Articles

Metals and DNA: Molecular Left-Handed Complements

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Chiral metal complexes provide unique molecular probes for DNA. Chiral reagents that "recognize" different local structures along the DNA strand have been designed by a process in which the asymmetry in shape and size of the complex is matched to that of the DNA helical groove. As a result, the chiral metal complexes provide very sensitive probes for local helical structure, both left- and right-handed. Direct coordination of chiral complexes to the DNA bases adds an element of sequence selectivity to the probe design. With a suitable reactive metal center, reagents that target chemically specific sites along the strand may be developed. One such chiral reagent, which cleaves left-handed DNA sites with photoactivation, has been useful in mapping this distinct conformation and examining its biological role. The conformation-specific molecular cleaver, much like a DNA-binding enzyme, recognizes and reacts at discrete sites along the DNA strand. These site-specific chiral metal complexes provide exciting new tools for probing the local variations in DNA structure and its role in the regulation of gene expression.

IN BIOLOGICAL SYSTEMS THERE IS A HIGH LEVEL OF SPECIFICity in the interaction between molecules and in their chemical reactions. In achieving precise molecular recognition in nature, extensive use is made of molecules and molecular shapes that are dissymmetric in three dimensions, that is, molecules that are chiral. Chirality, a word coined by Lord Kelvin, refers to the property that a structure is not superimposable on its mirror image (*1*). Some examples of chiral structures are given in Fig. 1.

"Chiral" is derived from the Greek word "cheir" for hand. The similarity but at the same time inherent difference between our left and right hands may easily convey the notion of chirality and also perhaps the importance of chirality to specific interactions between shapes and structures, as specific as two right hands clasped in a handshake. It is in fact the introduction of the second chiral center, so that a right hand is bound to either a left or another right, that is needed to distinguish the chirality of an object. A mirror-image enantiomeric pair of molecules share identical chemical properties. Yet the association with a second asymmetric center, forming the diastereoisomer, facilitates the resolution of the chiral isomers. Two right hands in a handshake are clearly distinct from a left and right hand held together. This is seen chemically in the differential crystallization of diastereoisomeric salts, in the use of chiral shift reagents to distinguish isomers in a nuclear magnetic resonance (NMR) experiment, or even in the detection of optical isomers by means of circularly polarized light. It is surely the interaction of one chiral object with another that leads to the remarkable specificity in chemical reactions seen by chiral proteins on chiral substrates. The natural enzyme chymotrypsin reacts efficiently with only one stereoisomer, the L-tryptophan methyl ester, to catalyze its hydrolysis. The mirror-image substrate, D-tryptophan methyl ester, is instead a potent competitive inhibitor, oriented in the active site with a scissile bond poised away from the reactive groups in the asymmetric protein pocket (*2*).

Chirality in chemistry is not simply confined to the asymmetric tetrahedral carbon atom. The isolation of optical isomers of ethylenediamine complexes of cobalt(III) by Werner (*3*) was essential to his proof that octahedral coordination geometries are adopted by transition metals. To quell the skeptics, the isolation had to be repeated with the cation tris[tetraammine-μ-dihydroxocobalt(III)]cobalt(III) so as to generate an optically active compound devoid of carbon atoms (*4*). Among the chiral metal complexes first synthesized by Werner were enantiomers of tris(phenanthroline)-iron(II) (*5, 6*). In the 1950's Dwyer (*7*) looked for differential biological activity between isomers of these simple metal complexes, and, perhaps not surprisingly, differential effects were detected. In the structures of enantiomers of the tris(phenanthroline) metal complexes (Fig. 2), the chirality around the octahedral metal center arises from the disposition of chelating groups clockwise or counterclockwise in a left- or right-handed spiral about the C_3 axis.

Handedness in molecular structures may also emanate simply from the helicity of the polymeric structure. Protein α-helices exist predominantly in a right-handed spiral. Double-stranded DNA also commonly adopts a right-handed helical conformation, that of B DNA, but it was discovered recently that certain sequences of DNA have the propensity to undergo conformational transitions to a left-handed helical form, termed Z DNA (*8, 9*). Whether this conformation of opposite helix chirality is exploited by nature is a subject of much debate.

In this article I focus on the use of simple chiral metal complexes, the tris(phenanthroline) metal cations looked at by Werner and Dwyer, to examine structures and conformations along the DNA helix. My co-workers and I were interested in exploring the heterogeneity in conformations along a DNA duplex; we were interested in whether a variety of secondary structures, differing in shape and handedness, exist along the strand, and whether these differing structures might serve some biological role. In contrast to traditional spectroscopic analysis of the average polymer structure, small molecular probes are uniquely suited to detect structural variations local to a site. Could small reporter molecules be targeted specifically to these sites? The use of various chiral metal complexes makes it possible to study specific recognition and reactions. In

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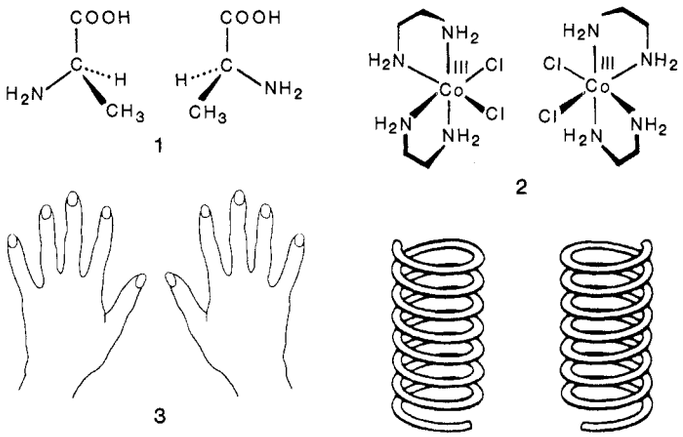


Fig. 1. Enantiomeric pairs.

some respects the specificity is not surprising, since interactions in nature show that specific binding depends on the stereochemistry. The DNA helix is, after all, an asymmetric molecule.

Stereoselective Intercalation in B DNA

A simple binding interaction of small molecules to double-stranded DNA is that of intercalation (*10*). Intercalative binding is a noncovalent stacking interaction resulting from the insertion of a planar heterocyclic aromatic moiety between the base pairs of the DNA helix. It is the most common mode of binding small spectroscopic probes, such as ethidium, and small drugs, such as actinomycin, to DNA (*11*). The flat intercalator is held rigidly in a well-defined orientation perpendicular to the axis of the helix. Metal complexes also may bind to DNA by intercalation, as was demonstrated by Lippard and co-workers (*12, 13*). It was shown that platinum(II) complexes containing a heterocyclic aromatic ligand, such as phenanthroline (phen) or terpyridine (terpy), bind to the helix noncovalently, inserting between the base pairs of the polynucleotide. Platinum(II) complexes adopt primarily square planar coordination geometries, and thus the crystal structure of (terpy)$PtCl^+$ bound to $(dCpG)_2$ revealed that the flat metal complex, not just the aromatic ligand, could insert between the base pairs of the dinucleotide (*14*). These complexes have been useful as electron-dense probes in x-ray diffraction experiments on nucleic acids (*15*).

Indeed, transition metal chemistry offers a rich variety of coordination geometries that can be exploited in the design of rigid, structurally well-defined metallointercalators. Through hydrodynamic experiments measuring DNA helical unwinding in topologically constrained closed circular DNA, evidence was obtained (*16*) that tetrahedrally and octahedrally coordinated phenanthroline complexes of zinc(II) bind to DNA by intercalation as well. For the nonplanar complex, however, only a partial insertion of the aromatic ligand is possible. That constraint is actually an advantage, since orienting the rigid molecule perpendicular to the helix axis through the intercalating ligand affords a means to design specific interactions between additional coordinated ligands and the base pairs along the groove of the DNA helix. A simple illustration of this principle is found in a comparison of the binding of enantiomers of $Zn(phen)_3^{2+}$, a chiral intercalator, to DNA. Since the nonintercalated ligands are chemically identical, any selectivity between enantiomers must depend solely on the disposition of ligands about the metal.

Is there enantiomeric discrimination evident in binding tris(phenanthroline) metal complexes to DNA? Dialysis of a calf thymus DNA solution against the racemic $Ru(phen)_3^{2+}$ solution yields a dialysate enriched in the Λ isomer (*17*). Clearly, then, enantiomeric selectivity accompanies binding of $Ru(phen)_3^{2+}$ complexes to DNA. Binding favors the Δ isomer, leaving upon equilibrium dialysis an imbalance in the less favored Λ isomer outside the dialysis bag. Identical results have been obtained for $Zn(phen)_3^{2+}$ (*16, 18*) and $Os(phen)_3^{2+}$. The binding and chiral discrimination depends not on the electronic structure or properties of the metal, but instead on the stereochemistry of the complex. The level of stereoselectivity varies as a function of the relative size of the complex and that of the DNA groove. To my knowledge, comparative investigations with DNA of binding by small chiral molecules had not been conducted previously. Indeed, DNA intercalation by chiral molecules had been described only in the case of the naturally occurring peptide-containing drugs (*11*), and for these drugs the importance of the chiral centers to specific DNA binding had not been delineated.

The basis for the chiral discrimination found with tris(phenanthroline) metal complexes becomes evident in viewing models of the interaction (Fig. 3). With one ligand intercalated, for the Δ isomer the remaining ligands lie along the groove of the right-handed DNA, complementing the groove in terms of disposition. For Λ-$Ru(phen)_3^{2+}$, however, with one ligand intercalated, steric repulsions are evident between the nonintercalated phenanthroline hydrogen atoms and phosphate oxygen atoms. For the Λ isomer, as indicated by the arrows, the disposition of nonintercalated ligands opposes the right-handed DNA groove. The chiral discrimination then depends on matching the chirality of the complex to that of the DNA helix.

A Probe for DNA Handedness

Because the formation of diastereoisomers between metal complexes and B DNA provided a ready means to distinguish the chirality of the metal complex, it seemed reasonable to use instead a given metal enantiomer, perhaps Λ-$Ru(phen)_3^{2+}$, to distinguish the chirality of DNA helices. The discovery of Z DNA by Rich and co-workers brought into sharp focus the notion (*9, 19*) that double-stranded DNA helices may be flexible and variable structurally. Does DNA secondary structure serve a biological role? How frequently

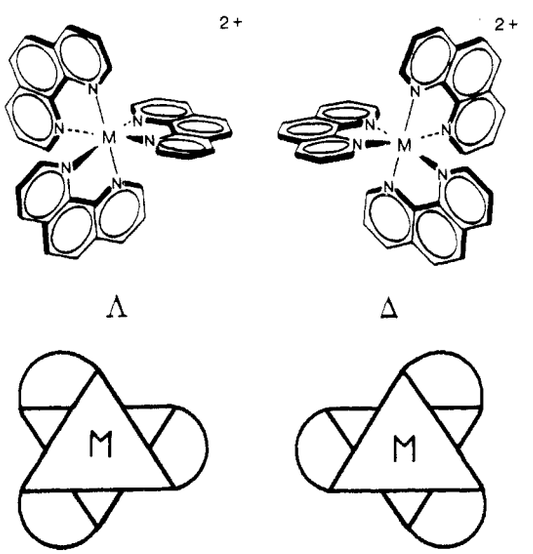


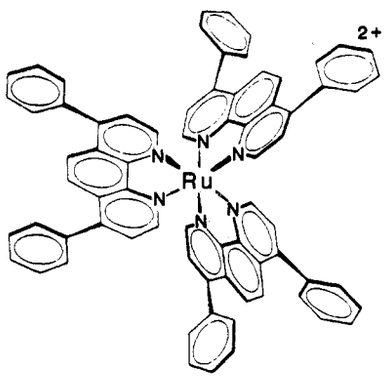
Fig. 2. The Λ- (left) and Δ- (right) enantiomers of a tris(phenanthroline) metal complex. The lower scheme is along the C_3 axis of the complex, illustrating how the absolute configurations are classically assigned, with helical screws counterclockwise for Λ and clockwise for the Δ isomers.

and where is Z DNA found? Is this left-handed DNA conformation exploited by nature in the chemical regulation of gene expression? The application of simple chiral metal complexes as molecular probes for Z DNA seemed a sensitive and specific route from which to approach these questions.

The structures of Z DNA and B DNA differ dramatically. In contrast to the right-handed B form, Z DNA is a left-handed antiparallel double helix. But Z DNA is not simply a left-handed B-like structure. Z DNA is a zigzag helix, with a dinucleotide repeating unit resulting from alternation along the strand in sugar puckering (C2′-*endo*/C3′-*endo*) and torsion angle (*syn*/*anti*) about the glycosidic bond. This zigzag structure probably accounts for the observation that alternating purine-pyrimidine sequences adopt the Z form most readily. The Z DNA conformation was originally detected through spectroscopic results (*20*) obtained for poly[d(GC)] and through the crystal structure of $d(CpG)_3$ (*8*). More recent results indicate that some mistakes in purine-pyrimidine alternation are permissible as long as the *syn*/*anti* alternation is maintained (*21*). The transition to the Z DNA conformation, therefore, probably represents the most dramatic example of a sequence-specific conformational change for DNA. Of particular importance for molecular probe design, the surface topology of Z DNA differs substantially from that of B DNA. Although B DNA has distinct right-handed major and minor grooves of well-defined width and depth, the Z DNA helix has for its left-handed major groove a very wide and shallow, almost convex, surface and a minor groove that is extremely narrow, resembling a deep, sharp crevice (Fig. 4). More detailed information about Z DNA is given in recent reviews (*9, 19*).

Chiral tris(phenanthroline) complexes of ruthenium(II) do indeed provide sensitive spectroscopic probes to distinguish DNA conformations based on their chirality (*17, 22, 23*). The rich photophysics of ruthenium(II) complexes has been extensively described (*24*). Tris(phenanthroline)ruthenium(II) complexes and their derivatives are characterized by intense ($\epsilon = 3 \times 10^4\ M^{-1}\ cm^{-1}$) metal-to-ligand charge transfer (MLCT) bands in the visible region. In addition, the complexes luminesce with high-quantum efficiency. Importantly, the MLCT band is perturbed upon DNA binding. Hypochromism and enhancements of luminescence accompany DNA binding (*17, 23*). For $Ru(phen)_3^{2+}$ in aerated buffer solution the lifetime of the excited state varies from 0.6 to 2.0 μsec in the presence of DNA. These perturbations with DNA binding therefore provide useful spectroscopic handles.

The utility of a chiral probe for DNA handedness is limited not only by the sensitivity with which binding may be detected but also by the sensitivity with which the probe may distinguish enantiomers. The level of enantiomeric discrimination is not high for the phenanthroline complexes; both Λ- and Δ-$Ru(phen)_3^{2+}$ intercalate into B DNA. The binding interaction becomes enantiospecific, however, for complexes of 4,7-diphenylphenanthroline (DIP), in which the steric bulk is markedly increased. It is this complex (**I**) that is a sensitive probe for Z DNA (*22*).



I

Two assays to demonstrate the use of $Ru(DIP)_3^{2+}$ as a probe for DNA handedness are given in Fig. 4. Binding of $Ru(DIP)_3^{2+}$ isomers to B DNA is enantiospecific. Hypochromism in the MLCT band is seen only for Δ-$Ru(DIP)_3^{2+}$ upon titration with B form DNA from calf thymus. Little reduction in visible absorbance in the presence of B DNA is found for Λ-$Ru(DIP)_3^{2+}$, the left-handed isomer, which does not bind intercalatively to the right-handed helix (*25*). This same enantiospecificity is seen in a luminescence-quenching experiment (*23*). A plot of inverse luminescence intensity (quenching) for Δ-$Ru(DIP)_3^{2+}$ bound to B DNA as a function of increasing concentrations of ferrocyanide, $Fe(CN)_6^{4-}$, an anionic quencher, gives a biphasic curve, reflecting one ruthenium component that is easily accessible to the solution quencher, and a second component that is tightly bound to the DNA polyanion and hence protected from the anionic ferrocyanide quencher. For Λ-$Ru(DIP)_3^{2+}$ with B DNA, a linear Stern-Volmer plot is obtained; one component only, easily accessible to ferrocyanide, exists. Quite different results are obtained with Z DNA, here poly[d(GC)] in the presence of cobalt hexammine. Equivalent hypochromism is displayed by Δ- and Λ-$Ru(DIP)_3^{2+}$ in the presence of Z DNA, which indicates that both isomers bind essentially equally to this helical form. Furthermore, in the quenching assay both isomers in the presence of Z DNA display curved Stern-Volmer plots, also indicating that both isomers bind to the Z DNA helix. This assay actually made apparent a somewhat reduced accessibility to quencher for Λ-$Ru(DIP)_3^{2+}$ relative to the Δ isomer. In other words, it appears that the Λ enantiomer is favored slightly in binding to the left-handed helix. Hence enantiospecific binding of Δ-$Ru(DIP)_3^{2+}$ is evident with the right-handed B DNA helix, and a small enantioselectivity for Λ-$Ru(DIP)_3^{2+}$ is associated with binding to the left-handed Z DNA.

These results are understandable after considering the structural models and the basis for the chiral discrimination. In binding to B DNA only the Δ isomer may intercalate easily. Model building studies based on the use of the crystallographically determined coordinates for Δ-$Ru(DIP)_3^{2+}$ (*26*), with phenyl groups rotated out of the phenanthroline plane, show that a close association, maximizing hydrophobic contacts, is possible for Δ-$Ru(DIP)_3^{2+}$ intercalated from the major groove (*27, 28*), with one phenyl group of the intercalated ligand darting through the helix to the minor groove. For the Λ isomer, in contrast, steric interactions between the nonintercalated ligands and the right-handed DNA-phosphate backbone completely preclude its similar intercalation. A left-handed helix favors the left-handed isomer, but only a slight stereoselectivity in binding Λ-$Ru(DIP)_3^{2+}$ to Z DNA is observed. Z DNA is not a

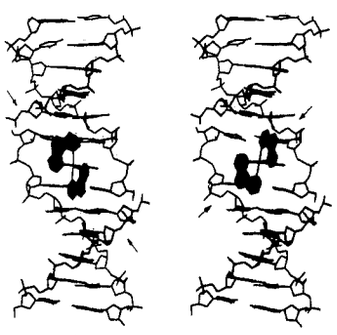


Fig. 3. Models of the intercalation of Λ- (left) and Δ- (right) Ru-$(phen)_3^{2+}$ in a B DNA helix. The arrows indicate the disposition of the nonintercalated ligands with respect to the helical groove. The ligands are aligned along the groove for the Δ isomer and opposed to the groove for the less favored Λ isomer.

left-handed B DNA. Because of its very shallow and wide major groove, the Z form helix provides only a poor template to discriminate between enantiomers.

The comparative binding by $Ru(DIP)_3^{2+}$ enantiomers then serves as the probe for helix conformation. If only the Δ isomer binds to the polynucleotide, the conformation is likely to be right-handed and B-like. Since Λ-$Ru(DIP)_3^{2+}$ does not bind to B DNA, then if the unknown DNA binds the Λ isomer, the helix conformation is probably left-handed. If binding occurs for both Δ- and Λ-$Ru(DIP)_3^{2+}$, but to a lesser extent with the Δ isomer, the unknown conformation is likely to be Z DNA. If no binding occurs with either isomer, then the base pairs are either inaccessible or perhaps unstacked; spectroscopic variations are not found with single-stranded DNA. Also, tris(phenanthroline) complexes of ruthenium(II) do not bind detectably to double-stranded RNA (*28*). Studies with these probes have supported Shafer's finding of a non-Z but left-handed conformation for poly[d(m^5-CG)] in 2 m*M* NaCl (*29*). The reagents, both left- and right-handed, are sensitive and detailed stains for fluorescence microscopy. These probes also seem well suited to studies of drug-DNA complexes and protein-bound DNA sites. Their relative binding and inhibitory characteristics have been used to demonstrate that a DNA conformational change occurs with the specific binding of a restriction enzyme to its DNA recognition site (*30*).

Tuning the Stereoselectivity to Local Helical Structure

The degree of stereoselectivity for the binding of chiral complexes to DNA provides not only an assay for absolute helical handedness but also for variations in helical structure and in particular for helical groove size in the vicinity of the bound complex. The chiral complexes are therefore useful in probing right-handed as well as left-handed helical conformations (*28*). The level of stereoselectivity or chiral discrimination, *S*, may be defined as the ratio of the amounts of Δ isomer bound to the DNA to total metal bound to the helix. Then if $S = 1$, as for $Ru(DIP)_3^{2+}$ with B DNA, we conclude that the helix is right-handed and has a groove size significantly smaller than the diameter of the metal complex. It is because of these relative dimensions that the Λ isomer sterically cannot intercalate into a right-handed helix. What if $S = 0.5$? Then the groove size of the helix must be wider than the complex, and no chiral discrimination is evident. Intermediate values of *S*, therefore, may be applied as a gauge, or "molecular yardstick," for the groove size of the helix in solution.

One test of this idea is the consideration of variations in *S* for $Ru(phen)_3^{2+}$ with DNA, as a function of increasing sodium concentrations. As the salt concentration increases, cations bind more to the phosphate backbone, neutralizing phosphate-phosphate repulsions, and permitting a compression along the helical axis. As the helix compresses as a function of salt concentration and the groove size decreases, *S* does increase for $Ru(phen)_3^{2+}$ with DNA (*28*). Variations in *S* are best detected by means of a reagent of size comparable to the helical groove size (not much larger or much smaller). The phenanthroline complexes in particular may be used to advantage here.

Variations in *S* for $Ru(phen)_3^{2+}$ in binding to DNA's of differing base composition can also be seen. If the binding constants of $Ru(phen)_3^{2+}$ to DNA's of differing guanine-cytosine (GC) contents are determined, no variations with GC content are detected. Examination of the degree of stereoselectivity for intercalation, however, reveals that, as the GC content increases, so too does the stereoselectivity. These results show that interactions can be described with sensitivity through measurements of the chiral discrimination; simple trends were not apparent in the examination of variations in binding affinity. The results may also suggest that GC base pairs stack more closely together than adenine-thymine (AT) base pairs, that a helical compression occurs with increasing GC content of the DNA.

Measurements of chiral discrimination also provide a means to characterize other binding modes of the metal complexes to the helix. A secondary binding mode of $Ru(phen)_3^{2+}$ to the helix, less loosely held than the intercalative species, has been found through luminescence assays (*28*). This binding mode appears to favor the Λ isomer.

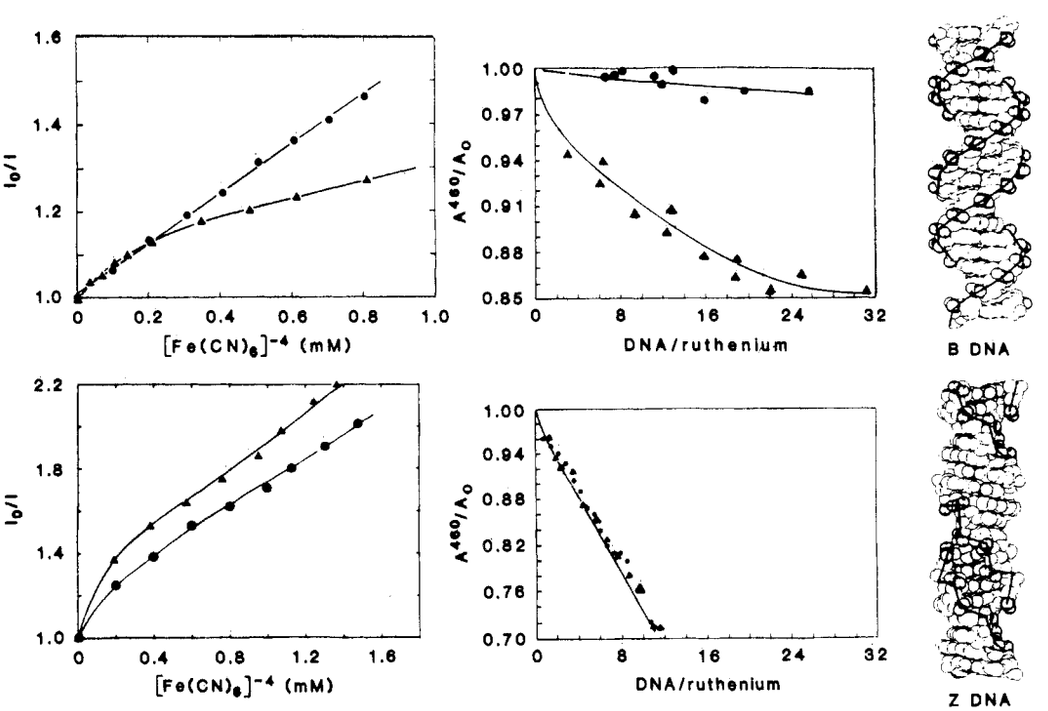


Fig. 4. Spectroscopic assays for helical handedness using Λ- (●) and Δ- (▲) $Ru(DIP)_3^{2+}$. (Left) Luminescence quenching (I_0/I) by ferrocyanide of solutions containing ruthenium enantiomers bound to B DNA (top) and Z DNA (bottom) [adapted from (*23*)]; (middle) hypochromism (A/A_0) in the charge transfer band of the ruthenium isomers in the presence of B DNA (top) and Z DNA (bottom) [adapted from (*22*)]; and (right) B (top) and Z (bottom) DNA conformations [adapted from (*9*)]. The assays indicate an enantiospecific association of the Δ isomer with the right-handed helix and an enantioselective association of the Λ isomer with the left-handed helix.

On the basis of the luminescence results, it is likely that Λ-Ru(phen)$_3^{2+}$ also interacts with right-handed DNA as a hydrophobic B DNA groove binder. Hydrophobic groove binding, even more than intercalation, is a common mode of noncovalent binding of small molecules to DNA. A new probe, based on such a groove association, that recognizes the A DNA conformation is an interesting possibility (*31*); one distinguishing feature of right-handed A DNA is the large and shallow surface of its groove.

Covalent Binding by the Chiral Complexes

Metal ions and complexes often show some biological activity, such as cytotoxicity or mutagenicity, which results from direct coordination of the metal to the nucleotide bases (*32*). In addition to the harder phosphate anion sites along the backbone, the endocyclic nitrogen lone pairs on purines and pyrimidines provide favorable sites for coordination to a transition metal ion, and particularly so for a softer heavier metal. In fact, DNA is an extremely good ligand for metals. This fact may be best illustrated by the clinical success of the simple coordination complex, *cis*-diamminedichloroplatinum(II), *cis*-DDP, as an antitumor drug (*33*). Its mode of action is thought to involve direct coordination of *cis*-diammineplatinum(II) to neighboring guanines through their N-7 nitrogen atoms (*34*), thus forming an intrastrand cross-link. Thus, metal coordination provides a route to the incorporation of sequence selectivity into the design of DNA site-specific agents.

Dichloro-*bis*(phenanthroline)ruthenium(II) (Fig. 5) shares with *cis*-DDP characteristics of both structure and reactivity. Like *cis*-DDP, the neutral (phen)$_2$RuCl$_2$ contains two *cis*-oriented chloride ions that are good leaving groups. Furthermore, like platinum(II) and other softer metal ions, ruthenium complexes tend to favor coordination to purine N-7 sites (*35*). With respect to targeting covalently guanine sites along the helix, (phen)$_2$RuCl$_2$ represents an octahedral analog for *cis*-DDP, but one that is chiral.

Racemic (phen)$_2$RuCl$_2$ does bind covalently to DNA (*36, 37*). Incubation of (phen)$_2$RuCl$_2$ with DNA followed by ethanol precipitation shows the association of the ruthenium with the polynucleotide in the precipitate. In contrast, intercalated Ru(phen)$_3^{2+}$ remains in the ethanol supernatant. The time scale for the covalent binding of (phen)$_2$RuCl$_2$ to DNA is several hours. Hydrodynamic experiments indicate that structural changes seen in the DNA helix as a result of ruthenium binding resemble in detail those seen upon platination. Moreover, binding experiments indicate a preference for guanine-rich DNA's and, in particular, for poly(dG) · poly(dC) (*37*).

Enantiomeric selectivity accompanies also the covalent binding of this chiral complex to the asymmetric helix (*36*). After incubation with racemic (phen)$_2$RuCl$_2$ and separation by ethanol precipitation, optical enrichment is evident in the supernatant (*38*). The stereoselectivity varies as a function of sequence and may point to a preference for homoguanine sites since the highest stereoselectivity is obtained for poly(dG) · poly(dC) (*37*). A complete reversal of selectivity is seen for binding to poly(dGC), which is a sequence known to favor transitions to a left-handed structure upon platination or alkylation. The variation in stereochemical preference with sequence suggests that various sequence-dependent structural adducts must be present. Coordination by the metal monofunctionally to a single base position is likely to display a lower level of stereoselectivity than that found in a bifunctional adduct with the chiral metal complex cross-linking nearby base sites.

The stereochemistry furthermore provides clues on how the complex coordinates to the helix. Unlike intercalation, where a right-handed isomer stacks best with a right-handed helix, here it

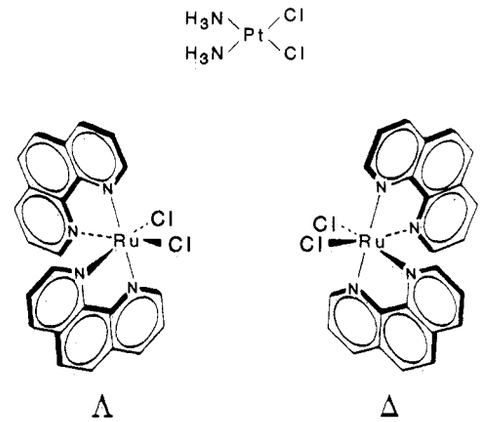


Fig. 5. *cis*-Diamminedichloroplatinum(II) (top) and Λ- and Δ-*bis*(phenanthroline)dichlororuthenium(II) (bottom).

appears that the left-handed (phen)$_2$RuCl$_2$ is favored. The stereochemical constraints of coordination obviously differ from those governing intercalation. Coordination of (phen)$_2$Ru^{2+} to DNA seems instead to resemble the groove-bound mode (*28*), mentioned above, which favors the Λ isomer in binding to a right-handed helix. The absolute configuration observed can be understood in terms of the steric constraints inherent in a model involving bifunctional coordination to neighboring bases in the major groove of an intact right-handed double helix.

Perhaps some general rules of symmetry that govern the various structural interactions possible with a helix are now being discovered. Intercalators, which stack with and thus resemble base pairs, seem to favor helices of like symmetry. In contrast, binding modes in which the complex fits against the helix, hydrophobically binding to the surface of the groove or coordinating to sites along the groove, favor instead structures that fit well one with another, a right hand with a left, as structures of complementary symmetry.

Coupling Recognition to Reactivity

In addition to the rigid asymmetric framework provided by metal coordination geometries and the spectroscopic sensitivity to assay binding obtained through the metal's electronic transitions, a metal complex can also provide a rich source of reactivity, often through redox chemistry. Therefore the means do exist to couple modes of recognition to site-specific reactions of metal complexes along the helix. This notion was illustrated quite elegantly in work by Dervan and co-workers (*39–41*). When ferrous EDTA is tethered to methidium, an intercalator, giving methidiumpropylEDTA · Fe(II) [MPE · Fe(II)], a DNA binder becomes instead a DNA cleaver. Tethering the EDTA/Fe(II) to the intercalator brings to the intercalation site Fe(II)/Fe(III) redox activity, and presumably then also hydroxyl radicals, generated through Fenton chemistry, which cleave the sugar-phosphate backbone. Tethering a redox active metal to specific DNA binders, such as distamycin, yields an "affinity cleaver," that is, a reagent that cuts the DNA in the vicinity of the binding site (*41*). Cu(phen)$_2^+$ is also quite an efficient artificial nuclease (*42, 43*). Here, too, cleavage occurs when the redox active metal (the cuprous/cupric ion redox reaction) is delivered to the site, generating locally high concentrations of hydroxyl radical. Variations in cleavage, which may reflect differences in binding or cleavage efficiencies or both, are found as a function of the DNA conformation (*44*). Cu(phen)$_2^+$ has been particularly useful in elucidating accessible regions in chromatin (*45*). For both the iron

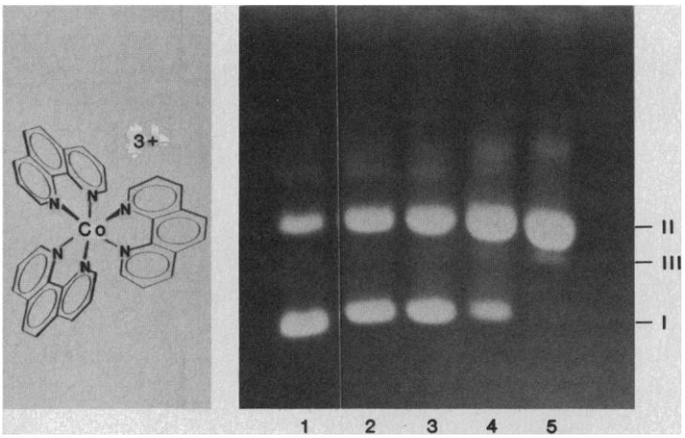


Fig. 6. Photoactivated cleavage of plasmid ColE1 DNA with $Co(phen)_3^{3+}$. The gel electrophoresis pattern shows the distribution of DNA forms (I, supercoiled; II, nicked circular; and III, linear) in the absence (lane 1 and scheme at left) and presence (lanes 2–5) of the cobalt complex with increasing times of irradiation. Photolysis in the presence of the cobalt intercalator results in single-strand cleavage (*49*).

and copper reagents cleavage specificity is limited by two factors: the specificity of the binding interaction and diffusion of hydroxyl radicals along the DNA strand before reaction. Both these complexes represent analogs of the natural product bleomycin, an antitumor drug that in the presence of coordinated iron cleaves DNA site-specifically (*46*). Fenton chemistry is not likely to operate here.

It seemed possible that the conformation-specific recognition obtained for tris(phenanthroline) metal complexes might be converted to conformation-specific cleavage reactions through the suitable selection of a redox-active metal. Prompted by the findings that cobalt(III) bleomycin cleaves DNA in the presence of light (*47*) and that cobalt(III) polyammine complexes undergo photoreduction (*48*), cleavage reactions of $Co(phen)_3^{3+}$ were examined (*49*) by gel electrophoresis (Fig. 6). A mixture of nicked (II) and supercoiled (I) closed circular DNA is shown in the absence of $Co(phen)_3^{3+}$. Single-strand cleavage occurs as a function of irradiation in the presence of $Co(phen)_3^{3+}$; the supercoiled plasmid is converted to the nicked circular form. The DNA intercalator becomes a DNA cleaver, coupling photoreduction of the metal complex to local oxidative cleavage of the sugar phosphate backbone. Although the quantum efficiency for the photoreaction is quite poor (*50*), the concentration of $Co(phen)_3^{3+}$ local to the helix is quite high and hence effective cleavage is obtained. A reaction need not be highly efficient in homogeneous solution to be effective when delivered specifically to the DNA helix.

Importantly, the recognition properties associated with the chiral phenanthroline complexes are also preserved in these cleavage reactions. Cleavage by $Co(DIP)_3^{3+}$ enantiomers is enantiospecific (*49*). Although Λ-$Co(DIP)_3^{3+}$ undergoes photoreaction in solution, in the absence of close association to the DNA helix, coupling to oxidative DNA cleavage does not occur. Although Δ-$Co(DIP)_3^{3+}$ appreciably cleaves plasmid DNA that is somewhat relaxed and therefore lacks left-handed helical regions, Λ-$Co(DIP)_3^{3+}$ does not. Plasmid DNA's containing left-handed inserts are, however, cleaved efficiently by the Λ isomer. Conformation-specific recognition by the chiral complexes is therefore coupled to photoactivation to yield conformation-specific cleavage.

The inorganic photochemistry of a variety of cationic transition metal complexes may be harnessed to provide a range of DNA photocleavers (*51*). These molecules should be useful for footprinting and mapping experiments both in vitro and in vivo. Moreover, photoreactivity at the metal center may be coupled to various modes of recognition, covalent or noncovalent, to yield a family of site-specific DNA cleaving reagents.

Left-Handed DNA Sites Along the Strand

Molecular cleavers provide sensitive tools to probe variations in DNA structure along the strand, or in other words, to detect the presence of a small region of one conformation or sequence in a polymer consisting mostly of another form. The chiral metal complexes, as they recognize particular conformations based on their asymmetry and then cut the DNA strand at these sites, resemble enzymes that cleave DNA at specific sites (*52*). Restriction endonucleases, for example, bind to specific sequences with 4 to 8 base pairs and cleave both strands site-specifically. Other less specific DNA-binding enzymes, such as mung bean and micrococcal nucleases, even appear to show some conformational selectivity in their cleavage reactions (*53*). As with the DNA-binding enzymes, then, one may use the chiral metal complex Λ-$Co(DIP)_3^{3+}$, which binds to the left-handed Z DNA conformation, to map its specific cleavage sites, and hence to learn something about the frequency and occurrence of the conformationally distinct sites that it recognizes.

Λ-$Co(DIP)_3^{3+}$ cleaves specifically at left-handed DNA sites in supercoiled plasmids (*54*). In the plasmid pLP32, constructed by inserting a $d(CG)_{16}$ fragment into pBR322, it had been shown by two-dimensional gel electrophoresis that the $d(CG)_{16}$ insert adopts the left-handed conformation (*55*). Digestion of pLP32 by Λ-$Co(DIP)_3^{3+}$ results in specific cleavage at this left-handed Z DNA insert (*54*). If this insert is removed, cleavage by the cobalt complex at the site is not obtained. Thus far, cleavage by the cobalt complex has been identified with only a left-handed conformation. Single-stranded DNA's are not cleaved by the reagent, and A-like double-stranded RNA's do not bind detectably the analogous ruthenium complexes. As one would expect, conditions found to promote transitions to the Z conformation, such as negative supercoiling or very low sodium concentrations (<50 m*M*), enhance cleavage by Λ-$Co(DIP)_3^{3+}$ (*56*).

For both pLP32 and pBR322, however, Λ-$Co(DIP)_3^{3+}$ cleaves at specific sites in addition to the Z form $d(CG)_{16}$ insert, and these sites might therefore similarly be in a left-handed conformation. In mapping new cleavage sites with Λ-$Co(DIP)_3^{3+}$, it is important to keep in mind that Λ-$Co(DIP)_3^{3+}$ may bind also to other uncharacterized, open conformations. These "conformationally distinctive" sites may be mapped by gel electrophoresis after photocleavage by Λ-$Co(DIP)_3^{3+}$, linearization with a restriction enzyme, and digestion with S1 nuclease (to convert the cobalt-produced single-stranded nicks into double-stranded cuts). Much as can be seen with an enzyme, discrete bands, which reflect cleavage at specific sites along the strand, are found in the gel. The four cleavage sites in pBR322 map to long (11 to 14 base pairs) alternating purine-pyrimidine stretches (given one base out of alternation) in the plasmid. One of these sites has been shown to bind antibodies to Z DNA (*57*). These sites share no sequence homology. The results instead suggest that the discrete sites cleaved by Λ-$Co(DIP)_3^{3+}$ may share conformational homology.

Do these conformationally distinct segments share a common biological function in the plasmid? Figure 7 shows the biological map of pBR322 comparing coding regions with the positions along the plasmid recognized by Λ-$Co(DIP)_3^{3+}$. Plasmid pBR322 contains three distinct coding elements, the β-lactamase gene, which confers resistance to ampicillin, a gene coding for tetracycline resistance, and a segment serving as the origin of replication (*58*). There appears to be a remarkable correspondence evident between

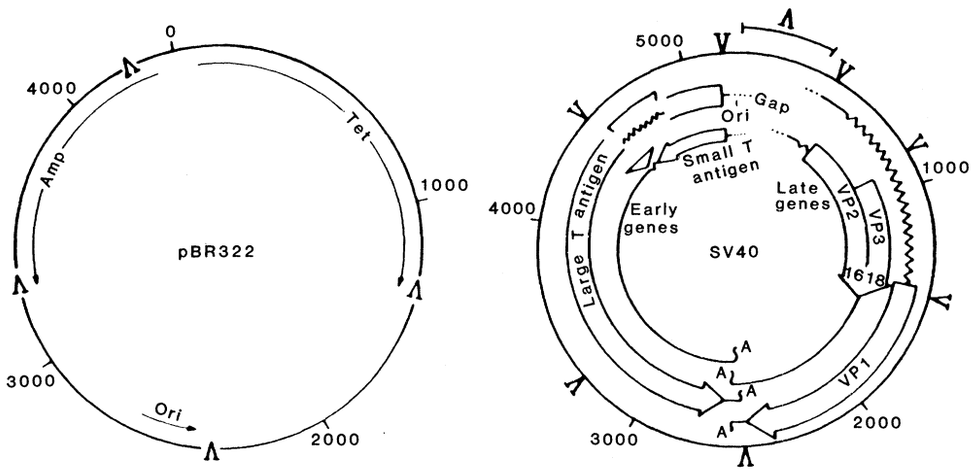


Fig. 7. Biological maps of pBR322 and SV40 showing positions (Λ) of specific cleavage by Λ-Co(DIP)$_3^{3+}$.

sites cleaved by Λ-Co(DIP)$_3^{3+}$ and the ends of the genetic coding elements. Λ-Co(DIP)$_3^{3+}$ cleaves at sites bordering both ends of the *amp*-gene and directly downstream of the *ori*-region and the *tet*-gene. These conformationally distinct sites recognized by Λ-Co(DIP)$_3^{3+}$ appear then in some sense to be like a conformational punctuation mark, demarcating the ends of genetic coding elements.

This same fascinating correspondence between conformation and biological function is evident with simian virus-40 (SV40) DNA (*59*). The positions of the Λ-Co(DIP)$_3^{3+}$ cleavage sites obtained in vitro for this eukaryotic viral DNA have also been mapped (Fig. 7) (*60*). Several distinct cleavage sites for Λ-Co(DIP)$_3^{3+}$ are seen within the regulatory gap, as if the many biological control sites in this region mirror a heterogeneity in conformations. Further, an intriguing correlation emerges between specific sites throughout the circular DNA recognized by the chiral cobalt complex and gene-splicing sites established in this virus. Higher resolution mapping has indicated that Λ-Co(DIP)$_3^{3+}$ cleaves at distinct interbase pair sites, which flank the polyadenylate tailing consensus sequences near the 3′-termini both for early and late viral messenger RNA's (mRNA's). Indeed one of the sites cleaved by Λ-Co(DIP)$_3^{3+}$ has been demonstrated (*61*) through deletion experiments to be essential in vivo for intact late mRNA formation.

The correlations between Λ-Co(DIP)$_3^{3+}$ cleavage sites and positions of biological function are therefore beginning to provide some exciting hints at roles for the left-handed conformation. The results revealed by mapping with chiral probes at least suggest that DNA secondary structure may play some part in the regulation of gene expression. Other probes, enzymatic and chemical, also have pointed to the regions between genetic coding elements as being particularly rich in structural heterogeneity. While much remains to be done, both in vitro and in vivo with these chemical probes, it is clear at this stage that chiral metal complexes are able to target conformationally distinct sites along the DNA strand and that simple molecular probes provide a sensitive route to establish relationships between DNA structure and biological function.

Conclusions

Chiral metal complexes have been designed that recognize different structures along the DNA helix. The chirality of the metal complex and the various structural interactions of these rigid enantiomers with the asymmetric DNA helix dictate the modes (stereoselective intercalation, groove binding, direct coordination) and therefore specific sites of binding. By coupling reactivity at the metal center to any of these probes for helical structure, reagents that chemically target specific sites along the strand can be developed.

For example, a chiral complex that recognizes and cleaves left-handed DNA sites has been obtained. By mapping the sites cleaved by this small molecule, both in vitro and in vivo, much will be learned about the occurrence of the left-handed conformation along the DNA strand. It is found that these conformationally distinct sites occur at biologically important sites at the ends of genetic coding elements. Simple chemical probes, which target different local conformations along the strand, offer exciting tools to explore roles for local DNA secondary structure in chemically regulating genetic expression.

It is intriguing, finally, to consider that, much like DNA-binding enzymes, these small chiral molecules actually recognize and react at highly specific sites. Lacking even hydrogen-bonding donors or acceptors, the simple coordination complexes undergo their site-specific reactions solely on the basis of matching of shapes and symmetries. Indeed, as is common in nature, the interactions of chiral molecules one with another provide uncommon specificity, a specificity useful to exploit in the design of artificial complements for DNA.

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Conservation in South America: Problems, Consequences, and Solutions

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Lack of solid data on rates of habitat conversion and on the basic biology of Neotropical organisms makes predictions of massive waves of extinction in South America premature. South America's problems regarding the use of natural resources are a result of historical, sociological, economic, and scientific factors. Most countries in South America have done a great deal to encourage conservation efforts, but the magnitude of the problem is well beyond their limited economic means to solve. The problems of species disappearance in South America are of global importance. A successful solution will involve a coordinated and massive effort of governments and specialists in all aspects of conservation biology from throughout the world. There is still time to resolve these problems. Unnecessarily dire predictions of species extinction may be counterproductive to the development of a long-term conservation strategy that is needed to manage Neotropical conservation problems.

ECOLOGISTS HAVE BEEN AWARE FOR DECADES THAT THE largest known ecosystem is the biosphere. The biospheric concept holds that all life is ecologically interrelated because the global ecosystem has many biotic and abiotic components that affect numerous subsets of the system. The world's weather patterns, for example, illustrate how quickly perturbations in one part of the biosphere can have repercussions throughout the entire system (*1*). Recent ideas regarding the global effects of nuclear war (*2*) are based primarily on climatic models showing atmospheric mixing around the world, as well as on a model devised to account for global extinctions resulting from a localized meteor impact (*3*). A nuclear winter scenario is feasible because of the interrelatedness of the ecosystems of the biosphere.

I place this article in a biospheric context because broad-scale ecological problems have little to do with national boundaries. In our complex world, where multiple links of commerce, communica-

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