8.1: The Basics

Nucleic-acid Structures[1]

Figure 8.1 displays a single deoxyribonucleotide and the four different nucleic-acid 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.

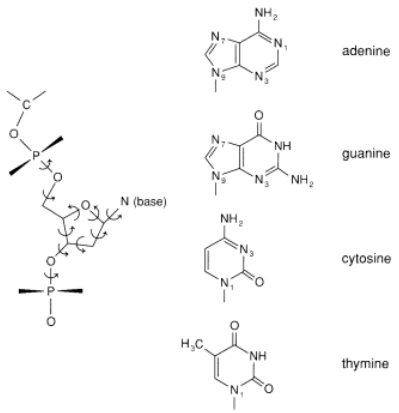

Figure 8.1 - Illustration of a mononucleotide unit. Arrows indicate the various torsional angles within each unit that together generate the wide range of conformations available in the polymer. Also shown are the individual bases as well as the commonly employed numbering scheme.

The monomeric units strung together in a polynucleotide furthermore provide an array of polymeric conformers. Figure 8.2A (See color plate section, pages C-14, C-15.) shows three crystallographically characterized structures of double-helical DNA oligonucleotides,[2-4] Figure 8.2B a schematic illustration of other conformations of DNA, and Figure 8.2C the crystal structure[5] of yeast $tRNA^{Phe}$. In double-helical DNA,[1] the two antiparallel polynucleotide strands are intertwined in a helix, stabilized through Watson-Crick hydrogen bonding between purines and pyrimidines, and through $\pi - \pi$ stacking interactions among the bases arranged in the helical column. There are electrostatic repulsions between the anionic phosphate backbones of the polymer, causing a 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 a 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.[4] 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 purinepyrimidine 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.

These crystal structures, shown in Figure 8.2A (see color plate section, page C-15), in fact each represent a family of conformations. 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, some of which are illustrated schematically in Figure 8.2B (see color plate section, page C-15). Double-helical DNA can bend,[6] form loops and cruciforms,[7] and fold back on itself into intramolecular triple helices, termed H-DNA.[8] 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.[9]

This complexity in DNA structure is in fact small compared to that of RNA. Figure 8.2C (see color plate section, page C-15) shows the first crystallographically characterized structure[5] of an RNA polymer, yeast $tRNA^{Phe}$. Ostensibly single-stranded RNAs do not exist as random coils, but instead fold up into well-defined 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,[10] the finding that RNAs can indeed catalyze nucleolytic reactions, makes our need to understand these structures even greater. Again transition-metal 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 8.3. Both strong covalent interactions and weak noncovalent complexes are observed.[11] 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.

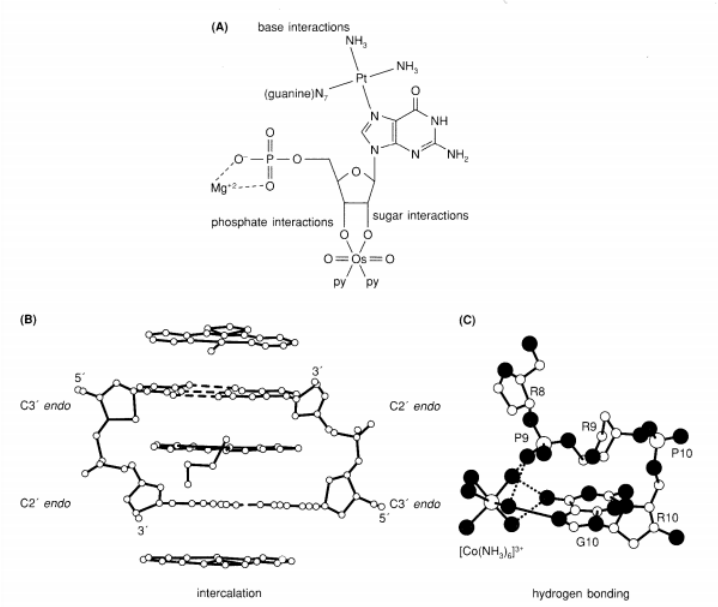

Figure 8.3 - Covalent and noncovalent binding modes of metal complexes with DNA. (A) Representative covalent interactions. Shown schematically are examples of coordination to the DNA base, sugar, and phosphate moieties given by the covalent binding of cis-(diammine)platinum to the N7 nitrogen atom of neighboring guanine residues, the formation of an osmate ester with ribose hydroxyl groups, and the primarily electrostatic association between $Mg(H_2O)_6^{2+}$ and the guanosine phosphate, respectively. (B) Noncovalent intercalative stacking of a metal complex. Shown is the crystal structure[20b] of (terpyridyl)(2-hydroxyethanethiolate)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[19] of Z-form $d(CG)_3$ with $Co(NH_3)_6^{3+}$ hydrogen-bonded both to the guanine base (G10) and phosphate backbone (P9).

1. Coordination

Most prevalent among covalent complexes with DNA are those involving coordination between soft metal ions and nucleophilic positions on the bases. The structure[12] 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 (see Chapter 9). 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.[12,13] Some additional covalent binding to the N1 positions of the purines has also been observed. Indeed, coordination by the metal to one site on the heterocyclic base lowers the pK_a 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).[13] 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 8.4).[14] 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.

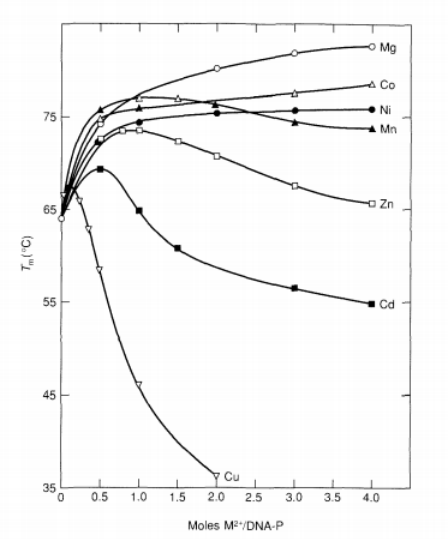

Figure 8.4 - The effects of various metal ions on the melting temperature (T_m) of calf thymus DNA.[14] Reproduced with permission from Reference 14.

Also of interest, but less common, are covalent interactions with the sugar moiety.[15,16] 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.
