TRANSITION METAL COMPLEXES

There are many practical motivations behind the study of how metal ions and complexes interact with nucleic acids. Heavy-metal toxicity in our environment arises in part from the covalent interactions of heavy-metal ions with nucleic acids. In addition, these heavy metals interfere with metalloregulatory proteins and in so doing disrupt gene expression. We need to understand the functioning of the natural metalloregulators of gene expression and we need to design new metal-specific ligands, which, like the proteins themselves, capture heavy metals before their damage is done. Heavy-metal interactions with acids indeed have provided the basis also for the successful application of cisplatin and its derivatives as anticancer chemotherapeutic agents. The design of new pharmaceuticals like cisplatin requires a detailed understanding of how platinum and other metal ions interact with nucleic acids and nucleic-acid processing. Furthermore, we are finding that metal complexes can be uniquely useful in developing spectroscopic and reactive probes of nucleic acids, and hence may become valuable in developing new diagnostic agents. Finally, Nature itself takes advantage of metal/nucleic-acid chemistry, from the biosynthesis of natural products such as bleomycin, which chelates redox-active metal ions to target and damage foreign DNA, to the development of basic structural motifs for eukaryotic regulatory proteins, the zinc-finger proteins, which bind to DNA and regulate transcription. In all these endeavors, we need first to develop an understanding of how transition-metal ions and complexes interact with nucleic acids and how this chemistry may best be exploited.

In this section, the focus on tris(phenanthroline) complexes serves as a springboard to compare and contrast studies of other, more intricately designed transition-metal complexes with nucleic acids. Last we consider how Nature uses metal ions and complexes in carrying out nucleic-acid chemistry. Here the principles, techniques, and fundamental coordination chemistry of metals with nucleic acids provide the foundation for our current understanding of how these fascinating and complex bioinorganic systems may function.

THE BASICS

Nucleic-Acid Structures

The fig 1displays a single deoxyribonucleotide and the four different nucleicacid bases. As may be evident, each mononucleotide along a nucleic-acid polymer contains a variety of sites for interactions with metal ions, from electrostatic interactions with the anionic phosphate backbone to soft nucleophilic interactions with the purine heterocycles. The different nucleic-acid bases

furthermore offer a range of steric and electronic factors to exploit. Coordination of a metal complex to the N7 nitrogen atom of a purine, for example, would position other coordinated ligands on the metal center for close hydrogen bonding to the O6 oxygen atom of guanine, but would lead to clashes with the amine hydrogen atoms of adenine. The monomeric units strung together in a polynucleotide furthermore provide an array of polymeric conformers.

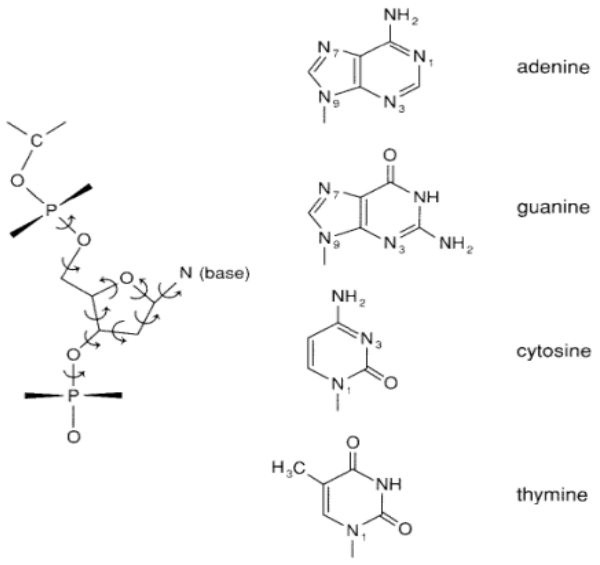


Figure 1

Illustration of a mononucleotide unit. Arrows indicate the various torsional angles within each unit that together generate the wide range of conformations available in the polymer. Also shown are the individual bases as well as the commonly employed numbering scheme.

There are electrostatic repulsions between the anionic phosphate backbones of the polymer, causing stiffening; each double-helical step has two formal negative charges. An atmosphere of metal ions condensed along the sugar-phosphate backbone serves partially to neutralize these electrostatic interactions. In the B-DNA conformation, the bases are stacked essentially perpendicular to the helical axis, and the sugars are puckered in general, with a C2'-endo geometry (the C2' carbon is to the same side as the C5' position relative to a plane in the sugar ring defined by the C1', C4', and O atoms). This conformer yields a right-handed helix with two distinct, well-defined grooves, termed the major and minor. The A-form helix, while still right-handed, is distinctly different in structure. The sugar rings are puckered generally in the C3'-endo conformation, causing the bases to be pushed out from the center of the helix toward the minor groove, and tilted relative to the helix perpendicular by almost 20°. What results is a shorter and

fatter helix than the B-form; the helical pitch is 28.2 Å in A-DNA for an 11-residue helix and 33.8 Å for 10-residue helix in B-DNA. The A-form helical shape is best characterized by the very shallow minor groove surface; what was the major groove in the B-form has been pulled deeply into the interior of the A-conformer and is really not accessible to binding by small molecules in solution. Transitions to the A-conformation are promoted by hydrophobic solvents or solutions of high ionic strength. The Z-conformation is perhaps most distinctive, owing to its left-handed helicity. The conformer was dubbed Z-DNA because of the zig-zag in the helix. Alternations both in sugar puckering, between C2'-endo and C3'-endo, and in the rotation of the base about the glycosidic bond, anti or syn relative to the sugar, are evident, and lead to a dinucleoside repeating unit versus a mononucleoside repeat in the A- and B-helices. Alternating purine-pyrimidine sequences have the highest propensity to undergo transitions into the Z-form. It is actually this syn conformation of purines that leads to the lefthanded helicity of the polymer. But it is not only its left-handedness that distinguishes the Z-conformation. The polymer is long and slender (the pitch is 45 Å for a 12-residue helix), and the major groove is a shallow and wide, almost convex, surface, whereas the minor groove is narrowed into a sharp and small crevice.

The bases in a base pair often do not lie in the same plane, but are instead propeller-twisted with respect to one another. The local unwinding of the helix and tilting of the base pairs furthermore tend to vary with the local nucleic-acid sequence so as to maximize stacking or hydrogen-bonding interactions among the bases. Hence there is a variety of structures within each conformational family.

Our understanding of these structural variations as a function of solution conditions and importantly of local sequence is still quite poor. But surely these structural variations affect and are affected by the binding of metal ions and complexes.

Even less defined structurally are other conformations of DNA, Double-helical DNA can bend, form loops and cruciforms, and fold back on itself into intramolecular triple helices, termed H-DNA. At the ends of chromosomes, four strands may even come together in a unique conformation. These structures, characterized thus far by means of biochemical techniques, arise because of sequence and local torsional stress, or supercoiling. Many of these structures are stabilized by the binding of highly charged metal ions, probably because the highly charged metal center in a small volume can neutralize the electrostatic repulsions between polyanionic

strands that are bundled together. Metal complexes can furthermore be extremely useful in targeting and characterizing these structures, as we will see. In chromosomes the DNA is packaged by histone proteins into even tighter bundles, with helical segments wrapped about the basic proteins to form superhelical nucleosomal units which are then arranged like beads on a string of more loosely packed DNA. This complexity in DNA structure is in fact small compared to that of RNA polymer, yeast $tRNA^{phe}$. Ostensibly single-stranded RNAs do not exist as random coils, but instead fold up into well-de fined three-dimensional structures, much like proteins. The structural variety, of course, bears some resemblance to that found in DNAs. Double-helical regions in the tRNA are A-like in conformation; helices fold together as one might imagine to occur in cruciforms, and even triple-helical segments are evident where three strands fold together in the polymer. But overall our ability to characterize structures of RNA thus far is lower than that with DNAs. RNAs are less stable in solution than is DNA, and fewer chemical as well as enzymatic tools are available for structural characterization. Yet the recent discovery of ribozymes, the finding that RNAs can indeed catalyze nucleolytic reactions, makes our need to understand these structures even greater. Again transitionmetal chemistry may participate in stabilizing, promoting, and probing these structures.

Fundamental Interactions with Nucleic Acids:

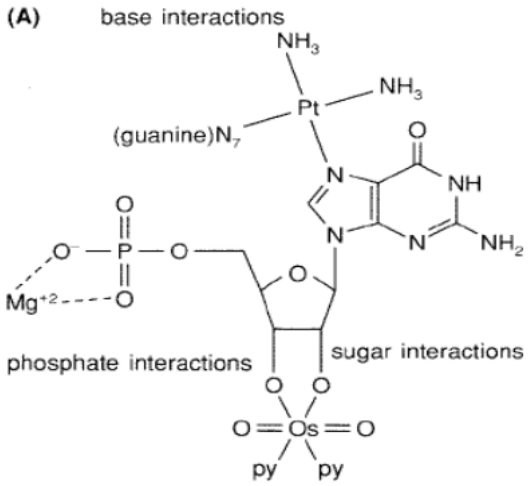
Metal ions and complexes associate with DNA and RNA in a variety of ways, as illustrated in. figure.2. Both strong covalent interactions and weak noncovalent complexes are observed. Each may yield a significant perturbation in the nucleic acid and/or may be exploited to obtain a site-specific response. Clearly there are some general guidelines, based on principles of coordination chemistry that may be helpful in sorting out these interactions.

Coordination

Most prevalent among covalent complexes with DNA are those involving coordination between soft metal ions and nucleophilic positions on the bases. The structure of cis-$(NH_3)_2$Pt-dGpG is an example: its platinum center coordinates to the N7 position of the guanine bases. In terms of interactions with the full polynucleotide, it is likely that the cis-diammineplatinum center, with two coordination sites available, would yield an intrastrand crosslink between neighboring guanine residues on a strand . Other nucleophilic sites targeted by soft metal ions on the bases include the N7 position of adenine, the N3 position on cytosine, and the deprotonated N3

position on thymine and uracil. Some additional covalent binding to the Nl positions of the purines has also been observed. Indeed, coordination by the metal to one site on the heterocyclic base lowers the *pKa* and increases the metal-binding affinity to secondary sites. It is noteworthy, however, that in base-paired double-helical DNA only the N7 positions on the purines are easily accessible in the major groove of the helix. Base binding at the purine N7 position is, of course, not limited to soft metal ions such as Pt(II), Pd(II), and Ru(II). Coordination at these sites has been evident also with first-row transition-metal ions such as Cu(II) and Zn(II). For these, as is consistent with basic coordination chemistry, the lability of complexes formed is higher.

Transition-metal ions with decreasing softness are capable of coordinating also to the phosphate oxygen atoms. The ionic versus covalent character of these complexes clearly depends on the metal ions involved. In a classic study, examining the melting temperature of double-helical DNA in the presence of different metal ions and as a function of their concentration, Eichhorn and coworkers established the preference of the metal ions for base versus phosphate binding (Figure 3). The preference for phosphate over base association was found to decrease in the order Mg(II) > Co(II) >Ni(II) >Mn(II) >Zn(II) >Cd(II) > Cu(II). This series arises from examination of DNA helix-melting temperatures, since base interactions in general should destabilize the helical form [except where interstrand crosslinking occurs, as may happen with Ag(I)], whereas phosphate coordination and neutralization would increase the helix stability and hence the melting temperature.



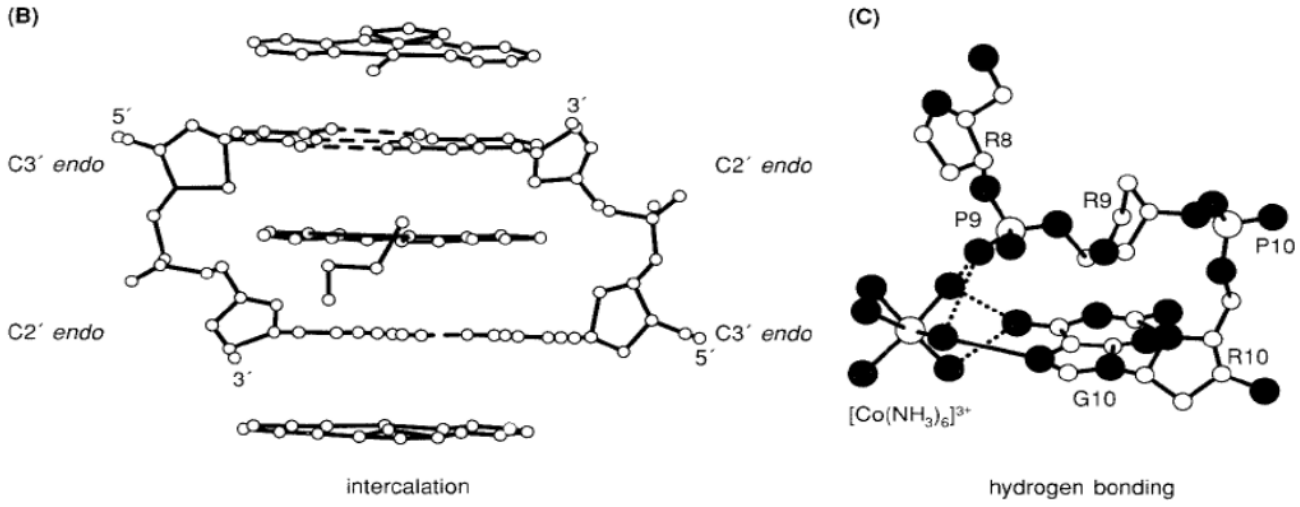


Figure 2

Covalent and noncovalent binding modes of metal complexes with DNA. (A) Representative covalent interactions. Shown schematically are examples of coordination to the DNA base, sugar, and phosphate moieties given by the covalent binding of cis-(diammine)platinum to the N7 nitrogen atom of neighboring guanine residues, the formation of an osmate ester with ribose hydroxyl groups, and the primarily electrostatic association between $Mg(H_20)_6^{3+}$and the guanosine phosphate, respectively. (B) Noncovalent intercalative stacking of a metal complex. Shown is the crystal structure of (terpyridyl)(2-)platinum(II) intercalated and stacked above and below the base-paired dinucleotide d(CpG). (C) An illustration of hydrogen bonding of coordinated ligands. Shown is a partial view of the crystal structure of Z-form $d(CG)_3$ with $Co(NH_3)_6^{3+}$ hydrogen-bonded both to the guanine base (G 10) and phosphate backbone (P9).

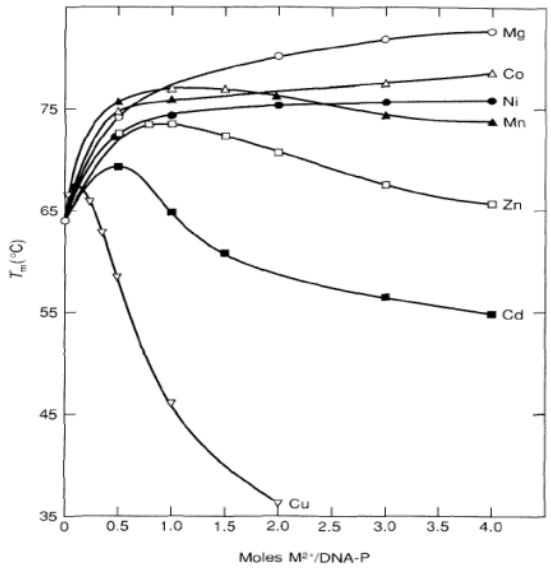


Figure:3

The effects of various metal ions on the melting temperature (T_m) of calf thymus DNA.

Also of interest, but less common, are covalent interactions with the sugar moiety. Although the pentose ring in general provides a poor ligand for metal ions, osmate esters can form quite easily across the C2'-C3' positions in ribose rings. This particular interaction has been suggested as a basis for heavymetal staining of RNA. In fact, OsO_4 is not restricted in its reactivity with the sugar positions. Cisoid osmate esters form as well upon reaction of OsO_4 across the electron-rich C5-C6 double bonds of accessible pyrimidines on DNA.

Intercalation and hydrogen bonding

But important interactions of metal complexes with polynucleotides are not restricted to those involving direct coordination of the metal center to the polymer. Instead, an abundance of highly selective interactions arise from an ensemble of weaker noncovalent interactions between the ligands of coordinatively saturated metal complexes and the nucleic acid. Two primary examples of noncovalent association are given by metallointercalation and hydrogen-bonding interactions of coordinated ligands. Planar aromatic heterocyclic ligands such as phenanthroline and terpyridine can stack in between the DNA base pairs, stabilized through dipole-dipole interactions. Here, depending on the complex and its extent of overlap with the base pairs, the free energy of stabilization can vary from ~2to10 kcal. Non-intercalative hydrophobic interactions of coordinated ligands in the DNA grooves also can occur, as we will see. Hydrogen bonding interactions of coordinated ligands with the polynucleotide are quite

common, and arise in particular with the phosphate oxygen atoms on the backbone. With cobalt hexaammine, for example, hydrogen bonding to an oligonucleotide occurs between the ammine hydrogens and both phosphate oxygen atoms and purine bases. A mix of covalent and noncovalent interactions is also possible. With *cis*-diammineplatinum(II) coordinated to the guanine N7 position, the ammine ligands are well-poised for hydrogen-bonding interactions with the phosphate backbone. The steric constraints on the molecule must be considered, however. With $Pt(terpy)Cl^{+}$, both intercalation of the terpy ligand and direct coordination of the platinum center (after dissociation of the coordinated chloride) are available, but not simultaneously; coordination of the platinum to the base would likely position the terpyridylligand away from the base stack in the DNA major groove, precluding intercalation. Sigel and coworkers have studied the thermodynamics of noncovalent interactions coupled to direct coordination of simple first-row transition-metal complexes with mononucleotides, and these results illustrate well the

interplay of weak noncovalent interactions and direct coordination in generating geometric specificity in complex formation.

Fundamental Reactions with Nucleic Acids

The reactions of transition-metal complexes with polynucleotides generally fall into two categories: (i) those involving a redox reaction of the metal complex that mediates oxidation of the nucleic acid; and (ii) those involving coordination of the metal center to the sugar-phosphate backbone so as to mediate hydrolysis of the polymer. Both redox and hydrolytic reactions of metal complexes with nucleic acids have been exploited with much success in the development of tools for molecular biology.

Redox chemistry

The simplest redox reaction with polynucleotides one might consider as an illustration is the Fenton reaction, which indirectly promotes DNA strand scission through radical reactions on the sugar ring. The reaction with $Fe(EDTA)^{2-}$ is shown in Figure 4A. As do other redox-active divalent metal ions, ferrous ion, in the presence of hydrogen peroxide, generates hydroxyl radicals, and in the presence of a reductant such as mercaptoethanol, the hydroxyl radical production can be made catalytic. Although ferrous ion itself does not appear to interact appreciably with a nucleic acid, especially when chelated in an anionic EDTA complex and repelled by the nucleic-acid polyanion, the hydroxyl radicals, produced in appreciable quantities catalytically, attack different sites on the sugar ring, indirectly yielding scission of the sugar-phosphate backbone. One such reaction that has been characterized in some detail is that involving hydroxyl radical reaction at the C4' position, the position most accessible to the diffusible radical in the minor groove of the helix. As illustrated in Figure 4B, the products of this reaction include a 5'-phosphate, a mixture of 3'-phosphate and phosphoglycolates, and a mixture of free bases and base propenals

Reactions of the hydroxyl radical at other sites on the sugar ring are now being identified as well by isotope-labeling studies. Comparable reactions with RNA have also been described. The application of this Fenton chemistry to promote site-specific or sequence- neutral cleavage of DNA was first demonstrated by Dervan and coworkers, and has provided the basis for the design of a tremendous range of new and valuable DNA cleavage agents. The development of

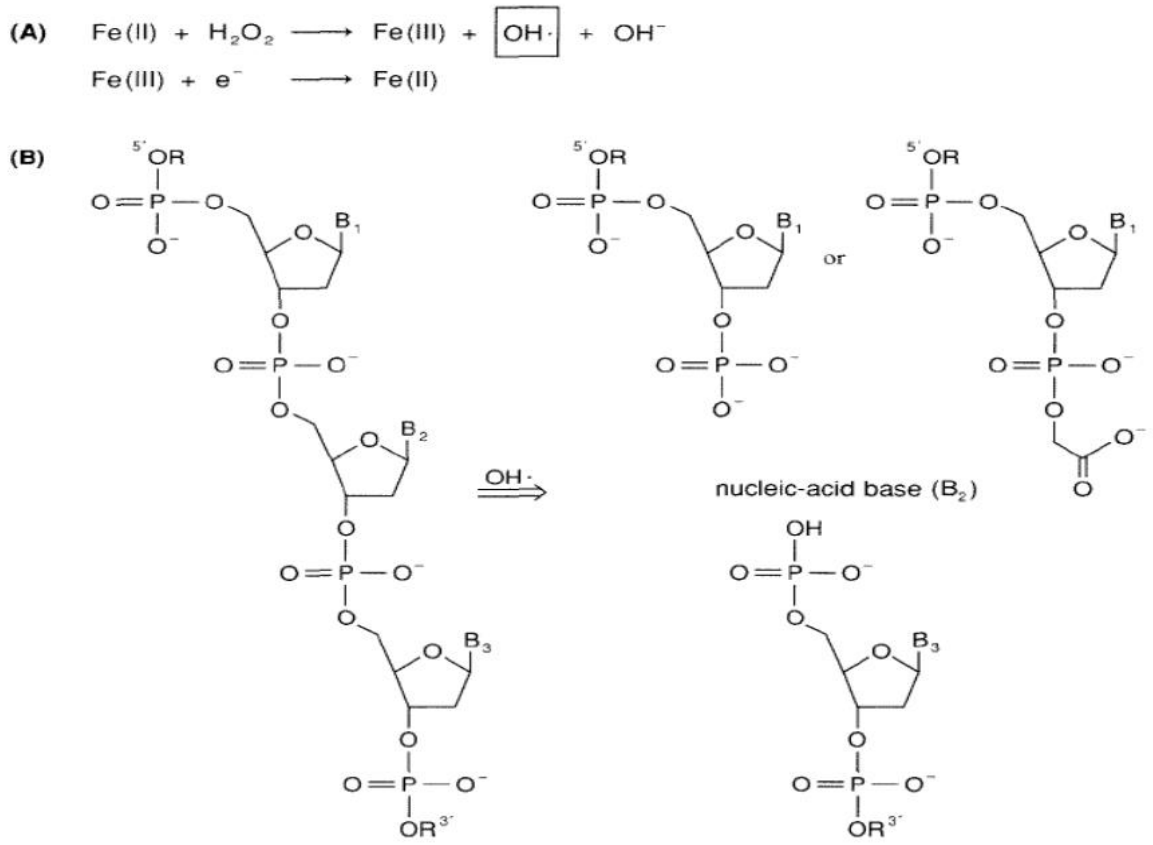


Figure :4

The figure 4 shows DNA strand cleavage mediated by hydroxyl radicals produced by Fenton reaction (A) of $Fe(EDTA)^{2-}$ with hydrogen peroxide. The cleavage scheme (B) shows the products obtained as aresult of initial C4'-H abstraction by the hydroxyl radicals.

this chemistry was originally based on modeling Fe-bleomycin, a natural product with antitumor and antibiotic activity, which binds and cleaves DNA. The chemistry mediated by Fe-bleomycin, as we will discuss later, is likely to be far more complex, however, involving direct reaction of an intimately bound ferryl intermediate species with the nucleic acid, rather than net oxidation of the sugar mediated by a diffusing hydroxyl radical. Other metal ions such as Cu(Il) can also promote redox-mediated cleavage of DNA through reactions on the sugar ring; whether the oxidizing radical is still coordinated to the metal or is a dissociated and diffusing species is a topic of much debate.

Metal ions can also be used to generate other oxidizing intermediates in aerated aqueous solution, such as superoxide ion and singlet oxygen. DNA strand cleavage reactions mediated by superoxide have not thus far been demonstrated, however. Singlet oxygen may be

produced by photosensitization of $Ru(phen)_3^{2+}$,and indeed photolysis of $Ru(phen)_3^{2+}$ bound to DNA yields oxygen-dependent, alkaline-sensitive strand cleavage. For singlet oxygen, the oxidation occurs on the nucleic-acid base rather than on the sugar ring. As such, the reaction varies with base composition; guanine residues are most reactive. Furthermore, since the primary lesion is that of a base modification, piperidine treatment, or other weakly basic conditions, are needed to convert the base lesion into a strand scission event.

Another scheme for oxidative cleavage of DNA mediated by metal complexes involves formation of a coordinated ligand radical bound to the helix that directly abstracts a hydrogen atom from the sugar ring. The photoreaction of $Rh(phen)_2phi^{3+}$(phi = 9, l0-phenanthrenequinone diimine) exemplifies this strategy. Here photolysis promotes a ligand-to-metal charge transfer with formation of a phi-centered radical. Isotope-labeling studies and product analysis have shown that this phi radical bound intercalatively in the major groove of DNA directly abstracts the C3'-H (which sits in the major groove of the helix); subsequent hydroxylation or dioxygen addition at this position promotes DNA strand scission without base treatment.

Some potent photooxidants can also produce outer-sphere electron transfer from the DNA. Here it is the guanine bases, likely those stacked with neighboring purines, that are most easily oxidized and hence most susceptible to attack. Again, this base modification requires alkaline treatment to convert the lesion to a strand breakage. The DNA double helix can furthermore alsomediate electron-transfer reactions between bound metal complexes. The DNA polymer has, for example, been shown to catalyze photoinduced electron-transfer reactions between $Ru(phen)_3^{2+}$ and $Co(phen)_3^{3+}$ bound along the DNA strand. Table 1 summarizes different redox reactions of metal complexes bound to DNA.

Hydrolytic chemistry

Hydrolysis reactions of nucleic acids mediated by metal ions are important elements in natural enzymatic reactions; chemists would like to exploit them in the design of artificial restriction endonucleases. Hydrolysis reactions of the phosphodiester linkage of polynucleotides appear preferable to redox-mediatedcleavage reactions, since in the hydrolytic reaction all information is preserved. In redox cleavage by sugar oxidation, for example, both a sugar fragment and free nucleic-acid base are released from the polymer, and, in contrast to hydrolytic chemistry, the direct religation of the fragments becomes practically impossible.

Examples of metal complexes that cleave DNA through redox chemistry.

Complex	Target[a]	Chemistry[b]	Diffusibility[c]	DNA Binding[d]	Site Selectivity[e]
$Fe(EDTA)^{2-}$	sugar	OH·, Fenton	diffusible	none	none
MPE-Fe(II)	sugar (C4′-H)	OH·, Fenton	diffusible	sequence-neutral	none
$Co(NH_3)_6^{3+}$*	base	photoelectron transfer	[f]	hydrogen-bonding	5′-G-pur-3′
$Cu(phen)_2^{+}$	sugar	Cu^{2+}-OH·	slight	AT-rich	AT-rich
Mn-Porphyrin	sugar	M=O	none	AT-rich	AT-rich
$U(O_2)(NO_3)_2$*	[f]	[f]	diffusible	[f]	none
$Ru(TMP)_3^{2+}$*	base	$^1O_2$	diffusible	A-form	A-form, G
$Ru(phen)_3^{2+}$*	base	$^1O_2$	diffusible	sequence-neutral	G
$Co(DIP)_3^{3+}$*	sugar	ligand radical	none	Z-form (non-B)	Z-form (non-B)
$Rh(DIP)_3^{3+}$*	sugar	ligand radical	none	Z, cruciforms	Z, cruciforms
$Rh(phen)_2phi^{3+}$*	sugar (C3′-H)	ligand radical	none	open major groove	5′-pyr-pyr-pur-3′
$Rh(phi)_2bpy^{3+}$*	sugar (C3′-H)	ligand radical	none	sequence-neutral	none

[a] DNA may be modified by attack either at the sugar or at the nucleotide base position.
[b] The reactive species involved in DNA cleavage, if known.
[c] Some reactive species are diffusible, producing broad patterns of DNA damage along the strand. Others are nondiffusible, resulting in cuts at single discrete sites.
[d] The site of metal complex binding to DNA, if known.
[e] The sites cleaved by the metal complex.
[f] Not known.
* Indicates an excited-state reaction requiring photoactivation.

TABLE1

Metal ions can be effective in promoting hydrolysis of the phosphodiester, since they can function as Lewis acids, polarizing the phosphorus-oxygen bond to facilitate bond breakage, and can also deliver the coordinated nucleophile to form the pentacoordinate phosphate intermediate. Figure 5 illustrates one crystallographically characterized model system developed by Sargeson and coworkers, where hydrolysis of a model phosphodiester was enhanced dramatically by taking advantage of both the acidic and the nucleophilic characteristics of the bound cobalt(III) species. A whole series of model systems utilizing both cobalt and zinc ions has been designed to explore the hydrolytic reactions of simple phosphodiesters. This strategy coupled to a DNA binding functionality has also been exploited, albeit inefficiently, in the hydrolytic cleavage of

doublehelical DNA by $Ru(DIP)_2$ Macro with Zn^{2+},Cd^{2+}, or Pb^{2+} added *in situ.* In this complex (see Figure 5), the central portion of the molecule, held together by the ruthenium(II), is responsible for DNA binding. Tethered onto the coordinatively saturated ruthenium complex are two diethylenetriamine functionalities (in the Macro ligand), however, and these serve to coordinate hydrolytically active metal ions such as Zn(II) and Co(II) , which promote DNA hydrolysis once delivered to the sugar-phosphate backbone by the DNA-binding domain.

Perhaps simpler and certainly better understood are the hydrolytic reactions of RNAs mediated by metal ions. More than twenty years ago Eichhorn and coworkers showed that simple metal ions such as Zn(II) and Pb(II) promote the hydrolysis of RNA. Figure 5 illustrates also the crystallographically characterized site-specific hydrolyis in tRNA by plumbous ion. In tRNA, Pb(II) occupies three quite specific high-affinity binding sites, and at one of these sites, the metal ion becomes poised to promote strand cleavage. The crystal structure with bound Pb^{2+} suggests that the lead-coordinated hydroxide ion deprotonates the 2'-hydroxyl of one residue, so that the resulting 2'-oxygen nucleophile may attack the phosphate to give a pentavalent intermediate that decays to form the 2' ,3'-cyclicphosphate and after reprotonation, the 5'-hydroxide. This very specific cleavage reaction is already being used by biologists as a tool in probing structures of mutant tRNAs, since the reaction is exquisitely sensitive to the stereochemical alignment of the nucleic-acid residues, phosphate backbone, and associated metal ion. In hydrolytic reactions on RNA, it is commonly considered, though certainly not established, that the job of the metal ion may be simpler than with DNA, since the ribose provides a nearby nucleophile already in the 2'-hydroxide. The reaction of tRNA with Pb(II) nonetheless illustrates how a metal ion may be utilized in promoting highly specific chemistry on a nucleic-acid polymer.

Last, it must be mentioned that metal coordination to the purine N7 position can also indirectly promote strand cleavage, although not through direct hydrolytic reaction on the sugar-phosphate backbone. Metal ions such as Pd^{2+} and Cu^{2+} ,through coordination at N7, promote depurination. The depurinated site then becomes easily susceptible to hydrolysis upon treatment with mild base.

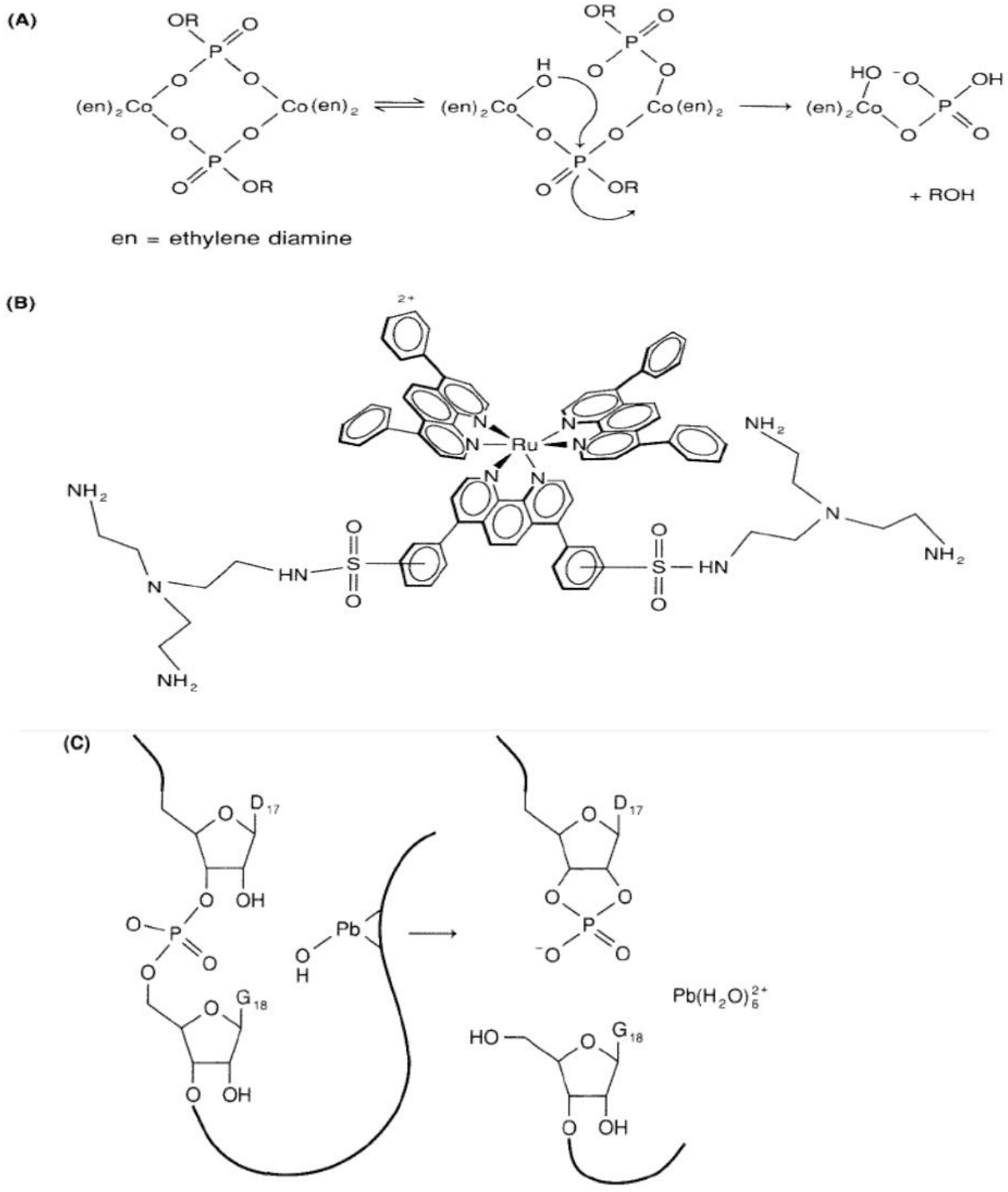


FIGURE:5

Hydrolysis reactions catalysed by metal ions and complexes.

(A)Illustration of a phosphate ester hydrolysis in a binuclear model complex catalysed by coordinated cobaltic ions, with one metal ion functioning as a Lewis acid and the other functioning to deliver the coordinated hydroxide.

(B) $Ru(DIP)_2$Macro, a metal complex constructed to contain a central DNA binding domain $Ru(DIP)_3^{2+}$ with two thethered amine arms to chelate additional metal ions (Zn^{2+}) to deliver to the sugar-phosphate backbone and promote hydrolytic strand cleavage.

(C) RNA site-specificially hydrolysed by lead ion. Diagramof the proposed mechanism of sugar-phosphate backbone cleavage between residue D^{17} and G^{18} in yeast RNA^{Phe}.

A CASE STUDY: TRIS(PHENANTHROLINE) METAL COMPLEXES

Now we may examine in detail the interaction of one class of metal complexes with nucleic acids, how these complexes bind to polynucleotides, the techniques used to explore these binding interactions, and various applications of the complexes to probe biological structure and function. Tris(phenanthroline) metal complexes represent quite simple, well-defined examples of coordination complexes that associate with nucleic acids. Their examination should offer a useful illustration of the range of binding modes, reactivity, techniques for study, and applications that are currently being exploited and explored. In addition, we may contrast these interactions with those of other transition-metal complexes, both derivatives of the tris(phenanthroline) family and also some complexes that differ substantially in structure or reactivity.

Binding Interactions with DNA

Tris(phenanthroline) complexes of ruthenium(II), cobalt(III), and rhodium(III) are octahedral, substitutionally inert complexes, and as a result of this coordinative saturation the complexes bind to double-helical DNA through a mixture of noncovalent interactions. Tris(phenanthroline) metal complexes bind to the double helix both by intercalation in the major groove and through hydrophobic association in the minor groove. Intercalation and minor groove-binding are, in fact, the two most common modes of noncovalent association of small molecules with nucleic acids. In addition, as with other small molecules, a nonspecific electrostatic interaction between the cationic complexes and the DNA polyanion serves to stabilize association. Overall binding of the tris(phenanthroline) complexes to DNA is moderate ($\log K = 4$).

The extent of intercalative versus groove binding is seen to depend upon environmental conditions, such as temperature and ionic strength, the charge of the metal center, and the DNA base sequence; groove binding is favored at AT-rich sequences. Second-generation mixed-ligand derivatives of the tris(phenanthroline) series have been prepared, and their interactions with DNA have provided useful insight into the factors important for promoting either intercalation or groove binding. Aromatic heterocyclic ligands with increased surface areas that are planar bind DNA with increasing avidity through intercalation, irrespective of the charge on the metal center. Intercalative binding constants greater than $10^7 M^{-1}$ can be easily achieved with planar heterocyclic ligands that jut out from the metal center. Not surprisingly, complexes containing ligands of increasing hydrophobicity that are not planar favor minor-groove binding.

Critically important as well in determining the binding mode is the chirality of the metal complex. Intercalation into the right-handed helix favors the Δ-isomer, whereas groove binding favors the A-isomer. Figure 6 illustrates these symmetry-selective interactions. In intercalation, we consider that one phenan throline inserts and stacks in between the base pairs, essentially perpendicular to the helix axis. For the Δ-isomer, once intercalated, the ancillary non-intercalated ligands are aligned along the right-handed groove of the helix. For the A-isomer, in contrast, with one ligand intercalated, the ancillary ligands are aligned in opposition to the right-handed groove, and steric interactions become evident between the phenanthroline hydrogen atoms and the phosphate oxygen atoms. Increasing the steric bulk on these phenanthrolines furthermore increases the enantioselective preference for intercalation of the Δ-isomer. *For intercalation,* then, *the chiral discrimination depends on matching the symmetry of the metal complex to that of the DNA helix.*

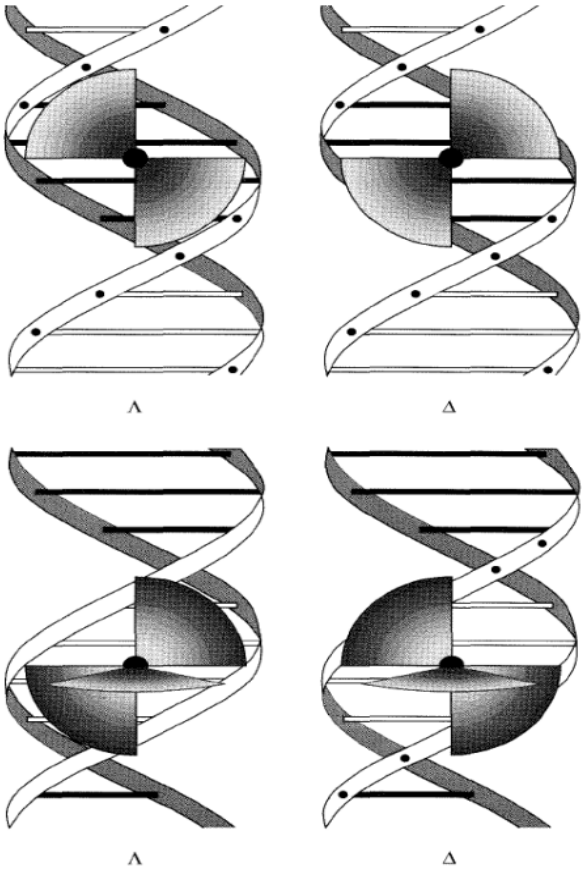


Figure 6
Enantiomeric discrimination in binding to DNA. Shown above is the basis for the preference for Δ-Ru(phen)$_3^{2+}$ upon intercalation and Λ-Ru(phen)$_3^{2+}$ for surface binding against a right-handed helix. With intercalation (top) the symmetry of the metal complex *matches* the symmetry of the helix; steric interactions preclude a close association of the Λ-isomer. With groove binding (bottom), where the metal complex binds *against* the minor-groove helical surface, complementary symmetries are required, and it is the Λ-isomer that is preferred.

For groove binding, where the metal complex is thought to bind *against* the helix, instead *it is a complementary symmetry that is required.* In our model for groove binding of the tris(phenanthroline) metal complex, two phenanthroline ligands are likely bound against the right-handed helical groove, stabilized through hydrophobic association. For the Λ-isomer, bound in this fashion, the ligands lie against and complement the right-handed groove; with the Δ -isomer, the ligands oppose the groove, and no close surface contacts are made.

Intercalation of metal complexes in DNA is not uncommon. Lippard and coworkers first established metallointercalation by Pt(II) complexes in the 1970s. Square-planar platinum(II)

complexes containing the terpyridyl ligand were shown to intercalate into DNA. In an elegant series of x-ray diffraction experiments on DNA fibers, Lippard illustrated the requirement for planarity in the complex. Although (phen)$Pt(en)^{2+}$ and (bpy)$pt(en)^{2+}$ were shown to intercalate into the helix, $(pyr)_2Pt(en)^{2+}$, with pyridine ligands rotated out of the coordination plane, could not. Complex planarity is in itself insufficient to promote intercalation, however. Cis-$(NH_3)_2PtCl_2$ or even cis-$(NH_3)_2Pt(en)^{2+}$ does not appear to intercalate into a helix, despite full planarity. Instead, aromatic heterocyclic ligands must be included in order to promote dipole-dipole interactions with the heterocyclic bases stacked in the helix. Indeed, planarity of the full complex is not required. Intercalation is not restricted to coordination complexes that are square planar. The tris(phenanthroline) complexes represented the first examples of "three-dimensional intercalators" and illustrated that octahedral metal complexes could also intercalate into the helix. Here one can consider the partial intercalation of one ligand into the helix, providing the remaining ligands on the complex an opportunity to enhance specificity or reactivity
at a given site.

Curiously, one unique and apparently general characteristic of metallointercalators is their preference for intercalation from the *major groove* of the helix. Most small molecules associate with DNA from the minor groove, but metallointercalators, both those that are square planar, such as (terpyridyl) platinum(II) complexes, and those that are octahedral, such as the tris(phenanthroline) metal complexes, appear to intercalate into the major groove. This then mimics quite well the association of much larger DNA-binding proteins with the helix; DNA regulatory proteins generally appear to target the major groove. The reason why metallointercalators favor major groove association is still unclear.

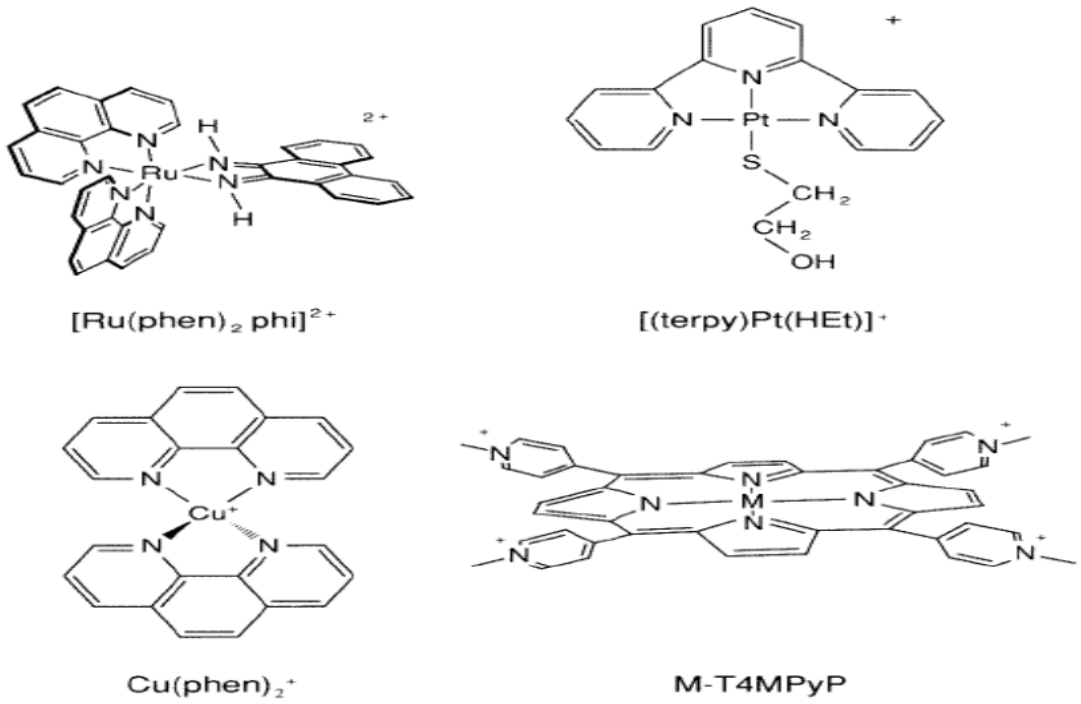


FIGURE7

Some metal complexes that bind DNA noncovalently primarily through intercalation (top) or binding in the minor groove (bottom). Some metalloporphyrins also primarily associate via intercalation.

Transition-metal complexes with aromatic ligands also generally associate by minor-groove binding or through the mix of intercalative and groove-bound interactions. $Cu(phen)^{+}$, a tetrahedral complex, appears to favor minor-groove binding over intercalation. Perhaps the tetrahedral coordination does not permit appreciable overlap of the phenanthroline ring with the bases in an intercalative mode. Metalloporphyrins, despite their large expanse and the presence commonly of nonplanar substituents, appear to bind to double-helical DNA both by intercalation and by minor-groove binding at AT-rich sequences. Occupation of the porphyrins by transition-metal ions, such as Cu(II), which bind axial ligands, leads to the favoring of groove binding over intercalation. Figure 7 illustrates some of the complexes that bind DNA noncovalently. The tris(phenanthroline) metal complexes themselves do not offer an illustration of hydrogen-bonding interactions with the helix, since these ligands lack hydrogen-bonding donors and acceptors, but as mentioned already, hydrogen bonding of coordinated ligands to the helix can

add some measure of stabilization, comparable to, but likely no greater in magnitude than, that provided by intercalative stacking, hydrophobic, or dispersive interactions. Indeed, mixed ligand derivatives of the phenanthroline complexes have been prepared that include hydrogen-bonding groups (amides, hydroxyls, and nitro substituents) on the ancillary phenanthroline ligands, and these have shown no greater avidity for double-helical DNA than their counterparts with hydrophobic substituents. A large number of weak hydrogen-bonding interactions to DNA by one complex can be stabilizing, however, as with, for example, hexaamminecobalt(III) or hexaaquoterbium(III) .

Tris(phenanthroline) metal complexes also do not offer an opportunity to explore covalent binding interactions with the helix in greater detail, but these interactions are, in fact, a major focus, concerned with the mode of action of cisplatin. One derivative of the tris(phenanthroline) series, $Ru(phen)_2Cl_2$, has been shown to bind to DNA covalently. In aqueous solution the dichlororuthenium(II) complex undergoes hydrolysis to form an equilibrium mixture of bis(phenanthroline) diaquo and chloroaquo species. These species bind covalently to DNA, with preferential reactivity at guanine sites. It is interesting that the same structural deformations in the DNA evident upon binding cis-diammineplatinum units become apparent upon coordination of bis(phenanthroline)ruthenium(II). It is also noteworthy that the chiral preference in coordination is for the A-isomer. As with groove binding, direct coordination to base positions requires a complementary symmetry, with the the A-isomer binding *against* the right-handed groove. This preference for the A-isomer reaffirms that, rather than noncovalent intercalation (which would favor the Δ isomer), covalent binding dominates the interaction. The energetic stabilization in direct coordination of the ruthenium(II) center is certainly more substantial than the weaker stabilization derived from intercalation. $Rh(phen)_2Cl_2^+$ and its derivatives have also been shown to bind covalently to DNA but only upon photoactivation, since light is needed to promote dissociation of the coordinated chloride and substitution of the nucleic acid base as a ligand.

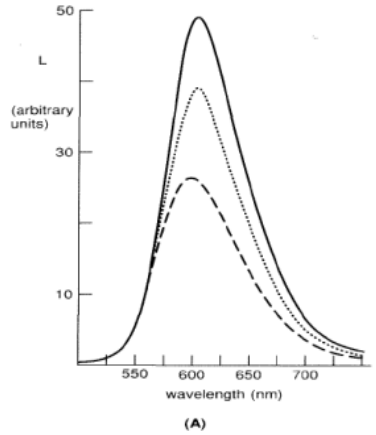
B. Techniques to Monitor Binding

Many of the same techniques employed in studying the basic chemistry of coordination complexes can be be used in following the binding of transition-metal complexes to nucleic acids, but biochemical methods, with their often exquisite sensitivity, become valuable aids as well in delineating specific binding interactions.

Tris(phenanthroline) metal complexes are particularly useful to illustrate this point, since here the metal center in the complex is selected in terms of the technique used for examination. Coordination complexes are often visibly colored, and these colorations provide a useful and sensitive spectroscopic handle in following fundamental reactions. This notion holds as well with tris(phenanthroline) metal complexes in their interactions with nucleic acids. $Ru(phen)_3^{2+}$ and its derivatives are highly colored because of an intense metal-to-ligand charge-transfer band (λ_{max} = 447 nm, € = 1.9 x104 $M^{-1}cm^{-1}$). Furthermore, the complexes are highly photoluminescent (λ_{em} = 610 nm, τ = 0.6 μs in aerated aqueous solution). On binding to nucleic acids these transitions are perturbed. Hypochromism is observed in the charge-transfer band, and intercalation leads to an increase in lifetime of the charge-transfer excited state. Indeed, single-photon counting experiments show a biexponential decay in emission from $Ru(phen)_3^{2+}$ bound to double-helical DNA. The longer-lived component (τ = 2μs) has been assigned as the intercalated component and the shorter-lived 0.6 μs component has been attributed to a mixture of free and groove-bound species. These spectroscopic perturbations permit one to define equilibrium-binding affinities for the different components of the interaction as a function of metal-center chirality and under different solution conditions. One can also follow the polarization of emitted light from the complexes after excitation with polarized light, and these studies have been helpful in describing the dynamics of association of the complexes on the helix. Mixed-ligand complexes of ruthenium(II) show similar spectroscopic perturbations, and these have been used to characterize binding affinities and chiral preferences, as well as the extent of intercalation versus groove binding as a function of ligand substitution on the metal center. The spectroscopic handle of the metal center therefore affords a range of experiments to monitor and characterize the binding of the metal complexes to polynucleotides.

Binding interactions of metal complexes with oligonucleotides can also be followed by NMR, and here as well the metal center offers some useful characteristics to exploit. As with organic DNA-binding molecules, shifts in the ^{1}H- NMR resonances of both the DNA-binding molecule and the oligonucleotide become apparent as a function of increased association with the helix. These shift variations can be used empirically to watch the dynamics of association and to gain some structural insights into the binding modes of the complexes on the helix. These kinds of experiments have been performed with tris(phenanthroline) complexes of ruthenium(II) and rhodium(III), where it was observed that the double-helical oligonucleotide is an

exceedingly good chiralshift reagent to separate resonances in an enantiomeric mixture of the (phenanthroline) complexes. For covalent binding molecules, such as *cis*diammineplatinum(II), furthermore, the lowering of the pK_a of purine positions and therefore shifting of resonances as a function of coordination to an alternate site has been helpful as well in assigning the sites of covalent binding on the oligonucleotide. But also in NMR experiments, special advantage can be taken of the metal center. For tris(phenanthroline) metal complexes, ^{1}H-NMR experiments were performed on the paramagnetic analogues, $Ni(phen)_3^{2+}$ and $Cr(phen)_3^{3+}$. It was reasonable to assume the binding characteristics would be identical with their respective diamagnetic analogues, Ru(II) and Rh(III); yet paramagnetic broadening by the metal complexes of nearby resonances on the oligonucleotide would allow one to deduce where along the helix the complexes associate. Using this method the groove-binding interaction of the complexes was identified as occurring in the minor groove of the helix. Figure 8 illustrates the monitoring of DNA binding by tris(phenanthroline) metal complexes using both the luminescence characteristics of ruthenium(II) complexes and the paramagnetic characteristics of nickel(II).



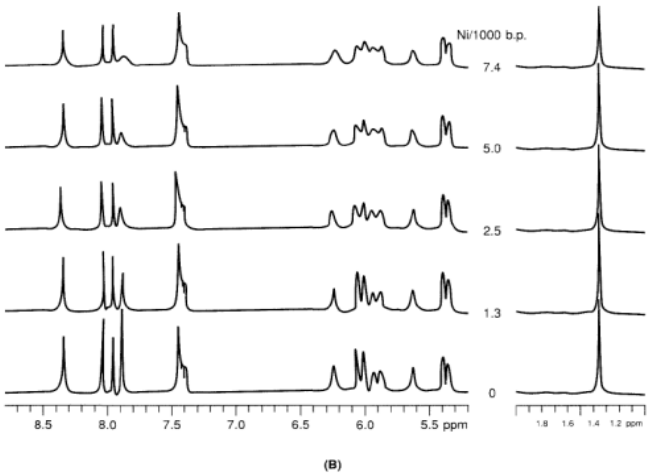


Figure 8

An illustration of two spectroscopic techniques used to probe DNA.
(A) The variation in luminescence characteristics of $Ru(phen)_3^{2+}$ with DNA binding. Shown is the emission spectrum of free $Ru(phen)_3^{2+}$ (----), A-$Ru(phen)_3^{2+}$ in the presence of DNA (.....), and Δ-$Ru(phen)_3^{2+}$ in the presence of DNA (--) illustrating the spectroscopic perturbation with DNA binding as well as the associated enantioselectivity in binding of the complexes to the helix. As is evident from the greater luminescence of the Δ- isomer on binding, it is this Δ-isomer that intercalates preferentially into the right-handed helix.
(B) An application of paramagnetic broadening by metal complexes in NMR experiments to obtain structural information on their association with nucleic acids. Shown is the ^{1}H-NMR spectrum of d(GTGCACh with increasing amounts of A-$Ni(phen)_3^{2+}$. Note the preferential broadening of the adenine AH2 resonance (7.8 ppm), indicating the association of this enantiomer in the minor groove of the helix.

heavy-metal derivatives to solve the structure. Such has certainly been true for the crystal structure of $tRNA^{Phe,}$ where heavy-metal ions such as platinum, osmium, and mercury were targeted to specific base positions, and lanthanide ions were used to label phosphate positions around the periphery of the molecule.

Other techniques can also be exploited to monitor and characterize binding. A recent novel illustration is one from electrochemistry, which has been applied in monitoring the binding of $Co(phen)_3^{3+}$ to DNA. Surely other techniques, from EXAFS to scanning tunneling microscopy, will be exploited in the future.

Biochemistry also provides very sensitive techniques that have been invaluable in characterizing interactions of metal complexes with nucleic acids. First are simply gel

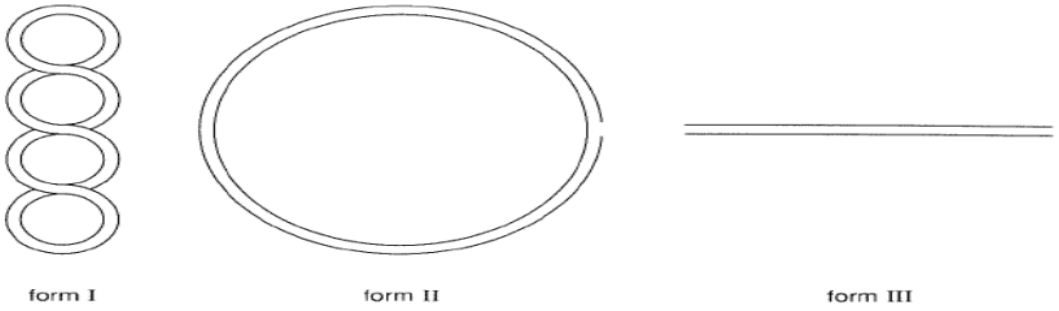
electrophoresis experiments, which permit an assessment of changes in the nucleic-acid conformation, through its changes in gel mobility, as a function of metal binding. A classic illustration is that of the unwinding of superhelical DNA as a function of intercalation. Closed circular DNA has much the same topological constraints on it as does a rope or a telephone cord; the DNA helices can wind up in coils. We define the duplex turning in a double helix as the secondary helical turns, and turns of the helices about one another as the supercoils or tertiary turns. As long as a DNA double helix is closed in a circle (form I), the total winding, that is, the total number of secondary and tertiary turns, is fixed. Molecules with differing extents of winding have different superhelical densities. In a circular molecule with one strand scission, what we call form II (nicked) DNA, the topological constraints are relaxed, and no supercoils are apparent. The same, by analogy, can be said of a telephone cord off the phone receiver, which can turn about itself to relax its many supercoils. Now let us consider a DNA unwinding experiment, monitored by gel electrophoresis. Supercoiled form I DNA can be distinguished from nicked DNA (form II) in an agarose gel because of their differing mobilities; the wound-up supercoiled molecule moves easily through the gelatinous matrix to the positive pole, whereas the nicked species is more floppy and thus is inhibited in its travels down the gel. A closed circular molecule with no net supercoils (form I_o) comigrates with the nicked species. Consider now the supercoiled molecule in the presence of an intercalator. Since the intercalator unwinds the DNA base pairs, the number of secondary helical turns in the DNA is reduced. In a negatively supercoiled, closed circular DNA molecule, the number of supercoils must be increased in a compensatory fashion (the total winding is fixed); hence the total number of negative supercoils is reduced, and the molecule runs with slower mobility through the gel. As the intercalator concentration is increased still further, the mobility of the supercoiled species decreases until no supercoils are left, and the species comigrates with the nicked form II DNA. Increasing the bound intercalator concentration still further leads to the positive supercoiling of the DNA and an increase in mobility. Figure 9 illustrates the experiment with tris(phenanthroline)ruthenium(II) isomers. This kind of unwinding experiment is an example of the sensitivity with which DNA structural changes can be monitored using biochemical methods; only low quantities($<\mu g$) of materials are needed to observe these effects. DNA strand scission can also be sensitively monitored, and even more importantly the specific nucleotide position cleaved can be pinpointed by biochemical methods. This methodology has been applied

successfully in monitoring both the efficiency of DNA strand scission by metal complexes and the specific sites cleaved, and hence where the complexes are specifically bound on the helical strand.

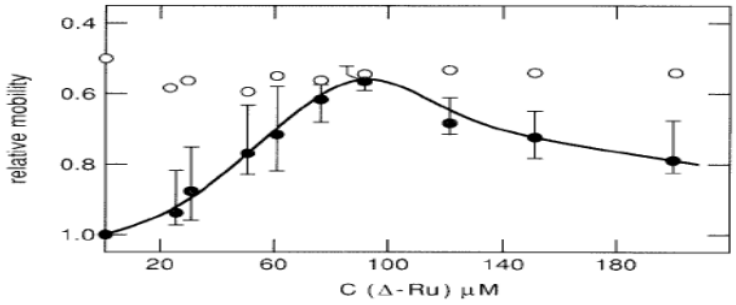
Relative extents of cleavage of DNA by different metal complexes can be easily assayed in an experiment that is an extension of the unwinding experiment described above. One simply measures the conversion of supercoiled form I DNA to nicked form II species. One strand cleavage on the DNA circle releases the topological constraints on the circular molecule and relaxes the supercoils. Two cleavage events within 12 base pairs on opposite strands will convert the DNA to a linear form (III), which also has a distinguishable gel mobility. Photoactivated cleavage of DNA by tris(phenanthroline) complexes of cobalt(lll) and rhodium(III) was first demonstrated using this assay. Given the high sensitivity of this assay, redox-mediated cleavage of DNA by a wide range of metal complexes can be easily demonstrated. However, other techniques are required to analyze whether appreciable and significant cleavage results, and, if so, what products are obtained. Since the assay can monitor, in a short time using little sample, a single nick in a full 4,000-base-pair plasmid, reactions of very low, almost insignificant yield can be detected. The assay provides, however, a simple scheme to assess *relative* extents of cleavage by different metal complexes, as well as a first indication that a cleavage reaction by a given metal complex occurs at all.

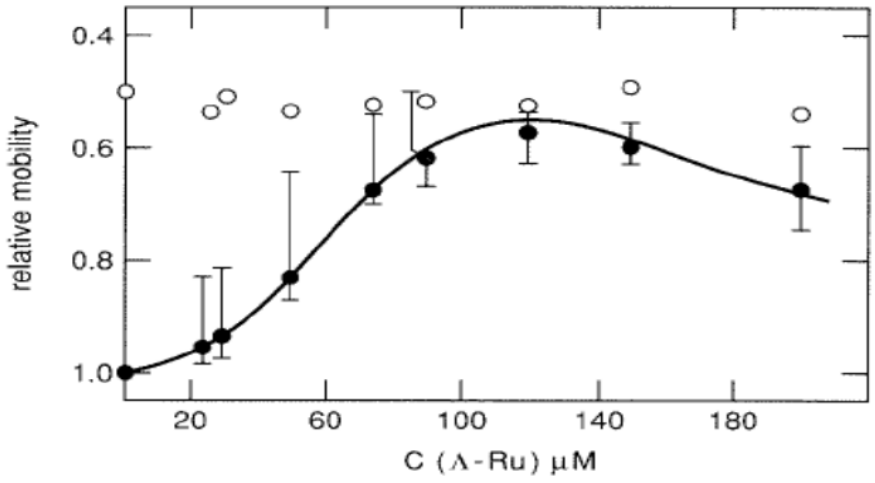
More informative are experiments on ^{32}P-labeled DNA fragments using highresolution polyacrylamide gel electrophoresis, since these experiments allow one to find the exact nucleotide where the complexes break the sugar-phosphate backbone. Consider a cleavage reaction by a given metal complex on a DNA fragment of 100 base pairs in length that has been labeled enzymatically with ^{32}p on one end of one strand. If the metal complex cleaves the DNA at several different sites, then one can arrive at conditions where full cleavage is not obtained, but instead a population of molecules is generated where single cleavage events per strand are obtained, and cleavage at each of the sites is represented. After denaturation of the fragment, electrophoresis through a high-density polyacrylamide gel, and autoradiography, only fragments that are radioactively endlabeled are detected, and hence the population of sites cleaved is determined. The denatured cleaved fragments move through the gel according to their molecular weight. By measuring their length, using molecular-weight markers, one can find the specific position cleaved relative to the end of the fragment. By this route the specific sites cleaved by a

molecule that binds and cleaves DNA, or end-labeled RNA, at unique positions may be identified. In a complementary experiment, using footprinting, where a molecule cleaves DNA nonspecifically at all sites along a fragment, one can find the binding positions of other molecules such as DNA-binding proteins. In this experiment, cleavage with the sequence-neutral cleaving reagent is carried out both in the presence and in the absence of the other



(A)





(B)

figure 9

The application of DNA supercoiling to probe metal-complex interactions with DNA. (A) A schematic representation of supercoiled DNA (form I), nicked DNA (form II) that, as a result of the single-strand scission, relaxes to a circular form lacking supercoils, and linear (form III) DNA. (B) Plots of the relative electrophoretic mobilities of form I (●) and form II (○) DNA as a function of increasing concentration of Δ-(top) and Λ-(bottom) $Ru(phen)_3^{2+}$ in the gel.[46] Increasing concentrations of bound intercalator unwind the negatively supercoiled DNA. Given the higher intercalative binding affinity of the Δ-isomer, slightly lower concentrations of this isomer are needed to unwind the plasmid to a totally relaxed state (where form I and II comigrate).

other binding molecule. In the absence of the protein, cleavage ideally occurs at all sites; hence a ladder of cleaved fragments is apparent on the autoradiograph. If cleavage is carried out in the presence of the protein, however, those sites that are bound by the protein are protected from cleavage by steric considerations, producing a shadow or footprint of the protein-binding site on the gel. Both the site-specific and footprinting experiments are illustrated schematically in Figure 10. This very powerful technology was first applied by Dervan and coworkers in demonstrating the application of methidium-propylFeEDTA (MPE-Fe) as a chemical footprinting reagent. Tris(phenanthroline) metal complexes have been shown to cleave DNA nonspecifically, and their derivatives have been applied either as sensitive photofootprinting reagents, or as site-specific cleaving molecules, as we will see.

APPLICATIONS OF DIFFERENT METAL COMPLEXES THAT BIND NUCLEIC ACIDS

Both the spectroscopy and the chemical reactivity of transition-metal complexes, coupled to biochemical assays, can therefore be exploited to obtain a wide range of useful reagents to probe nucleic acids. Here some specific applications are described.

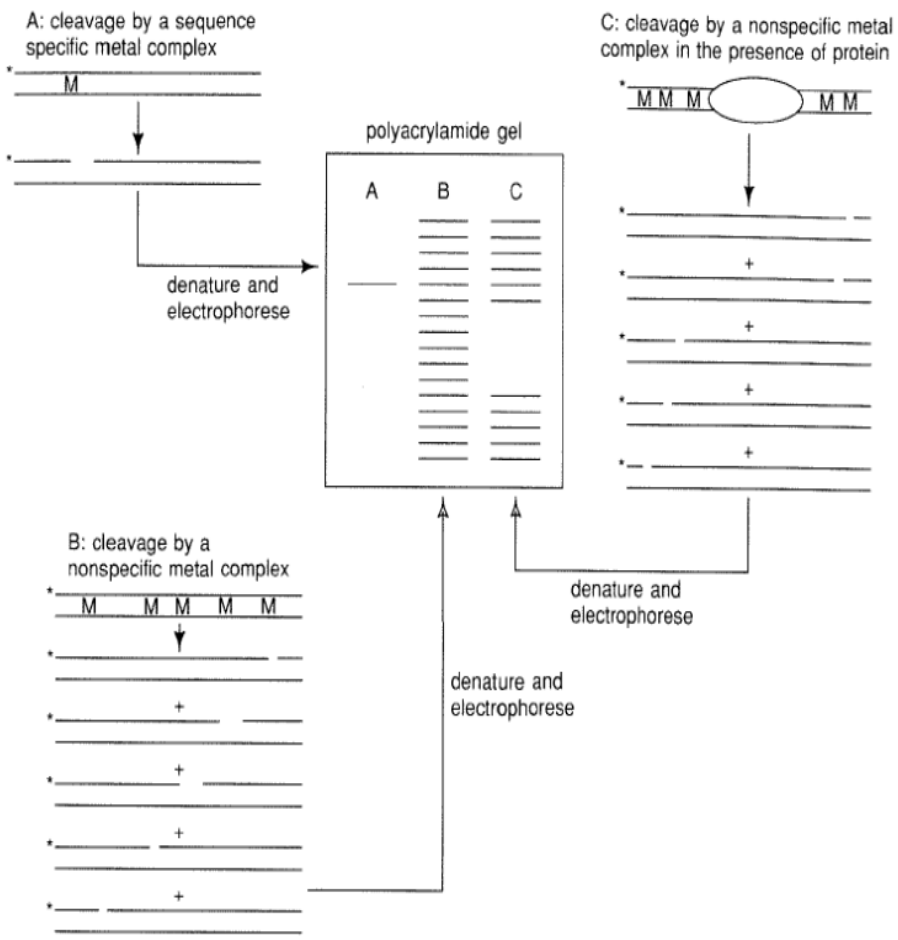


FIGURE 10

DNA cleavage by metal complexes. Shown schematically is the method used with single-base resolution to discover the sites where metal complexes are bound on double helical DNA. After the metal complex is bound to several sites on a radioactively end-labeled (*) DNA fragment and activated to permit strand cleavage at the binding sites, the nicked DNA is denatured and electrophoresed on a high-resolution polyacrylamide gel, and the gel submitted to autoradiography. From the molecular weights of the end-labeled denatured fragments, the positions of cleavage and therefore binding by the metal complex may be deduced. The results in lane A show the cleavage pattern observed for a metal complex that binds to a specific site. The results in lane B show cleavage observed for a nonspecifically bound metal complex that binds and therefore cleaves at every base site. Lane C illustrates a footprinting experiment. When protein is bound to DNA at a specific site, it protects the DNA from cleavage by the metal complex at its binding site, thus producing a ''footprint'' in the gel: the absence of end-labeled fragments of those lengths that are protected from cleavage as a result of protein binding.