some time or the other in the cell cycle. Several drugs have been designed that target the protein-DNA complexed state and thereby inhibit the enzyme function. Among this class of drugs, the best-studied are the topoisomerase poisons.

Topoisomerases (I & II) are enzymes that modify the DNA topology by a complex catalytic cycle involving DNA strand cleavage, strand passage and religation \( \text{[Osheroff et al., 1991]} \). The necessity of topoisomerases in the cell arises from the fact that the DNA double helix is normally stored in a highly supercoiled complexed state in chromatin which has to be unwound for processing. The cytotoxicity of topoisomerase poisons is due to stabilization of the enzyme-DNA covalent ‘cleavage complex’ \( \text{[Nelson et al., 1984]} \). Topoisomerase I inhibitors stabilize a covalent bond between a tyrosine residue on the protein and the 3’ phosphoryl end of the single strand it breaks \( \text{[Hsiang et al., 1985]} \), while topoisomerase II inhibitors stabilize a covalent bond between a tyrosine residue on the protein and the 5’ phosphoryl end of each broken strand of DNA with a 4 bp stagger between cleavage sites on complementary strands. In either case, it leaves the topoisomerase molecule covalently bound to DNA, masking the cleavage site.

The major class of topoisomerase I inhibitors comprise of the camptothecins, while topoisomerase II inhibitors fall into several classes – anthracyclines (e.g. doxorubicin), anthracyclenediones (mitoxantrone), anthrapyrazoles (bianthrazole), actinomycins (actinomycin D), acridines (m-AMSA), ellipticines (9-hydroxy-ellipticine) and epidophyllotoxins (Etoposide (VP-16) and VM-26). The chemical
Chromatin as a Target for the DNA-Binding Anticancer Drugs

Chemical structures of some topoisomerase inhibitors

structures of some topoisomerase inhibitors are shown below. Except for the camptothecins and epidophyllotoxins, the drugs bind to DNA through intercalation and then form a ternary drug/DNA/enzyme complex that inhibits the DNA resealing activity of the enzyme, stabilizing the cleavage complex and resulting in DNA double strand breaks. Camptothecins and epidophyllotoxins are believed to bind primarily to the protein followed by ternary complex formation.

However, topoisomerase poisons show a limited sequence preference. Especially the topoisomerase II targeted drugs trigger random double stranded breaks throughout the genome, which at times induce chromosomal translocations and in turn cause secondary leukaemia. With the intention to impart greater specificity to topoisomerase II poisons, Duca et al., 2006 have designed derivatives of VP16 conjugated to triplex forming oligonucleotides. These molecules induce cleavage 13—14 bp from the triplex end where the drug was attached. Hence, the molecules are expected to offer great promise in cancer chemotherapy.

Apart from the topoisomerase poisons, other drugs that trap proteins in ternary complexes include Cisplatin, and Et-743.

Cisplatin (cis-diamminedichloroplatinum (II)) initially binds covalently to adjacent guanines in the major groove of DNA and bends DNA in the direction of the major groove (Bellon et al., 1991). This distortion facilitates protein binding in the minor groove and stabilizes the interactions between DNA and its binding proteins such as TATA box binding protein (TBP), High mobility group 1 (HMG1), High mobility group 2 (HMG2), human upstream binding factor (hUBP) and sex-determining region Y protein (SRY) (Gniazdowski and Czyz, 1999). These proteins bind to DNA and enforce bending of DNA. So, trapping of these proteins, either at their natural or unnatural binding sites imposes architectural changes that are eventually hazardous to the cell. The above review is a glimpse of the activities with the platinum containing anticancer drugs. There has been some excellent reviews in this area in the last year (Sedletska et al., 2005; Wheate and Collins, 2005).
Ecteinascidin 743 (Et-743) is a complex natural product, which also seems to work by trapping DNA-binding proteins at sites where structural distortion of the DNA is recognized. The chemical structures of Cisplatin and Et-743 are shown earlier in this review.

Blocking an essential enzyme in its DNA bound state may be an intriguing mechanism to cause cell death, but in reality this feat has rarely been achieved. The drugs designed to target DNA/protein complexes have high cytotoxicity and low clinical efficacy. So, efforts are being made to handle these problems as far as possible and at the same time, newer classes of drugs, the ‘epigenetic’ drugs are coming up with greater promise.

6. EPIGENETIC THERAPY OF CANCER

‘Epigenetic’ is a term used to describe a state of gene expression that is mitotically and meiotically inherited without any change in the sequence of DNA. Epigenetic mechanisms are mainly of two classes: (1) the DNA may be modified by the covalent attachment of a moiety that is then perpetuated. (2) a self-perpetuating protein state may be established \( [Zelent \ et \ al., \ 2004] \). The two most studied epigenetic phenomena are DNA methylation and histone tail modifications \( [Mai \ et \ al., \ 2005] \).

Methylation is the most commonly occurring epigenetic modification of human DNA. Under normal conditions, it helps to maintain transcriptional silence in non-expressed or non-coding regions of the genome. Methylation results from the activity of a family of DNA methyl transferases (DNMTs) that catalyze the addition of a methyl group to the 5-position (C5) of the cytosine ring, almost exclusively in the context of CpG dinucleotides. Low levels of DNA methylation in the promoter region of genes are linked to active gene expression. On the contrary, methylation near the transcription start site stand in the way of gene expression.

Apart from gene silencing, other effects of DNA methylation include spontaneous deamination, enhanced DNA binding of carcinogens and increased UV absorption by DNA, all of which increase the rate of mutations, DNA adduct formation and subsequent gene inactivation.

DNA methylation is one such epigenetic phenomenon, which is abnormal in tumor cells \( [Szyf, \ 2003; \ Lund \ and \ van \ Lohuizen,\ 2004] \). Methylation of CpG islands, a feature of cancer cells, occurs rarely in normal tissue. Hence, methylation provides a tumor specific therapeutic target. De novo methylation as well as maintenance of methylation is carried out by DNMTs. There are 5 known human DNMTs – DNMT1, DNMT2, DNMT3a, DNMT3b and DNMT3L. Apart from DNMT2 and DNMT3L, which lack the amino terminal regulatory domain and the catalytic domain respectively \( [Goll \ and \ Bestor, \ 2005] \), the remaining DNMTs all have enzymatic function. Inactivation of DNMTs is the most effective means of inhibiting DNA methylation and restoring normal patterns of methylation. However, targeting the methyl transferase enzyme leads to loss of specificity and hypomethylation of the genome. The overall decrease in methylation level may even activate the potentially deleterious oncogenes \( [Szyf \ et \ al., \ 2004] \). DNA methylation inhibitors are of two broad classes: nucleoside and non-nucleoside analogues.
7. THERMODYNAMICS AND CRYSTALLOGRAPHY OF DRUG–DNA INTERACTION IN CHROMATIN AND NUCLEOSOME

An elucidation of the energetics of association of the non-covalently binding drugs to DNA as an integral part of chromatin and nucleosome is required to understand the structural basis of their association. It tells about the alteration in the state of the DNA in the chromatin (or nucleosome) as a result of the association with the drug. The scenario will be more complex for the covalently binding drugs. However, there has been very few studies aimed at understanding the energetics of drug–chromatin (nucleosome) interaction.

Haq, 2002, has described the overall observed drug–DNA binding free energy as being composed of at least five component free energy terms: $\Delta G_{\text{conf}}$ (the free energy contribution arising from conformational changes in DNA and drug); $\Delta G_{\text{r+t}}$ (the unfavorable contribution to free energy arising from losses in translational and rotational degrees of freedom upon complex formation); $\Delta G_{\text{hyd}}$ (free energy for the hydrophobic transfer of the drug from bulk solution to the DNA binding site); $\Delta G_{\text{pe}}$ (the polyelectrolyte contribution, mainly due to coupled polyelectrolyte effects, the most important of which is the release of condensed counterions from the DNA helix upon drug binding); $\Delta G_{\text{mol}}$ (the contribution to free energy from weak non-covalent interactions such as hydrogen bond formation, van der Waals interactions, specific electrostatic bond formation, dipole–dipole interactions, etc. between the drug and DNA). In the case of chromosomal DNA, the additional contributions from the reshuffling of histone–DNA interactions and the potential contribution from histones need to be considered.

The general trend for minor groove binders is that binding is driven largely by hydrophobic effect and favorable entropy is derived from the release of bound water and counterions from DNA and drug upon complex formation. Intercalators, on the other hand, have slightly lower affinities for DNA than minor groove binders. As with minor groove recognition the interaction of intercalators with DNA results from a delicate balance of opposing energetic factors. There is a free energy cost associated with deforming the DNA lattice in order to accommodate the intercalating chromophore. There are also energetic penalties that arise from losses in rotational and translational freedom in the DNA and drug upon complex formation. However, in case of intercalation, drug – binding results in additional van der Waals interactions between drug and adjacent bases that are capable of providing a significant favorable contribution to the overall free energy.

Detailed reports of the energetics of binding of an antibiotic to nucleosomal DNA under different conditions have been done only with aureolic acid group of antibiotics from our laboratory [Mir and Dasgupta, 2001a, b; Mir and Dasgupta, 2003]. The thermodynamic parameters were evaluated from temperature dependence of the affinity constants with native chromatin, nucleosomal DNA and naked DNA. The results showed that there is a gradual reduction in the free energy as we go down the series-naked DNA, native chromatin and nucleosomal core particle. It originates from an alteration in the extent of enthalpy-entropy compensation for each
system. A continuation of the studies with the same group of antibiotics showed that chopping of the N-terminal tails leads to a decrease in the free energy of association of the drugs with nucleosome. The effect of lowering in free energy is more pronounced in the case of intercalator like daunomycin (Mir et al., 2004). While these studies have thrown light upon the energetics of association, the isothermal titration calorimetric approach will provide an incisive picture. There is a report of calorimetric investigation of ethidium and netropsin binding to chicken erythrocyte chromatin (Taquet et al., 1998). They demonstrated that the presence of histones on DNA still allows the ligand binding that takes place according to a simple one single-site model. The results show the enthalpic origin of the association with little variation of heat capacity change with temperature. Knowledge about the variation of heat capacity change with temperature for the DNA will be a necessary input for understanding the structural basis of drug-chromatin/nucleosome association. Such studies are in progress in our laboratory. Melting studies and differential scanning calorimetry are other potential thermodynamic tools to get an idea about the effect of the drugs upon the energetics of histone–DNA interactions at the nucleosomal and linker level. There have been many differential scanning calorimetric studies to understand the fine structure of nuclei, chromatin and nucleosome. Differential scanning calorimetry of nuclei was used as test for the effects of strand breakers like bleomycin on human chromatin (Almagor and Cole, 1989). We have employed melting studies to examine the effect of aureolic acid group of antibiotics upon DNA in the chromatin, nucleosome and H1-depleted chromatin (Mir and Dasgupta, 2003).

Evaluation of crystal structure will provide insight to the site-specific nature of the drug-DNA interaction at the nucleosomal level. In this regard, a recent report on the crystal structures of three nucleosome core particles in complex with site-specific DNA-binding ligands, the pyrrole-imidazole polyamides has been the first study (Suto et al., 2003). The notable feature emerging from this study shows that the minor groove of nucleosomal DNA is capable (within limits) of adjusting its parameters to allow recognition and binding of small ligands while retaining a full complement of histone–DNA interactions. However, nucleosomal DNA undergoes significant structural changes at the ligand-binding sites and in adjacent regions to accommodate the ligands. Notwithstanding the importance of this result, appropriate control studies should be done to check that association of the ligands does not lead to disruption of the nucleosome.

8. EFFECT OF REVERSIBLE DNA-BINDING OF ANTIBIOTICS UPON CHROMATIN CONDENSATION

The ability of DNA-binding drugs to induce chromatin condensation calls for in-depth studies, because the biological consequence of chromatin condensation is its loss of transcriptional ability. Therefore it might be a mechanism of transcription inhibition by the reversible DNA binders. The first report in this line (Sen and Crothers, 1986) employed transient electric dichroism to study the ability of the drugs to induce folding of chromatin from the 10- to 30-nm fiber either by
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themselves or in conjunction with multivalent cations. It was found that charge on the drug is a factor for its condensation potential. Interestingly these drugs, irrespective of the groove binder nature or intercalative nature, inhibit compaction of chromatin; they rather induce condensation. Physicochemical studies with metal complexes of aureolic acid group of antibiotics, chromomycin A3 and mithramycin, and some other intercalators like daunomycin from our laboratory also suggest that these drugs induce condensation of the chromatin. Based on these results we propose a model for the effect of these drugs upon chromatin structure. At present we are examining the validity of this mechanism for other reversible binders to DNA. However, studies need to be done to examine whether the condensation ability differs for normal and neoplastic cells.

9. CONCLUDING REMARKS

There have been excellent review articles related to the present topics (e.g. Muenchen and Pienta 1999; Nelson et al. 2004; Inche and La Thangue 2006). Our review has provided an overview of the research activities to understand the molecular basis of the function of the various classes of DNA-targeting drugs at
the chromatin level. By no means, the review has covered all reports in this area. We have emphasized upon the salient features.

The rationale that has driven cancer drug design over the years is the specific targeting of tumorigenous cells, leading to induction of cell death. In that run, the DNA targeting drugs have occupied an important position. The earliest anticancer agents were the DNA alkylators/cross-linkers which modify the strands of DNA and thereby inhibit their templating properties. Later, utilizing this same principle, the DNA strand cleaving molecules were developed. Along with them reversible binders of DNA were also examined and employed as drugs (Martinez and Chacon-Garcia, 2005).

DNA secondary structures and protein-DNA complexes were subsequently targeted for specific drug design. The modern era of chemotherapeutics holds prospects for the DNA secondary structures and protein-DNA complexes as anticancer drug targets. This not only imparts high specificity to the drug, but also establishes the importance of the anti-gene approach and the drug target.

At present, cancer treatment schemes mostly use combination chemotherapy which (1) kills maximum number of cells within the range of toxicity tolerated by the host for each drug; (2) offers a broader range of coverage of resistant cell lines in a heterogeneous tumor population; and (3) prevents or slows the development of new drug-resistant cell lines. This strategy will perhaps remain the method of choice in future as well. Since, DNA damage and subsequent apoptosis induction is physiological effect of many DNA-binding anticancer drugs, therefore, nontoxic amplification of DNA-cleaving activity of anticancer drugs would effectively reduce drug dose and side effects, leading to development of improved chemotherapy. In a recent survey (Kawanishi and Hirak, 2004), the enhancing effects of DNA-binding ligands (‘amplifiers’), especially minor groove binders and intercalators, on anticancer drug-induced apoptosis and DNA cleavage were made using human cultured cells and (32)P-labeled DNA fragments obtained from the human genes. The mechanism of amplification of DNA cleavage has been ascribed to the fact that binding of amplifier changes the DNA conformation to allow anticancer drug to interact more appropriately with the specific sequences, resulting in enhancement of anticancer effect. This study on amplifiers of anticancer agents shows a novel approach to the potentially effective anticancer therapy.

A major objective of the present-day synthetic, chemical and structural biology and molecular medicine is to find natural or synthetic small molecules with the DNA – binding potential, so that their site-specific binding potential to DNA can be utilized to regulate the DNA-templated biological processes. The major problem confronting the above task is the inability to define their full range of specific targets in the cell nucleus (Kim et al., 2003) and therefore to predict the target sites and their effectiveness to kill specifically the target cells afflicted with neoplasia. A recent report (Warren et al., 2006) has been a pathfinder to address this problem at a global level. The group has developed a comprehensive high-throughput platform that can rapidly and reliably identifies the cognate sites of DNA-binding molecules. This platform provides an unbiased analysis because it
consists of a double-stranded DNA array that displays the entire sequence space represented by 8 bp (all possible permutations equal 32,896 molecules) and can currently be extended to as many as 10 variable base pair positions. This report has featured the importance of chemical genomics approach as one of the tools to achieve the target (Jung et al., 2003). Delivery of the drugs to the appropriate site is another challenge for chemical biology. There has been considerable progress in this area too.

The focus of research interest in my laboratory has been to elaborate the effect of DNA-binding class of anticancer drugs upon the chromatin structure related to its function during the process of gene expression. As the literature survey shows, there has been a lacuna of information in this area. A judicious combination of biophysical, biochemical and genetic approaches would definitely unfold the intricacies behind the different steps responsible for the mode of action of these drugs. In order to focus upon the site of action, the expression of genes and proteins, which are suppressed or enhanced as a consequence to the use of the drugs, also need to be identified in order to assess their efficacy as anticancer drugs. However, from the knowledge accrued from the research in the last decade on chromatin structure and function it is becoming progressively clear that effect may not be confined to a singular locus. A network of steps might be responsible for the ultimate cellular function of these DNA-binding drugs. In this connection identification and validation of a single (or more) target is an absolute prerequisite to understand the molecular pharmacology of the drugs.

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