Part A

Basic Structural and Kinetic Aspects
1

Sequence-Selective Binding of Transition Metal Complexes to DNA

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1.1 Introduction

The biological significance of the interaction between metal ions and nucleic acids has become a rather well-established fact. One may mention the observed necessity for the presence of metal ions in many natural processes where nucleic acids play the dominant role. The effect of platinum-based chemotherapeutic drugs probably originates from their attack on DNA. Another aspect of metals in biological systems is the increased flux of metals in the environment during the last decades. An assessment of the toxic effect of an unnatural metal ion concentration must include information on the processes in which the metal can participate. In a comparison of metal carcinogenicity in humans based on several experimental factors, Cr and Ni turned out to be the most potent carcinogens.1

The nucleic acid monomers, guanine (G), adenine (A), thymine (T) and cytosine (C) have different metal ion affinities. The order of stability of 3d transition metal ion–nucleobase complexes are: G > A, C > T.2 At physiological pH the preferred binding sites on the nucleobases are: guanine N7, adenine N1 and/or N7, cytosine N3, thymine O4. For nucleotides the relationship between phosphate and base binding is dependent on the type of metal ion. Eichhorn and Shin3 studied the effect of various metal ions on the melting temperature of DNA (Figure 1.1). The authors suggest that magnesium ions increase $T_m$ by binding to phosphate and
stabilizing the double helix, whereas copper ions decrease $T_m$ by binding to the bases and destabilizing the double helix. Based on the metal-induced variation in $T_m$ they suggested that the relative metal affinity to the phosphate backbone of DNA follows the order $\text{Mg}^{2+} > \text{Co}^{2+} > \text{Ni}^{2+} > \text{Mn}^{2+} > \text{Zn}^{2+} > \text{Cd}^{2+} > \text{Cu}^{2+}$.

This implies that the binding of an individual metal ion may involve phosphate and base on the same molecule or form a linkage between two different nucleotides. An example of the latter situation is the mononucleotide–metal ion binding pattern observed for the Cu-(GMP) complex, where Cu$^{2+}$ ions are bridging the GMP ligands through alternating N7–Cu–phosphate bonds (Figure 1.2).

When nucleobases are incorporated into a duplex DNA matrix, the affinities towards metal ions are modified. It has been shown that several divalent metal ions, like Mn$^{2+}$, Cu$^{2+}$ and Pt$^{2+}$ prefer GC-rich regions, while Hg$^{2+}$, for example prefer AT-rich regions.$^{5,6}$ A more detailed picture indicates that metal binding to base residues is sequence-dependent, i.e. not all guanines in a particular sequence show identical affinity towards a specific type of metal ion.$^{7-10}$ As a consequence, one may envisage designing metal complexes that can bind selectively to chosen sequences of DNA.

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**Figure 1.1** Variation of $T_m$ of solutions of DNA as a function of divalent metal ion concentration. (Reprinted with permission from J. Am. Chem. Soc., 1968, 90, 7327. Copyright 1968 American Chemical Society.)
Such complexes may be used as drugs that block specific gene expression associated with a certain disease.

Metal ions can interact with nucleic acids in two distinct modes of binding: diffuse binding and site binding, both of which are important for the structure and function of nucleic acids.\textsuperscript{11a,b} In the diffuse binding mode the metal and the nucleic acid retain their hydration layer and the interaction is through water molecules.\textsuperscript{11c} This is a long-range Coulombic interaction, in which positive metal ions accumulate around the nucleic acid in a delocalized manner; for example, the counterion atmosphere that all nucleic acids possess is made up of diffusely bound positive ions. In the site-binding mode the metal is coordinated to specific ligands on the nucleic acid; the coordination can either be direct (termed inner-sphere) or through a water molecule (termed outer-sphere). In the outer-sphere binding mode only the innermost hydration layer of the metal is kept intact, and the metal and the nucleic acid ligand(s) to which the metal is coordinated share solvation shells. In inner-shell binding there is direct contact between the metal and the nucleic acid. Dehydration of the metal ion and the nucleic acid binding site therefore has to occur before an inner-shell bond is formed.

The mechanism of inner-sphere binding is likely to be initiated by a diffuse binding mode, in which the metal and the nucleic acid are separated by no more than two layers of solvent molecules.\textsuperscript{11d} This step is diffusion controlled. The next step is that the metal ion and the nucleic acid form an outer-sphere complex, separated only by one layer of solvent molecules. This step primarily depends on electrostatic attractions and hydrogen bonding between the metal and the nucleic acid.\textsuperscript{12} In the final step the metal and the nucleic acid come into direct contact (inner-sphere binding). Here the nucleophilicity of the coordination site plays a crucial role. In the last two steps steric effects are also important. Several attempts have been made to quantify the importance of accessibility and molecular electrostatic potential (MEP) at the site where the inner-sphere complex/covalent bond is formed.\textsuperscript{13,14} In these studies a reasonable correlation between these two important
factors exists, and has been used to predict which DNA site is the most reactive to metalation or methylation.

In this chapter we present data on sequence-selective interactions between metal complexes and nucleic acids. In the outline we will distinguish between (i) site-selective inner-sphere metal coordination of nucleobases, and (ii) the selectivity of fully hydrated species located in the minor or major groove through hydrogen bonding and electrostatic interaction. In the former case a further distinction will be made between labile and nonlabile metals.

1.2 *Ab initio* Calculations and Photo-Cleavage Studies

The highest occupied molecular orbital (HOMO) of DNA nucleobases plays a crucial role in metal coordination by interacting with the lowest unoccupied molecular orbital (LUMO) of metal ions. The calculations of HOMOs of macromolecules such as duplex DNA are extremely difficult. Consequently, there has been little focus on the role of the HOMO/LUMO in studies of DNA–metal ion interactions. Theoretical calculations of DNA bases have mostly focused on ionization potentials (IP) of monomeric nucleobases and the stability of the nucleobase pair in neutral and radical cation states. About ten years ago the Saito group published the first extensive studies on the variation of nucleobase IPs and localization of HOMOs as a function of base stacking, using high-level *ab initio* calculations. The IPs of four base monomers and 16 sets of nearest-neighbour stacked nucleobases in the B form were calculated. It was found that the GG/CC system has the lowest IP among ten possible stacked nucleobase pairs and that approximately 70% of the HOMO is localized on the 5′-G of 5′-GG-3′. These calculations indicate that the 5′-G of 5′-GG-3′ is the most electron-donating site in B-DNA. The origin of IP lowering as a result of base stacking was further investigated by calculations of HOMO energy distribution as a function of the twist angle of a GG dimer. Within a normal range of twist angles for B-DNA (−25° to −45°), IP values of GG are between 7.2 and 7.4 eV and the HOMO is predominantly localized on 5′-G. This implies that in B-form DNA the 5′-side G of the 5′-GG-3′ sequence is the most strongly interacting site with electrophiles. This principle may be very important in governing sequence-selective metal binding to DNA.

According to the theoretical calculations, HOMOs of GG sites in duplex DNA should serve as the most reactive one-electron oxidation sites. In order to verify this assumption experimentally, Saito et al. performed laser flash photolysis of duplex DNA oligonucleotides with added photosensitizer (photocleaving aminoacid, PCA) and subsequent hydrolysis dephosphorylation with alkaline phosphatase (Scheme 1.1). The G₃:G₄ ratio (84:16) determined by HPLC implies that the major degradation pathway of the hexamer involves the G₃ site – the most readily oxidizable site according to theoretical calculations. It should be noted that photo-irradiation of the single-strand alone results in a nonselective cleavage at G₃ and G₄ in the ratio 1:1.
Further work by the Saito group involved *ab initio* calculations of HOMOs for a wide variety of double-stranded G-containing 5-mer sequences with B-form geometry using Gaussian 9x1 at the HF/6-31G* level.19,20 For the quantum mechanical studies, all of the sugar backbones of the 5-mers were removed from the coordinate file and replaced by methyl groups. A few examples of the distributions of HOMOs in the duplex 5-mers are shown in Figure 1.3.

The general trend for HOMO distribution is that the HOMO of stacked GG doublets is localized overwhelmingly on the 5'-G, regardless of the 3'- and 5'-flanking residues (A, C or T). Bearing in mind that the model used is rather crude (all of the sugar backbone replaced by methyl groups), further discussion of more subtle sequence-specific differences is not warranted.

Further experimental support for the theoretical results was obtained by studying the oxidation of oligodeoxynucleotides (ODN) with Co^{2+} ions and benzoyl peroxide using PAGE analysis of the reaction mixture after hot piperidine treatment. Sequence-dependent G-cleavage was observed for double-stranded ODN, whereas nonselective equal G cleavage was observed for single-stranded ODN. The relative rates of sequence oxidation were determined by densitometric assay of the ODN cleavage bands. Experimentally observed relative rates of G oxidation matches well with the calculated HOMOs of the G-containing sequences, implying that the Co^{2+} ion is coordinated more strongly to the G having the larger HOMO.20

Comparable theoretical calculations by Senthikumar *et al.*21 on stacked XGY triplets with B-form geometries, including sugar and phosphate groups, show that the site energy is strongly influenced by the type of nucleobase at the 3' position.

![Scheme 1.1](image)

**Figure 1.3** Distribution of HOMOs (normalized to the largest value) in B-form duplex 5-mers obtained by ab initio calculations. The arrows indicate the distribution of the HOMOs normalized to the largest HOMO as 100.20

\[
5\:'- T_1T_2G_3G_4T_5A_6 \quad (T_1T_2 + G_3TA) \quad (TTG_4 + TA) + PCA \rightarrow \\
\]

**Ab initio Calculations**
When C or T is present at the 3' position, the site energy at the guanine was found to be up to 0.44 eV higher than for A or G at this position. The influence of the base at the 5' position was much smaller, the variation in site energy being less than 0.1 eV. The amount of charge on a certain G was calculated from the coefficients of the guanine fragment orbitals. It was concluded that the neighbouring base at the 3' position to a large extent determines the charge distribution and therefore the oxidative damage on a sequence of guanine bases.

Further work by the Saito group on experimental mapping of G-rich hot spots included photo-induced cleavage of double-stranded $^{32}$P-end-labelled oligodeoxynucleotides (ODNs) 30-mers possessing two different G-containing sequences (5'-TXGYT-3') and a 5'-TTGCT-3' step as a standard on the same strand. Under low photo-irradiation conditions, only the cleavage bands of 5'-Gs of the two GG steps and the middle step of the GGG triplet were observed by hot piperidine treatment. Quantitative densitometric assay was used to determine the relative amounts of cleavage products. The experimental results were compared to IP values calculated for 16 sets of base-paired G- and GG-containing 5-mers. A plot of the log of the relative reactivity ($k_{rel}$) toward photo-induced one-electron oxidation versus calculated IP is shown in Figure 1.4.

A different explanation for the enhanced reactivity of the 5'-G of a GG step compared to the 3'-G is presented by the Schuster group. Theoretical calculations of base-paired quartets, $d(5'-XGGX-3')/d(5'-YCCY-3')$, suggested that electronic factors may not be the primary determinant of the reaction selectivity for GG steps. Instead the authors propose, based on molecular dynamic (MD) simulations on B-DNA oligomers, $d(5'-GXXGGXXG-3')/d(5'-GYGGYYYYG-3')$, where X = A,T,U and Y is the complementary base, that ‘there is an important steric contribution to the preference for reaction at the 5'-G in the GG doublets’. Photo-cleavage experiments carried out on the series of B-DNA oligomers showed that for GG steps in

![Figure 1.4](image-url)
the context AGGA, the ratio of 5′ to 3′ reactivity was 1.8 ± 0.1, and for GG in the context TGGT, the ratio was 6.1 ± 0.3. The authors propose that the accessibility of H₂O to the reaction site determined by the steric blocking by the methyl group plays the dominant role for the observed sequence-selectivity, rather than electronic effects.¹³

1.3 NMR Spectroscopic Studies of Metal Binding to DNA Oligonucleotides

1.3.1 NMR Methodology

Most early nuclear magnetic resonance (NMR) studies on DNA involved complementary homopolymers and self-complementary, alternating copolymers, e.g. poly(dA)/poly/dT). The development of efficient and rapid methods of large-scale oligonucleotide syntheses has made it possible to design heteropolymeric sequences of high purity. Dodecamer (12 base pair) sequences adopting a normal B-DNA double-helical conformation, are assumed to complete a full turn of a right-handed helix. The structure of such a mini-helix is probably sufficiently close to that of real DNA to serve as a realistic model for determining preferred metal binding sites.

The effects of adding paramagnetic metal ions to an aqueous solution of DNA fragments may be monitored by observing the decrease in spin-lattice (T₁) and spin-spin (T₂) relaxation times (related to line-broadening) for protons close to the metal centres. Paramagnetic metal ions may be classified according to their electronic correlation times, i.e. as relaxation probes producing broad lines or as paramagnetic shift probes producing narrow lines. Divalent manganese is a typical relaxation probe with an estimated electronic relaxation time (tₛ = T₁c = T₂c) of 10⁻⁸ – 10⁻⁹ s, while nickel, which has a shorter tₛ in the range 10⁻¹⁰ – 10⁻¹² s, is a typical chemical shift probe. Cobalt(II) in a low-spin coordination environment has an estimated tₛ between that of Mn²⁺ and Ni²⁺ in kinetically labile metal complexes. At low metal to nucleotide ratios paramagnetic shift effects of Ni²⁺ are difficult to detect. In this case geometric information about metal binding sites is most effectively obtained by measuring proton spin-lattice relaxation times (T₁).

Paramagnetic relaxation arises in NMR spectroscopy when an unpaired electron spin interacts with a nuclear spin. The large magnetogyric ratio of the electron compared to that of the proton makes the dipolar coupling to the electron spin a very effective means of relaxation for the nuclear spin. Scalar interactions between the electron and nuclear spins have similar effects. In the simplest possible case, a ligand molecule exchanges between a paramagnetic environment (e.g. bound to Mn(II), S = 5/2) and a ‘free’ state, when the ligand is present in solution in vast excess to the paramagnetic centre (e.g. 10² – 10⁴). The effect of paramagnetic metal ions located at specific binding sites on DNA is observed as differential line-broadening of proton signals close to the binding site. Often, in 1D spectra of oligonucleotide molecules containing ten base pairs or more, key proton resonances may be severely overlapped, preventing an accurate assessment of the influence of
the added metal ions. In these cases, 2D NOESY experiments may be used to obtain sufficient resolution.

For diamagnetic metal ions (no unpaired spin) the formation of a chemical bond is usually found to cause changes in the chemical shifts of proton resonances of hydrogen atoms in the proximity of the metal binding site. However, the coordination of Hg$^{2+}$ ions to single nucleobases induces only rather insignificant $^1$H shift changes, as shown for thymidine and guanosine. This could be explained by a down-field chemical shift change, induced by metal binding, being cancelled by an up-field chemical shift caused by changes in ring current effects due to altered nucleobase stacking. The heteronuclei $^{13}$C, $^{15}$N and $^{31}$P may experience large shift-effects when a metal ion binds to nucleobase or phosphate groups.

### 1.3.2 Model Systems

One of the first oligodeoxynucleotides studied by X-ray crystallography and subsequently by NMR spectroscopy was the famous Dickerson–Drew sequence, d(C$_1$G$_2$C$_3$G$_4$A$_5$A$_6$T$_7$T$_8$C$_9$G$_10$C$_11$G$_12$) (seq. I). The 1D and 2D NMR spectra used as bases for the three-dimensional NMR structure showed an unexpectedly strong line-broadening of the G$_4$-H8 resonance. As a consequence, the G$_4$-H8 to C$_3$-1'H cross-peak was barely detectable in the 2D NOESY spectra, and the corresponding interproton distance was scored as $>4$ Å by using the usual $r^6$ dependency to calculate the proton–proton distance from the intensity of the cross-peaks. Structure determination based on protom–proton distance constraints showed a distinct bend of the helix axis at the G$_4$ residue. However, at a later stage, when EDTA was added to the solution the G$_4$-H8 resonance was found to exhibit normal line width. Since trace amounts of paramagnetic impurities are often found to be present in nucleotide preparations it is recommended to always run samples through a CHELEX column to remove such impurities before NMR metal ion titration experiments are performed.

### 1.3.3 Oligonucleotide–Transition Metal Adducts

**Mn$^{2+}$ Ions**

The first metal ion titration NMR experiment on a DNA oligonucleotide was carried out by Frøystein and Sletten on the Dickerson–Drew duplex d(C$_1$G$_2$C$_3$G$_4$ A$_5$A$_6$T$_7$T$_8$C$_9$G$_10$C$_11$G$_12$) (seq. I) which contains the Eco-RI recognition sequence GAATTC. Since most of the base proton signals are well resolved in the 1D spectrum, the effect of adding aliquots of a solution of MnCl$_2$ was easily observed. A plot of metal-induced paramagnetic line-broadening of G-H8 showed a clear preference for G$_4$ compared to G$_2$, G$_10$ and G$_12$ (Figure 1.5). At a Mn$^{2+}$: duplex ratio of 1:10$^4$ substantial line-broadening was already detected for the G$_4$-H8 signal, while insignificant effects were observed for the other base proton resonances in the duplex. Of particular importance was the observation that the central adenine A-H2 protons showed no broadening effect, indicating that Mn$^{2+}$ ions were not located in
the minor groove (see discussion below). The amount of broadening was consistent with inner sphere coordination to G-N7. The binding to guanine by 3d transition metals was not surprising, considering the differences in thermodynamic stability of the corresponding complexes of the nucleoside and nucleotide monomers. However, the sequence-selective metal binding pattern manifested through the apparent preference for G<sub>4</sub> rather than the residues G<sub>2</sub>, G<sub>10</sub> or G<sub>12</sub>, was highly unexpected.

Further NMR spectroscopic evidence for apparent sequence-selective metal binding to DNA is presented for the duplex d(G<sub>1</sub>C<sub>2</sub>C<sub>3</sub>G<sub>4</sub>A<sub>5</sub>T<sub>6</sub>A<sub>7</sub>T<sub>8</sub>C<sub>9</sub>G<sub>10</sub>C<sub>11</sub>G<sub>12</sub>) (seq. II) containing the Eco-RV recognition sequence GATATC. The 1D spectra with and without MnCl<sub>2</sub>, shown in Figure 1.6, exhibit differential G-H8 line-broadening in the order: G<sub>10</sub> > G<sub>4</sub> > G<sub>1</sub> ~ G<sub>11</sub>. It is evident that the exposed terminal residues both in sequence II and the previous sequence I, although offering favourable accessibility, are not the preferred binding sites. One may also notice that the A<sub>5</sub>-H2 and A<sub>7</sub>-H2 protons, residing in the minor groove, are completely unaffected.

A systematic search for a selectivity pattern was initiated by designing three self-complementary sequences: [d(TATGGTACCATA)]<sub>2</sub> (III), [d(TATGGATCCATA)]<sub>2</sub> (IV) and [d(TATGGCCATA)]<sub>2</sub> (V), where the central triplet is GGX (X = A, T or C). Mn<sup>2+</sup>-induced line-broadening for G-H8 versus r = [Mn<sup>2+</sup>]/[duplex] is plotted in Figure 1.7a–c. The line-broadening of G-H8 for the 5'-G is practically the same for all three sequences. However, the line-broadening of 3'-G-H8 is seen to depend on the adjacent residue X following a
distinct sequence-selective pattern: GA > GT >> GC. The adenine A-H8 resonances, plotted as references, are not influenced by the paramagnetic ions.

Elmroth and coworkers have shown that metalation of single-stranded oligonucleotides of the type d(T\textsubscript{n}GT\textsubscript{16−n}) reach a maximum in the central part of the oligomer indicating lack of sequence-selective influence on the reaction rate.\textsuperscript{33,34} This result is in accordance with NMR and photo-cleavage studies on metalation of single-stranded DNA oligomers.\textsuperscript{18} Based on Monte Carlo descriptions of the oligoelectrolyte properties of double-stranded DNA oligomers it has been postulated that outer-sphere Coulombic interactions cause cations to be localized preferentially in the interior rather than the terminal part of the DNA oligomers.\textsuperscript{35} For inner-sphere metalation other factors may dominate, as demonstrated for the palindromic hexanucleotide d(G\textsubscript{1}G\textsubscript{2}C\textsubscript{3}G\textsubscript{4}C\textsubscript{5}C\textsubscript{6}) (seq. VI).\textsuperscript{36} Addition of MnCl\textsubscript{2} induces selective line-broadening on the terminal G\textsubscript{1}-H8 with no significant effect on G\textsubscript{2} and G\textsubscript{5}, as shown in the plot of line-broadening versus added MnCl\textsubscript{2}. In contrast, the terminal G\textsubscript{1}-H8 in duplex II (see above) shows almost no line-broadening.

Labiuk et al.\textsuperscript{37} have reported X-ray determinations of Co\textsuperscript{2+}, Ni\textsuperscript{2+} and Zn\textsuperscript{2+} complexes of d(G\textsubscript{1}G\textsubscript{2}C\textsubscript{3}G\textsubscript{4}C\textsubscript{5}C\textsubscript{6}) (seq. VI), where the metal ions are coordinated only to the terminal guanine G\textsubscript{1}-N7 position, with no metal ions binding to nonterminal guanine positions. The authors concluded that in the regular B-DNA conformation the internal binding sites are not accessible to Co\textsuperscript{2+}, Ni\textsuperscript{2+} and Zn\textsuperscript{2+}, and that consequently these metal ions bind exclusively to the terminal region of double-helical B-DNA, irrespective of base sequence. This is in contrast to our studies, which show a clear sequence-selective binding pattern for 3d metal ions with no special

Figure 1.6 400MHz \textsuperscript{1}H NMR spectra, the aromatic region, of the dodecamer [d(GCCGATATCGGC)]\textsubscript{2}: (a) the spectrum of Mn\textsuperscript{2+}-free solution, and (b) with added MnCl\textsubscript{2} at a Mn\textsuperscript{2+}/duplex ratio of approximately 10\textsuperscript{−3}. The H8 and H6 proton resonances of purine and pyrimidine residues are labelled according to their sequential assignment.\textsuperscript{8}
preference for terminal base residues. Based on the NMR results from several DNA sequences the d(GGCGCC) duplex is expected to bind preferentially to the 5'G in the 5'GG step and that the 5'G in the 5'GC step should have negligible affinity, in agreement with the X-ray result.

Crystallographic data of a metal–DNA complex containing an internal GG step would probably show binding at this site.

Table 1.1 is a compilation of available line-broadening data including both controlled titration experiments and the effect of paramagnetic impurities (reversed by addition of EDTA). One may notice that only Gs in the context G-purine exhibit maximum broadening. For sequence no. 6, which contains no G-purine step, the 5'-G-H8 resonance in the context of the 5'-GT step exhibits maximum broadening. A close inspection of Table 1.1 reveals that the line-broadening of 5'-G-H8 follows a consistent pattern, where the influence on the 5'-G-H8 is dependent on the residue.

Figure 1.7 Line-broadening versus Mn$^{2+}$/Duplex ratio of the H8 resonances of the guanosines 5'-G4 (*) and 3'-G5 (□) with the terminal adenine H8 protons (○) for comparison. (a) d(TATGGTACCAT)2; (b) d(TATGGATCCATA)2; (c) d(TATGGCCATA)2. (J. Vinje, J.A. Parkinson, P.J. Sadler, T. Brown, E. Sletten, Sequence-selective metalation of double-helical oligodeoxyribonucleotides with PtII, MnII and ZnII ions. Chem. Eur. J., 2003, 9, 1620–1630. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.)
on the 3′-side according to the simple rule: 5′-GG ≥ 5′-GA > 5′-GT >> 5′-GC. Apparently, the residue on the 5′-side is less important. At higher metal ion concentrations most 1 H NMR resonances undergo varying degrees of broadening. It must be stressed, however, that only relative broadening effects for each sequence are taken into account. For sequences 12, 13 and 14 the GGX (X = A, T, G) triplet is placed in an almost identical environment. The effects of varying the X-residue are clearly shown in Figure 1.7. In sequence no. 12 containing a GGG triplet, both line-broadening and T1 data show the following order: G3 = G4 > G5 consistent for, not only Mn2+, but also for Ni2+ and Co2+ (see Ni2+ ions below).

**Fe2+ Ions**

In early investigations of Fe2+-DNA binding using NMR, the T1 spin–spin relaxation time technique indicated that both the base and the phosphate groups interact with Fe2+ ions.38 Later on, Bertoncini et al.39, by using X-ray absorption near edge structure spectroscopy (XANES), demonstrated that while Fe3+ tends to associate with oxygen ligands, Fe2+ prefers to form inner-sphere coordination with nitrogen ligands on DNA. Linn and coworkers have made extensive studies on sequence-specific DNA cleavage by Fe2+-mediated Fenton reactions.40–42 Nicking of duplex DNA by the iron-mediated Fenton reaction occurs preferentially at a limited number of nucleotide sequences. Most notable are a purine nucleotide followed by three or more G residues, [RGGG], and purine nucleotides flanking a TG combination, [TTGR].42 The preferred reaction sites are probably a consequence of sequence-selective localization of the ferrous ions. Using 1H NMR to characterize Fe2+ binding within the duplex CGAGTTAGGGTAGC/GCTACCCTAACTCG it was shown that Fe2+ binds preferentially at the GGG sequence, most strongly towards its 5′-
end. These studies are especially interesting because of the presence of RGGG in a large majority of telomere repeats. Recent studies have implicated that telomere shortening during human aging may be accelerated by oxidative stress.

**Co**\(^{2+}\) **Ions**

The Co\(^{2+}\) ion in a low-spin environment has an estimated electronic relaxation time \((t_s)\) between those of Mn\(^{2+}\) and Ni\(^{2+}\). Selective line-broadening was observed when CoCl\(_2\) was added to the duplex, d(A\(_1\)T\(_2\)G\(_3\)G\(_4\)T\(_5\)A\(_7\)C\(_8\)C\(_9\)C\(_10\)A\(_11\)T\(_12\) \) VII. At \(r = \text{Co}^{2+}/\text{phosphate} = 1.8 \times 10^{-3}\) Co\(^{2+}\) was found to bind preferentially at the GGG sequence with almost equal affinity based on selective line-broadening; for G\(_3\) and G\(_4\) (35 Hz) and significantly less (12 Hz) for G\(_5\). NOESY spectra show that the intensities of cross-peaks were reduced by paramagnetic effects from the nearby bound Co\(^{2+}\) ion. The differential H8 broadening of G\(_3\), G\(_4\) and G\(_5\) observed in the 1D spectra is reflected as quenched NOE effects for the \(\text{intra- and inter-}\) proton cross-peaks involving G-H8 \(\ldots\) G-H1\(^{'\prime}\) connectivity. As a caveat we should mention that minute paramagnetic impurities in DNA samples used for NMR structure determination seriously affect the validity of the structural analysis since calculations of proton-proton distances are based on the magnitude of cross-peak intensities.

**Ni**\(^{2+}\) **Ions**

The electronic relaxation time \((t_s)\) for Ni\(^{2+}\) in the range \(10^{-10}\) to \(10^{-12}\) s is much shorter than for Mn\(^{2+}\). Consequently, spin-spin T\(_1\) relaxation time measurements will give more reliable information on metal binding sites than line-broadening (T\(_2\)) data. Figure 1.8a shows that the pattern for Ni\(^{2+}\) binding to the Dickerson–Drew sequence deduced from T\(_1\) data is almost identical to that observed for Mn\(^{2+}\) binding based on line-broadening data. The T\(_1\) results of NiCl\(_2\) titration of the d(A\(_1\)T\(_2\)G\(_3\)G\(_4\)T\(_5\)A\(_7\)C\(_8\)C\(_9\)C\(_10\)A\(_11\)T\(_12\) \) duplex, which contains a triple GGG sequence are plotted in Figure 1.8b. The affinities for G\(_3\) and G\(_4\) are identical within experimental errors, while the affinity of G\(_5\) in the context GT is, as expected, significantly lower. The T\(_1\) relaxation pattern follows exactly the same trend as was shown for the Co\(^{2+}\) line-broadening measurements.

Abrescia et al.\(^{45}\) reported an X-ray structure determination of the Ni\(^{2+}\)/DNA complex of the sequence d(CGTATATACG)\(_2\). The Ni\(^{2+}\) ions were associated with N7 atoms of all the guanines and with none of the N7 atoms of adenines. Subsequently the same group published a low resolution X-ray structure determination of the duplex d(CGTTGTAACAG), where they found Ni\(^{2+}\) ions associated only with the terminal Gs and the phosphate backbone.\(^{46}\) This last result is not in accordance with the sequence-selectivity observed in solution. However, the discrepancy may be due to crystal packing effects, which lead to cross-linking of guanines by Ni\(^{2+}\) ions.

**Cu**\(^{2+}\) **Ions**

One of the first detailed UV spectroscopic studies on the influence of metal ions on DNA stability was published by Eichhorn and Shin.\(^{3}\) While some metal ions tend...
to stabilize the double-helical structure, Cu$^{2+}$ salts were shown to induce a dramatic lowering of the melting temperature (Figure 1.1). Recent NMR studies of Cu$^{2+}$ and Cu(en)$_2^{2+}$ binding to the Dickerson–Drew duplex show the same selectivity pattern as for the other transition metals. The increase in line width at an $r = [Cu^{2+}]/[Phosphate]$ of $2.5 \times 10^{-3}$ was approximately 10 Hz, a corresponding increase in line width for [Cu(en)$_2$]$^{2+}$ was obtained at $r = 15 \times 10^{-3}$.

Oikawa and Kawanishi have investigated the telomere shortening induced by H$_2$O$_2$ plus Cu$^{2+}$ and found predominant DNA damage at the 5′-site of 5′-GGG-3′ in a 48-base fragment of the telomere duplex. In contrast, when single-stranded DNA was used, the damage induced by oxidative stress occurred at every guanine. The difference in site specificity of DNA damage between double-stranded DNA and single-stranded DNA could be explained in terms of the lower ionization potentials of stacked guanine base pairs in double-stranded DNA, on the basis of theoretical calculations.

**Zn$^{2+}$ Ions**

UV spectroscopic titration studies of ctDNA by ZnCl$_2$ indicate that Zn$^{2+}$ ions bind both to phosphate groups and base nitrogen atoms. Marzilli and coworkers carried out Zn$^{2+}$ titration of duplex d(ATG$_3$G$_4$G$_5$TACCCAT) (VII) containing a GGG triplet, and found the chemical shift variation for the G-H8 in the order G$_4$ > G$_3$ >> G$_5$. Zn$^{2+}$ titration experiments analogous to those using Mn$^{2+}$ (see above) have been carried out for sequences III, IV and V. In contrast to paramagnetic metal systems, where very low metal/DNA ratios were employed, excess salt was added to the duplex solutions until an upper limit of Zn$^{2+}$-induced chemical shift changes
was reached. This upper level was observed at \( r = [\text{Zn}^{2+}]/[\text{duplex}] \approx 5 \), which corresponds to a \([\text{Zn}^{2+}]/[\text{G}]\) value of \( \approx 1.2 \). Plots of chemical shifts versus \( r \) exhibit the same trend as for the Mn\( ^{2+} \) titration data (Figure 1.9).

In a recent multinuclear NMR study on Zn\( ^{2+} \) binding to oligonucleotides the variation in \( ^{1}H, ^{15}N \) and \( ^{31}P \) chemical shifts was monitored as a function of added ZnCl\(_2\). Measurements for three different sequences were carried out: d(GGCGCC) (III); d(GGTACCGGTACC) (VIII); d(GGTATATACCGGTATA) (IX). The chemical shift pattern for sequence III followed closely the line-broadening pattern determined for Mn\( ^{2+} \) (see above). For sequence VIII large chemical \(^{15}N\) shift effects were observed for G\(_7\)-N7 at natural abundance, a clear proof of direct metal binding to this site. \(^{31}P\) spectra also show large chemical shifts for the central G\(_7\) residue indicating that at excess ZnCl\(_2\) concentration the Zn\( ^{2+} \) ions have direct contact with the G\(_7\) phosphate group and/or that a change in the phosphodiester backbone conformation has occurred.\(^{36}\)

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**Figure 1.9** Chemical shift versus Zn\( ^{2+} \)/duplex ratio plots for the H8 resonances of 5'G4 (*) and 3'G5 (□), with the terminal adenine H8 protons (o) for comparison: (A) d(TATGGTACCATA)\(_2\); (B) d(TATGCCATCCATA)\(_2\); (C) d(TATGCGCCATA)\(_2\). (J. Vinje, J. A. Parkinson, P.J. Sadler, T. Brown, E. Sletten, Sequence-selective metalation of double-helical oligodeoxyribonucleotides with PtII, MnII and ZnII ions. Chem. Eur. J., 2003, 9, 1620–1630. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.)
Zn$^{2+}$ titration of IX was carried out to check if the expected accumulation of negative electrostatic potential in the interior of a long sequence$^{33,34}$ (compared to shorter ones) would lead to a higher Zn$^{2+}$ affinity for the internal GG step, compared with that observed for VIII.$^{36}$ The results clearly showed that there is no enhanced selectivity for the internal Zn$^{2+}$ binding for the long sequence.

$[^{15}\text{Pt(dien)}]^{+}$ Ions

Numerous publications have dealt with the binding pattern of cisplatin anticancer drugs to DNA (see Chapters 5–9 in this book). One of the first studies on this topic involved enzymatic digestion of platinated DNA using the well known anticancer drug cisplatin ($\text{cis}$-$[\text{PtCl}_2(\text{NH}_3)_2]$).$^{50}$ HPLC analysis of the cleavage products showed that cisplatin forms chelates spanning G and/or A residues with the following percentages: Pt-GG (65%), Pt-AG (25%), Pt-GA (0%). Apparently, the rule of sequence-selectivity proposed for labile metal ion complexes based on $^1$H NMR and photochemical cleavage studies does not apply to nonlabile platinum complexes. However, the mechanism of chelate formation does not necessarily follow the sequence-selectivity of the initial formation of monofunctional adducts. The fact that the 5$'\prime$-monoadducts are formed more rapidly and chelate more slowly than the 3$'\prime$-monoadducts might reflect the inherently greater reactivity of the 5$'\prime$-G compared to the 3$'\prime$-G. The common view is that the monoqua species of cisplatin make the initial attack on DNA bases, although recent results have lead Chottard and coworkers to suggest that cisplatin may undergo double hydrolysis before reacting with DNA.$^{51}$

In most reported studies on the kinetics of monofunctional platination reactions of single- and double-stranded oligonucleotides, both NMR spectroscopic and chromatographic methods have been used to determine kinetic parameters. The reactions involving bifunctional platinum complexes have usually been carried out with single-stranded oligonucleotides. In these reactions an initial monofunctional adduct is formed, which subsequently ring-closes to form a bifunctional chelate. The proposed selectivity rule is based on the intrinsic binding properties of duplex DNA. Sadler and coworkers showed that in a reaction between $^{15}$N-labelled $[^{15}\text{PtCl(dien)}]^+$ and a single-stranded 14-mer d(ATACATGGTACATA), little kinetic preference for platination of either 5$'\prime$G or 3$'\prime$G sites was observed, while the single-stranded 8-mer d(ATACATGG) showed a distinct preference for 5$'\prime$-G platination.$^{52}$ Chottard and coworkers, using hairpin-forming oligomers as duplex models, concluded that the selectivity for monofunctional attack by Pt$^{2+}$ on 5$'\prime$ and 3$'\prime$ G-residues is dependent on the ligand in the $\text{trans}$ position (e.g. Cl$^-$, H$_2$O, OH$^-$, NH$_3$).$^{51}$ In a later study they proposed a model for sequence-selective binding of cisplatin to DNA duplexes, involving a combination of molecular potentials and N7 accessibility.$^{53}$ In a study on selectivity of adduct formation between $^{15}$N-cisplatin and 14-mer duplexes containing central AGT and GAT residues, respectively, Hambley and coworkers concluded that ‘the purine base on the 3$'\prime$side of the pair exerts substantially greater influence on the rate of binding at the 5$'\prime$-base than does the 5$'\prime$-base on the rate of binding at the 3$'\prime$-base’.$^{54}$ However, monofunctional binding of cisplatin to the TGAT
sequence was found to be approximately an order of magnitude slower than binding to TAGT sequences, a result which does not agree with the proposed selectivity rule for labile metal ion complexes.

Vinje et al.\textsuperscript{10} have investigated the monofunctional reaction kinetics of \([\text{PtCl(dien)}]^+\) (dien = diethylenetriamine), with the oligodeoxyribonucleotides, III, IV and V, used for Mn\textsuperscript{2+} and Zn\textsuperscript{2+} titration (see above). The reaction mixtures were separated by HPLC and the chromatographic profiles showed a clear difference in the amount of 3′ versus 5′ monoplatinated species between the three duplexes (Figure 1.10). The reaction pathway of platination of seq. III is shown in Scheme 1.2.

Reaction rates for Pt(dien) were determined based on 2D [\(^1\text{H}, \text{\textsuperscript{15}N}\)] HSQC/HMQC NMR spectroscopy using \textsuperscript{15}N-labelled Pt(dien). The time courses for reaction rates of each of the three duplexes are shown in Figure 1.11. Comparison of the three different duplexes containing the central sections GGX (X = A, T, C), respectively,\textsuperscript{10} shows that the selectivity for covalent platination matches that for adducts with labile metal ions Mn\textsuperscript{2+} and Zn\textsuperscript{2+}. The reaction rate for platination is faster for the 5′-G than for the 3′-G. The reactivity of the 3′-G depends on the adjacent residue X in the following order: X = A > T >> C. For GGA the reaction rate is 1.2 times faster for 5′-G than for 3′-G, for GGT the rate of 5′-G platination is about eight times faster than that of 3′-G, and for GGC there is no significant adduct formation at the 3′-G (Table 1.2).

When the reaction mixture of the platinated species was aged over several weeks, the relative amounts of 5′ and 3′ HPLC fractions changed. This was also confirmed by comparison of the NMR spectra of the aged solutions with those recorded in the initial kinetic experiments. It was suggested that Pt-N7 bond cleavage and isomerization had taken place, similar to that observed for platinated single-
Leng and coworkers have suggested a catalytic property of the DNA double helix to explain the rearrangement that is observed when a stable \trans-\platinated single strand is annealed with its complementary strand.\textsuperscript{54} The occurrence of Pt–N bond cleavage may influence the results of kinetic analysis that are based on HPLC techniques, in which aliquots of the reaction mixture are collected at several time points and quenched with large amount of potassium chloride.
**Figure 1.11** Experimental concentrations (NMR data) and theoretically-fitted curves for the reactions between [Pt(dien)]³⁺ and DNA: (a) d(TATGGTACCATA)₂⁺; (b) d(TATGGATCCATA)₂⁺; (c) d(TATGGCCATA)₂⁺. Symbols: (□) Pt(dien); (○) Pt-G5’ and (△) Pt-G3’. (J. Vinje, J.A. Parkinson, P. Sadler, T. Brown, E. Sletten, Sequence-selective metalation of double-helical oligodeoxyribonucleotides with PtII, MnII and ZnII ions. Chem. Eur. J., 2003, 9, 1620–1630. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.)

**Table 1.2** Rate constants (standard deviations in parentheses) for reactions of [PtCl(dien)]³⁺ with DNA oligonucleotides. pH 5.6, 0.1 M NaClO₄.

<table>
<thead>
<tr>
<th>Sequence</th>
<th>$k_{3'}$ (M⁻¹ s⁻¹)</th>
<th>$k_{5'}$ (M⁻¹ s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-GGTA⁻⁻⁻</td>
<td>4.3 (6)</td>
<td>0.5 (1)</td>
</tr>
<tr>
<td>-GGAT⁻⁻⁻</td>
<td>4.0 (6)</td>
<td>3.4 (5)</td>
</tr>
<tr>
<td>-GGCC⁻⁻⁻</td>
<td>7.6 (6)</td>
<td>(c)</td>
</tr>
</tbody>
</table>

Temperature: (a) 288 K; (b) 298 K; (c) Negligible 3'-G platination.
1.4 Summary of Theoretical and Experimental Evidence for Sequence-Selective Binding to DNA

Paramagnetic and diamagnetic ions are shown to form both labile and nonlabile adducts with DNA duplexes, and in a series of G-containing duplexes a certain selectivity pattern emerges where the metal prefers the 5'-G in the following order: 5'-GG > 5'-GA > 5'-GT >> 5'-GC. The adjacent residue (Y) on the 5'-side (5'-YGG) is found to exert a negligible influence on the selectivity. For the monofunctional binding of [PtCl(dien)]^+ to double helical DNA the variation in reaction rates follows qualitatively the same selectivity pattern as for the labile metal ions. Experimentally observed relative rates of G-oxidation are found to match well with the NMR results. Nonselective equal G cleavage is observed for single-stranded DNA, in line with the NMR experimental data. *Ab initio* molecular orbital calculations of stacked DNA bases with B-form geometry clearly indicated that the highest occupied molecular orbital (HOMO) localization on the 5'-G is highly sequence-dependent. The degree of \( \pi-\pi \) interaction between the stacked bases influences the HOMO energies. However, it should be emphasized that for metal complexes the stereochemistry of the ligands also plays a significant role in determining the most favourable binding site.

1.5 Sequence-Specific Groove Binding

Among the large number of contributions to the field of cation–groove interaction, probably a majority is based on X-ray crystallography. Sequence-specific nucleic acid conformation and dynamics are directly influenced by metal ions. DNA conformational heterogeneity has been explained by an electrostatic model where the local position and transient fluctuation of ions act through asymmetric neutralization of phosphate charges. Evidence from NMR spectroscopy, X-ray crystallography and molecular dynamics simulations has revealed that B-form duplexes interact in a sequence-specific manner with fully hydrated mono and divalent cations.55

1.5.1 Groove Geometry

A noteworthy feature of B-DNA is the presence of two kinds of grooves, called the major groove (12 Å wide) and the minor groove (6 Å wide) (see figures in biochemistry text books). They arise because the glycosidic bonds of a base pair are not diametrically opposite each other. The minor groove contains the pyrimidine O2 and the purine N3 of the base pair, and the major groove is on the opposite side of the pair. In the minor groove, N3 and O2 can serve as hydrogen bond acceptors, and the amino group attached to guanine can be a hydrogen donor. In the major groove N7 is a potential hydrogen bond acceptor, as are O4 of thymine and O6 of guanine. The amino groups attached to adenine and cytosine, respectively, can serve as hydrogen donors.
The width of the grooves is not constant, but is found to be sequence-dependent. Alternative models have been presented to provide explanations for this sequence-dependent variation. NMR spectroscopic analyses of a series of DNA duplexes have shown that alternating (AT)_n sequences are characterized by a rather wide minor groove.\textsuperscript{56,57} From a number of experimental and theoretical studies, it has been concluded that on average the minor groove of DNA A-tracts (several consecutive adenine residues) is significantly narrower than the minor groove of G-tracts.\textsuperscript{58-60}

1.5.2 Monovalent Cations

In the report of the first X-ray structure of a double-helical DNA duplex, [d(CGCGAATTCCGCG)]\textsubscript{2}, published by Dickerson and Drew,\textsuperscript{58} it was proposed that a ‘spine of hydration’, composed of localized, geometrically arranged water molecules, in the minor groove, is an important structural component of A-tract DNA. However, this result is not fully conclusive since Na\textsuperscript{+} and H\textsubscript{2}O, being isoelectronic, are not easily distinguishable by X-ray diffraction. Egli and coworkers repeated the structural analyses of the same duplex using X-ray diffraction data to near-atomic resolution with crystals grown in the presence of Rb\textsuperscript{+} cacodylate.\textsuperscript{61} They found that a single Rb\textsuperscript{+} ion, with partial occupancy, was localized at the central ApT step at the bottom of the AATT minor groove (Figure 1.12). The ion replaces the water molecules that link the keto oxygen of thymines from opposite strands.

The authors suggest that minor groove ion coordination appears to be an isolated event, highly sequence-dependent and unlikely to significantly affect the particular geometry of the A-tract in the Dickerson–Drew dodecamer. Further studies by Williams and coworkers on the same duplex using Tl\textsuperscript{+} to mimic K\textsuperscript{+} showed that none of the observed Tl\textsuperscript{+} sites surrounding the duplex were fully occupied.\textsuperscript{62} The

![Figure 1.12 Coordination of Rb\textsuperscript{+} at the central ApT step in the Dickerson–Drew Duplex.\textsuperscript{61} (Reprinted from Chem. Biol., 9, 3, M. Egli, DNA-cation interactions: Quo vadis?, 10. Copyright 2002, with permission from Elsevier.)](image-url)
most highly occupied sites (20–35%) were located within the G-tract major groove while the occupancy in the minor groove were estimated to be around 10%.

The situation concerning penetration of Na⁺ ions into the spine of hydration is still an open question among X-ray crystallographers. The existence of just a single alkali metal ion coordination site in the Rb⁺-form crystal structure argues against a view that ions can invade the minor groove hydration spine along the entire length, or the existence of a mixed water-ion spine of hydration. In a recent high-resolution (1.1 Å) structure determination of the Dickerson–Drew duplex no experimental evidence for the presence of Na⁺ ions in the minor groove was found.⁶³–⁶⁵

A series of ²³Na NMR quadrupolar relaxation studies have been carried out on B-DNA.⁶⁶,⁶⁷ A general conclusion from these studies is that monovalent counterion binding to DNA is loose and delocalized, without any dehydration or sequence-specific features. In an optimally designed test for sequence-specific Na⁺ binding in the minor groove Denisov and Halle have used a magnetic relaxation dispersion (MRD) technique, where the ²³Na relaxation rate is measured over nearly two decades of resonance frequency.⁶⁷ Comparison of Na⁺ MRD data from three dodecamers with different nucleotide sequences: CGCGAATTCGCG (abbreviated A₂T₂), CGAAAATTTTCG (A₄T₄) and CGCTCTAGAGCG (TA) showed that the most tightly bound Na⁺ ions reside in the minor groove. However, the occupancies are quite low corresponding to a binding constant $K_{Na}$ of 0.03 M⁻¹ for TA and 0.1 M⁻¹ for the other two dodecamers, and imply that Na⁺ binding in the minor groove is a rare event and is not likely to be detected by X-ray diffraction. These results are not necessarily inconsistent with higher occupancy at the cryogenic temperature (120–160 K) used in recent crystallographic studies. Even a modest binding enthalpy of 5 kJ mol⁻¹ could increase the binding constant from 0.1 M⁻¹ at 277 K to 1.7 M⁻¹ at 120 K, which is sufficient to give 50% occupancy in a single binding site, as found for Rb⁺ at the ApT step in A₂T₂.⁶¹ The authors conclude that groove bound Na⁺ ions, with an occupancy of only a few percent at room temperature, are not likely to contribute importantly to the ensemble of DNA structures under physiological conditions.⁶⁷

### 1.5.3 Divalent Cations

At physiological concentrations the binding of divalent cations to DNA is both cation dependent and sequence dependent. From a sequence standpoint, specificity is contributed to by both the local molecular nucleophilicity (see above) and the hydrogen bond environment. These H-bond interactions reflect the greater hydration properties of divalent cations over monovalent. Generally, it is difficult to assess how contributions of base sequence or cation type influence groove specificity because of crystal packing effects. Chiu and Dickerson have examined a database of 28 cation-bound B-DNA structures spanning ten different crystal packing environments and showed that there is a correlation between experimental conditions and the number of observed cations.⁶⁸ Hence, the locations of cations can be compared safely only between structures having similar crystallizing conditions, data collection methods and resolutions. Despite these difficulties, the authors find a
strong correlation between divalent cation binding and base sequence for the 28 structures examined. For four high-resolution (0.99 Å) structures, the minor groove affinity for Mg$^{2+}$ is GG > AG > AC and for Ca$^{2+}$ is GG > AT ~ AC, with cations positioned in the centre of the groove. For the major groove, the order for Mg$^{2+}$ is GG > AG ~ GT and for Ca$^{2+}$ is GG > AG.$^{68}$

Treshko et al.$^{65}$ have redetermined the crystal structure of the Dickerson–Drew duplex at 1.1 Å resolution. Three ordered Mg$^{2+}$ ions are present in the asymmetric unit, two hexahydrates and one pentahydrate complex. One Mg$^{2+}$ is located in the major groove, close to the end of the duplex (Figure 1.13). The ion contacts the N7 and O6 edges of residue G$_2$ and G$_{22}$ from opposite strands via coordinated water molecules. None of the contacts between Mg$^{2+}$ ions and DNA atoms on the floor of the major groove involve inner-sphere coordination.$^{65}$

Hud and Feigon have studied the localization of Mn$^{2+}$ in A-tract DNA by $^1$H NMR spectroscopy, using a series of self-complementary dodecamer oligonucleotides that contain the sequence motifs A$_n$T$_n$ and T$_n$A$_n$, where n = 2, 3 or 4 flanked by 5′-CG or 5′-GC base pairs.$^{69}$ At an Mn$^{2+}$ to duplex ratio of 10$^{-3}$ most of the aromatic base protons are severely broadened, however, in contrast to the Mn$^{2+}$ NMR data referred to above, the adenine H2 resonances exhibit the largest broadening. The authors conclude that Mn$^{2+}$ is localized in the minor groove with the position and degree of localization being highly sequence-dependent. In addition, G$_2$-H8 in the sequence CGT is broadened by 7Hz/µM MnCl$_2$ while G$_1$-H8 in the sequence GCT is broadened less than 2 Hz, a finding that is in agreement with the observation that the affinity of 5′-GT >> 5′-GC (see above).

Figure 1.13 Coordination of Mg$^{2+}$ at the CpG step near one end of the Dickerson–Drew Duplex.$^{63}$ (Reprinted from Chem. Biol., 9, 3, M. Egli, DNA-cation interactions: Quo vadis?, 10. Copyright 2002, with permission from Elsevier.)
NMR spectroscopy\textsuperscript{70} and molecular dynamics (MD)\textsuperscript{71} studies have shown that A\textsubscript{n}T\textsubscript{n} and T\textsubscript{n}A\textsubscript{n} duplexes have unusual structures and dynamics. Anomalous broadening of the TpA adenine H2 resonance indicative of large amplitude base motion, has been observed for a series of nine unique four-nucleotide sequences.\textsuperscript{70} In A\textsubscript{n}T\textsubscript{n} sequences, the DNA assumes a unique structure characterized by a gradual and increasingly compressed minor groove, which reaches a minimum at the ApT step. In conclusion, this type of sequences does not exhibit a regular B-form conformation and thus minor groove cation binding may be unique to A-tract sequences.

1.5.4 Conclusion on Groove Binding

The general trend is that ions bind in the minor groove of DNA A-tracts and in the major groove of DNA G-tracts. NMR spectroscopic studies indicate that sequence-specific binding of alkali and alkali earth ions appears to have only minor influence on the heterogeneity of DNA structures.\textsuperscript{67} The relative lack of hydration of alkali cations makes their interactions with base atoms largely electrostatic and relatively nonspecific. Divalent alkali earth cations tend to be fully hydrated and their interactions with duplex DNA are more sequence-specific through formation of hydrogen bonds to base atoms.\textsuperscript{55,67}

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>MEP</td>
<td>Molecular electrostatic potential</td>
</tr>
<tr>
<td>HOMO</td>
<td>Highest occupied molecular orbital</td>
</tr>
<tr>
<td>LUMO</td>
<td>Lowest occupied molecular orbital</td>
</tr>
<tr>
<td>ODN</td>
<td>Oxidation oligodeoxynucleotides</td>
</tr>
<tr>
<td>MD</td>
<td>Molecular dynamics</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene diamine tetraacetic acid</td>
</tr>
<tr>
<td>XANES</td>
<td>X-ray absorption near edge structure</td>
</tr>
<tr>
<td>NOESY</td>
<td>Nuclear overhauser effect spectroscopy</td>
</tr>
<tr>
<td>MRD</td>
<td>Magnetic relaxation dispersion</td>
</tr>
</tbody>
</table>

References


31. Hare, D.R.; Reid, B.R.; Three-dimensional structure of a DNA hairpin in solution: two-dimensional NMR studies and distance geometry calculations on d(CGCGTGTGTGCGCG); *Biochemistry*, 1986, **25**, 5341–5350.


34. Snygg, Å.S.; Brindell, M.; Stochel, G.; Elmroth, S.K.C.; A combination of access to preassociation sites and local accumulation tendency in the direct vicinity of G-N7 controls the rate of platination of single-stranded DNA; *Dalton Trans.*, 2005, 1212–1227.


68. Chiu, T.K.; Dickerson, R.E.; 1 Å crystal structures of B-DNA reveal sequence-specific binding and groove-specific bending of DNA by magnesium and calcium; *J. Mol. Biol.*, 2000, **301**, 915–945.
69. Hud, N.V.; Feigon, J.; Characterization of divalent cation localized in the minor groove of AnTn and TnAn DNA sequence elements by 1H NMR spectroscopy and managnese(II); *Biochemistry*, 2002, **41**, 9900–9910.