8.2: The Basics (Part 2)

2. 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.[17,18] 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 ~2 to 10 kcal. Nonintercalative hydrophobic interactions of coordinated ligands in the DNA grooves also can occur, as we will see. Hydrogenbonding 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.[19]

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.[12] The steric constraints on the molecule must be considered, however. With $Pt(terpy)CI^{+}$, 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 terpyridyl ligand away from the base stack in the DNA major groove, precluding intercalation.[20] Sigel and coworkers[21] 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.

1. 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 8.5A. 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.[22] As illustrated in Figure 8.5B, the products of this reaction include a 5'-phosphate, a mixture of 3'-phosphate and phosphoglycolates, and a mixture of free bases and base propenalso 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.[23]

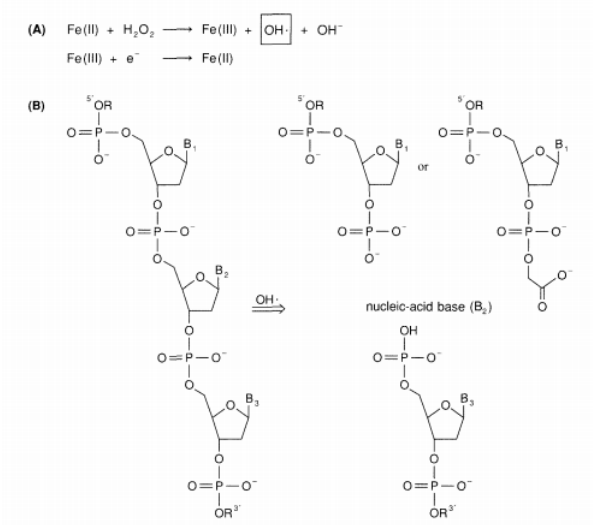

Figure 8.5 - An illustration of DNA strand cleavage mediated by hydroxyl radicals produced by the Fenton reaction (A) of $Fe(EDTA)^{2-}$ with hydrogen peroxide. The cleavage scheme (B) shows the products obtained as a result of initial C4'-H abstraction by the hydroxyl radicals.

The application of this Fenton chemistry to promote site-specific or sequence-neutral cleavage of DNA was first demonstrated[24] 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 this chemistry was originally based on modeling Fe-bleomycin, a natural product with antitumor and antibiotic activity, which binds and cleaves DNA.[25] 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 ferryI 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[26,27] 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.[26]

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.[28,29] 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 = 9,10-phenanthrenequinone diimine) exemplifies this strategy.[30] 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);[31] 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.[11b,17]The DNA double helix can furthermore also mediate 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.[32] Table 8.1 summarizes different redox reactions of metal complexes bound to DNA.

Table 8.1 - Examples of metal complexes that cleave DNA through redox chemistry.

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.

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	phtoelectron 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

2. 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.[33] Hydrolysis reactions of the phosphodiester linkage of polynucleotides appear preferable to redox-mediated cleavage 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.

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 8.6 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.[34] A whole series of model systems utilizing both cobalt and zinc ions has been designed to explore the hydrolytic reactions of simple phosphodiesters.[35] 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*.[36] In this complex (see Figure 8.6), 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.

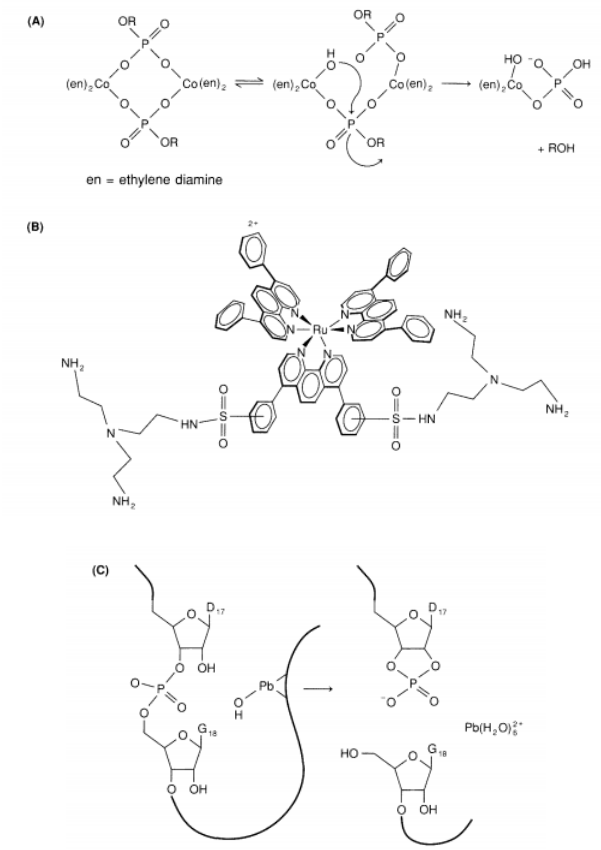

Figure 8.6 - Hydrolysis reactions catalyzed by metal ions and complexes. (A) Illustration of a phosphate ester hydrolysis in a binuclear model complex catalyzed by coordinated cobaltic ions, with one metal ion functioning as a Lewis acid and the other functioning to deliver the coordinated hydroxide.[34] (B) $Ru(DIP)_2$Macro, a metal complex constructed to contain a central DNA-binding domain ($Ru(DIP)_3^{2+}$) with two tethered amine arms to chelate additional metal ions (Zn^{2+}) to deliver to the sugar-phosphate backbone and promote hydrolytic strand cleavage.[36] (C) RNA site-specifically hydrolyzed by lead ion. Diagram of the proposed mechanism of sugar-phosphate backbone cleavage between residue D_{17} and G_{18} in yeast RNA^{Phe}.[38]

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.[37] Figure 8.6 illustrates also the crystallographically characterized site-specific hydrolyis in tRNA by plumbous ion.[38] 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'-cyclic phosphate 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.