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Simple Coordination Complexes: Drugs and Probes for DNA Structure

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Simple Coordination Complexes: Drugs and Probes for DNA Structure

Specific interactions of some simple transition metal complexes with DNA are described. Several binding modes and reactions, intercalation, covalent modification, and oxidative cleavage, are discussed and all have in common the specific recognition by metal complexes of subtle features of DNA helical structure. The versatility of the metal center, from its spectroscopic properties to stereochemistry, suggests that coordination complexes may be promising candidates to exploit in the design of drugs and probes for DNA.

INTRODUCTION

Metal complexes are uniquely suited for specific interaction with DNA. Their shapes, charges, and propensity for binding to nucleic acid sites make the coordination complexes attractive probes to examine DNA structure and potentially useful reagents in site-specific drug design.[1,2] This area of research, while still in its infancy, is burgeoning. The youth and yet strength of the field is probably best illustrated by the fact that the simple coordination complex *cis*-dichlorodiammineplatinum(II) (*cis*-DDP), isolated more than 100 years ago by inorganic chemists, is only today becoming one of the most widely used antitumor drugs.[3]

Why is the design of molecules targeted specifically for DNA sites important? The DNA molecule contains all the genetic information necessary for cellular function.[4] The control or regulation of what genetic information is expressed on a chemical level must depend upon binding proteins or small molecules to specific sites along the DNA strand. We and others are interested in understanding how

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that recognition process takes place, how the DNA structure varies along the strand to direct specific binding at some loci and not others, and what the factors are that determine specificity in binding. How is a local sequence of bases, adenine, thymine, guanine, or cytosine recognized and what are the consequences of specific modifications?

Metal complexes can be particularly useful probes for the DNA structure.[5] The spectroscopic sensitivity of intense metal to ligand charge transfer bands, for example, makes metal complexes bound to the helix good reporters of the local DNA environment. The high electron density of heavy metals similarly provides useful probes in x-ray scattering experiments. Moreover, features intrinsic to metal complexes result in avid binding to the DNA helix. DNA is a polyanion, and electrostatic considerations alone account for a high association of cationic metal centers with nucleic acids. DNA also contains a rich selection of coordination sites, both the hard phosphate oxygen atoms and softer, polarizable heterocyclic base nitrogen positions. Importantly, coordination complexes offer many unique, well-defined geometries that can be useful in designing reagents to match specific local DNA conformations. And finally the high redox activity of many metal complexes can be exploited in directing chemistry at specific sites along the DNA strand and not at others.

In this Comment we will describe some examples of how these features of the interactions of metal complexes with DNA have been exploited in specific probe and drug design. We will focus on the important characteristics in the interaction that are unique to the coordination complex. This discussion is therefore not intended as a general review of metal–nucleic acid interactions. Descriptive accounts of metal–nucleic acid chemistry and systematic structural studies may be found elsewhere.[1-3,6-9] Detailed discussions of possible modes of action for the platinum antitumor drugs and studies of metal chemotherapeutic agents in general have also recently been written.[1,2,10-12] Our intention here is instead to illustrate a variety of contributions that have been made to our understanding of DNA structure and recognition by taking advantage of a metal center.

NONCOVALENT INTERACTIONS

A simple noncovalent binding mode of small molecules to DNA is that of intercalation.[13] Planar heterocyclic chromophores can insert and stack between the base pairs of the DNA helix. Figure 1 shows

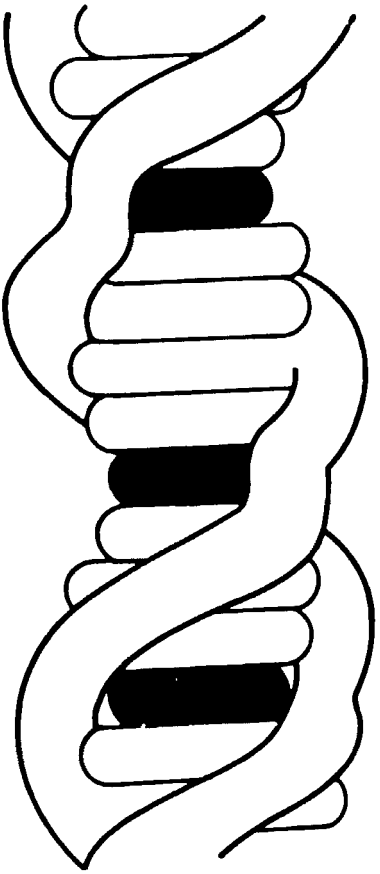


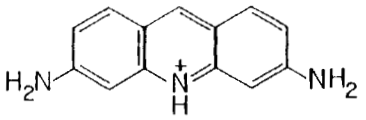
FIGURE 1 Schematic illustration of intercalation. (Reproduced with permission of John Wiley & Sons, Ltd. from Ref. 14.)

a schematic illustration of intercalation. The base pairs must separate and the duplex unwind to accommodate the planar, aromatic intercalator. Notice that the orientation of the small molecule is precisely defined with respect to the DNA helix, since the stacking interaction in some sense anchors the molecule parallel to the plane of the base pairs. Intercalation is a common binding mode for organic cationic dyes.[14] Many are frame-shift mutagens and display antibiotic and antifungal activity.[15] Several natural antibiotic anthracyclines such as daunomyucin and echinomycin contain a planar chromophore and show potent antitumor activity.[16] Besides the pharmacological utility

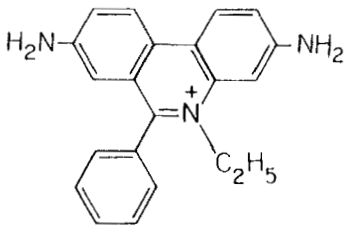
of intercalating agents, many are useful probes and stains for DNA structure. Probably the most common stain for DNA in electrophoresis experiments is ethidium; the fluorescence of ethidium is enhanced by approximately a factor of 20 upon DNA intercalation.[17] The structures of some intercalators are given in Figure 2.

Shown also in Figure 2 is the first example of a metallointercalation reagent, (2-hydroxyethanethiolate)terpyridylplatinum(II), [(terpy)Pt-(HET)]$^+$.[18,19] Lippard and co-workers demonstrated that a cationic metal complex containing a flat, aromatic ligand can also intercalate into the DNA helix. DNA melting temperature studies showed that (terpy)Pt(HET)$^+$ stabilized the duplex versus single stranded form. Viscosity studies showed the increase in DNA length necessary for insertion of the complexes, and on binding to DNA, the sulfur–platinum charge transfer band exhibited hypochromicity, a characteristic common to the organic stacked chromophores. The electron-dense platinum intercalator was used to identify general stereochemical constraints on the intercalative binding mode. X-ray diffraction studies of DNA fibers saturated with bound [(terpy)Pt(HET)]$^+$ showed a regular distribution of platinum atoms at 10.2 Å intervals along the helix corresponding to occupancy of the intercalator in every other interbase pair site at saturation.[20] The diffraction photographs thus provided direct evidence for the "neighbor exclusion" model[21] of drug binding to DNA, and suggested an alternating conformation about the sugar phosphate backbone. The electron-dense platinum "addition probe" was critical to these experiments. Since the electron density of an organic intercalator is equivalent to that of a base-pair, diffraction studies[14] of drug-bound DNA fibers had previously shown only disorder, and yielded no information.

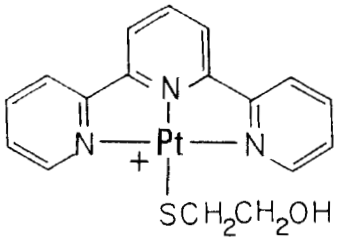
The different effects seen in binding these simple coordination complexes to DNA provided a striking illustration of the most important stereochemical requirement for intercalation, that is that the intercalative moiety be flat.[22] Figure 3 shows fiber diffraction patterns of calf thymus DNA in the presence of [(phen)Pt(en)]$^{2+}$, [(bipy)Pt(en)]$^{2+}$, and the nonintercalating [(py)$_2$Pt(en)]$^{2+}$. The intense 10.2 Å layer lines characteristic of the intercalated platinum species are evident in photographs of bound [(bipy)Pt(en)]$^{2+}$ and [(phen)Pt(en)]$^{2+}$. In the presence of the bis(pyridine) species, however, the fiber diffraction pattern for an ordered DNA helix is seen; the DNA helix is unperturbed by the platinum species. The patterns illustrate nicely that both the (phenanthroline)platinum and (bipyr-



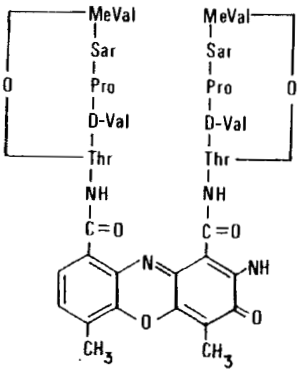
proflavine



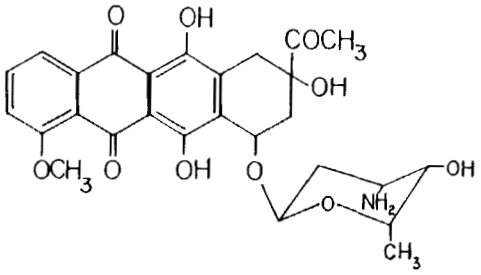
ethidium



$[(terpy)Pt(HET)]^{+}$

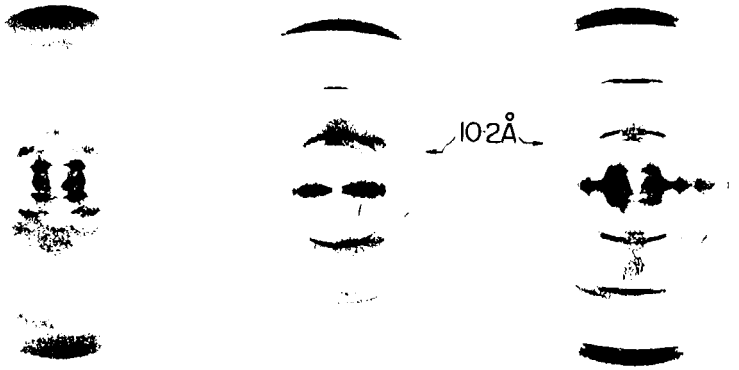


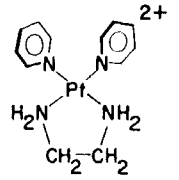
actinomycin



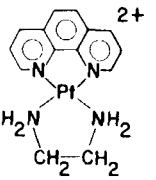
daunomycin

FIGURE 2 Some intercalating agents.

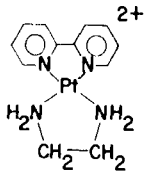




$[(py)_2Pt(en)]^{2+}$



$[(o\text{-}phen)Pt(en)]^{2+}$



$[(bipy)Pt(en)]^{2+}$

FIGURE 3 X-ray diffraction patterns of calf thymus DNA with bound platinum reagents. Note that reflections occur at the 10.2 Å repeat characteristic of intercalation for the planar platinum reagents. (Reproduced with permission from Ref. 22. Copyright 1976, AAAS.)

idyl)platinum cations that have planar aromatic ligand systems intercalate easily into DNA. However, for $[(py)_2Pt(en)]^{2+}$ nonbonded steric interactions rotate the pyridine ligands out of the coordination plane and thus prevent intercalation. Closed circular DNA unwinding experiments also supported these findings.

Among the most common and well-characterized planar aromatic metal complexes are surely the metalloporphyrins. Do these complexes intercalate into DNA? Several physical–chemical studies, including absorption spectroscopy, viscometry, circular dichroism, and superhelical DNA unwinding, are consistent with DNA intercalation by the large cationic porphyrin ligands.[23–25] Copper(II), zinc(II), cobalt(III), iron(III), and manganese derivatives have also been examined spectroscopically.[26] However, it is difficult to imagine the full

insertion of the large tetra-(4-*N*-methylpyridyl)porphine complexes into the helix, especially in view of the wide expanse of the complex, the steric barrier of the four *meso*-pyridyl substituents, and the high local charge on the metal derivatives. Nonetheless some specific hydrophobic interaction with the DNA helix, possibly involving a partial insertion of the ligand in between the base pairs, seems likely.

These studies raise the question of whether in fact full insertion of the metal complex into the helix is necessary. Is a planar coordination geometry about the metal center a requirement for stable intercalation or is the stacking interaction obtained upon the partial insertion of a flat aromatic ligand sufficient? The platinum(II) complexes, for which the intercalative binding mode had been characterized in detail, all possess a square planar configuration and in fact the crystal structure of $[(terpy)Pt(HET)]^+$ intercalated in *d*(CpG) revealed the platinum center sandwiched between base pairs with an axial interaction with the guanine 06 atoms.[27]

To address this question, we examined binding of tetrahedral (phen)$ZnCl_2$ to duplex DNA.[28] Figure 4 shows a plot of the electrophoretic mobility of DNA through agarose gels containing increasing concentrations of (phen)$ZnCl_2$. The helical DNA when closed in a circle possesses topological constraints that fix the total number of duplex turns plus tertiary supercoils. Just as one might find in a tightly wound rubber band or telephone cord, the total winding of the helix (duplex plus tertiary) is a constant as long as the circle is closed. The torsional strain is however released if even one strand is cleaved, yielding the nicked circular form which contains no supercoils. The topological relationship can be used advantageously in assaying for intercalation or duplex unwinding, since supercoiling alters the hydrodynamic properties of the polymer in an easily detectable manner.[29,30] As can be seen in Figure 4, without zinc present, the compact superhelically wound closed circle has a greater mobility through the gel than the floppy nicked species. As the concentration of (phen)$ZnCl_2$ is increased, the DNA duplex is unwound, releasing the negative supercoils in the native plasmid, and therefore decreasing the mobility. At an added concentration of 0.5 mM zinc, the nicked and closed circles comigrate; sufficient metal complex is bound to unwind completely the supercoiled DNA. Increasing the zinc concentration still further, then, leads to an introduction of positive supercoils, and the mobility increases once again. This electrophoretic assay had been applied earlier in platinum studies[19] and is a very

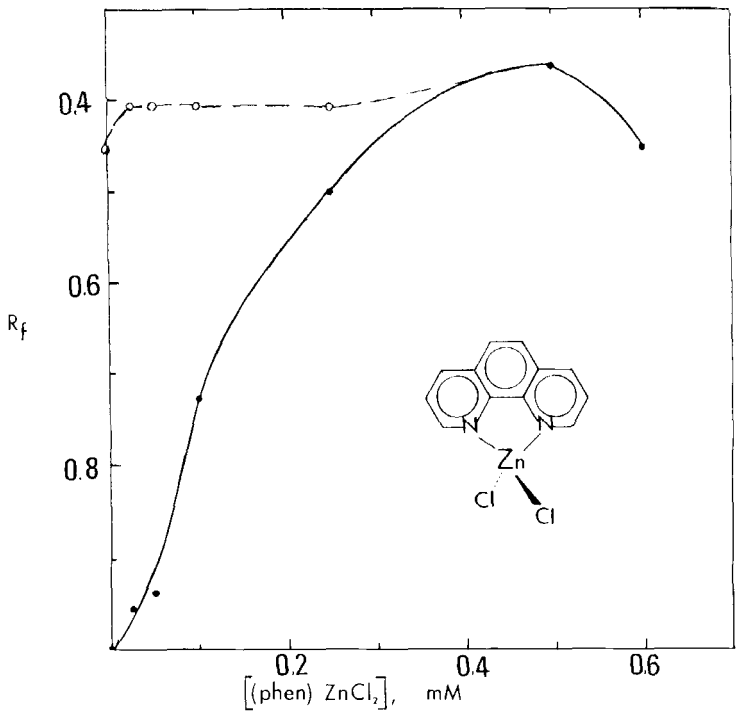


FIGURE 4 Plot of the electrophoretic mobility of closed (●) and nicked (○) circular DNAs through gels containing increasing concentrations of Zn(phen)Cl_2. This electrophoretic profile reflects the duplex unwinding that is characteristic of intercalation.

strong indication of the duplex unwinding that is characteristic of intercalation. Free phenanthroline at comparable concentrations showed no effect on the DNA structure. Since in solution (phen)$ZnCl_2$ yields a mixture of hydrolysis products, electrophoretic titrations of supercoiled DNA with mono(phenanthroline)dichloro zinc, bis-(phenanthroline)zinc, and the coordinatively saturated tris(phenanthroline)zinc cation were conducted and all revealed a similar unwinding profile. Moreover, the level of binding, amount of unwinding per zinc, increased with phenanthroline substitution. The experiments therefore demonstrated that metallointercalators required only a planar aromatic ligand, not a planar coordination geometry.

Advantage can therefore be taken of additional nonintercalated ligands in designing complexes that bind to the helix with some degree of specificity. One possible mode of discrimination is that

based upon stereochemistry. Figure 5 shows structures of the two enantiomers of an octahedral tris(phenanthroline) metal complex. From equilibrium dialysis of calf thymus DNA against racemic $(phen)_3Zn^{2+}$, an optically active dialysate, enriched in the enantiomer having a poorer affinity for DNA, was prepared.[28] [Interestingly, the DNA template permitted the isolation of optically enriched $(phen)_3Zn^{2+}$ for the first time. The racemization of the zinc complex is not instantaneous but occurs over several days at 4°C.] This experiment demonstrated that binding the chiral metal complex to the asymmetric DNA helix is stereoselective. The DNA helix discriminates between metal isomers. This selectivity is best understood in the framework of the intercalation model.[31] As Figure 5 illustrates, with one ligand intercalated, and thus fixed perpendicular to the helix axis, the two remaining nonintercalated ligands of the delta isomer are disposed along the right-handed helical groove. For the Λ-isomer, intercalation is inhibited by steric repulsions between phenanthroline hydrogen atoms and the DNA-phosphate oxygen atoms; the disposition of the nonintercalated ligands opposes the screw of the helix. Despite studies of many natural product intercalators with DNAs, these experiments represented a first description of enantiomeric selectivity in DNA intercalation. The location of the asymmetric center (the metal), proximal to the site of intercalation, (at the ligand) was an important advantage.

The stereoselective binding model was confirmed in experiments with tris(phenanthroline)ruthenium(II) complexes.[31,32] Another advantage of the coordination chemistry rests in the ability to vary the metal for the application required. The low spin d^6 ruthenium(II) complexes are inert to racemization, have been well characterized, and the absolute configurations have been assigned.[33-35] CD spectra of $(phen)_3Ru^{2+}$ solutions optically enriched after dialysis against DNA and of Δ-$(phen)_3Ru^{2+}$ isolated independently mirror one another, thus making clear that the Λ-isomer is less favored in binding to the helix. The intense metal to ligand charge transfer band and strong luminescence that are characteristic of aromatic amine ruthenium(II) complexes futhermore provide a sensitive, spectroscopic handle with which to monitor the binding process.[36] Figure 6 shows absorption and luminescence spectra of $(phen)_3Ru^{2+}$ in the absence and presence of DNA.[32] On stacking within the helix, both hypochromicity and enhanced luminescence are evident. The excited state lifetime of aerated $(phen)_3Ru^{2+}$ solutions increases from 0.6 to 2.0 μs

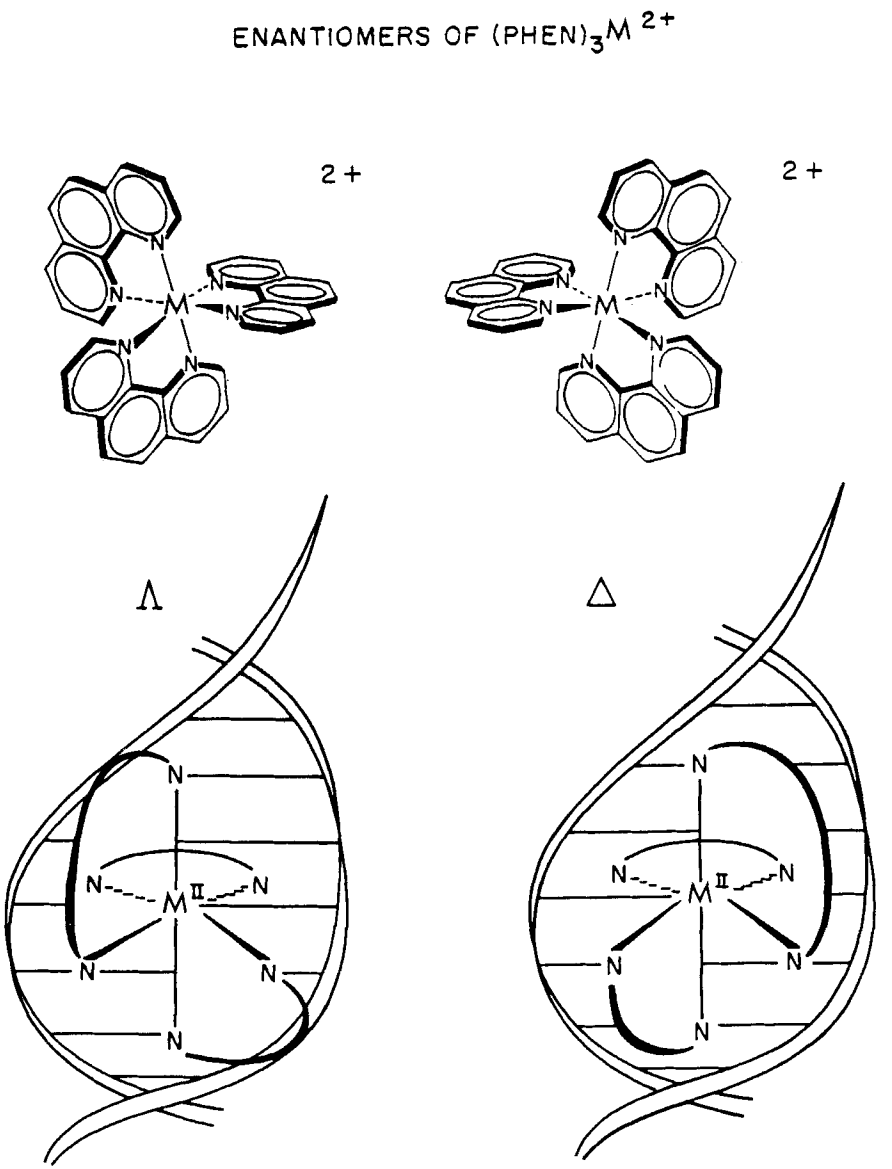


FIGURE 5 Lambda and delta tris(phenanthroline) metal complexes, their structures and schematic illustration intercalated in right-handed DNA. (Redrawn from Ref. 32.)

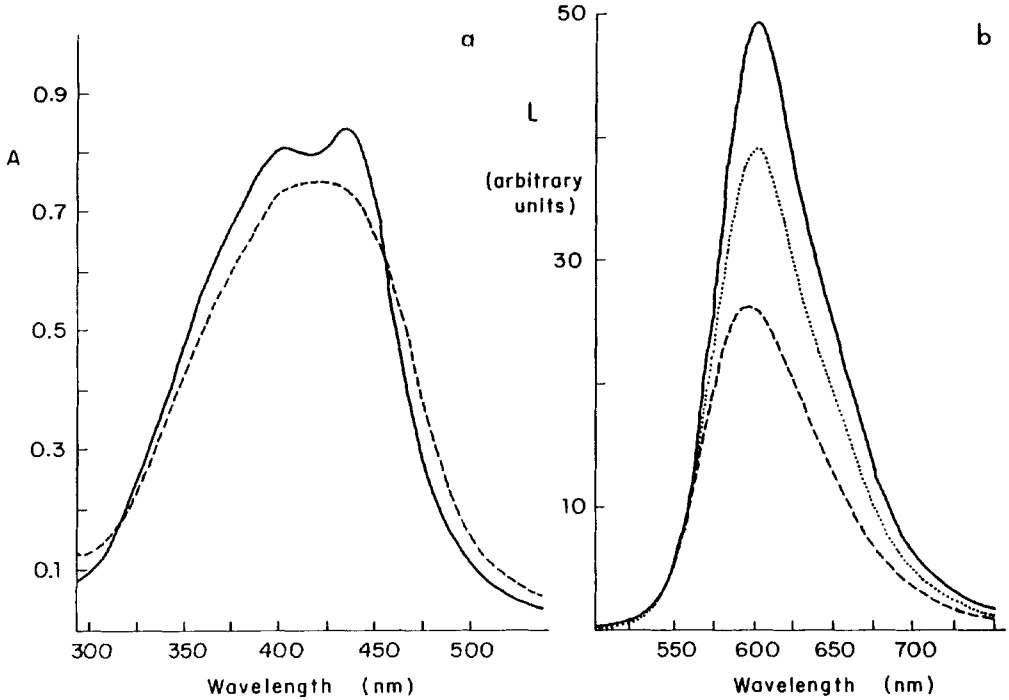


FIGURE 6 (a) Absorption spectra of racemic $(phen)_3Ru^{2+}$ in the absence (——) and presence (– – – –) of calf thymus DNA. (b) Emission spectra of free (– – – –) $(phen)_3Ru^{2+}$ and of Λ-$(phen)_3Ru^{2+}$ (····) and Δ-$(phen)_3Ru^{2+}$ (——) in the presence of calf thymus DNA. (Reproduced with permission from Ref. 32, Copyright 1984, American Chemical Society.)

on binding. Studies are now underway, using this luminescence probe, to obtain information about the dynamics of the cation's interaction with the DNA polymer. Binding isotherms obtained spectroscopically and by equilibrium dialysis were consistent with the intercalative model given in Figure 5. The ruthenium cation binds to a four base pair site and the affinity is approximately one-third that of $[(phen)Pt(en)]^{2+}$,[37] presumably because the nonintercalating phenanthroline ligands in the octahedral case hang over and therefore limit the surface area of the phenanthroline which stacks between the base pairs. The enantiomeric preference is approximately 1.3.

The well-defined structure of the rigid metal complex intercalated in the DNA helix suggested a means to improve the stereoselectivity and thereby provide a sensitive probe for double-helical DNA conformations.[31] Phenyl substitution onto each of the phenanthroline ligands yields a significantly bulkier structure, tris(4,7-diphenylphenanthroline)ruthenium(II) ($Ru(DIP)_3^{2+}$), which is still rigid, inert, and has the same stereochemical and spectroscopic advantages as the parent phenanthroline complex. $Ru(DIP)_3^{2+}$ binds to DNA. Presumably the phenyl groups rotate into the plane of the intercalated ligand to facilitate stacking. Moreover, the interaction of $Ru(DIP)_3^{2+}$ with right-handed B-form DNA is completely stereospecific.[38] Figure 7 shows an absorption titration of the resolved enantiomers with calf

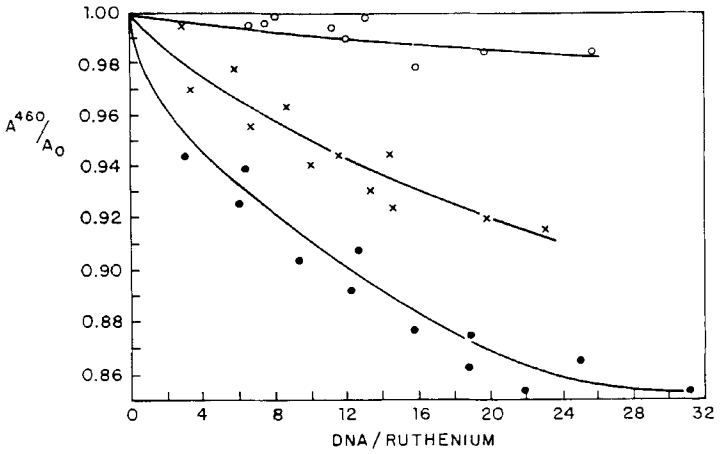


FIGURE 7 Absorption titration of Λ-(○), racemic-(×) and delta- (●) $Ru(DIP)_3^{2+}$ with calf-thymus DNA. The binding to the right-handed helix is stereospecific. (Redrawn from Ref. 38.)

thymus DNA. Hypochromicity is evident only with the Δ-isomer. The decrease in absorption for all samples tested in fact reflects the amount of Δ-isomer present in the preparation. This enantiomeric specificity of binding may be again understood in terms of the intercalation model. The steric bulk of the nonintercalated diphenylphenanthroline ligands completely precludes the interaction of Λ-$Ru(DIP)_3^{2+}$ with the DNA helix. The ~20 Å expanse of the complex considerably exceeds the width of the helical groove. The relative ability of enantiomers to bind to a particular DNA therefore provides a probe for the groove size of the helix in solution and importantly for the chirality of the helix.

Figure 8 shows the conformations of right-handed B form DNA and left-handed Z-DNA.[39-42] Alternating purine–pyridine sequences show a tendency to adopt the Z-DNA conformation under extreme solvent conditions, if heavily alkylated, or in highly underwound superhelical structures. Z-DNA represents the most dramatic example thus far of a sequence-dependent DNA conformational change and it has been proposed that Z-DNA structures may be important in mechanisms for the regulation of gene expression.[43] We have been using Λ-$Ru(DIP)_3^{2+}$ as a specific chemical probe for left-handed DNA in solution.[38] Although Λ-$Ru(DIP)_3^{2+}$ does not bind to right-handed B-DNA, it binds quite easily to the left-handed form. Absorption titrations with Z-DNA unlike those in Figure 7 therefore show hypochromicity for the Λ-isomer. Actually no stereoselectivity is seen in binding experiments with Z-DNA since the wide and shallow major groove of its helix provides a poor template to discriminate between ruthenium enantiomers. Thus we can use the relative binding of Δ- and Λ-$Ru(DIP)_3^{2+}$ to a given DNA as a sensitive spectroscopic assay for the helical conformation. We are currently using these simple coordination complexes as probes for helicity in drug-DNA adducts and protein-DNA complexes. In fact, $Ru(DIP)_3^{2+}$ even turns out to be sensitive stain for helix conformation in chromosome studies by fluorescence microscopy.[31]

COVALENT MODIFICATION

The antitumor drug *cis*-DDP is thought to exert its cytotoxic effect by coordination to base positions on the DNA strand.[9-12,44] Once bound if not repaired by cellular enzymes, the lesion blocks DNA

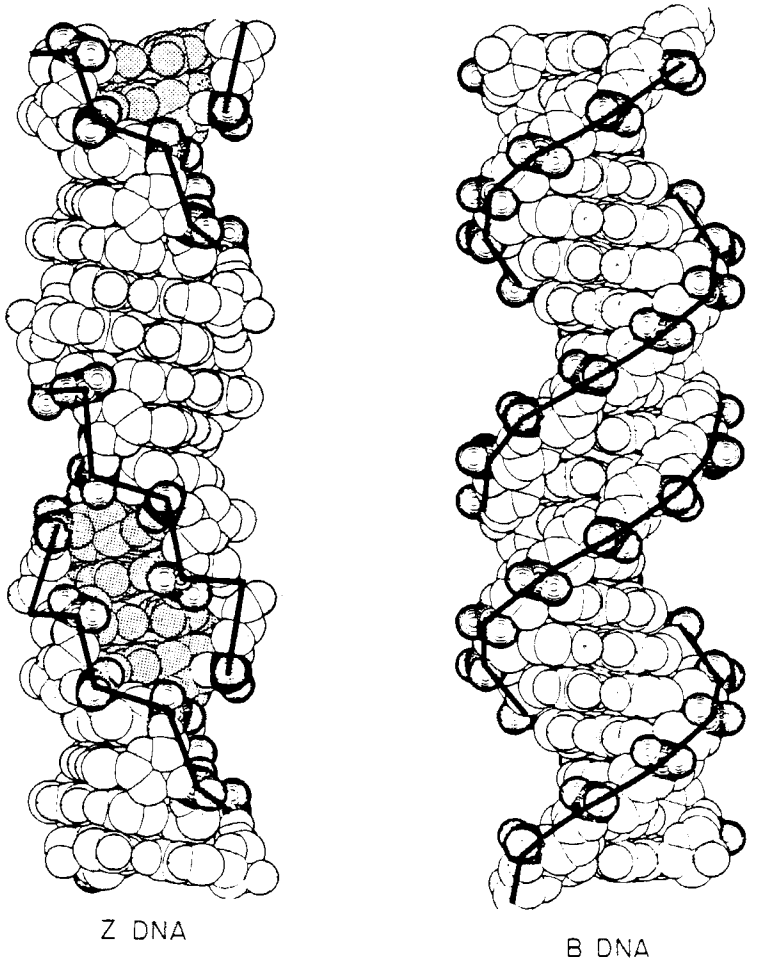


FIGURE 8 Van der Waals diagrams for left-handed Z-DNA and right-handed B-DNA. (Reproduced with permission from Ref. 41, Copyright 1983, Adenine Press.)

replication. The corresponding *trans*-isomer is inactive (Figure 9). It is remarkable that so simple a coordination complex could provide such potent chemotherapeutic activity. Several subtle features of the transition metal chemistry appear to contribute to the efficacy of *cis*-DDP including ligand affinities, rates of hydrolysis, and again the coordination geometry and stereochemistry.

FIGURE 9 *Cis*- and *trans*-dichlorodiammineplatinum(II) (DDP).

Just as with the metallointercalators described above, here too the coordination chemistry must determine the activity of the drug. Activity appears to require two substitutionally inert amine ligands and two *cis*-oriented labile chloride sites. The hydrolytic equilibria of the platinum species seem well-balanced for optimal reactivity within the cell.[12,45] Table I shows estimates based on thermodynamic data for the closely related[46] [enPtCl$_2$] for the relative concentrations of the various hydrolyzed forms of the complex in human plasma and cytoplasm. It is generally thought that the complex remains in the unreactive, neutral dichloro form in the plasma where the chloride concentration is high (~103 mM). After diffusion across the cell membrane, the complex, in the presence of ~4 mM chloride ion in the cytoplasm, is hydrolyzed to various cationic aquated complexes. With water a good leaving group, the platinum drug may then readily bind the DNA polyanion. Spectroscopic studies of *cis*-DDP with oligonucleotides reveal heterocyclic nitrogen atoms of the bases, and in particular the N7 of guanine, to be the most favorable DNA binding sites.[47–50]

The differential cytotoxicity of *cis*- and *trans*-DDP implies that an

TABLE I
Estimates of Hydrolysis Products of [enPtCl$_2$]

Species	Relative Concentration	
	Plasma, mM	Cytoplasm, mM
Cl	103	4
enPtCl$_2$	37.3	1.45
[enPt(OH$_2$)Cl]$^+$	1	1
[enPt(OH$_2$)$_2$]$^{2+}$	0.00134	0.0345
[enPt(OH)Cl]	1	1
[enPt(OH$_2$)(OH)]$^+$	0.053	1.38
[enPt(OH)$_2$]	0.033	0.86

important discrimination in DNA binding between stereoisomers also occurs. *Trans*-DDP displays hydrolytic equilibria that are comparable to that of the *cis*-isomer and shares as well ligand affinities for the lone pairs of nitrogens on purines and pyrimidines. Gel electrophoretic studies demonstrate that both *cis*- and *trans*-DDP unwind supercoiled plasmids and in fact equivalent amounts of bound platinum result in equivalent changes in DNA conformation.[51,52] One qualitative difference in binding was evident with in vitro experiments with nucleosomes.[53] *Cis*-DDP at low concentrations binds almost exclusively to the nucleosomal DNA, while *trans*-DDP forms DNA–histone crosslinks. The orientation of reactive sites in *cis*-$(NH_3)_2Pt^{2+}$ is however particularly well poised for intrastrand crosslinking. *Cis*-DDP binds to regions of DNA rich in $(dG)_n \cdot (dC)_n$ sequences, a result consistent with the formation of intrastrand crosslinks between neighboring guanine bases.[50,54–57] Larger bouyant changes are found for (poly*d*G · poly*d*C) than for the alternating heteropolymer poly(*d*GC).[57] NMR studies of oligonucleotides with bound *cis*-DDP also support that conclusion.[47,50,58] ^{1}H-NMR spectra of the reaction of platinated [*d*(ApGpGpCpCpT)$_2$] as a function of pH show deprotonation of guanine N1, consistent with a lowered pK_a with platinum binding at N7, as well as large downfield shifts in guanine H8.[50] These observations indicate that in the hexanucleotide the *cis*-$(NH_3)_2Pt^{2+}$ species is chelated by the N7 atoms of adjacent guanine residues. The spectra also indicate that with platination normal base stacking is disrupted.

At low binding levels, then, *cis*-DDP appears to form a specific adduct with neighboring (or near neighboring) intrastrand guanine residues (Figure 10). This coordination comp[illegible]x is recognized by DNA binding enzymes. Cleavage of plasmid DNAs after reaction with *cis*-DDP by the restriction enzyme Pst I, for example, is preferentially inhibited at sites flanked by oligo *d*G sequences.[56] Bound *cis*-DDP also stops digestion of DNA by exonuclease III, which degrades exonucleolytically from the 3′ end.[59,60] This inhibition of the enzyme has been used to specifically map sites of platination along the DNA strand and again oligo(*d*G) sites appear to be recognized preferentially by *cis*-DDP. Why this specific DNA lesion results in potent antitumor activity is not clear. Perhaps the lesion is particularly difficult to repair.[61] Another possibility is that these oligopurine domains are associated with important regulatory sites in the genome, and *cis*-diamminplatinum(II) binding preferentially

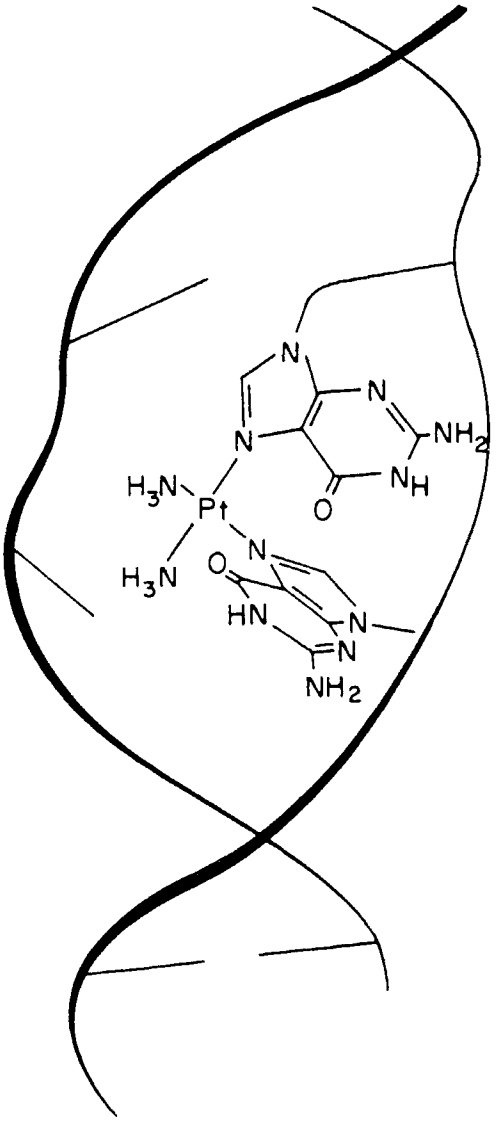


FIGURE 10 Illustration of an intrastrand crosslink by cis-$(NH_3)_2Pt^{2+}$ of adjacent guanine bases. (Reproduced with permission from J. K. Barton and S. J. Lippard, Ann. N.Y. Acad. Sci. **313**, 686 (1978)).

to these sites is therefore particularly harmful to the cell. Antibodies have been elicited against DNA modified with *cis*-DDP and remarkably the antiserum exhibits good specificity only for DNA modified by platinum analogs having the *cis* stereochemistry; poor immunoreactivity is seen within both $[(dien)PtCl]^+$ and *trans*-DDP.[62,63] Perhaps this biochemical probe which specifically recognizes the active coordination complex can provide the link between the in vivo activity and in vitro structural chemistry.

The clinical success of *cis*-DDP and our increasing understanding of its chemistry in the cell is stimulating research into other metal-containing antitumor drugs. Some studies have been conducted using palladium analogues of *cis*-DDP, but it seems likely that the greater lability of palladium(II) in comparison to platinum(II) accounts for its unavoidable toxicity; the palladium analog may not even reach nuclear DNA, given the possibility of extensive side reactions along the way. Titanocene dichloride (Figure 11) represents an interesting example of an organometallic antitumor agent.[65,66] Marked tumor inhibition is found in vivo using bis(cyclopenadienyl) dichloride complexes of titanium, niobium, and molybdenum. Only sporadic activity was found with the heavier tantalum and tungsten analogs and none with zirconocene and hafnocene dichlorides.[67] The similarity in bite distance between *cis*-DDP and the active tetrahedrally coordinated metallocene dichlorides suggests that a structurally similar metal-

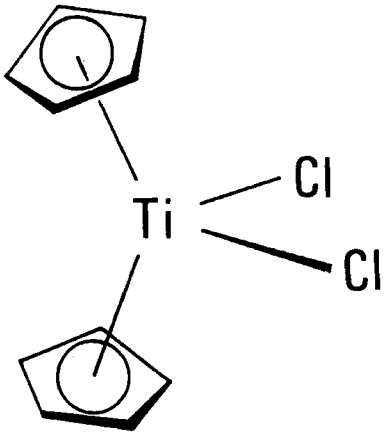


FIGURE 11 Titanocene dichloride.

DNA adduct may be important in vivo. Numerous other coordination complexes may be designed with this skeletal feature in mind and it seems likely that these simple metal complexes represent only a prelude to many inorganic DNA site-specific pharmaceutical agents.

REDOX ACTIVITY

DNA binding drugs commonly exert their activity by altering the DNA structure, either by covalent modification, as described above, forming adducts which inhibit DNA processing enzymes, or by the introduction of lesions that damage the DNA in ways which cannot be repaired. Electron transfer reactions of metal complexes offer pathways for oxidative cleavage of the DNA strand. Moreover drug-dependent damage to the DNA is probably the most sensitive marker of binding, not only of the number of bound sites but also of their location along the strand. Redox active metal complexes have therefore become very useful in the design of specific DNA cleaving agents and drugs.

The simplest illustration of the delivery of metal/oxygen chemistry to the DNA helix is given by reactions of (methidiumpropyl-EDTA)iron(II) (MPE · Fe(II))[68] shown in Figure 12. Dervan and co-workers cleverly attached the DNA intercalator methidium to the EDTA chelator with a short hydrocarbon tether. In the presence of micromolar concentrations of ferrous ion, MPE and oxygen, single-strand DNA scission is observed. Cleavage is sensitively assayed by measurement of the conversion of supercoiled (form I) DNA to nicked circles (form II). The intercalator that binds tightly to the duplex but without sequence specificity delivers high concentrations of ferrous EDTA to the helix. Ferrous ion can then activate oxygen to yield either oxygen radicals or iron-bound oxygen species which at their high local concentrations can produce appreciable cleavage of the sugar phosphate backbone. Hydroxyl radicals are suggested to be one species that may be degrading the DNA, but for this system DNA products have not been characterized. Superoxide dismutase and catalase both inhibit the reaction. Also ethidium bromide and ferrous ion, not tethered, or Fe(II)-EDTA alone do not yield cleavage at concentrations below 0.1 mM. Interestingly, the efficiency of the Fe(II)–MPE reaction is dramatically increased in the presence of 1

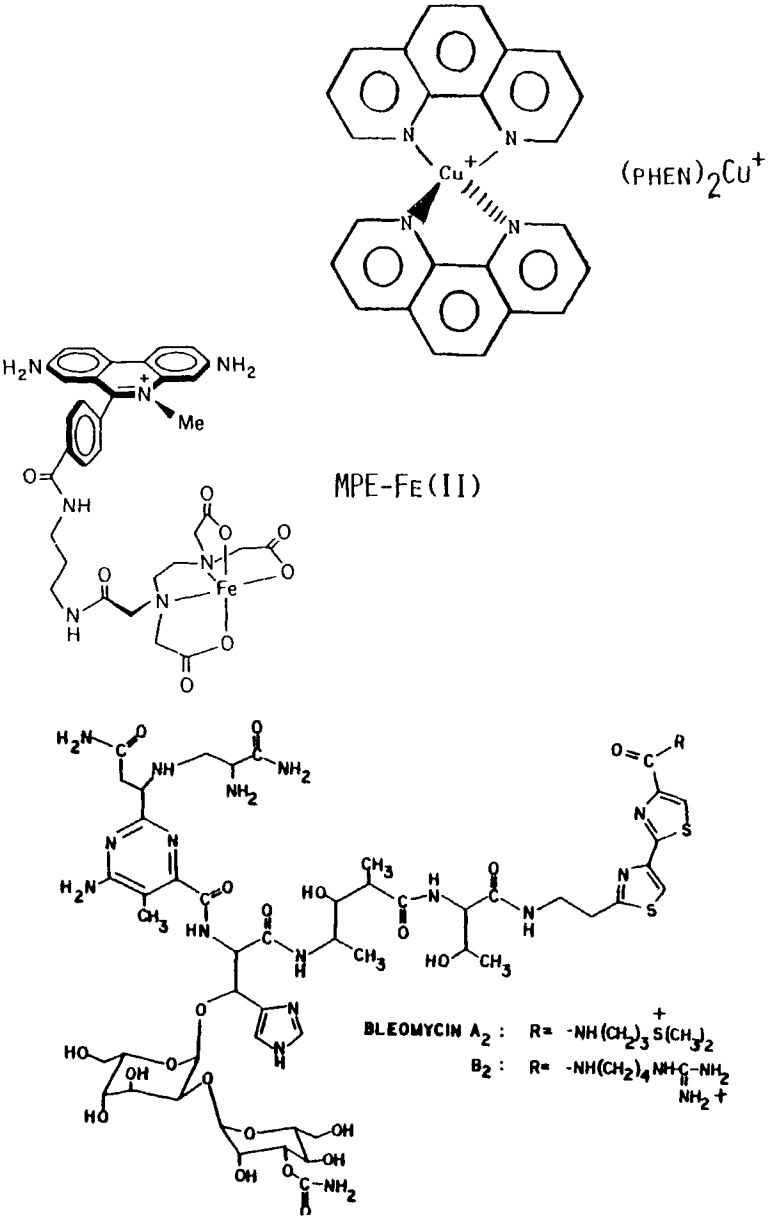


FIGURE 12 DNA-cleaving molecules.

mM dithiothreitol. Presumably the thiol acts as a reducing agent to regenerate Fe(II) from Fe(III), producing a catalytic source of active metal ion.

Cuprous phenanthroline complexes also exhibit nuclease activity.[69-72] Bis(phenanthroline)cuprous ion, generated in situ with the addition of phenanthroline, cupric ion, and thiol, cleaves double-stranded DNA rapidly in the presence of oxygen or hydrogen peroxide. Hydroxyl radical scavengers inhibit the reaction. Although stereochemical details of binding are not known, it is reasonable that, like the phenanthroline complexes of zinc(II) and ruthenium(II), $(phen)_2Cu^+$ can bind to the duplex by intercalation. Strand scission can then result from oxidative destruction of the deoxyribose by hydroxyl radicals generated by peroxide oxidation of DNA-bound cuprous ion. Unlike MPE · Fe(II), here it is the redox active coordination complex itself that is intimately bound to the helix. An interesting dependence of $(phen)_2Cu^+$ nuclease activity on the DNA secondary structure has been found.[73] The A, B, and Z forms of DNA are cleaved at different rates, the most common B form being most susceptible to cleavage. Apparently the Z and A forms do not promote formation of a comparably stable noncovalent complex with either the optimal geometry or conformational susceptibility to cleavage.

Both MPE · Fe(II) and $(phen)_2Cu^+$ as artifical DNases have served as extremely useful probes of DNA structure. Gel electrophoresis patterns of end-labelled DNA, containing a bound drug or protein, after digestion with the metal reagent reveal the sites of drug or protein binding. The metal reagents are small, nonspecific DNA cleavers. However, at those points along the DNA strand occupied by a given drug or protein, cleavage by the metal reagent is blocked. This "footprinting" technique, illustrated in Figure 13, has been applied already in determining the sequence-specific binding sites of several antitumor antibiotics.[74-76] Both MPE · Fe(II) and $(phen)_2Cu^+$ have also been useful in analyses of chromatin structure.[77-79] Partial digestion of Drosophila nuclei with $(phen)_2Cu^+$ leads to nucleosomal ladders identical to those obtained with micrococcal nuclease. Surprisingly, the preferential site of cleavage recognized by both the coordination complex and enzyme are highly nonrandom with major sites appearing in the spaces between genes. Digestions with MPE · Fe(II) show a similar cleavage pattern of the nucleosome array but

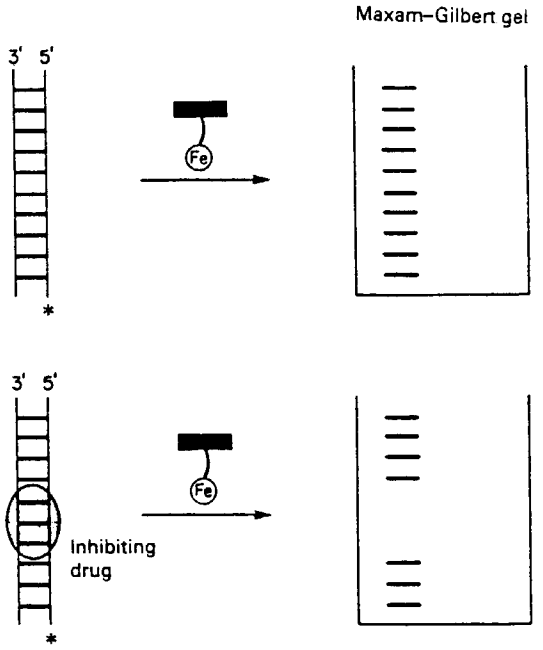


FIGURE 13 Scheme for footprinting drug binding with a nonspecific DNA-cleaving molecule. (Reprinted with permission from Ref. 74.)

analyses of the protein-free DNA reveal considerably lower sequence specificity.

Perhaps the clinically most important example of a site-specific redox-active DNA cleaving molecule is the antitumor antiobiotic bleomycin.[80–83] Since bleomycin is not really a simple synthetic co-ordination complex and its mode of action in vivo is still far from clear, it is best only to mention it in the context of this discussion. The structure is given also in Figure 12. The natural product can however coordinate either iron or copper to give an activated complex which degrades DNA preferentially at guanine-rich sequences. It is likely that its antineoplastic activity is derived from this oxidative reaction with the nucleic acid.

Coordination complexes are now being designed which, like DNA-binding proteins, recognize and then cleave specific high affinity sites along the strand. Dervan and co-workers tethered ferrous EDTA first to distamycin, a natural tripeptide containing three *N*-methyl-

pyrrolecarboxyamides which binds to the minor groove of DNA with a strong A-T preference.[84,85] Attachment of EDTA · Fe(II) to a sequence-dependent DNA binding molecule yields a molecule that cleaves DNA based upon the site affinity of the tethered group. Because of its high DNA affinity, the pentapeptide penta-*N*-methylpyrrolecarboxyamide-EDTA · Fe(II) (P5E · Fe(II)) achieves double-strand cleavage of DNA catalytically adjacent to a six–seven base pair A + T recognition site.[86] Moreover in the presence of O_2 and dithiothreitol, nanomolar concentrations of bis(EDTA-distamycin · Fe(II)), shown in Figure 14, cleave the duplex to form discrete fragments with cleavage contiguous to the eight base pair sequence 5′-TTTTTATA-3′.[87]

Conformation-specific DNA cleaving molecules have been designed by taking advantage of the stereospecificity of tris(diphenyl-

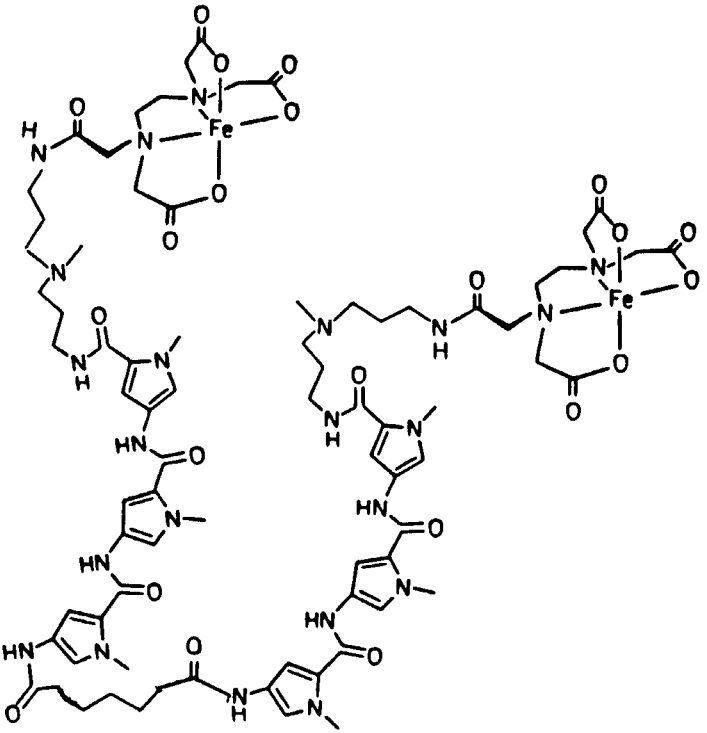
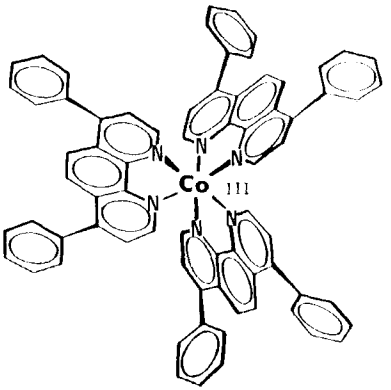


FIGURE 14 Bis(EDTA · Distamycin · Fe(II)).

phenanthroline) metal complexes.[88] Since cobalt(III) polyamine complexes can be reduced photochemically, we thought the delivery of redox activity to the DNA helix might similarly be obtained by irradiation of $(phen)_3Co^{3+}$ bound to the duplex. Tris-(phenanthroline)cobalt(III) does indeed promote DNA strand scission in the presence of light. Interestingly, unlike for the ferrous and cuprous ion systems, here it must be a metal-center *reduction* that leads to the oxidative DNA cleavage; the route is as yet unclear. The cleavage reaction can however be made stereospecific. Figure 15 shows a plot of the cleavage of two supercoiled DNAs incubated with either Λ- or Δ-$Co(DIP)_3^{3+}$ and irradiated. Significant cleavage of the native ColE1 plasmid is seen only with the delta isomer. The Λ-isomer does however cleave pBR322 at low salt concentration and native superhelical densities. The Λ-isomer must then recognize and bind to a structure in pBR322 that is not present in the ColEl plasmid. Given the Z-DNA specific recognition by Λ-$Ru(DIP)_3^{2+}$, it is likely that Λ-$Co(DIP)_3^{3+}$ recognizes and cleaves a specific left-handed segment in pBR322. Z-DNA antibody results are consistent with this conclusion.[41] This coordination complex should therefore be useful in mapping very sensitively Z-DNA sites in genes; no other method of comparable sensitivity is currently available. It is noteworthy that here it was the introduction of cobalt(III), which has octahedral coordination, is inert to racemization, and at the same time redox-active photochemically, that permitted the design of this conformation-specific DNA cleaver.

CONCLUSION

In this Comment, we have described reactions of inorganic compounds no more complicated than Fe(II) · EDTA, $(phen)_3Ru^{2+}$, and *cis*-$(NH_3)_2PtCl_2$. And yet the interactions of these coordination complexes with DNA are subtle and in some cases highly specific. Their utility ranges from chromosomal stains to antitumors drugs, artificial restriction enzymes, and probes for gene regulation. The somewhat prosaic nature of these complexes illustrates, we think, that the application of transition metal chemistry to study DNA is only just beginning. The work presented hopefully hints at how powerful that application might be. As inorganic chemists begin to explore the



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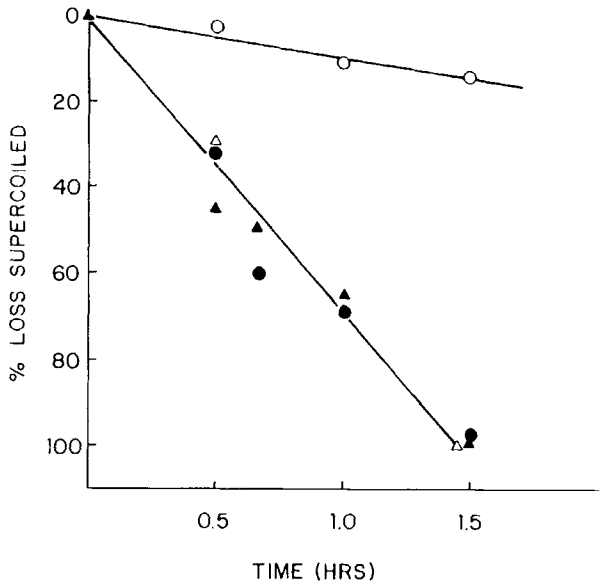

FIGURE 15 Stereospecific cleavage of supercoiled DNA in the presence of $Co(DIP)_3^{3+}$ with irradiation as a function of time. Shown is cleavage of ColEl plasmid (open) and pBR322 (closed) DNAs in the presence of Δ (triangles) or Λ (circles) isomers. In the presence of light, Λ-$Co(DIP)_3^{3+}$ recognizes and cleaves left-handed DNAs. (Redrawn from Ref. 88.)

interaction of more complex and finely tuned molecules with DNA, the results will become all the more exciting.

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