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Mechanism of DNA–Drug Interactions

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ABSTRACT

Over the last two decades many strategies have been planned to design specific drugs for rare diseases to target their action at the DNA level. Advancements in our understanding of the interactions of small nonpeptide molecules with DNA have opened the doors for "rational" drug design. Special methods have now been developed to give accurate account of the precise location of ligand–DNA adducts on target DNA. We are now in a position to think of designing ligands that recognize particular sequences of base pairs. This work will allow us to enter into a new era of gene therapy for diseases like Cystic fibrosis, Alzheimer's disease and many related disorders at genetic level. These ligands can also be employed in the treatment of various types of cancers. They may also be useful as highly specific probes to locate particular sequences in the genomic DNA.

Index Entries: Antibiotics; intercalators; pseudonuclease; bisintercalators; antitumor agents.

INTRODUCTION

There are certain drugs that are shown to effect the very vital functions of living organisms, such as protein biosynthesis, nucleic acid replication and gene expression, collectively called antibiotics.

Over the past 20 years, enormous advancement has been made in our understanding of the interaction of these small molecules (antibiotics) and in general cytotoxins, with DNA. The interest derived not only from

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a pharmacological point, i.e., how a drug acts at molecular level, with the expectation that relating its structure to its action would contribute to the specific design of clinically useful agents, but also from the appreciation that antibiotics of determined specificity of action can be used as powerful tool for elucidating the biochemistry of nucleic acids and their physiological involvement.

The recognition of one type of molecule by another is fundamental and a very common feature of all living organisms (*1*). The replication of DNA and its transcription to RNA, which provides the template for protein synthesis, is perhaps one of the best known example of molecular recognition. These nonprotein molecules that are known to bind DNA molecules includes natural products such as antitumor antibiotics, viz.; anthracyclins (duanomycins, nogalomycin, cinnerubins, and so on) (*2–4*) and other secondary metabolites from bacteria and fungi (*5*) and plants, synthetic compounds, viz.; CC 1065 (*6*), copperphenanthroline complex (*7*), Alkylnitroso-urea (*1*), and so on, and also heterocyclic and multi ring heterocyclic aromatic compounds (*5*) and homopyrimidine oligonucleotides (*1*). The interaction of relatively small molecules of this type with DNA may lead to a "useful" results such as antibacterial and anticancer activity, but may also involve undesirable biological responses, such as carcinogenesis or mutagenesis.

For the preliminary confirmation of binding and/or bonding of a ligand to a DNA, spectrophotometry, circular dichroism, optical rotatory dispersion, IR, Raman spectroscopy, viscometric measurements, thermal denaturation studies, or association studies with radiolabeled version of the ligand, are carried out. Assays are available to evaluate the extent of DNA binding and to determine the sequence specificity. Calf thymus DNA is often used for physical studies because of its ready availability in a purified form and contains a relatively even distribution of AT and GC base pairs.

Three main techniques have an importance in determining the interactions, and are preferentially employed, viz., footprinting, NMR and X-ray crystallography.

DNA "footprinting" can be used for covalent and noncovalent binders, intercalators, and other types of adducts such as coordination complexes and triple helices (*8*). The ligand and radiolabeled DNA are incubated to allow binding and/or bonding to occur, and the adduct is then treated with methidiumpropyl.EDTA.Fe(II) (MPE.Fe(II)), which is capable of cleaving DNA nonselectively at every base pair (*9,10*). However, the regions of DNA bound to drug are protected from cleavage, which appear as gaps in the sequence of bands on the electrophoresis gel. The enzyme DNase I can also be used instead of MPE.Fe(II), but differs in that it produces larger footprints and reveals information about ligand-induced conformational changes of DNA. At sites of ligand attachment, the progress of enzyme is impeded and a cut may occur. Identifiable "stop sites" appear as intense bands on the gel that can be related to ligand binding sites.

NMR and X-ray crystallography are other powerful techniques for studying DNA binding with short-length of DNA; which can provide precise structural information about the types of interactions occurring and the functional groups involved (*11*). Ligands can be interacted with oligomers ranging from 6–15 basepairs or more, and two dimensional ^{1}H- or ^{31}P-NMR experiments such as NOSEY or COSY can be used to locate precisely the ligand on the strand. Data from X-ray crystallography and NMR can be used in conjunction with computational methods to generate useful 3-D models of ligand DNA complexes (*12,13*).

In this review our main aim is to discuss the mechanisms of action at a fundamental level of a limited number of an inexhaustible list of a large series of agents known to exert their biological activity via interactions with DNA.

MODES OF DRUG INTERACTION

Such type of interactions can inhibit the processes related to DNA, viz.; replication and transcription in the following ways:

1. Inhibition of Template Functions:
   a. Intercalators;
   b. Noncovalent complex formers;
   c. Covalent complex formers; and
   d. Type of interaction uncertain.
2. Inhibition of polymerase function.
3. Inhibitor complexing with template and inhibits polymerase function.
4. Triple-helix formers.

The drug that interacts with defined sequence of base pairs are termed "sequence selective" or "sequence specific." However, there are three levels of selectivity.

1. A compound may be selective for either the minor or major groove for an intercalative mode, between base pairs.
2. For covalent binders, a second level of selectivity results from a choice of a number of possible sites for noncovalent modifications, such as N-7 or G in the major groove or the N-3 of A or C-2-NH_2 of G in the minor groove.
3. The third level of selectivity relates to the length and type of sequence to which the ligand binds. Factors that contribute to base-pair recognition for small nonprotein ligands include the size of the ligand and the number and type of chemical interaction involved (*17*).

Table 1
DNA Intercalating Drugs

Compound/antibiotic	Mode of intercalation	Other notes
Actinomycin D	Binds to G-C base pair in the minor groove of ds-DNA. Binds with DNA only.	Transcription inhibitor
Anthracylins e.g., Duanomycins Nogalomycins Cinnerubins Pyrromycins Rhodomycins	Duanomycins binds to self complementary hexanucleotide d(CGTACG). Other drugs show a preference for a base pair triplet containing adjacent GC base pairs of variable sequences flanked by an AT base pair.	Used as anticancer drugs
Profavine, Acridine, and Ethidium	These fit snugly between adjacent base pair in the DNA helix.	Mutagens

The groove geometry and molecular electrostatic potential (MEP) at a particular sequence, helix distorsion energy and ligands solvation may also play a role in DNA binding and sequence selectivity (*17*).

Inhibition of Template Function

Intercalators

Intercalators are planar molecules usually consisting of 3–4 fused aromatic rings that are capable of intercalating between stacked nucleic acid base pairs. Table 1 gives some examples of intercalating drugs.

ACTINOMYCIN-D. A potent inhibitor of transcription in eukaryotes and prokaryotes produced by *Streptomycis antibioticus,* consisting of two cyclic pentapeptides bound to a phenoxazone ring (Fig. 1). The formation of a complex between DNA and drug has been detected with the advent of the techniques such as perturbation of the visible spectrum of DNA, elevation of DNA melting temperature, equilibrium dialysis with radioactive actinomycin, and inhibition of the transcription by RNA polymerase. Actinomycin inhibits synthesis of almost all fractions of RNA, with r-RNA being most susceptible (*5*).

The antibiotic specifically inhibits DNA-directed RNA polymerase of various organisms at the elongation level (*5*).

Using various synthetic and natural actinomycins, it has been shown that the integrity of several groups, however, are essential for its biological activity and ability to form stable complexes with DNA. The NH_2 group at position 3 of the chromophore, the unreduced quinonoid oxygen, and the

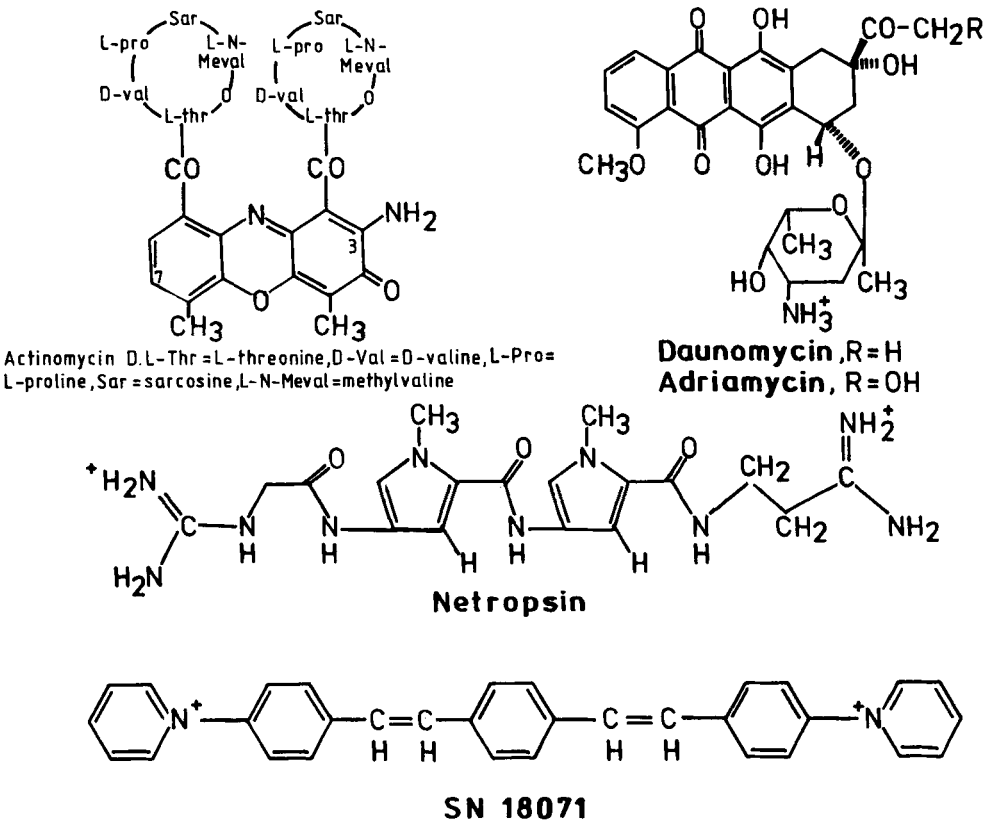

Fig. 1. The structure of the DNA interactive ligands.

intact cyclic pentapeptides are indispensible. A certain minor change in the amino acid sequence may cause minor activity change, but a major change leading to change in its conformation is not allowed (*5*).

Actinomycin D reacts with DNA only and forms a stable complex and reversible complex that can be isolated by various physical separation methods (*3,8,15*). Several studies have shown that for stable complex formation the DNA must be helical and possess G residues (*15*). The binding of actinomycin to DNA depends on G content but it is not proportional (*5*).

This antibiotic was considered to be located in the minor groove of helical DNA, with which it can form seven hydrogen bonds. This was also supported by X-ray diffraction studies (*16*). One hydrogen bond is formed between the quinonoid oxygen and 2-NH_2 group of G-residues; the 3-amino group of drug chromophore forms one hydrogen bond each with ring N-3 and the deoxyribose ring oxygen of the dG. The cyclic peptides of actinomycines are packed into the minor groove of the DNA-helix. The lactone stabilizes the peptide chain in a conformation such that -NH- group of peptide bonds can form hydrogen bonds with phosphodiester

oxygen of the strands opposite to that containing the guanosine that interacts with the actinomycin chromophore. In this manner it provides a considerable stabilization against DNA strand separation and thereby inhibits the movement of the RNA polymerase during transcription. Whereas, in the case of DNA replication the strands are separated by helicases, which reduce the binding or increase the rate of dissociation of drug from the DNA (*17*). Thus, this is not affected.

ANTHRACYCLINS. Antibiotics such as duanomycin (Rubidomycin), nogalomycin, cinnerubins, pyrromycins, rhodomycins, and the reticulomycins contain a tetrahydrotetracenquinone ring system linked to a sugar and are considered as belonging to the anthracyclin group of antibiotics.

The duanomycins and adriamycins represent one of the best known classes of intercalators and are important drugs in cancer. Duanomycin is effective for treating acute leukemia, whereas adriamycin, is more effective for treating solid tumors (*23*). They predominantly bind to duplex DNA and inhibit both its transcription and replication. These antibiotics act by interfering with the passage of both RNA and DNA polymerases, and the affinity of these drugs for DNA increases with increasing GC content. Footprinting studies demonstrate a preference for a base pair triplet containing adjacent GC base pairs of variable sequences flanked by an AT base pair (*10,17*).

The X-ray crystal structure of a complex of duanomycin with self complementary hexanuclotide (dCGTACG) reveals that duanomycin's planar ring system (B-D) (Fig. 1) is intercalated between the CG pairs at both ends of the double helical fragment (*18*). The nonpolar A ring extends into the minor groove where the side groups stabilize the complex through hydrogen bonding interactions with DNA (*8*). The additional C-14 OH functional group of duanomycin appears to enhance binding affinity, possibly through hydrogen bonding to an oxygen of a sugar residue or to a phosphate group (*10,17*).

There are also bisintercalators such as echinomycin that consists of two intercalating moieties joined by a linker capable of intercalation at two sites separated by a distance defined by the linker length (*18*). It is a cyclic decipeptide antibiotic which is active against gram-positive bacteria and displays a potent antitumor activity also (*20,21*). The first physical characterization of the drug DNA complex showed that this drug binds tightly as a bisintercalator (*21*).

Low et al. (*22*) have shown a maximum binding site of about 6–8 bases with DNase I and II footprints, whereas MPE-Fe(II) footprinting studies showed smaller binding sites of 4 bases with sequence NCGN. van Dyke and Dervan have identified the sequence of binding by restriction fragment DNA sampling. (*8*).

Nogalomycin strictly inhibits DNA-directed RNA synthesis in vivo (*4,24,25*), and this drug is more effective against gram-positive bacteria

(*24,26,27*). In spite of the mechanistic obstacle presented by the dumbbell shaped nogalomycin the drug forms a stable intercalative complex with the DNA (*28–35*). The rate of association and dissociation of DNA and drug are very slow (*4,36*) and these observations are consistent with the suggestion that transient DNA melting is required for entry of nogalomycin into the duplex (*37*). An alternative mechanism has also been proposed that an opening to allow entry of nogalomycin could be created by unstacking and buckling of DNA base pairs without hydrogen bond disruption (*23,24*). Liaw et al. (*38*) has shown that nogalomycin binds with higher affinity to AT rich regions on DNA.

Profavine, acridine and ethidium are also archetypal cationic DNA intercalating molecules. They are potent mutagens and cause frame shift mutations (*3,33*). Profavine intercalates into DNA with essentially all of its aromatic system inserted between base pairs that forms the top and bottom of the intercalation site (*39*). Detailed crystallographic and NMR studies have shown that many well-characterized intercalators such as ethidium (*40,41*), actinomycin (*42,43*), and duanomycin (*44*) intercalate with DNA such that their bulky substituents are in the minor groove.

The anticancer drug amsacrine, also binds with its bulky substituents in the minor groove (*45,46*). The insertion of a simple aromatic ring system such as anthracene between DNA base pairs to form an intercalation complex requires separation of adjacent base pairs by 3.4 Å (*39*). These mutagens, once intercalated, distort the DNA double helix and disrupt DNA polymerase so that an addition or deletion of one or more nucleotides occurs (*3*).

Noncovalent Minor Groove Binders

There are a group of compounds that have been included in this class. These are netropsin, distamycin A_3, SN18071, chromomycin A_3, mithramycin, olivomycin, rubiflavin, hedamycin and pluramycin.

Netrospin (Fig. 1) and the related distamycin A_3 are relatively long, flexible, molecules with one or more positively charged end groups and a number of proton donor and acceptor groups along their length. Minor groove binders are typically isohelical with B-DNA and fit snugly within the minor groove held in position by a combination of hydrogen bonds, van der Waals forces, and electrostatic interactions (*10*).

The sequence selectivity of netropsin-like binders for A-T rich region of DNA is a common feature, and one explanation is that steric hindrance may result from protrusion of guanine C2-NH_2 group into the minor groove at GC sites (*45*). Alternatively, A-T rich sequences may correspond to sites of maximum molecular electrostatic potential (MEP) in the minor groove (*17*), which may attract ligands, especially those with cationic functional groups. The bisquaternary heterocycle SN18071 cannot form hydrogen bonds but still binds in the minor groove with a similar AT selectivity, apparently confirming the role of electrostatic attraction

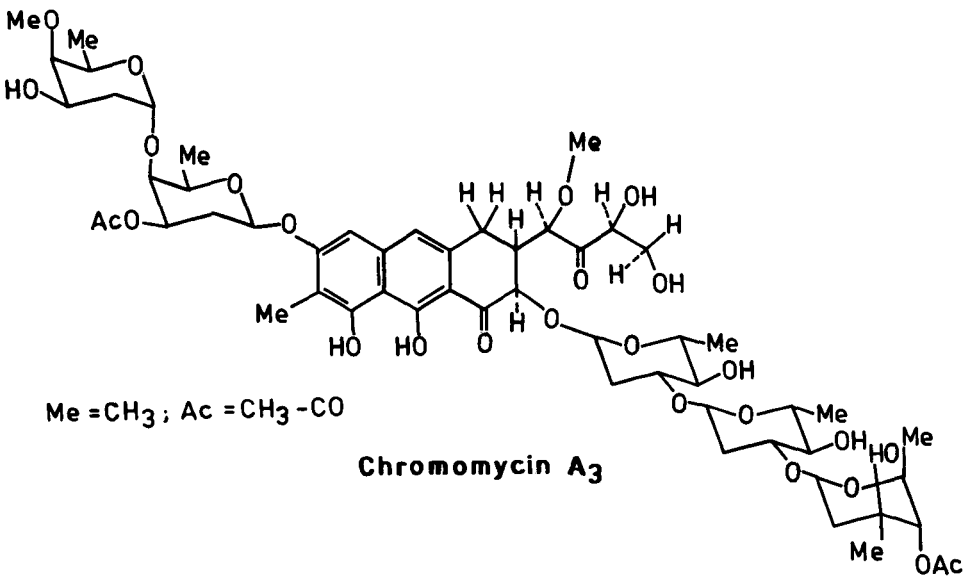

Fig. 2. The structure of Chromomycin A_3.

(*10*). However, a netropsin derivative devoid of cationic end groups can still bind to poly(dA)-poly(dt) oligomers, demonstrating that electrostatic attraction or interactions are not essential for binding (*10*). Recently, it has been suggested that the preference for binding is for a more equal distribution of GC and AT base pairs.

Chromomycin A_3 (Fig. 2), mithramycin and olivomycin are also intercalated into DNA and are closely related antibiotics having identical mechanisms of action and are considered as a group. The sugar sidechains differ in three antibiotics and the chromophore of olivomycin is slightly different.

These agents are potent inhibitors of transcription in both prokaryotes and eukaryotes. Stable complexes are formed between DNA and antibiotic but these can be dissociated with organic solvents which indicates that covalent bonds are not formed (*28,48–50*). Like actinomycin D these drugs are also requiring 2-NH_2 groups of G in helical DNA for complex formation but differ in requiring the presence of divalent cation for interaction like Mg^{2+} ions (*48,49*). The amount of Mg^{2+} required to promote maximum interaction between DNA and antibiotic corresponds to a 1:1 molar equivalence with antibiotic concentration (*48*). The bulky sidechains appear not to influence the rate of association reactions but the rate of complex dissociation increases with a decrease in the size of the chains (*48*). Unlike actinomycin these antibiotics do not cause uncoiling of the DNA double helix, which suggests that these antibiotics do not intercalate into DNA.

The noncovalent cationic complex *bis*(1,10-phenanthroline) Copper(I) $(phen)_2[Cu^I]^I$ binds noncovalently exclusively in the minor groove of the

DNA molecule (*53,54*). In the presence of molecular oxygen and a reducing agent this complex acts as an effective nuclease with a high preference for ds.DNA. $(Phen)_2[Cu^I]^I$ complex has been used as a small molecular "pseudonuclease" for examining the sequence specificity of DNA binding of a variety of ligands by the "footprinting" technique (*56–58*).

Phenanthroline complexed with copper has also been used as the active moiety in synthetic restriction endonuclease constructed by covalently coupling phenanthroline to polynucleotides or sequence specific DNA binding proteins (*59–61*).

Covalent Complex Formation

Electrophilic functional groups such as epoxide, aziridines, carbinolamines, imines, and natural products are capable of covalent interaction with DNA. Nitrogen mustards such as mechlorethamine represent one of the simplest classes of covalent binders, and many compounds of this family are clinically useful anticancer drugs. They act by crosslinking DNA through covalent bonds to G N-7 atoms in the major groove and exhibit selectivity for a guanine flanked by two other guanines (*64*). Table 2 gives some examples of covalent complex formers.

The alkylating species is generally considered to be the cyclic aziridinium ion and the selectivity can be seen by calculation showing that the highest MEP at a G N-7 of a central G in a GGG sequence is greater than other representative triplets such as AGA or TGT (*10*).

Mitomycin C (Fig. 3) is representative of antibiotics which bind to a DNA template by covalent linkage. Upon activation by chemical or enzymatic reduction mitomycin becomes a bisfunctional alkylating agent that can form covalent bonds with many types of biological molecules, including nucleic acids (*5*).

As a bisfunctional alkylating agent, mitomycin induces crosslinks between the complementary strands of the DNA, thus ensuring spontaneous renaturation of the DNA when the strands are partially separated. It binds to N-2 or N-7 of G in minor groove or major groove via carbocation species.

Type of Binding Uncertain

CARZINOPHYLLIN AND STREPTONIGRIN. These antibiotics, like mitomycin, selectively inhibit DNA synthesis, leading to degradation of DNA. Carzinophyllin, appears to induce crosslinking in DNA, as evidenced by the reversibility of heat denaturation. Unlike, mitomycin, however, carzinophyllin can accomplish this in vitro without requiring prior activation (*5*).

Streptonigrin (Fig. 3) is related to mitomycin in having an aminoquinone group but lacks the reactive aziridine ring characteristics of mitomycines. It does not crosslink DNA, but does bind to DNA in vivo evidenced by higher T_m value. It has been suggested that streptonigrin is

Table 2
Covalent Binding Drugs

Compounds	Binding	Other notes
CC1065	To N-3 of A in minor groove via Cyclopropane moiety (*23*)	Anticancer antibiotic preferred sequences include 5′-AAAAA
Aflatoxin B_1 oxide	To N-7 of G in minor groove via an epoxide (formed through metabolism) (*24*)	Carcinogen and mutagen
Benz(a)pyrenediol epoxide	To C2-NH_2 of G in minor groove via an epoxide (formed through metabolism) also to N-7 of G in major groove	Carcinogen
Anthramycin	To N_2 of G in minor groove via a carbinolamine moiety (*25–27*)	Antitumor antibiotic; preference for purine G triplet
Saframycin	Evidence for binding in N_2 of G in minor groove via a carbinolamine moiety	Antitumor antibiotic
Mitomycin C	To N_2 of N-7 of G in minor or major groove via carbocation species	Antitumor antibiotic
Alkylinitrosourea	To N-7 of G in major groove via carbocation species	Synthetic antitumor agent
Mitozolamides	To N-7 of G in major groove	Synthetic antitumor agent
Cisplatin	Coordination complexes with single atom crosslinks between N-7 atom of adjacent G bases in major groove; also crosslinks N-7 and O-6 of same (*47*)	Synthetic antitumor agent
Tris (phenantholine) Cu(II) complexes	To N-7 of G in major groove via chiral octahedral crosslinks (*28*)	DNA probes (left handed isomer bonds better than right)

reduced intracellularly and then interacts with the DNA to produce single-strand breaks. In this respect it differs from mitomycin.

The lethal event is accomplished, however, by the degradation of DNA. Since, both an electron source and oxygen were found to be necessary for streptonigrin to exert maximal lethality (*5*), it was postulated that a reaction product of oxygen and intracellularly reduced antibiotic perhaps a peroxide or peroxy free radical, is the lethal agent that interacts

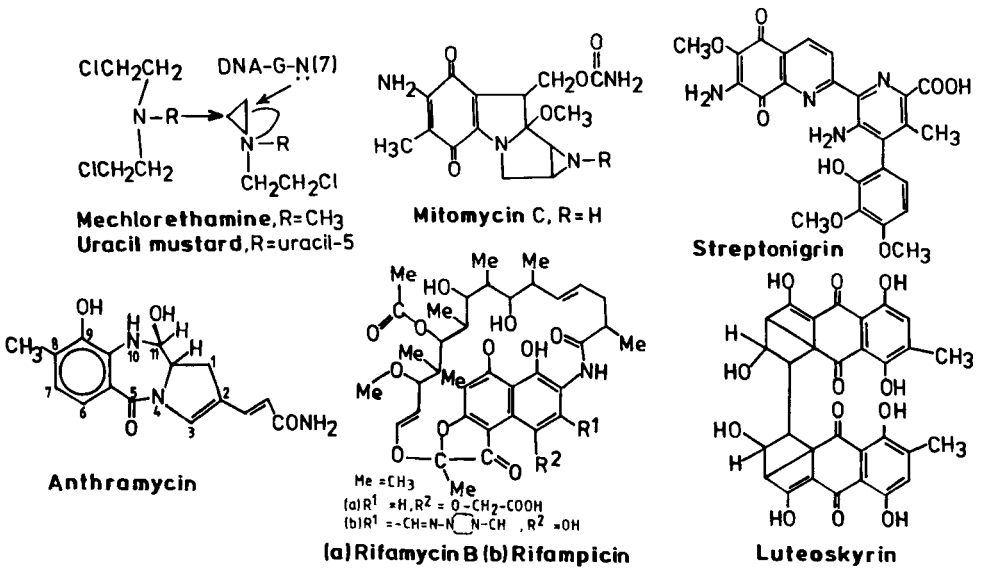

Fig. 3. The structure of the DNA interactive ligands.

with DNA to produce single-strand breaks with single hit kinetics, analogous to X-ray irradiation.

BLEOMYCINS AND PHLEOMYCINS. The bleomycins and phleomycins are closely related copper chelating antibiotics. These agents exhibit antibacterial and antitumor activities, and have been found to inhibit DNA synthesis in bacterial and mammalian cells. Both antibiotics are antimitotic agents.

Phleomycins selectively inhibit both the bacterial and mammalian DNA polymerase reaction and exonuclease I reaction by binding to DNA. The antibiotic appears to bind to the carbonyl oxygen at the 2 position of thymidine. Binding of these antibiotics are diminished in the presence of Cu^{2+}, Zn^{2+}, and Co^{2+} ions.

The bleomycins are a group of glycopeptide antitumor antibiotics that are used effectively in the treatment of several cancers (*65*). Two features of the bleomycin molecule are related to its biological action, an ability to bind DNA, and an ability to bind metal ions (*66,67*). When ferrous ions are complexed to bleomycin in the presence of oxygen, the complex can cleave DNA strands, largely at G-C(5′→3′) and G-T(5′→3′) sequences, and this iron ion dependent cleavage is thought to be the mechanism by which bleomycin exerts its antitumor effect (*68–70*).

DNA degradation by a bleomycin-Fe^{3+} complex can be brought about either by addition of a reducing agent, such as ascorbic acid, in the presence of oxygen or by addition of hydrogen peroxide (*71,72*). Ascorbic acid incubation of bleomycin with Fe^{2+} or Fe^{3+} in the presence of a reducing system has been suggested to produce highly reactive hydroxyl radicals (ȮH). However, DNA degradation by bleomycin/Fe^{3+} in the presence of

oxygen and ascorbic acid is not inhibited by catalase, and scavenger of OH, or superoxide dismutase (*73–77*).

It is now widely accepted that DNA cleaving species is an oxo-iron complex of bleomycin, such as bleomycin-Fe^{3+} O_2H^- (*78,79*). Gajewski et al. have shown that treatment of DNA with bleomycin/iron ions leading not only to cleavage of the DNA backbone but also to the formation of (8-OH guanine) in the DNA (*80,81*).

ANTHRAMYCINS. These are potent, cytotoxic antibiotics, that are active against bacteria and tumors and inhibit DNA and RNA synthesis (Fig. 3).

The anthramycins form a very tight complex with ds helical DNA. The firmness of binding of anthramycins with DNA appears to be greater than expected for the usual noncovalent type of interaction.

Both base composition and conformation of DNA are important factors in anthramycin DNA interaction. Anthramycins binds readily to *dGn–dCn* but not at all to $d(A–T)_n$. It has been found that free positions 9 and 10 are important for interaction of DNA (*5*).

Inhibition of Polymerase Function

RIFAMYCINS. Two closely related antibiotics, Rifamycin B and Rifampicin (Fig. 3) are potent inhibitors of prokaryotic RNA polymerase: Rifamycin B is produced by *Streptomyces mediterrani* and rifampicin is its semisynthetic derivative.

It is very specific for prokaryotic RNA polymerase but does not at all affect eukaryotic RNA polymerase (K_i amount required for 50% inhibition is $2 \times 10^{-8}M$). It is used against gram-positive bacteria and also in tuberculosis. The RNA polymerase consists of two α and β and one σ subunit: The β subunit has a binding site for this drug. This drug does not inhibit the elongation process, but the incorporation of the first purine nucleotide into RNA is blocked.

This inactivates RNA polymerase and this inactivated enzyme always remains bound to the DNA, thereby blocking the binding of uninhibited enzyme.

Inhibitor Complexing with Template and Inhibiting Polymerase Function

LUTEOSKYRIN. This agent is a potent hepatotoxic excreted from the fungus mat *Penicillium islandicum,* associated with yellow rice.

Luteoskyrin (Fig. 3) contains an identical pair of 4-membered ring systems. This interacts with DNA but only in the presence of stoichiometric quantities of Mg^{2+}. As with the chromomycin-like antibiotics, Luteoskyrin Mg^{2+} complex is formed first and this subsequently binds to DNA (*5*).

It can form two distinct kinds of complexes with DNA. Complex I is formed rapidly with single-stranded nucleic acids containing purine bases. To form complex I at least 5–6 nucleotide long oligonucleotides should be present, which suggests that a specific conformation is required for this binding.

Complex II is formed with double-stranded nucleic acids. For complex I formation any purine residue in a polynucleotide is a potential site but neither the nature of the purine nor the nature of the sugar is important. It is a potent inhibitor of *E. coli* RNA polymerase.

Triple-Helix Formation

Another mode of interaction involves the formation of a "Triple Helix." Triple helix formers or triplex DNA, a triple strand form of DNA was produced by Alexander Rich in 1957. Homopyrimidrine oligonucleotides of 10–20 base pairs long have been shown to form stable complexes in the major groove at homopurine with GC and AT complementarity (*83*). Researchers in several labs are exploring the use of triplex DNA to produce a new type of "molecular scissors" for cutting DNA. It has been demonstrated recently that triplex DNA can be generated at specific sites on naturally occurring genes, and this can disrupt gene transcription. The first experimental verification of the possibility of the use of triplex DNA in therapy came 5 years ago when Hogan and Hay Balor colleagues showed that, at least in a test-tube assay, triplex formation could selectively inhibit a target gene. This was correlated with repression of the activity of the human *myc* gene (*84*).

One of the goals of this type of research work is to develop specific probes that can recognize a particular sequence of base pairs with a view to modify gene expression (*85*).

There is particular interest in developing ligands with GC specificity because most of the known compounds are AT selective. This interest originated from the finding that regions of high GC content are common in genomes of mammals, including humans, and 5′ flanks of certain oncogenes.

CONCLUSION

With the advancement of analytical methods we have been able to understand the mode of recognition of DNA sequences by these drugs. This understanding can be utilized to design specific drugs for a particular disease. These drugs will be unique for a particular gene and of possible use in therapy (*86*). The ability of selectively varying substituent size and thus, *intercalation kinetics*, whereas maintaining fairly constant

binding free energy offers the chance to develop important medicinal agents as well as probes for large-amplitude DNA dynamics.

However, before making use of these drugs just by understanding the nature of interaction with DNA, we will also have to take into account the mutagenicity and carcinogenicity of that drug. For example, in Alzheimer's disease, β-amyloid proteins are deposited in the brain. So, to check its synthesis specific drugs can be designed or specific homopolymers can be made that can recognize specific sequences of β-amyloid protein gene (*86*). Triplex DNA is turning to have therapeutic potential as well.

Similarly, in many autoimmune disease, viz., rheumatoid arthritis, antibodies are formed for the self-antigen of synovial fluids. By identifying such a gene, which is coding for the synthesis of these antibodies, specific antibiotics that can recognize a very vital part of the gene can be used to prevent expression of these genes or specific probes can be developed that will form a triple helix and thereby will stop expression (*87*).

The newest and perhaps most exciting development in rational drug design is the use of molecular graphics. A few computer-aided drugs have already reached the market (e.g., antihypertension agents). Many drug companies are betting on this powerful tool as their best weapon in the fight against viral infections, cancer, and other ills.

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