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DNA-Cation Interactions: Quo Vadis?

Review

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The interactions between double helical DNA and cations, specifically mono- and divalent metal ions, have recently received increased attention. Molecular dynamics simulations, solution NMR, and X-ray crystallography have all shed light on the coordination of ions in the major and minor grooves of DNA. Metal ion interactions may play key roles in the control of DNA conformation and topology, but despite progress in locating the ions and determining their precise binding modes, it remains difficult to figure out just how important ions really are. What have we learned and what remains to be done?

Introduction

The last five years have seen a flurry of publications regarding sequence-specific and conformation-specific interactions of DNA with counterions [1]. The renewed interest in the ionic environment of DNA was in part stimulated by a fascinating paper by Beveridge and colleagues [2] who proposed that strongly electronegative pockets in the Dickerson-Drew dodecamer (I will use the abbreviation DDD here, first introduced in [3]) B-DNA were harboring Na^+ ions. Their insights were based on molecular dynamics (MD) simulations of the DDD and its solvent hull, suggesting relatively long residence times for ions, in some cases matching or exceeding those of well-ordered water molecules. Remarkably, this work showed that a Na^+ ion could intrude the central portion of the so-called minor groove "spine of hydration" [4]. Given that this spine has possibly been investigated in greater detail than any other features of nucleic acid structure (at least judging from the number of papers dedicated to the subject), the above finding was certainly quite sensational. Shortly thereafter, Hud and Feigon [5] reported on the preferential binding of Mn^{2+} in the minor groove of A tract DNA. A tracts are DNA sequences of the form homo-$(A)_x$/homo-$(T)_x$ or $[5'\text{-}(A)_x(T)_x\text{-}3']_2$ that induce bending in the direction of the minor groove [6]. Thus, the DDD with sequence CGCGAATTCGCG does contain a short A tract. Although quite well characterized, the debate with regard to the origins of A tract-related bending is still ongoing and includes sequence-dependent (e.g., [7]) and electrostatic models (e.g., [8]) and variations thereof. The sequestering of ions in one groove could lead to a model for the type of bending observed with sequences containing A tracts. The growing literature on DNA-cation interactions has been reviewed recently [1] and I have summarized key insights of selected papers in the field since 1997 in chronological order in Table 1.

That metal cations are an intricate part of nucleic acid structure and can occupy particular sites, reflecting both sequence and topological specificity, is really nothing radically new. Early X-ray crystal structures of RNA revealed a Na^+ ($[r(AU)]_2$, [9]) and Mg^{2+} ions as well as the polyamine spermine ($tRNA^{Phe}$, [10]). In the large majority of the structures, the retrieved cations do not account for charge neutrality. Typically, only one or two ordered Mg^{2+} ions could be located in crystal structures of DNA duplexes. Owing to its regular octahedral coordination chemistry, Mg^{2+} can be quite easily located in electron density maps of good resolution. That most cations appeared to be missing was commonly explained with limited resolution of the diffraction data or low occupancy of the majority of ion binding sites. However, crystal structures of left-handed Z-DNA constituted a notable exception. The three basic forms of the hexamer CGCGCG, the Mg/spermine-form [11], the Mg-form [12], and the spermine-form [13, 14], all determined to 1 Å resolution, revealed metal ions and spermines that fully neutralize the 10 negative charges of phosphates per crystallographic asymmetric unit. Indeed, in terms of DNA-cation interactions, the Z-DNA lattice is a veritable treasure trove, as summarized here.

(i) Mg^{2+} ions were observed to coordinate mainly to the exposed O6/N7 edges of guanines in the convex surface (the major groove equivalent) [11, 12]. Occasionally, the coordination sphere of divalent ions comprises base atoms and phosphate groups. (ii) In the Mg/spermine form, the spermine molecule also contacts the convex surface and is shared between molecules, thus providing the mortar between "DNA bricks" [11, 15]. (iii) Mg^{2+} ions and primary and secondary amino groups of spermine molecules can replace water molecules in the solvent shell of Z-DNA [16]. (iv) Coordination of Mg^{2+} can lead to conversion between the Z_I and Z_{II} backbone geometries [12]. (v) In the spermine form, one of the spermine molecules present in the structure was bound in the cavernous minor groove, effectively replacing a string of water molecules, and hydrogen bonding to both base atoms and phosphate groups [14]. As a consequence, the minor groove is more narrow compared with the structures where spermine was absent from the groove. (vi) Atomic resolution crystal structures revealed considerable local flexibility of the Z-DNA backbone [13] and metal ions may stabilize a particular conformation. The observation of spermine in the minor groove of Z-DNA was particularly intriguing in view of the efficiency with which polyamines can induce the B- to Z-DNA transition [14]. Micromolar concentrations of spermine can fully convert the right-handed to the left-handed duplex form with CG-rich sequences, while mM and M amounts of Mg^{2+} and Na^+ are necessary, respectively, to achieve the same result [17]. This is just one example of the importance of cations for modulating or perhaps controlling nucleic acid conformation. When judging the results and claims of more recent work on DNA-cation

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Table 1. Chronology of Key Structural Studies Regarding DNA-Cation Interactions over the Last Five Years

Publication Date	First, Last Author	Method and Highlight	Reference
January 1997	Young, Beveridge	MD simulation of DDD establishes Na^+ binding site of partial occupancy at ApT step in minor groove.	[2]
June 1997	Hud, Feigon	NMR solution study maps Mn^{2+} ions to minor groove of A tracts.	[5]
May 1998	Berger, Egli	One Mg^{2+} observed near junction of G and A tracts in major groove of DDD, carrying a single 2′F-ANA T (resolution 1.55 Å).	[36]
June 1998	Shui, Williams	Independent observation of above Mg^{2+} in major groove of native DDD at 1.4 Å resolution.	[37]
January 1999	Tereshko, Egli	First atomic resolution crystal structure of the DDD; five Mg^{2+} ions observed per asymmetric unit.	[3]
February 1999	Hud, Feigon	Ammonium ions reside in the minor grooves of B-DNA duplexes with A tracts of various lengths (NMR).	[24]
April 1999	Tereshko, Egli	Cocrystallization of the DDD with Rb^+ allows observation of one partially occupied binding site at the central ApT step in the minor groove.	[26]
August 1999	Minasov, Egli	Comparison of the Mg^{2+} and Ca^{2+} forms of the DDD finds evidence of ion dependence of B-DNA minor groove width by X-ray crystallography.	[29]
September 1999	Chiu, Dickerson	Absence of monovalent cations in the minor groove of a crosslinked DDD at 1.4 Å provides evidence against invasion of the entire spine of hydration by ions.	[7]
October 1999	Feig, Pettit	MD simulations suggest that localizations and residence times of Na^+ ions in the major grooves of A and B form duplexes differ significantly.	[21]
January 2000	Denisov, Halle	Magnetic relaxation dispersion shows preferential binding of alkali metal ions and ammonium ions in the minor groove of A tracts, albeit with low occupancy.	[23]
February 2000	Woods, Williams	Structure of the DDD crystallized in the presence of Cs^+ reveals coordination sites in the minor groove A tract.	[27]
March 2000	Halle, Denisov	Find relatively long residence times for Na^+ ions in the minor groove of the DDD using solution NMR.	[45]
July 2000	Auffinger, Westhof	K^+ binds to major groove GpC but not CpG step and does not bind in the minor groove, based on MD simulations of DNA and RNA $d(GC)_{12}$ duplexes.	[22]
August 2000	Chiu, Dickerson	Alternative backbone conformations and minor groove coordination modes of Mg^{2+} and Ca^{2+} are observed in crystal structures of B form decamer duplexes lacking A tracts.	[30]
November 2000	Hamelberg, Wilson	A 10 ns MD simulation suggests that the DDD minor groove narrows as Na^+ ions bind either in the groove or at its periphery.	[20]
March 2001	Tereshko, Egli	First application of SAD-type diffraction experiments establishes binding of alkali metal ions in the major and minor grooves of A-DNA.	[33]
August 2001	Howerton, Williams	Crystals of the DDD grown in the presence of $T1^+$ allow determination of multiple ion binding sites in the major groove and confirm the site in the minor groove seen earlier.	[34]

MD = molecular dynamics; DDD = Dickerson-Drew dodecamer; SAD = single-wavelength anomalous diffraction; 2′F-ANA = 2′-fluoro-arabinonucleic acid.

interactions, it definitely seems worthwhile to bear in mind these earlier insights, gained a decade and in some cases two decades ago. In science and elsewhere, one is occasionally reminded of a statement that is attributed to Marie Antoinette: "There is nothing new except what has been forgotten."

Here I will briefly review progress in the understanding of the ionic environment of DNA and its effects on the structure of B and A form double helices. Among the recent contributions to the field, probably a slight majority is based on X-ray crystallography (Table 1). For this reason and given my own interest and background, I have focused mostly on crystallographic studies. However, wherever possible, the results from solid state studies are discussed in the context of research employing a variety of other techniques.

Methods

The main techniques that were applied in recent investigations of DNA-cation interactions are molecular dynamics, solution NMR, and X-ray crystallography. These efforts have been complemented very recently by capillary electrophoresis [18]. MD simulations were carried out over the nanosecond range [19] and involved the DDD [2, 8, 20] as well as a variety of decamers and dodecamers [21, 22], some containing longer A tracts. All simulations were performed in solution, or in other words, an isolated double helix was immersed in a solvent box composed of water and either Na^+ or K^+ ions.

Many of the solution NMR experiments also focused on the DDD, in the presence of either divalent [5] or monovalent cations including ammonium [23]. The latter experiments employed magnetic relaxation dispersion for ^{23}Na with the DDD, a dodecamer with an A_4T_4 portion, and another one lacking an A tract (Table 1). Moreover, the exchange rate of Na^+ in the case of the DDD was measured with the drug netropsin as a competitor; netropsin is known to bind in the minor groove AATT portion. Earlier, ^1H and ^{15}N NMR solution experiments with duplexes featuring A tracts or reverse 5′-T_xA_x-3′ stretches of various lengths in combination with Mn^{2+} [5] or alkali metal ions and ammonium [24] had been used to ad-

Table 2. Recent X-Ray Crystal Structure of DNA-Metal Ion Complexes Determined to Atomic Resolution

Sequence	Duplex Form	Ion	Resol. [Å]	NDB [61] Code	Reference
CCAGTACTGG	B	Ca^{2+}	0.74	BD0023	[32]
GCGAATTCG	B	Mg^{2+}	0.89	BD0016	[31]
CGCGAATTCGCG	B	Mg^{2+}	0.95	BD0030	[28]
CCAACGTTGG	B	Mg^{2+}	1.00	BD0033	[30]
CCAACGTTGG	B	Ca^{2+}	1.00	BD0034	[30]
CCAGCGCTGG	B	Mg^{2+}	1.00	BD0035	[30]
CCAGCGCTGG	B	Ca^{2+}	1.00	BD0036	[30]
CGCGAATTCGCG	B	Mg^{2+}	1.10	BD0007	[3]
CGCGAATTCGCG	B	Rb^{+}	1.20	BD0012	[26]
GGCCAATTGG	B	Mg^{2+}	1.15	BD0006	[62]
CGCGAATTCGCG	B	K^{+}	1.20	BD0041	[63]
CGCGAATTCGCG	B	Tl^{+}	1.20	BD0054	[34]
(C)GCGAATTCGCG	B	Ca^{2+}	1.30	BD0018	[29]
GCGTATACGC	A	Mg^{2+}	0.83	AD0007	[28]
GCGTATACGC	A	Na^{+}, K^{+}, Rb^{+}, Cs^{+}, Mg^{2+}, Ba^{2+}	max. 1.05	AD0012–19	[33]
ACCGGCCGGT	A	Mg^{2+}	1.40	AH0007	[40]
CGCGCG	Z	Na^{+}	0.60	ZD0004	[33]

All sequences shown from 5′- to 3′-end; for some sequences isolated bases carry chemical modifications. Ion refers to the metal ion that was the focus of the particular study; the crystallization conditions usually comprise additional cations, including, in some cases, the polyamine spermine.

dress the consequences of regio-specific ion binding for groove topology and bending.

Attempts to locate metal ions in the crystal structures of DNA duplexes have profited significantly from major breakthroughs regarding the resolution of X-ray diffraction data during the last couple of years. High intensity synchrotron radiation is the main reason for the increasing number of atomic resolution structures being reported recently (Table 2) [25]. However, cryoprotection of crystals, low-temperature data collection, optimized crystallization conditions, and high purity of the chemically synthesized oligodeoxynucleotides should also be mentioned in this respect. The possibility of selective binding of alkali metal ions in the minor groove of A tracts was studied exclusively in crystal structures of the DDD. To overcome the difficulties with respect to locating the lighter alkali metal ions in Fourier electron density maps, the DDD was cocrystallized with Rb^{+} [26] and Cs^{+} [27]. Significant advances were also made in terms of the determination of Mg^{2+} binding sites in the DDD. Up to five Mg^{2+} were observed in high-resolution structures of the DDD [3], and a maximum resolution of 0.95 Å has been obtained [28]. In addition, a Ca form of the DDD was determined at a resolution that almost matches that of the Mg form [29]. Moreover, crystal structures of decamer duplexes in the presence of Mg^{2+} or Ca^{2+} were investigated at atomic resolution [30] and structures at ultra high resolution of B-DNA nonamers [31] and decamers [32] have been reported (Tables 1 and 2). In most cases, the structures were determined with the molecular replacement technique, using coordinates from earlier refinements at lower resolution or standard B form model duplexes. It is worth mentioning that the improved resolutions of the diffraction data of nucleic acid duplexes now make possible the application of so-called direct methods for structure determination. This was initially demonstrated for the structure of an A form decamer duplex using data to a resolution of 0.83 Å [28].

While high resolution of the diffraction data is certainly a prerequisite for reliably establishing the locations of weakly scattering metal ions, it is by no means sufficient. However, the significant anomalous contribution to the X-ray scattering amplitude exhibited by all alkali and earth alkali metal ions with the exception of Na^{+} and Mg^{2+} can be exploited for facilitating their detection in electron density maps. This was proven with crystals of an A form DNA model system obtained in the presence of various concentrations of Na^{+}, K^{+}, Rb^{+}, or Cs^{+} [33] (Table 1). Thus, a combination of high-resolution and single-wavelength anomalous diffraction (SAD) data collections is more powerful for locating alkali metal ions than high resolution alone. Another example of the use of anomalous scattering for verifying the presence of ions with partial occupancies is the recent structure of the DDD cocrystallized with Tl^{+} [34], a metal ion that had previously been shown to be a K^{+} mimic [35]. Independent of the actual methodology for locating metal ions in DNA crystals, it is important to bear in mind that the outcome of such experiments can be critically dependent on the crystallization conditions. Thus, cocrystallization of DNA with cations, soaking of DNA crystals in solutions of metal ions at various concentrations, or combinations thereof may give rise to significantly different interpretations regarding the interactions between DNA and metal ions, the number of observed metal ions, and their individual occupancies.

Divalent Metal Ions

This section and the following one will give overviews of the binding modes of earth alkali and alkali metal ions, respectively, to A and B form DNA duplexes and how ion coordination affects the topologies of the major and minor grooves.

A magnesium ion in the DDD was first located in two different crystal structures of the dodecamer at resolutions of 1.55 [36] and 1.40 Å [37], respectively, the former featuring a single 2′-fluoro-arabinonucleic acid (2′F-ANA) residue per strand. The Mg^{2+} coordinates to guanines from opposite strands at the G2pC3 step near the transition

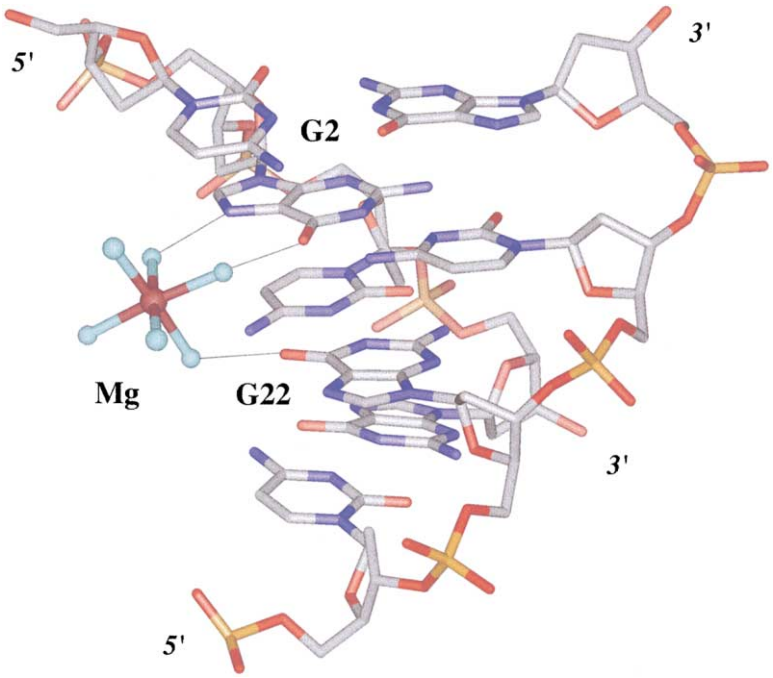

Figure 1. Coordination of Mg^{2+} at the CpG Step Near One End of the DDD [3]

to the A tract (Figure 1). None of the contacts between the ion and DNA atoms on the floor of the major groove involve inner sphere coordination. The site where Mg^{2+} binds is also marked by a kink into the major groove (reviewed in [38]), and it was proposed that the ion might actually cause this bend [29]. However, another possibility is that the ion simply stabilizes a particular conformation that is brought about by lattice forces in the first place. The environments of the helix ends are different in the DDD lattice and it is instructive that no kink exists at the other end, although a partially ordered spermine has now been observed in its major groove in several structures. Seven atoms out of fourteen were visible in electron density maps of the native DDD [37] and 11 spermine atoms were ordered in the structure of a DDD containing bicyclic nucleic acid residues in the central part [39]. None of the additional Mg^{2+} ions found in the DDD structure at atomic resolution are located in the major groove [3].

Mg^{2+} and Ca^{2+} ions were also observed in the major grooves of B-DNA decamer duplexes for which structures had been determined to atomic resolution. In $[d(CCAGCGCTGG)]_2$, Mg^{2+} and Ca^{2+} are both bound at the GpG step, but only the latter ion exhibits inner sphere coordination to the DNA [30]. In both duplexes, the major groove is compressed at the site of ion coordination. In the structure of the decamer with the closely related sequence CCAGTACTGG, Ca^{2+} was also observed at the GpG step but the ion exhibits some direct contacts to DNA atoms [32]. Interestingly, all other coordination sites of divalent metal ions in the two above decamers as well as the Ca and Mg forms of the duplex $[d(CCAACGTTGG)]_2$ comprise guanine as well [30, 32]. The ions reside either at ApG or GpT steps, thus establishing the major groove edge of guanine as the preferred binding sites of divalent metal ions.

The conclusions regarding the base pair steps preferred by Mg^{2+} in the major groove also appear to apply to ion coordination in the major groove of A form duplexes. Thus, Mg^{2+} was located at a GpG step in the A-DNA decamer duplex with sequence ACCGGCCGGT [40]. In crystal structures of an A-DNA duplex with sequence GCGTATACGC, we observed binding of earth alkali (Mg^{2+}, Ba^{2+}) and alkali metal ions (Na^+, K^+, Rb^+, and Cs^+) at the GpT step deep in the major groove [33]. The positions of all ions are nearly identical but the numbers and nature of ligands in their coordination shells differ. Interestingly, as in the case of the DDD above, the A form duplex displays a local bend into the major groove near the site of ion binding. Again, lattice interactions may be at the origin of the bend and the enhanced electrostatic potential as a result of the compression of the major groove may then serve as an ion trap. A further similarity to the situation in the DDD is the lack of a bend at the opposite end of the A duplex. However, no other ions were located there, although the decamer had also been cocrystallized with spermine. For now, we note that Mg^{2+} can coordinate at GpN steps in the major grooves of A- and B-DNA duplexes and that the binding of ions seems to be favored by a change in the local topology. This sequence-specific coordination of Mg^{2+} is further corroborated by the enhancement by Mg^{2+} ions of the reactivity of pyrimidines toward MnO_4^- attack at TC:GA and CC:GG steps in double-stranded DNA [41].

An important difference between the topologies of the major grooves in A and B form duplexes is the spacing of phosphate groups across strands. The A form major groove is deep, but phosphate groups from opposite strands are closely spaced at the groove periphery. As a consequence, Mg^{2+} hexahydrates can bridge these phosphates. An example of such a coordination mode was found in the crystal structure of a fully 2′-*O*-modified dodecamer duplex [42]. There, two adjacent Mg^{2+} ions

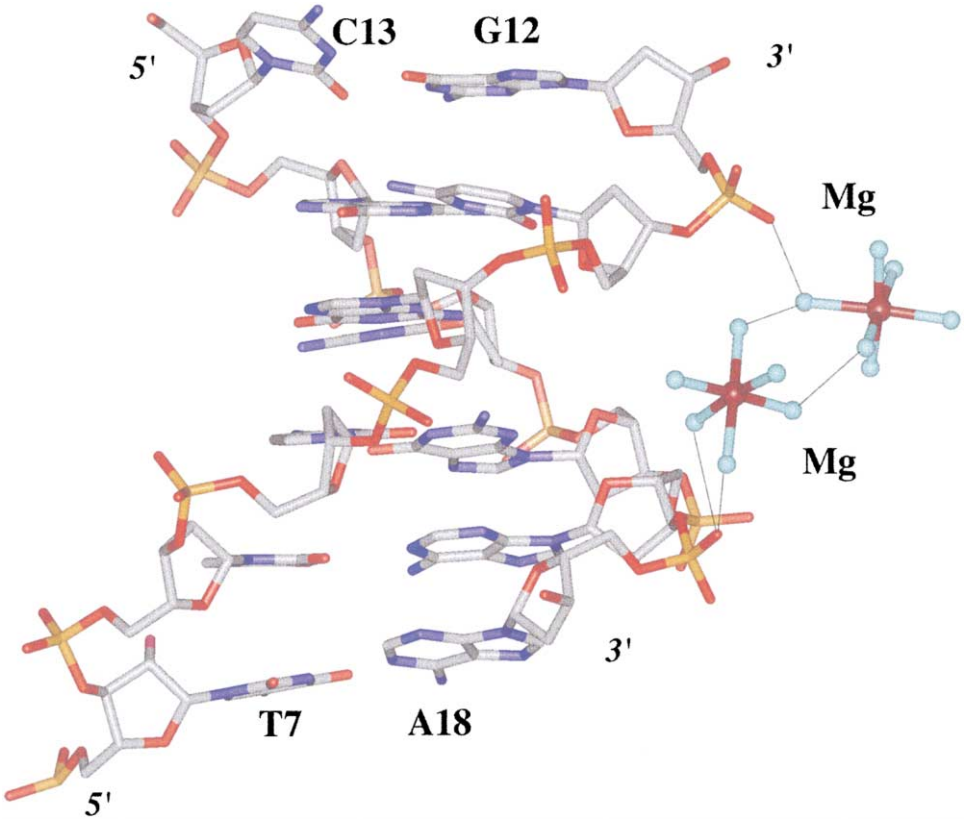

Figure 2. Bridging of Phosphate Groups by Two Mg^{2+} across the Minor Groove of the DDD [29]

zip up the central portion of the major groove. One of the ions is also engaged in a lattice contact and is shared by neighboring duplexes that face each other via their major grooves. Phosphate groups across the B-DNA major groove are too far apart to be bridged by a single hydrated ion and therefore metal ions bind deep inside that groove. However, with A form duplexes, we have to differentiate between ion binding at the floor of the major groove and a peripheral binding mode across the groove involving phosphate groups.

The minor groove of B form duplexes is more shallow compared with the major groove in the A form, and phosphate groups lie further apart across strands in the former. Nevertheless, in terms of the two above modes of ion coordination, inside the groove and at its periphery, the B-DNA minor groove resembles the A-DNA major groove. First crystallographic evidence for this emerged from the atomic resolution structures of the DDD Mg and Ca forms [3, 29]. In the Mg form, a tandem of Mg^{2+} ions links phosphate groups from opposite strands across the minor groove (Figure 2). Both are engaged in additional water-mediated contacts to DNA atoms from symmetry-related DDD duplexes. Except for the Mg^{2+} in the major groove that was discussed previously, all other Mg^{2+} ions in the DDD are stabilizing short contacts between phosphate groups from neighboring duplexes in the crystal [3]. Most interestingly, the site where the two ions cross the minor groove is characterized by a marked narrowing that amounts to about 1 Å relative to the average groove width of the DDD A tract [29]. Although the A tract minor groove is contracted relative to the rest of the DDD duplex, the Mg^{2+} ions bridge phosphates outside the AT-rich section. Quite a different picture emerges from the crystal structure of the Ca form. There, two Ca^{2+} ions are inserted into the groove near both ends of the duplex, leading to a local widening of the minor groove [29]. Therefore, these structures clearly demonstrated the plasticity of the minor groove as a function of divalent metal ions.

Earth alkali metal ions were subsequently also retrieved in the minor grooves of B-DNA decamer duplexes. Rees and coworkers found two fully hydrated Ca^{2+} ions in the structure of $[d(CCAGTACTGG)]_2$ determined to ultra high resolution [32] (Table 2). Interestingly, both ions occupy two closely spaced alternative positions and the disorder of ions is accompanied by shifts of phosphate groups in their vicinity. This observation provides additional evidence that binding of divalent metal ions and changes in the topology of the minor groove are coupled. Similar shifts in the positions of minor groove metal ions and correlated changes were also observed in the Mg and Ca forms of a decamer with closely related sequence [30]. Chiu and Dickerson assessed the influence of Mg^{2+} and Ca^{2+} on duplex structure for two related decamers in one and the same crystal lattice. For example, in the structure of the CCAGCGCTGG decamer, the alternative positions of three Mg^{2+} ions and associated altered backbone conformations go along with variations in the minor groove

width that amount to 1.5 Å. However, this phenomenon is absent in the crystal structure of the decamer with sequence CCAACGTTGG where two divalent ions are bound inside the minor groove of both the Mg and Ca forms. The sites of coordination for Mg^{2+} and Ca^{2+} are slightly shifted, but as in the above structures of the CCAGCGCTGG decamer, none of the ions exhibits contacts to phosphate groups mediated by inner-sphere water molecules.

In the crystal structures, divalent metal ions were found in the minor groove of GC-rich stretches. When they are near the periphery or, as observed in the structure of the DDD, when they bridge phosphates across the groove, the minor groove is narrower. However, divalent ions that reside near the floor of the groove appear to widen the minor groove (see for example [29]). In principle, this is consistent with the results of NMR studies in solution, revealing Mn^{2+} ions in the minor groove of the AT-rich portion of the $[d(CGTTTTAAAACG)]_2$ duplex [24]. Unlike A tracts that display narrow minor grooves (e.g., at the ApT step in the center of the DDD), the T_xA_x sequence features a relatively wide minor groove that can be penetrated by divalent metal ions. There is currently no data that would support appreciable entry of divalent metal cations into the minor groove near the ApT step of A tracts.

Monovalent Metal Ions

As in the preceding section on divalent metal ions and DNA, I will first focus on coordination of monovalent ions in the major grooves of A and B form duplexes and then finally on ion coordination in the minor grooves of these duplexes.

Crystal structures of the DDD at atomic resolution revealed a large number of water molecules in the major groove, with the solvent structure exhibiting regular patterns [29]. Due to the aforementioned difficulties regarding the determination of Na^+ ions in electron density maps even at high resolution, information about the coordination of alkali metal ions in the major groove initially remained limited. Even in the 0.89 Å crystal structure of GCGAATTCG that contains the same A tract as the DDD (Table 2), only a single disordered Na^+ was tentatively placed in the major groove [31]. To date, the most detailed insight on the coordination of monovalent metal ions in the B-DNA major groove comes from a study of the DDD cocrystallized with Tl^+ [34]. Both Fourier difference electron density maps and anomalous difference density maps were used to ferret out positions of Tl^+ ions (see Methods section). All of the 13 ions that were assigned to solvent peaks have occupancies that lie significantly below 50%. An analysis of the ion coordination modes in the major groove revealed a highly conserved pattern, either involving the O6 and N7 atoms of a single G or O6 atoms of adjacent guanines from opposite strands.

This preference for the G tract major groove by monovalent ions is consistent with the observations in MD simulations of the DDD and B-DNA duplexes with other sequences (Table 1). With simulations that focused on Na^+ coordination, most of the ions resided near G-C base pairs [8]. The locations of Na^+ ions were also assessed in simulations of the A and B form duplexes with the sequence A_5G_5-C_5T_5, containing adjacent A and G tracts [21]. The residence times of Na^+ ions near guanines were substantially longer compared with those for ions in the A tract. Moreover, the ions appeared more ordered in the major groove of the A form duplex than in the one of the B form duplex. This is hardly surprising given the depth and narrowness of the A-DNA major groove. These results are further corroborated by the MD simulation conducted for a B-DNA with sequence $(CG)_{12}$ in the presence of K^+ [22]. Cation binding was only observed in the major groove and O6 atoms of guanines were slightly more favored by K^+ ions than N7 atoms, and the base oxygen ranked about equally with phosphate oxygens directed into the groove.

The observation of an alkali metal ion binding site at the GpT step in the major groove of the A form duplex with sequence GCGTATACGC was mentioned above [33]. This site is actually shared by mono- and divalent metal ions. Thus, the ion positions are situated outside the plane defined by the guanine base. In that sense, the binding mode differs from the coplanar orientation preferred by the majority of Tl^+ ions in the DDD major groove. We took the presence of Cs^+, Rb^+, and K^+ at a single site as strong evidence that a Na^+ was also bound there in the corresponding Na form of the A form duplex. Moreover, the protonated terminal amino nitrogen of spermine was observed in close vicinity of the metal ion binding site. It is likely that an ammonium ion could occupy the same spot as the metal ions in the major groove of the A-DNA. Overall, the findings of theoretical and X-ray crystallographic investigations are in agreement and hint at a clear preference for G tract coordination by the oxophilic alkali metal ions.

Partly due to the possible consequences for groove topology (narrowing) and DNA conformation (bending at A tracts), the coordination of alkali metal ions in the minor groove of B-DNA has been examined in particularly close detail. In addition, it was the proposal that Na^+ ions could penetrate the minor groove of the DDD that initially stimulated the current interest in DNA-cation interactions [2]. Following these findings based on MD simulations and using a 1.4 Å resolution structure of the DDD crystallized in the presence of Na^+, Mg^{2+}, and spermine, Williams and coworkers postulated a "spine of water on sodium" [37]. The skepticism with which this claim was met did not stem so much from doubts whether Na^+ could enter the minor groove at all as from the way their experiment had been carried out [3, 7]. A more detailed account of the potential pitfalls encountered with inspections of solvent regions in Fourier electron density maps for the presence of Na^+ or K^+ can be found in [7]. Suffice it to say that it is basically impossible to locate Na^+ ions with tetrahedral coordination geometry and partial occupancies in electron density maps of virtually any resolution without resorting to metal ions mimicking Na^+ in combination with anomalous scattering techniques [33].

Clear evidence for the presence of a single alkali metal ion bound in the minor groove of the DDD was first provided by the structure of the dodecamer crystallized in the presence of Rb^+ [26]. The Rb^+ ion sits in the center of the minor groove at the ApT step and is engaged in

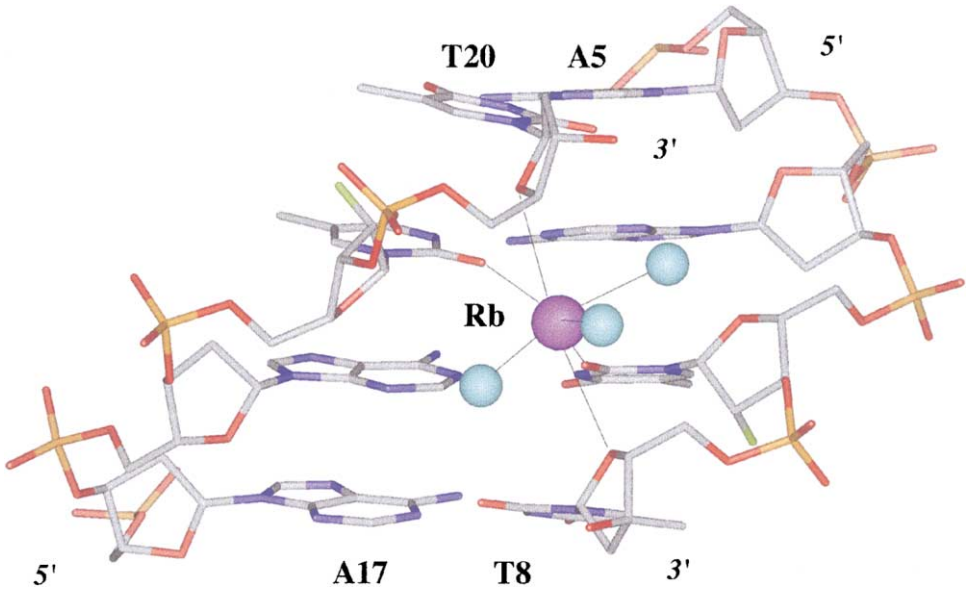

Figure 3. Coordination of Rb^+ at the Central ApT Step in the DDD [26]

inner sphere coordination to O2 atoms of thymines from opposite strands (Figure 3). The occupancy of the site was estimated to be on the order of 50% based on the crystallographic refinement parameters and no other ion was observed in the minor or major grooves. These results were considered flawed by some because the DDD that had been used in the structure determination carried a single 2′-F-arabinofuranosyl thymine (2′F-ANA) at position 7 [43]. Accordingly, the presence of fluorine will result in a local alteration of the electronic properties of the minor groove, thus preventing cation coordination. Two observations argue strongly against such a reasoning. Firstly, the fluorine is directed into the major groove and cannot therefore sterically hinder ion binding [36]. Secondly, RNase H cleaves the RNA strand of hybrid duplexes between RNA and fully modified 2′F-ANA strands with an efficiency that approaches the degradation of the corresponding wt-DNA/RNA hybrids [44]. RNase H probes the minor groove of DNA/RNA hybrids and is known to be exquisitely sensitive to any changes in the steric or electronic nature of the minor groove. The facts that substrate recognition and processivity of RNase H are virtually unaffected when the DNA in the hybrid duplex is substituted by a 2′F-ANA oligomer render a drastic change in the electrostatic potential of the minor groove by a single fluorine very unlikely. However, the best evidence for the correctness of the crystallographic results regarding Rb^+ coordination comes from subsequent structures of the Cs- and Tl-forms of the DDD [27, 34]. Both studies essentially confirm the preferred coordination of a monovalent metal ion at the central ApT step in the DDD. Incidentally, this ion binding mode in the DDD minor groove is identical to the one found over 25 years ago for a Na^+ bound to the $[r(AU)]_2$ mini-duplex [9].

Thus far, MD simulations [2], solution NMR [24], and the above crystallographic results are all in agreement with respect to the direct coordination of monovalent ions at the ApT step in A tract DNA. However, theoretical approaches and NMR draw a somewhat more complex picture of the distribution of ions in the minor groove and the consequences for groove topology. Several closely spaced Na^+ coordination sites appear to be present in the groove based on recent NMR solution work [45]. These findings are in line with the results of earlier NMR experiments that provided direct evidence for preferential binding of multiple Na^+ ions in the minor groove of A tract DNA [23] as well as minor groove monovalent cation (NH_4^+) binding to three different B-DNA duplexes [24]. Furthermore, the latter work is the only study to date that shows a difference in the locations in the minor groove of monovalent cations on DNA duplexes containing runs of A's which are known to exhibit macroscopic bending versus ones that don't. MD simulations consistently lead to recovery of several ion binding sites as well [20, 21]. For the DDD, these sites are (not quite unexpectedly) not limited to the positions occupied by hydration spine water molecules. Interestingly, the MD results suggest that entry of ions into the minor groove is accompanied by groove narrowing [20, 21]. The location of Na^+ within the groove does apparently not matter in this regard, and binding near the periphery along with contacts to phosphate oxygens was observed to narrow the groove as well [20]. However, no dependence of the groove width on the nature of the alkali metal ion was found in the crystal structures determined to date. In this respect, the consequences of binding by monovalent ions in the B-DNA minor groove seem to differ considerably from those seen in the case of divalent metal ions. It should be added that the relatively insignificant effects of monovalent cations on the structure of double helices cannot be taken as evidence that such ions are unimportant for nucleic acid systems with more complex folds, as was correctly pointed out by Chiu and Dickerson [30].

Information based on experimental data concerning

the locations of cations in the minor groove of A form duplexes is much more sparse by comparison. Rb^+ and Cs^+ were observed to bind at the periphery of the minor groove in crystal structures of an A form decamer duplex with sequence GCGTATACGC [33]. The ions are engaged in a water-mediated contact to a N3 of guanine at the GpT step and make an inner-sphere contact to O4′ of thymine. Both ions are coordinated to two further duplexes via phosphate groups and terminal hydroxyl groups.

Prologue and Epilogue

Theoretical and experimental efforts directed at a better understanding of the interactions between cations and DNA duplexes have furnished several principles regarding the preferred binding sites of mono- and divalent metal ions in the major and minor grooves. (i) Mg^{2+} and Ca^{2+} bind at GpN steps in the major grooves of A- and B-DNA. The preference for guanine in the major groove is also consistent with Mg^{2+} binding at GpG or GpU steps and to single guanines in recent crystal structures of RNA pseudoknots at atomic resolution [46, 47]. (ii) Mg^{2+} and Ca^{2+} bind to G tracts or at the G/A tract interface in the B-DNA minor groove. Binding of ions either inside the groove to base edges or at its periphery via phosphate groups results in changes of the groove width. Solution NMR provides evidence for binding of Mn^{2+} in the minor groove of 5′-T_xA_x-3′ stretches with B form geometry. (iii) Monovalent metal ions exhibit a strong preference for G in the major groove of B-DNA and were observed at a GpT step in A-DNA. MD simulations suggest that groove topology is altered by ion binding. In crystal structures of the DDD, the shape of the major groove does not seem to depend on the nature of the alkali metal ion (Tl^+ included) present in the lattice. (iv) In the B-DNA minor groove, alkali metal ions coordinate to thymines from opposite strands at the central ApT step of A tracts. MD simulations and solution NMR indicate multiple binding sites in the minor groove as well as narrowing as a result of ion binding either inside the groove or at its periphery. X-ray crystal structures are more supportive of the uniqueness of the ApT steps in terms of ion binding.

While much has been learned about DNA-cation interactions, it is clear that the progress concerns only structured ions. NMR and X-ray crystallography provide no information about delocalized ions. These are often important for stabilization and structure of nucleic acids, particularly in systems exhibiting more complex tertiary and quaternary structures [8, 48–50]. Despite a general trend—ions bind in the major groove of DNA G tracts and the minor groove of DNA A tracts—a quantitative correlation between ion binding and changes in duplex conformation (bending) and groove shape (narrower or wider) remains elusive. By comparison, in the case of a 58-nucleotide ribosomal RNA fragment, the role of monovalent cations in the stabilization of tertiary structure was assessed in significant detail [51]. Thermodynamic experiments pointed to a crucial contribution by a single K^+ and Draper and coworkers were subsequently able to identify this ion in a crystal structure [52].

Currently, X-ray crystallography (focusing mainly on the DDD) on the one hand and solution NMR as well as MD on the other provide a somewhat incoherent picture of the interactions between alkali metal ions and the A tract minor groove. All MD simulations were conducted for duplexes "in solution." A more meaningful comparison between the duplex models from crystal structures and simulations might be achieved by performing MD simulations of the DDD in the crystal lattice and then examining the trajectories of ions and potential alterations of the groove topology. It is important to bear in mind that the electrostatics of a duplex in a crystal are affected by neighboring molecules and therefore are quite different from the situation in solution. In crystal structures, ion binding to A tracts was exclusively studied with the DDD. The DDD has been a tremendously useful model system for DNA structure over the years. However, we also need to recognize that a thorough understanding of ion coordination in A tracts based on crystallography may require other sequences, i.e., oligonucleotides with the AATT portion imbedded in an alternative sequence [53] or with longer A tracts [54]. In terms of the application of anomalous diffraction methods for retrieving metal ions in DNA crystals, it is noteworthy that a limitation of such approaches arises from the relatively narrow range of wavelength tunability of most synchrotron beamlines dedicated to crystallography. Thus, a number of metals cannot be studied efficiently as they don't have an absorption edge at an accessible wavelength. However, this limitation can be passed over with the application of soft X-rays [55, 56].

Sequence–specific binding of ions appears to contribute significantly to the heterogeneity of DNA structure. While the consequences of ion coordination for the geometry of the duplex grooves may previously have been underestimated, it would obviously be wrong to treat the interactions between ions and DNA as the major determinant of DNA structure. Thus, binding to alkali and earth alkali metal ions cannot have been the driving force in the evolution of DNA as the genetic material and the choice of phosphate as the backbone linkage [57]. Moreover, helicality and thus groove topology are largely affected by the nature of the sugar moiety in the backbone [58]. Etiological studies of nucleic acid structure have revealed that several analogs with alternative sugars form stable duplexes [59], some of them pairing efficiently with DNA and RNA [60].

Clearly, an interplay of forces is at work in mediating nucleic acid structure as well as why nucleic acids (DNA and RNA) have evolved as life's genetic material. The study of DNA-cation interactions via molecular modeling, NMR, and X-ray crystallography has furnished additional insights on the effect of environment on nucleic acid structure and function.

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