8.5: Applications of Different Metal Complexes that Bind Nucleic Acids (Part 2)

Conformational Probes

Metal complexes are also finding wide application in probing the local variations in conformation that arise along nucleic-acid polymers. X-ray crystallography has been critical in establishing the basic conformational families of double-helical DNA, and to some extent how conformations might vary as a function of nucleic-acid sequence. Yet many conformations have still not been described to high resolution, and only a few oligonucleotides have been crystallized. Other techniques are therefore required to bridge the small set of oligonucleotide crystal structures that point to plausible structures and the large array of structures that arise as a function of sequence on long helical polymers. Furthermore, only a very small number of RNA polymers has been characterized crystallographically; hence other chemical methods have been needed to describe the folding patterns in these important biopolymers. Metal complexes, mainly through specific noncovalent interactions, appear to be uniquely useful in probing the structural variations in nucleic acids.

1. Nonspecific Reactions of Transition-metal Complexes

Hydroxyl radical cleavage with $Fe(EDTA)^{2-}$ illustrates again how simple metal complexes can be used in characterizing nucleic acids. One example involves efforts to describe the local structural variations in "bent" DNA. Biochemists had found that DNA fragments containing runs of adenines, such as in the tract dAAAAAA, possessed unusual gel-electrophoretic mobilities. Indeed, kinetoplast DNA isolated from mitochondria of trypanosomes showed a remarkable lacework pattern of structure, with loops and circles of DNA; these structures were found to be governed by the placement of these $d(A)_6$ tracts. By constructing a series of oligonucleotides with adenine runs positioned either in or out of phase relative to one another, researchers found that the adenine tracts caused a local bending of the DNA toward the minor groove.[6] But what were the detailed characteristics of these bent sites? Using hydroxyl radical cleavage of DNA, generated with $Fe(EDTA)^{2-}$, Tullius and coworkers found a distinctive pattern of cleavage across the adenine tracts, consistent with a locally perturbed structure.[68]Here the notion again was that $Fe(EDTA)^{2-}$ in the presence of peroxide would generate hydroxyl radicals at a distance from the helix, and thus careful densitometric analysis of the cleavage across ^{32}P-end-Iabeled DNA fragments would reveal any differential accessibility of sugar residues to cleavage mediated by the radicals caused by the bending. The cleavage patterns suggested a smooth bending of the DNA across the tract and indicated furthermore an asymmetry in structure from the 5'- to 3'-end of the adenine run.

The reactivities of other transition-metal reagents have also been used advantageously in probing nucleic-acid structures. As described in Section II, OsO_4 reacts across the 5,6 position of accessible pyrimidines to form a *cis*-osmate ester. Upon treatment with piperidine, this base modification can yield scission of the sugar-phosphate backbone. Hence DNAs containing unusual local conformations with prominent solvent-accessible pyrimidines can be probed with OsO_4. The junction regions of Z-DNA, the single-stranded loops in cruciform structures, and a segment of the dangling third strand in H-DNA, have all been probed by means of the differential reactivity of osmium tetroxide with DNA sites dependent upon their accessibility.[7,8,16,69] Surely other transition-metal chemistry will become similarly applicable.

2. Transition-metal Complexes as Shape-selective Probes

Transition-metal complexes have also been designed with three-dimensional structures that target complementary structures along the helical polymer. This recognition of DNA sites, based upon *shape selection*, has proved to be extremely useful both in demarcating and in characterizing structural variations along the polymer and in developing an understanding of those factors important to the recognition of specific polynucleotide sites. Complexes, basically derivatives of the tris(phenanthroline) metal series, have been designed that specifically target A- and Z-form helices, cruciforms, and even subtle variations such as differential propeller twisting within B-form DNA.[11c] By appropriate substitution of the metal at the center of the coordinatively saturated complex, complexes that cleave the DNA at the binding site are obtained. Figure 8.14 shows some of these shape-selective conformational probes.

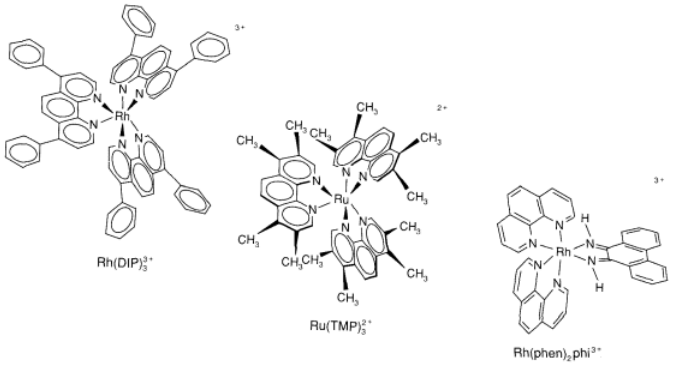

Figure 8.14 - Shape-selective probes that target local DNA conformations. $Rh(DIP)_2^{3+}$, which with photoactivation promotes double-stranded cleavage at cruciform sites; $Ru(TMP)_3^{2+}$, a photoactivated probe for A-like conformations; and $Rh(phen)_2phi^{3+}$, which targets openings in the DNA major groove.

One example of this shape-selective cleavage is apparent in reactions of $Ru(TMP)_3^{2+}$ (TMP = 3,4,7,8-tetramethylphenanthroline), a probe of the A-conformation.[28,29] The complex was designed by incorporating methyl groups about the periphery of each phenanthroline ligand to preclude intercalative binding of the complex to the helix, owing to the bulkiness of the methyl groups, and at the same time to promote hydrophobic groove binding, Importantly, however, this hydrophobic groove binding could not occur against the minor groove of B-DNA, given the width and depth of the groove versus the size of the complex. Instead, the shape of the complex was matched well to the shallow minor-groove surface of an A-form helix. Binding studies with synthetic polynucleotides of A, B, and Z-form were consistent with this scheme. Photolysis of the ruthenium complex, furthermore, as with $Ru(phen)_3^{2+}$, leads to the sensitization of singlet oxygen, and hence, after treatment with piperidine, to strand cleavage. Thus, photocleavage reactions with $Ru(TMP)_3^{2+}$ could be used to delineate A-like regions, with more shallow minor grooves, along a helical polymer. At such sites, $Ru(TMP)_3^{2+}$ would bind preferentially, and upon photolysis, generate *locally* higher concentrations of singlet oxygen to mediate cleavage of the sugar-phosphate backbone. This scheme revealed that homopyrirnidine stretches along the helix adopt a more A-like conformation.[29]

The targeting of altered conformations such as Z-DNA has been described earlier[58] in the context of a spectroscopic probe, Λ-$Ru(DIP)_3^{2+}$. Substitution of a photoredox-active metal into the core of the tris(diphenylphenanthroline) unit leads also to a complex that both binds and, with photoactivation, cleaves at the altered conformation.[55] Both Co(III) polypyridyl and Rh(III) polypyridyl complexes have been shown to be potent photooxidants. Coupled to site-specific DNA binding, these metal complexes, with photoactivation, become conformationally selective DNA cleavage agents. $Co(DIP)_3^{3+}$, for example, has been shown to cleave specifically at Z-form segments inserted into DNA plasmids.[55,70] Perhaps even more interesting, on both natural plasmids and viral DNAs, the various sites cleaved by $Co(DIP)_3^{3+}$, corresponding both to Z-form sites and to other locally altered non-B-conformations, coincide with functionally important regions of the genome, e.g., regulatory sites, gene termination sites, and intron-exon joints.[70,71] The altered structures recognized by the metal complexes, therefore, appear to mark biologically important sites, those presumably recognized also by cellular proteins. Cleavage studies with these metal complexes, therefore, are providing some insight also into how Nature specifically targets and accesses the sequence information encoded along the DNA polymer, sequence information encoded indirectly through local structure.

The most striking example of the specificity to be derived from shape-selective targeting has been given by the double-stranded cleavage induced by $Rh(DIP)_3^{3+}$ at cruciforms.[72] $Rh(DIP)_3^{3+}$, like its Co(III) and Ru(II) congeners, binds to locally unwound, non-B-conformations such as Z-DNA, but interestingly this potent photooxidant yields the specific cleavage of *both* DNA strands at cruciform sites. Lacking any crystallographic information, our understanding of the local structure of a cruciform is poor. In these palindromic sites, a torsionally strained DNA extrudes two intrastrand hydrogen-bonded helices from the main helix (see Figure 8.2B). Clearly the structure is grossly altered and locally unwound. $Rh(DIP)_3^{3+}$ appears to bind into a pocket generated by the folding of the extruded helix onto the main helix. The recognition is of this intricately folded structure, not of the sequence used to generate the cruciform. Studies with the transition-metal complex on different cruciforms should be useful in helping to characterize this interesting tertiary DNA structure.

Shape-selective transition-metal probes have also been useful in delineating more subtle variations in structure, such as the propeller twisting and tilting evident in B-form DNA.[30,73] $Rh(phen)_2phi^{3+}$ was found to target preferentially sites in the major groove where the DNA base pairs are more open; this preferential recognition arises from the steric constraints at more-closed intercalation sites because of the bulkiness of the ancillary phenanthroline ligands above and below the intercalation plane. Two

straightforward structural perturbations that lead to an opening of the major groove involve the propeller twisting of bases with respect to one another and the tilting of base pairs along the helix. Chiral discrimination in cleavage by $Rh(phen)_2phi^{3+}$ is now being used quantitatively to discriminate among these structural parameters. The goal in these studies is to use cleavage results with the shape-selective metal complexes to describe the three-dimensional structure of a long double-helical DNA sequence in solution. Probing structurally well-defined sequences with a whole family of shape-selective metal complexes may provide a route to this goal.

As mentioned above, describing the three-dimensional structures of RNAs is an even more complicated task than it is for double-stranded DNAs. Only a few tRNAs have been characterized crystallographically to high resolution, and for other larger RNA structures, such as 5S RNA, or any of the catalytic intervening-sequence RNAs, little is known about their folding characteristics. To understand the regulation and catalytic functions of these biopolymers, we need to develop chemical tools to explore these structures. Figure 8.15 shows the results of cleavage studies using the variety of transition-metal probes on $tRNA^{Phe}$. Hydroxyl-radical cleavage mediated by $Fe(EDTA)^{2-}$ reveals the protection of solvent-inaccessible regions, the "inside" of the molecule.[23] MPE-Fe(II) appears to demarcate the double-helical regions,[74] $Cu(phen)_2^+$ shows the loopedout single-stranded segments,[75] and $Rh(phen)_2phi^{3+}$ seems to delineate those regions involved in triple-base interactions, the sites of tertiary folding.[76] Taken together, the full structure of the tRNA can be described based upon cleavage data with transition-metal complexes. It therefore seems as if this full family of coordination complexes might be generally useful in delineating RNA structures. Still more work is needed quantitatively to compare the patterns obtained with the few well-characterized structures. Nonetheless, an important role for these and possibly other transition-metal reagents is indicated.

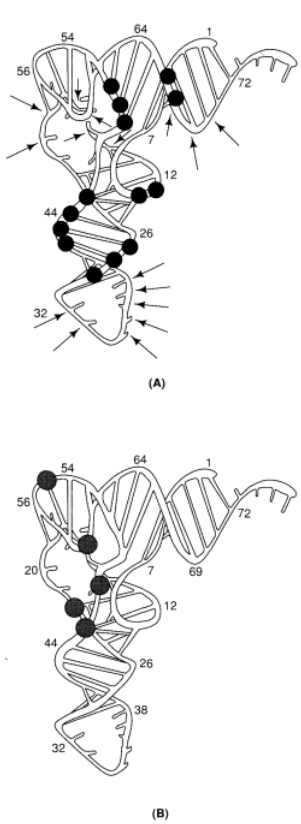

Figure 8.15 - The diversity of cleavage sites for metal complexes on $tRNA^{Phe}$. In (A) is shown cleavage by probes that primarily detect features of RNA secondary structure. $Cu(phen)_2^+$ (arrows), detecting single-stranded regions and MPE-Fe(II) (black dots), detecting double-helical segments. In (B) are shown probes that detect protected or more complex structures on tRNA. Inaccessible sites protected from OH• after treatment with $Fe(EDTA)^{2-}$ are shown as shaded portions of the molecule, and specific cleavage by $Rh(phen)_2(phi)^{3+}$ at tertiary folds is indicated by the circles.[11b]

Other Novel Techniques

Transition-metal ions can also be used advantageously tethered onto peptides, proteins, oligonucleotides, and other natural products, to provide a chemical probe for their binding interactions with nucleic acids. This strategy, termed *affinity cleavage*, was

developed by Dervan and coworkers in preparing and characterizing distamycin-Fe(II)EDTA.[24] Distamycin is a known natural product that binds in the minor groove of DNA at AT-rich sequences. By tethering Fe(II)EDTA onto distamycin, the researchers converted the DNA-binding moiety into a DNA-cleaving moiety, since, as with MPE-Fe(1I), in the presence of peroxide and a reductant, hydroxyl radical chemistry would be delivered to the distamycin binding site. Unlike MPE-Fe(II), however, the distamycin moiety shows preferential binding at some sites along the polymer, and hence only at those sites would the local hydroxyl-radical concentration be increased and cleavage be obtained. As a result the tethered $Fe(EDTA)^{2-}$ could be used as a cleavage probe, marking sites of specific binding.

Affinity cleaving has been generalized so that now $Fe(EDTA)^{2-}$ can be tethered onto both oligonucleotides and peptides to follow their interactions with nucleic acids. The sequence-specific binding of oligonucleotides to double-helical DNA through triple-helix formation is but one of many examples where the tethering of $Fe(EDTA)^{2-}$ has been applied advantageously.[77]

Other redox-active metals can be incorporated into DNA-binding moieties as well. Schemes have been developed to functionalize accessible lysine residues on DNA-binding repressor proteins with phenanthrolines, so that in the presence of copper ion, peroxide, and a reductant, the phenanthroline-bound copper on the protein would induce DNA strand cleavage. Through this scheme, again the conversion of a DNA-binding moiety into a cleaving moiety by incorporation of a redox-active metal, the specific binding sites of repressor proteins can be readily identified (far more quickly on large DNA than through footprinting).[78]

Another scheme, which perhaps takes advantage more directly of bioinorganic chemistry, involves engineering redox metal-binding sites into DNA-binding proteins and peptides. The DNA-binding domain of the protein Hin recombinase was synthesized chemically, and first, to examine the folding of the peptide on the DNA helix, EDTA was tethered onto the peptide for Fe(II) cleavage experiments.[79] But as is illustrated repeatedly in these chapters, Nature has already provided amino-acid residues for the chelation of metal ions into proteins. Thus the DNA-binding domain of Hin recombinase was synthesized again, now including at its terminus the residues Gly-Gly-His, a known chelating moiety for copper(II).[80] This chemically synthesized peptide, with now both DNA-binding and DNA-cleaving domains, as illustrated in Figure 8.16, specifically promotes cleavage at the Hin recombinase binding site in the presence of bound copper and ascorbate. Interestingly, the addition of nickel(II) also leads to specific strand cleavage, without diffusible intermediates. Using this approach, taking advantage of the chelating abilities of amino acids and the cleaving abilities of different metal ions, one may prepare new synthetic, functional metalloproteins that bind and react with DNA.

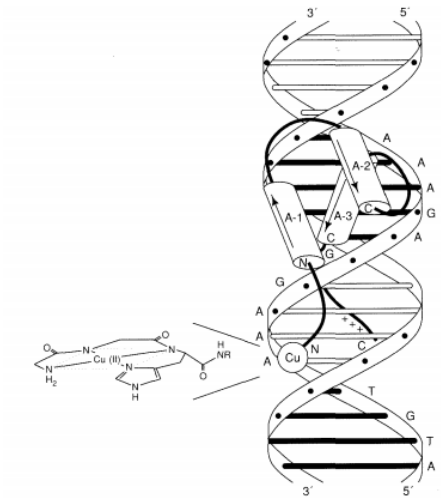

Figure 8.16 - A schematic of a synthetic DNA-cleaving peptide bound to DNA that was constructed by synthesis of the DNA-binding domain of Hin Recombinase with Gly-Gly-His at the N-terminus to coordinate copper.[80] Reproduced with permission from Reference 80.