A revisit of the mode of interaction of small transcription inhibitors with genomic DNA

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One class of small molecules with therapeutic potential for treatment of cancer functions as transcription inhibitors via interaction with double-stranded DNA. Majority of the studies of the interaction with DNA have so far been reported under conditions nonexistent *in vivo*. Inside the cell, DNA is present in the nucleus as a complex with proteins known as chromatin. For the last few years we have been studying the interaction of these DNA-binding small molecules at the chromatin level with emphasis on the drug-induced structural alterations in chromatin. Our studies have shown that at the chromatin level these molecules could be classified in two broad categories: single-binding and dual-binding molecules. Single-binding molecules access only DNA in the chromatin, while the dual-binding molecules could bind to both DNA and the associated histone(s). Structural effects of the DNA-binding molecules upon chromatin in light of the above broad categories and the associated biological implications of the two types of binding are discussed.

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1. Introduction

The structure of the DNA molecule makes it an extremely versatile target for anticancer drugs (Palchaudhuri and Hergenrother 2007; Panigrahi and Desiraju 2007; Nelson *et al.* 2007). It has a negatively charged phosphate backbone, hydrogen-accepting and hydrogen-donating functional groups in the major and minor grooves, phosphate oxygen atoms and aromatic hydrophobic components able to promote van der Waals interactions. Moreover, 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 a certain extent. Hence, the DNA molecule possesses a variety of features that may be exploited for the design of small molecules with unique targets. Consequently, the past few decades have witnessed the growth of a large number of DNA-targeted small molecules with anticancer properties.

DNA-targeted anticancer drugs bind to the same by either non-covalent forces or covalent interactions (Haq 2002). The primary and most important step in the drug–DNA interaction process is the specific recognition of DNA by the drug. This initial process of recognition is based on size and shape complementarities between the drug and its binding site. In general, the interaction of a drug molecule with its cognate DNA site results in the formation of a primary complex that is stabilized by non-covalent forces such as the Coulombic force, van der Waals interactions, and hydrogen bonding (Panigrahi and Desiraju 2007). For drugs that interact with DNA via non-covalent forces, the primary complex formed is the active complex, whereas for drugs that interact with DNA via covalent forces, the non-covalent reversible drug–DNA association (primary complex) is followed by covalent bond formation (figure 1).

In this review, we shall deal with non-covalent binding drugs that interact reversibly with DNA. Non-covalent binding of drugs to DNA occurs by two principal modes: intercalation and external groove binding.

2. Intercalators and external groove binders

Intercalating drugs possess flat, heteroaromatic ring systems that can insert between two adjacent base pairs in a helix

Keywords. Dual mode of binding; intercalators; groove binders; single mode of binding; transcription inhibitors

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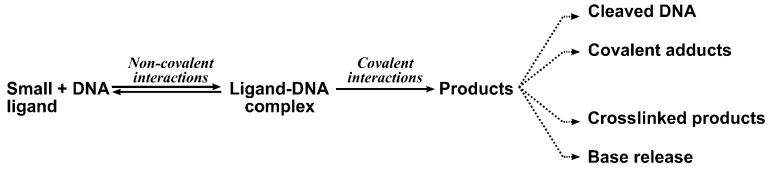

Figure 1. Chemical basis of action of DNA targeting anticancer drugs (Majumder *et al.* 2007; Ghosh *et al.* 2010).

(figure 2A). The complex is stabilized by π-π stacking interactions between the drug molecule and the DNA bases surrounding it. Intercalators are known to approach the DNA bases via both the minor and major grooves. 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. Examples of intercalators include ethidium bromide, daunomycin, actinomycin D, doxorubicin, sanguinarine, etc. Intercalation results in structural perturbations in DNA so as to lengthen it by ~1 bp spacing (i.e. ~3.4 Å) and there is also some unwinding of the DNA helix. The DNA is forced to adopt a C3′-endo-(5′, 3′)-C2′-endo mixed puckering conformation around the intercalation site.

External groove binders (figure 2B) recognize the difference in the pattern of hydrogen bond donors and acceptors in the DNA minor and major grooves, and utilize that information for sequence readout (Nelson *et al.* 2007). Minor groove binders are typically composed of several aromatic rings, such as pyrrole, furan or benzene, that are connected by bonds possessing 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 (Kopka *et al.* 1985). The molecules may alter their own structure ('induced fit' type of mechanism) or even induce structural changes in the DNA duplex for better structural complementarity between drug and DNA (Spolar and Record 1994; Chaires 1997). These drugs generally adopt a characteristic curved shape that is isohelical with the target groove. Van der Waals interactions, hydrophobic forces and hydrogen bonds stabilize the resulting DNA–drug complex.

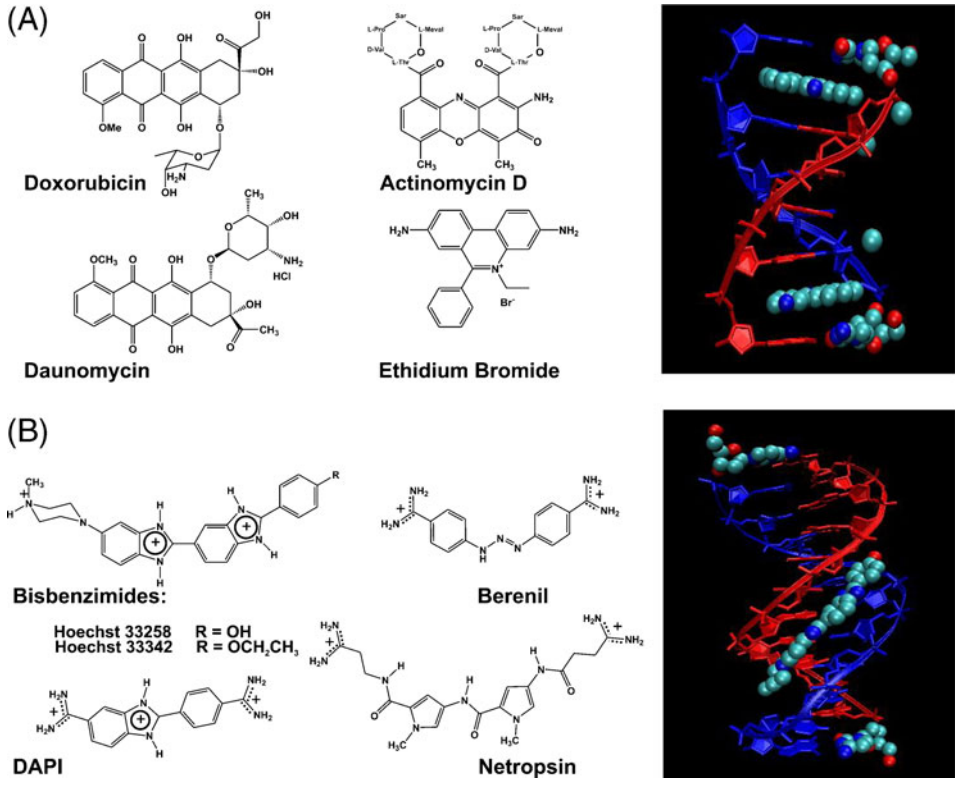

Figure 2. Chemical structures and the mode of binding of some common intercalators (**A**) and groove binders (**B**).

The DNA major groove is mainly recognized by proteins. Synthetic molecules targeted towards the major groove include oligomers called triplex-forming oligonucleotides (TFOs) (Thuong and Helene 1993). They can read polypurine–polypyrimidine duplex sequences and bind in the major groove to form hydrogen bonds with bases on the purine strand. TFOs bind within the existing major groove of DNA, whereby the orientation of the third strand relative to the duplex is dependent on the sequence. Major groove recognition is also achieved by peptide nucleic acids (PNAs) (Nielsen 1999) that have a peptide-like backbone. PNAs invade the helix to form a triplex (Lohse *et al.* 1999), which then results in the displacement of the non-complementary oligopyrimidine DNA strand.

The interaction of small molecules with double-stranded DNA has been thoroughly studied in the past few decades and extensively reviewed in the following references (Hurley 2002; Majumder *et al.* 2007).

In the cell nucleus, DNA does not exist free in solution, but is present in association with numerous proteins in the form of chromatin. The basic repeating unit of chromatin is the nucleosome (Kornberg 1977; Mcghee and Felsenfeld 1980; Luger *et al.* 1997), which is composed of 145–147 bp of DNA, wrapped around an octamer of histone proteins in 1.65 turns of a left-handed superhelix. The histone octamer consists of two copies each of histones H2A, H2B, H3 and H4 that are divided into four 'histone fold' dimers defined by H3-H4 and H2A-H2B histone pairs. Tandemly arranged nucleosome cores further assemble into higher-order structures that are stabilized by the linker histone. A complement of histone proteins therefore shapes physiological DNA and reduces both its accessibility and flexibility. As a result, the mode of interaction of DNA with ligands in the chromatin context is much more complicated as compared to free DNA. Figure 3 shows the general mechanism of action of DNA-targeting small molecules at the chromatin level.

In case of chromatin, the small molecules interact with both nucleosomal DNA and linker DNA. However, the affinity of binding to nucleosomal DNA and linker DNA may differ depending upon steric factors and sequence preferences. In either case, reversible drug binding may lead to structural changes in the DNA template. These changes, coupled with the inherent structural constraints of chromatin, give rise to global changes in the morphology of chromatin.

In our laboratory, we have shown the effect of some small molecules on chromatin structure. Among them, mithramycin, chromomycin and distamycin are minor groove binders. Sanguinarine and ethidium bromide are intercalators. Apart from sanguinarine, the remaining molecules inhibit transcription from DNA template. Sanguinarine does not inhibit DNA transcription, but inhibits transcription from chromatin template (Selvi *et al.* 2009).

2.1 *Mithramycin and Chromomycin*

Magnesium complexes of the two aureolic acid antibiotics mithramycin and chromomycin interact with double-stranded DNA in a GC-selective manner. They interact with chromatin to form a single type of complex (Mir *et al.* 2003). Binding isotherms with chromatin show a non-cooperative nature. Our results show that the trend in binding affinity and stoichiometry is independent of the source of chromatin. The complexes bind to both nucleosomal DNA and linker DNA and stabilize the same. However, the affinity towards histone-free chromosomal DNA is greater than chromatin (table 1). Binding of the drug complexes to chromatin leads to chromatin aggregation. Recent studies from our laboratory have shown that mithramycin interacts with histone octamer isolated from chicken erythrocyte chromatin. The presence of associated linker histones has a negative effect upon the DNA binding potential of both drugs. This is due to preoccupation of the minor grooves of DNA by the side chains of the amino acid residues in histones and alteration in the supercoiling of DNA. Depletion of linker histone H1 from chromatin enhances the accessibility of different ligands to chromosomal DNA (Mir and Dasgupta 2003). H1-depleted chromatin is more susceptible to aggregation and less accessible towards micrococcal nuclease in the presence of both antibiotics. Likewise, the tryptic removal of N-terminal tail domains in nucleosome core particles enhances the binding potential and accessibility of both antibiotics to nucleosomal DNA. In presence of the antibiotics, N-terminal-chopped nucleosomes are more susceptible to disruption compared to N-terminal-intact nucleosomes. Similar results are obtained in case of daunomycin (Mir *et al.* 2004).

2.2 *Distamycin*

Distamycin preferentially binds to AT-rich sites in DNA (Rentzeperis *et al.* 1995; Breslauer *et al.* 1987). Unlike mithramycin and chromomycin, distamycin binds to chromatin and chromosomal DNA with comparable affinity, indicating that the binding site for distamycin is equally accessible in both cases. The affinity towards chromatosome is slightly lower. Distamycin–chromatin association is accompanied by a negative change in the specific heat capacity (ΔCp), which indicates the predominance of hydrophobic interaction leading to surface compaction. Mechanistically, the compaction occurs due to bending of linker DNA and a simultaneous contraction of internucleosomal angle (Majumder and Dasgupta 2011). Previous studies with nucleosome core particles reconstituted on *tyrT* DNA fragment or on cloned sequences of synthetic DNA with phased $(A/T)_4$ stretches showed that distamycin changes the

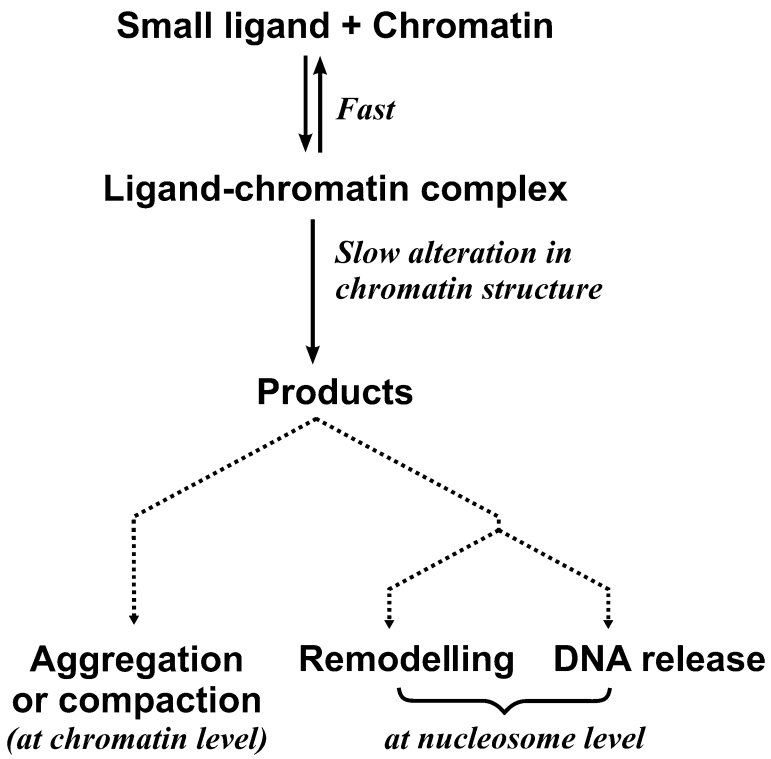

Figure 3. Mechanism of action of DNA-targeting anticancer drugs in the chromatin context.

rotational positioning of nucleosomal DNA (Low *et al.* 1986; Brown and Fox 1996). We have shown that distamycin causes remodelling of chromatosomes by sliding of the histone octamer along with release of some histone proteins. However, distamycin does not interact with core histones isolated from chromatin.

Table 1. Binding parameters for the interaction of complex I [Chromomycin A_3 (CHR) and Mithramycin (MTR)] with rat liver soluble chromatin, nucleosome core particle and naked DNA in 10 mM Tris-HCl buffer pH 8.0 at 20°C

System	K_d[a] (μM)	*n* (number of DNA bases/molecule of bound ligand)
CHR:Mg^{2+} complex		
Native chromatin	110	13±2.0
H1-depleted chromatin	85	11±1.2
Nucleosome core particle	116	18±2.0
Naked DNA	54	6±0.6
MTR:Mg^{2+} complex I		
Native chromatin	107	14±2.0
H1-depleted chromatin	85	13±1.5
Nucleosome core particle	154	18±2.0
Naked DNA	33	5.2±0.5

[a] The standard deviation from three sets of experiments is 15%

2.3 *Sanguinarine*

Like mithramycin and chromomycin, the plant alkaloid sanguinarine interacts with chromosomal DNA with higher affinity compared to chromatin and nucleosome. Binding of sanguinarine leads to aggregation of chromatin, and destabilization of mononucleosome *in vitro*, causing the release of nucleosomal DNA. The most important aspect of sanguinarine interaction is that it binds to core histones as well. It also inhibits histone methylation and acetylation (Selvi *et al.* 2009).

2.4 *Ethidium bromide*

Although highly mutagenic, ethidium bromide has been widely studied as a typical DNA intercalator for the investigation of DNA topology. In the chromatin context, studies of ethidium bromide are important, since they reveal how changes in local DNA structure are reflected in a higher-order packaged state in chromatin. Taquet *et al.* (1998) have

shown that ethidium bromide binds to calf thymus DNA, H1/H5-depleted chromatin and chicken erythrocyte chromatin with decreasing order of binding affinity. It destabilizes the nucleosome structure and dissociates the nucleosome core particle (Mcmurray and Van Holde 1986). We have shown that ethidium bromide interacts with rat liver soluble chromatin, chromatosome and chromosomal DNA. Binding of ethidium bromide induces aggregation of chromatin. It also releases DNA from chromatosomes. However, interaction with core histones has not been observed.

3. Dual mode of chromatin binding

Chromatin being an assemblage of DNA and proteins complicates the ligand–polymer interaction scenario. In the chromatin context, small molecules that interact via non-covalent forces may bind to either chromosomal DNA or chromosomal proteins. A number of molecules have been reported that bind to both DNA and histones (Ghosh *et al.* 2010). Examples include the well-studied anthracycline antibiotics daunomycin, and mitoxantrone (Hajihassan and Rabbani-Chadegani 2011). Daunomycin interacts with chromatin-bound linker histones without any noticeable binding to core histones. However, in solution it binds with higher affinity to core histones H3/H4 and H2A/H2B as free proteins in solution (Rabbani *et al.* 2004). Mitoxantrone, on the contrary, binds to linker histones with higher affinity compared to core histones. Unlike daunomycin, mitoxantrone perturbs the secondary structure of core histones (Hajihassan and Rabbani-Chadegani 2011). We have recently observed the binding of mithramycin with chicken erythrocyte histone octamer. We have also reported the binding of a plant alkaloid sanguinarine with core histones in solution (Selvi *et al.* 2009). Table 2 classifies the known transcription inhibitors in terms of their single- and dual-binding capabilities.

4. Concluding remarks

The DNA binding ability and subsequent architectural alteration of the chromatin/nucleosome confers transcription inhibitory potential to small molecules, thereby making them molecules with therapeutic potential. The biological effect of the dual-binding mode might have far-reaching consequences. The molecules may serve as modulators of epigenetic modifications (Adhya and Basu 2010). Our results, for example, have shown that sanguinarine interacts with chromatin and potently inhibits H3K9 methylation by G9a *in vitro* and H3K4 and H3 R17 methylation in cells. It also inhibits histone acetylation both *in vitro* and *in vivo*. Nevertheless, it inhibits chromatin transcription (Selvi *et al.* 2009). Mithramycin has also been labelled as an epigenetic modulator, in that it prevents huntingtin-specific histone H3 hypermethylation at lysine 9 (Ferrante *et al.* 2004).

At present there is serious concern regarding the cellular toxicity of these molecules. The mutagenic effects of some anticancer agents are reviewed in Singh and Gupta (1983). Studies of the inhibition of TNF-α-induced fractalkine mRNA expression have shown that mithramycin is not toxic at less than 30 nm/L, whereas concentrations of mithramycin greater than 100 nm/L may have toxic effects with significant reduction of DNA synthesis and induction of apoptosis. Similar results were observed in case of chromomycin (Ahn *et al.* 2004). Moreover, it has been shown from our laboratory that mithramycin possesses alternate targets within the cell, further highlighting the cytotoxicity issue (Devi *et al.* 2009; Lahiri *et al.* 2011).

Table 2. Classification of some small transcription inhibitors based on their DNA binding property and the ensuing effect upon chromatin structure

Small Molecules	Modes of binding to DNA	Effect upon chromatin structure
Mithramycin	Minor groove binder	Aggregates chromatin and causes DNA release (Mir *et al.* 2003); binds to histone octamer
Chromomycin	Minor groove binder	Aggregates chromatin and causes DNA release (Mir *et al.* 2003)
Distamycin	Minor groove binder	Compacts (Majumder and Dasgupta 2011) and remodels chromatin (no DNA release)
Sanguinarine	Intercalator	Aggregates chromatin and causes DNA release; binds to histone octamer (Selvi *et al.* 2009; Rabbani *et al.* 1999)
Ethidium bromide	Intercalator	Aggregates chromatin and releases DNA
Daunomycin	Intercalator	Unfolds and aggregates chromatin; releases DNA; binds to linker and core histones (Rabbani *et al.* 1999; Mir *et al.* 2004; Rabbani *et al.* 2004)
Mitoxantrone	Intercalator	Compacts chromatin; binds to linker and core histones (Hajihassan and Rabbani-Chadegani 2011; Hajihassan and Rabbani-Chadegani 2009).

Thus, research in the past few years has led to the discovery of the histone binding potential of many DNA-binding molecules. At present, it is therefore necessary to re-examine the well-known DNA-binding molecules in an attempt to screen for single- and dual-binding possibilities in them.

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