8.4: 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.

Spectroscopic Probes

As discussed previously, the tris(phenanthroline)ruthenium(II) complexes offer a novel spectroscopic probe of nucleic acids, since their luminescence is increased upon intercalation into the double helix. As a result the complexes provide a simple luminescent stain for DNA in fluorescent microscopy experiments. More interesting, perhaps, is the conformational selectivity of derivatives of tris(phenanthroline)ruthenium. $Ru(DIP)_3^{2+}$ (DIP = 4,7-diphenyl-1,10-phenanthroline) shows enantiospecificity in binding to B-form DNA.[40] Because of the steric bulk of the phenyl rings, detectable binding is seen only with the Δ-isomer in a righthanded helix; no binding is evident with the Λ-isomer. But with the left-handed Z-form helix, both isomers bind avidly.[40,58] The shallow left-handed major groove can accomodate the two enantiomers., A left-handed but more B-like helix shows selectivity instead for the Λ-isomer. Spectroscopic experiments that measure the chiral selectivity of $Ru(DIP)_3^{2+}$ isomers in binding to a given DNA then provide a novel probe for helical handedness. Indeed, Λ-$Ru(DIP)_3^{2+}$ was the first spectroscopic probe for Z-DNA (or other alternate conformations that are sufficiently unwound to permit binding by the bulky left-handed isomer).[58]

Another set of derivatives of the tris(phenanthroline) metal complexes that may become exceedingly useful as spectroscopic probes are $Ru(bpy)_2dppz^{2+}$ and $Ru(phen)_2dppz^{2+}$ (dppz = dipyridophenazine).[59] In these complexes the metal-to-ligand charge transfer is preferentially to the electron-accepting dppz ligand. In nonaqueous solutions, the complexes luminesce. However, in aqueous solution at pH 7, no luminescence is observed, likely because hydrogen bonding by water to the phenazine nitrogen atoms quenches the charge-transfer excited state. But the dppz ligand is also an expansive, aromatic heterocyclic ligand, and as a result both $Ru(bpy)_2dppz^{2+}$ and $Ru(phen)_2dppz^{2+}$ bind avidly to DNA by intercalation. Once intercalated, the phenazine ligand is protected from water. Therefore these complexes are luminescent when intercalated into DNA, whereas no luminescence is apparent from the complexes in the absence of DNA in aqueous solution. The enhancement factor is > 10^4 with DNA. One might consider the ruthenium complexes as true "molecular light switches" for DNA.

Both simpler bipyridyl and phenanthroline derivatives as well as dppz complexes of ruthenium are currently being tethered onto other DNA binding moieties, in particular onto oligonucleotides, so as to develop new, nonradioactive luminescent probes for DNA sequences. These transition-metal complexes may provide the basis for the development of new families of DNA diagnostic agents, and many industrial laboratories are currently exploring routes to accomplish these goals. Figure 8.12 illustrates Λ-$Ru(DIP)3^{2+}$ and $Ru(bpy)_2dppz^{2+}$, two complexes whose luminescence properties can be employed to probe nucleic acids.

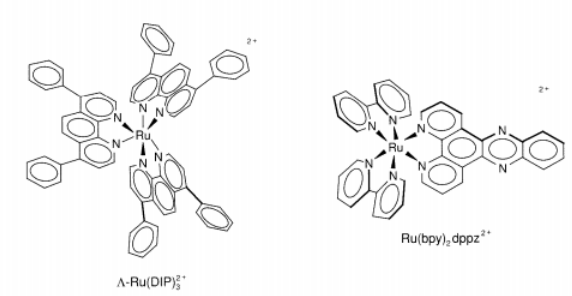

Figure 8.12 - Two spectroscopic probes of nucleic acids: Λ-$Ru(DIP)_3^{2+}$ and $Ru(bpy)_2dppz^{2+}$.

Other transition-metal complexes besides those of ruthenium have shown some promise in spectroscopic applications with nucleic acids. Lanthanide ions have been applied both in NMR experiments and in luminescence experiments to probe tRNAs, and more recently with synthetic DNAs of differing sequence and structure.[60] Lanthanide ions have been exceedingly useful in probing Ca^{2+} binding sites in proteins, and one would hope that a parallel utility would be achieved with nucleic acids. Their poor absorptivity have made luminescent experiments difficult, however, requiring relatively high concentrations of material. Nonetheless, the sensitivity of luminescent lifetimes to coordination and indeed solvation is providing a novel spectroscopic handle to explore binding sites and structures of the macromolecules. Another quite novel luminescent handle has been phenanthroline and diphenylphenanthroline complexes of copper(I).[61] These complexes are extremely valuable cleavage probes, as we will see later; to characterize better their interactions with the helix, luminescence experiments are being explored. A problem here has been the nonphysiological conditions necessary to achieve detectable luminescence. Nonetheless, studies with the copper complexes

demonstrate how the whole range of transition-metal chemistry and spectroscopy is beginning to be applied in sorting through nucleic-acid interactions.

Metallofootprinting Reagents

Probably the most widespread application of metal nucleic-acid chemistry in the biology community has been the utilization of metal complexes for chemical footprinting. The footprinting technique (Figure 8.11) was developed by biologists[62] as a means of locating protein-binding sites on DNA. ^{32}P-end-labeled double-stranded DNA fragments could be digested with a nuclease, such as DNAse, in the presence or absence of DNA-binding protein. After electrophoresis of the denatured digests and autoradiography, one would find a "footprint," that is, the inhibition of cleavage by DNAse, at the spot bound by protein, in comparison to a randomly cleaved pattern found on the DNA in the absence of binding protein. Although DNAse is still widely used, this footprinting reagent has some disadvantages: (i) the nuclease is not sequence-neutral in its cleavage, resulting in lots of noise in the footprinting background; and (ii) since the nuclease is itself a large protein, its ability to provide high-resolution footprinting patterns of smaller molecules is quite limited.

Several metal complexes now serve as high-resolution, sequence-neutral chemical footprinting reagents. Some of these reagents are shown in Figure 8.13. The first, as mentioned previously, was MPE-Fe(II).[57] The complex cotains a sequence-neutral DNA binding moiety, the intercalator methidium, and a tethered DNA redox cleaving moiety, Fe(EDTA). The methidium, in binding nonspecifically to DNA, delivers the hydroxyl radicals, generated via Fenton chemistry at the Fe(II) center in the presence of peroxide and a reducing agent, to the DNA backbone in a random manner. Since the complex is small, high resolution can be achieved. Indeed, MPE-Fe(II) has been shown to footprint small natural products that bind to DNA, in addition to footprinting much larger DNA-binding peptides and proteins.

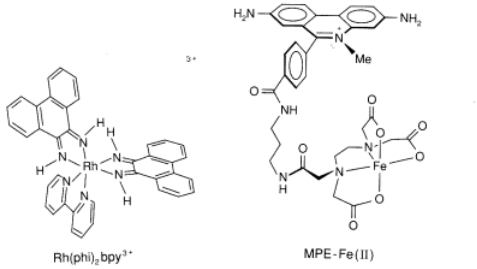

Figure 8.13 - Examples of metallofootprinting reagents. $Rh(phi)_2bpy^{3+}$, a photofootprinting intercalator, and MPE-Fe(II), a sequence-neutral intercalating agent.

Perhaps simpler still and now very widely used as a footprinting reagent is $Fe(EDTA)^{2-}$ itself.[63] The concept here is that $Fe(EDTA)^{2-}$, as a dianion, is unlikely to associate at all with the DNA polyanion. Hence hydroxyl radicals, generated via Fenton chemistry at a distance from the helix, would likely diffuse to the helix with a uniform concentration along the helix and provide a completely sequence-neutral pattern of cleavage. Tullius and coworkers have demonstrated[63] this to be the case. The resolution is furthermore extremely high since the hydroxyl radical is sufficiently small that it can even diffuse within the DNA-binding protein to delineate binding domains. Some difficulties are found, however, with the high concentrations of activating reagents needed to activate a cleavage reagent that *does not* bind to the helix, and problems of course arise in trying to footprint metalloproteins. Nonetheless, $Fe(EDTA)^{2-}$, a reagent easily found on the biologist's shelf, is now finding great utility in labs as a chemical substitute for DNAse.

Other transition-metal complexes are also finding applications in chemical footprinting. Both $Cu(phen)_2^+$ and manganese porphyrins have been used to footprint DNA-binding proteins.[64,65] These complexes likely cleave DNA through either Fenton chemistry or direct reaction of a coordinated metal-oxo intermediate with the sugar-phosphate backbone. The complexes, however, appear to bind DNA predominantly along the surface of the DNA minor groove, and with some preference for AT-rich regions. The patterns obtained are actually quite similar to those found with DNAse, and thus the lack of high sequence neutrality is somewhat limiting. Furthermore, the complexes are most sensitive to binding moieties in the minor groove, rather than those in the major groove, where proteins bind. Intercalators such as MPE-Fe(II) can sense binding species in both grooves. $Cu(phen)_2^+$ has nonetheless proved to be quite effective in detecting hyperreactivities in the minor groove, owing to DNA structural perturbations that arise from protein binding in the major groove. Whether this sensitivity emanates from the intimate interaction of $Cu(phen)_2^+$ in the minor groove of the helix, or because of the characteristics of the reactive radical formed, is not known.

Inorganic photochemistry has also been applied in developing metal complexes as photofootprinting reagents. Uranyl acetate, for example, at high concentrations, upon photolysis, promotes DNA cleavage.[66] It is thought that the ions interact with the phosphates, generating some excited-state radical chemistry, although no detailed characterization of this chemistry has been undertaken. The cleavage reaction is nonetheless remarkably sequence-neutral and therefore shows some promise for photofootprinting applications. In fact, the applicability of uranyl acetate typifies how simple coordination chemistry and now even photochemistry may be helpful in the design of a variety of reagents that interact and cleave DNA, both nonspecifically and specifically. The biochemical techniques used to monitor such processes are sufficiently sensitive that even quite inefficient reactions in solution can be harnessed in developing useful reagents. The better our understanding of the chemistry of the coordination complex, the more effectively it may be utilized.

The best derivative of a tris(phenanthroline) metal complex currently being applied in footprinting experiments is $Rh(phi)_2bpy^{3+}$, a second-generation derivative of the tris(phenanthroline) series[67] that binds DNA avidly by intercalation and in the presence of light promotes direct strand cleavage by hydrogenatom abstraction at the C3'-position on the sugar.[31] Since no diffusing intermediate is involved in this photocleavage reaction, the resolution of the footprinting pattern is to a single nucleotide. Here the excited-state transition-metal chemistry involves a ligand-to-metal charge transfer, producing a phi cation radical that directly abstracts the hydrogen from the sugar at the intercalated site. The high efficiency of this photoreaction and high sequence-neutral binding of the complex to double-stranded DNA add to the utility of this reagent in footprinting studies. Indeed, both DNA-binding proteins, bound in the major groove, and small natural products, associated with the minor groove, have been footprinted with $Rh(phi)_2bpy^{3+}$ to precisely that size expected based upon crystallographic results. One may hope that this and other photofootprinting reagents will soon find applications for footprinting experiments *in vivo*.

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