A "Hydrat-Ion" Spine in a B-DNA Minor Groove

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Abstract: The minor groove hydration spine is a key feature of the crystal structure of the B-DNA dodecamer duplex $[d(CGCGAATTCGCG)]_2$. At the floor of the groove, water molecules bridge bases from opposite strands by hydrogen bonding to N3 and O2 atoms of adenine and thymine, respectively. However, the interpretation that the series of electron density peaks lining the groove represents indeed water molecules, while generally agreed upon, remains an assumption. The limited resolutions of dodecamer crystal structures have thus far made it impossible to reliably distinguish between water and monovalent metal cations, such as Na^+, normally present in the crystallization buffer. Using X-ray diffraction data to near-atomic resolution of dodecamer crystals grown in the presence of either Rb^+ or Cs^+ cacodylate, we have tested the possibility of alkali metal ion coordination in the minor groove. The structural data are consistent with a single Rb^+ intruding the hydration spine at the central ApT step. The ion has partial occupancy and replaces the water molecule that links the keto oxygens of thymines from opposite strands. The observed dimensions of the binding site suggest preferred binding of Rb^+ or K^+, while Na^+ or Cs^+ may be prevented from binding stably. Therefore, 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. In addition to allowing a distinction between water and alkali metal ions, the high-resolution crystal structures provide a more complete picture of the minor groove water structure: four fused water hexagons dissect the central portion of the minor groove, with the inner corners of the hexagons coinciding with the original spine water positions. Thus, it may be more appropriate to refer to this arrangement as a ribbon of hydration instead of a spine of hydration.

Introduction

The first oligonucleotide for which a single-crystal structure was determined, the Dickerson−Drew DNA dodecamer CGCGAATTCGCG, has remained the focus of biophysical studies to the present day.[1−4] Intriguing features of this B-form double helix are the narrowing of the minor groove in the central A-tract and ordered water molecules that line this groove, referred to as a spine of hydration.[5,6] Five first-shell water molecules form hydrogen bonds to acceptor atoms of bases and deoxyribose sugars across the two strands. Further hydrogen bonds then exist between these inner water molecules and six second-shell waters, giving the spine its characteristic zigzag shape.

Based on MD simulations, it was suggested that sodium ions could intrude into the spine of hydration and remain there with fractional occupancies.[7,8] Moreover, an NMR solution study of double-stranded DNA provided evidence for the location of Mn^{2+} ions in the A-tract region of the minor groove.[9] The resolutions of many crystal structures of B-DNA dodecamers were limited to around 2.2 Å, precluding a reliable determination of the location of metal ions coordinated to the DNA. However, more recent structures of Dickerson−Drew type dodecamers display markedly higher resolutions of up to 1.4 Å,[10,11] and an atomic resolution structure of this dodecamer has revealed five Mg^{2+} ions per crystallographic asymmetric unit, two of them located at the periphery of the minor groove.[12] Despite many arguments in support of ion coordination in the minor groove, such as the existence of electronegative pockets[7,8] as well as the coordination modes and low *B*-factors of spine waters,[10,12] the proposition that sodium ions can invade the minor groove hydration spine presently lacks experimental proof.

Here, we present high-resolution crystal structures of the Dickerson−Drew dodecamer duplex and demonstrate that, while an alkali metal ion can, indeed, coordinate to bases in the minor groove, invasion of the hydration spine seems to occur only at one location and with partial occupancy.

Materials and Methods

The DNA dodecamer was synthesized and purified using previously described procedures.[11] The Rb^+-form crystals of the dodecamer duplex were grown by the sitting drop vapor diffusion method (see Table 1

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Table 1. Crystal Data and Refinement Parameters

	type 1 Rb^+-form crystal			type 2 Rb^+-form crystal		
Crystallization						
DNA (mM)	1.2			1.2		
$Mg(OAc)_2$ (mM)	25.0			6.2		
spermine (mM)	3.2			1.6		
rubidium cacodylate buffer	20 mM, pH 6.9			20 mM, pH 6.9		
MPD (%)	40			40		
Crystal Data						
space group	$P2_12_12_1$			$P2_12_12_1$		
a (Å)	25.11			25.07		
b (Å)	39.73			39.72		
c (Å)	65.83			65.87		
Data Collection and Refinement Statistics						
X-ray source/detector	Synchrotron-APS/MARCCD			Rigaku RU-200/R-axis IIc		
temperature (K)	120			120		
resolution (Å)	1.2			1.5		
R-merge[a]	0.068			0.084		
resolution range (Å)	*N*(unique)	%complete	*R*-factor[b]	*N*(unique)	%complete	*R*-factor[b]
20−3.0	1 462	98.2	0.190	1 484	99.9	0.186
3.0−2.5	1 016	99.9	0.178	1 013	100.0	0.177
2.5−2.0	2 276	99.5	0.167	2 278	99.9	0.170
2.0−1.8	1 676	98.5	0.163	1 699	99.7	0.166
1.8−1.6	2 641	98.4	0.151	2 617	98.4	0.159
1.6−1.4	4 274	97.7	0.170	1 722[c]	94.8	0.204
1.4−1.2	7 317	94.8	0.216			
all data	20 662	97.1	0.178	10 813	98.8	0.178
	occupancy = 1	partial occupancy		occupancy = 1	partial occupancy	
no. of waters	135	54		93	71	
no. of ions	1 Mg^{2+}	2 Mg^{2+}, 1 Rb^+		1 Mg^{2+}	1 Mg^{2+}, 1Rb^+	
rms distances (Å)	0.008			0.006		
rms angles (deg)	1.79			1.49		

[a] $R_{merge} = \sum_{hkl}\sum_i|I(hkl)_i - \langle I(hkl)\rangle|/\sum_{hkl}\sum_i\langle I(hkl)_i\rangle$. [b] *R*-factor $= \sum_{hkl}|F(hkl)_o - F(hkl)_c|/\sum_{hkl}F(hkl)_o$. [c] To a resolution of 1.5 Å.

for details). Crystals of the dodecamer in the presence of cesium cacodylate and different Mg^{2+} concentrations were grown in a similar fashion.

For data collection, crystals were picked up with a nylon loop and transferred into a cold nitrogen stream (120 K). Diffraction data for a type 1 crystal were collected on the insertion device beamline of the Dupont−Northwestern−Dow Consortium Access Team (DND-CAT) at the Advanced Photon Source, Argonne, IL, using a Mar-Research MARCCD detector. Diffraction data for a type 2 crystal were collected in-house, using an R-axis IIc image plate mounted on a Rigaku RU-200 rotating anode generator. All data were processed and scaled in the DENZO/SCALEPACK program suite[13] (Table 1).

The structures were refined with the program SHELX-97,[14] using the 1.1-Å structure of the dodecamer duplex[12] as an initial model. All DNA atoms, ions, and fully occupied water molecules were treated anisotropically. The R_{free} values obtained using 10% data subsets[15] were 23.2% (*R*-factor 19.1%) and 22.5% (*R*-factor 20.1%) in the case of the Rb^+-form type 1 and type 2 structures, respectively. All data were used for the final cycles (no σ cutoff), and final *R*-factors and rms deviations from standard parameters[16] are listed in Table 1. A sum electron density map around a portion of the molecule is depicted in Figure 1. Final coordinates and structure factors have been deposited in the Nucleic Acid Database (NDB entry codes BD0012 (type 1) and BD0013 (type2)).

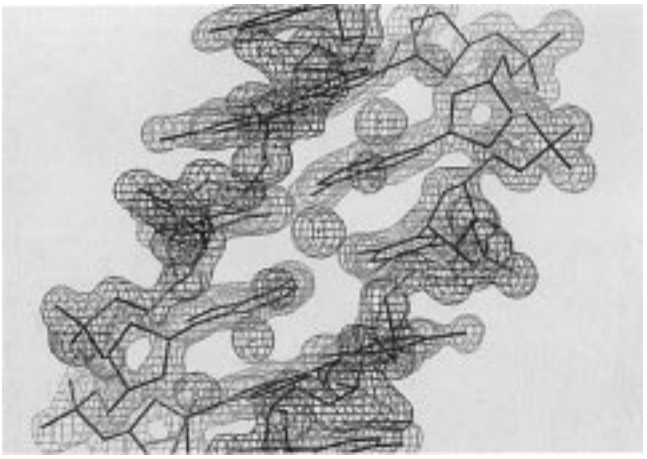

Figure 1. Final $(2F_o - F_c)$ sum electron density drawn at the 1.5σ level around the central section of the type 1 $[d(CGCGAATTCGCG)]_2$ duplex. The view is into the minor groove, and portions closer to the viewer appear darker. The central peak in the minor groove represents the Rb^+ ion.

Results and Discussion

Determination of Ion Binding Sites. To facilitate detection of alkali metal ions in electron density maps, we crystallized the Dickerson−Drew dodecamer from solutions that were buffered with either rubidium or cesium cacodylate. Crystals were grown at both high (termed type 1) and low (termed type 2) concentrations of magnesium acetate (Table 1). Type 1 crystals diffract to higher resolution than type 2 ones. X-ray diffraction data for type 1 crystals of the Rb^+- and Cs^+-forms were collected to a maximal resolution of 1.2 Å using synchrotron radiation. Data for type 2 crystals of both forms were collected to 1.5-Å resolution on an in-house rotating anode generator source (Table 1).

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Initially, all regions of superposed sum and difference electron density surrounding the DNA were treated as water molecules. After conventional positional and *B*-factor refinement, four criteria were applied to identify Rb^+ or Cs^+ ions among water molecules: (1) presence of sufficient difference electron density at a water position, (2) valence of a water molecule close to 1, (3) unusually low *B*-factor of a water molecule compared with those in its vicinity, and (4) changes in the distances between water molecules compared with the reference structure. To establish such changes, the 1.1-Å resolution structure of the dodecamer duplex crystallized in the presence of sodium cacodylate and magnesium acetate served as the reference (Nucleic Acid Database entry code BD0007).[12] The concentrations of mono- and divalent metal cations used for growing the reference and the type 1 Rb^+- and Cs^+-form crystals were identical (Table 1). To grow the type 2 Rb^+- and Cs^+-form crystals, the Mg^{2+} concentration was reduced 4 times.

All water molecules were screened in the above fashion. Figure 2 depicts sum and difference electron densities at several regions in the minor groove of the type 1 Rb^+-form and reference structures as well as a shift of a water molecule toward the presumed site of ion coordination in the former structure. Table 2 lists *B*-factor and valence data for minor groove water molecules forming the inner spine of hydration in the type 1 Rb^+-form and reference structures.

Several water molecules displayed a low *B*-factor and valences of around 1. For example, in the type 1 Rb^+-form crystal, water molecules W_4, W_6, and W_8 have reasonably low *B*-factors, and their valences exceed 0.9 (Table 2). However, buildup of difference electron density was observed only around W_4 (Figure 2c,d), consistent with the finding that W_4 is the strongest solvent peak (followed by W_2 and W_8). Another feature that distinguishes W_4 from all others is the 1.4-Å shift toward W_4 by $W_{4'}$ (relative to the reference structure, Figure 2a−c). W_8 has a relatively short average coordination distance of 2.8 Å (Table 2, 3.1 Å for W_4). In particular, one might consider its close contact to O4′ of residue A18 (3.0 Å; the average distance to O4′ atoms for W_4 is 3.4 Å) as an indication of ion coordination. However, comparison with the reference structure reveals that the minor groove is narrower in that region due to Mg^{2+} coordination at its periphery.[12] Thus, W_8 in the reference structure also displays a 2.9-Å distance to O4′ of A18. While the valence value of W_8 could support ion coordination, the lack of difference density and a conserved location of the adjacent water in the outer spine (ca. 5 Å from W_8 in both the reference and type 1 Rb^+-form structures) argue against Rb^+ ion coordination at its site. Thus, both the type 1 and type 2 Rb^+-form structures revealed one bound ion per DNA duplex, while no ordered Cs^+ ions were detected in either the type 1 or type 2 crystal structures.

We believe that our criteria are sufficiently stringent to differentiate between water and Rb^+ ions with significant occupancy. If the occupancy falls below 20%, the resulting levels of difference electron density will become very weak, and the ion will most likely evade detection. At least within the range tested in our experiments, the Mg^{2+} concentration in the crystallizations appears to only marginally affect Rb^+ ion coordination.

Rubidium Ion Coordination. The Rb^+ ion is located in the center of the minor groove and is coordinated to O2 oxygen atoms of thymidines 7 and 19 (Figures 2 and 3). The refinement results indicate that its occupancy is only fractional, ca. 0.5 in both crystal types, with *B*-factors of 24 (type 1) and 29 $Å^2$ (type 2). However, the existence of considerable difference electron densities even at the 5σ level at this site as well as a shift of more than 1.4 Å by a higher shell water toward the putative metal ion support the interpretation that this is, indeed, a partially occupied ion and not a fully occupied water (Figure 2).

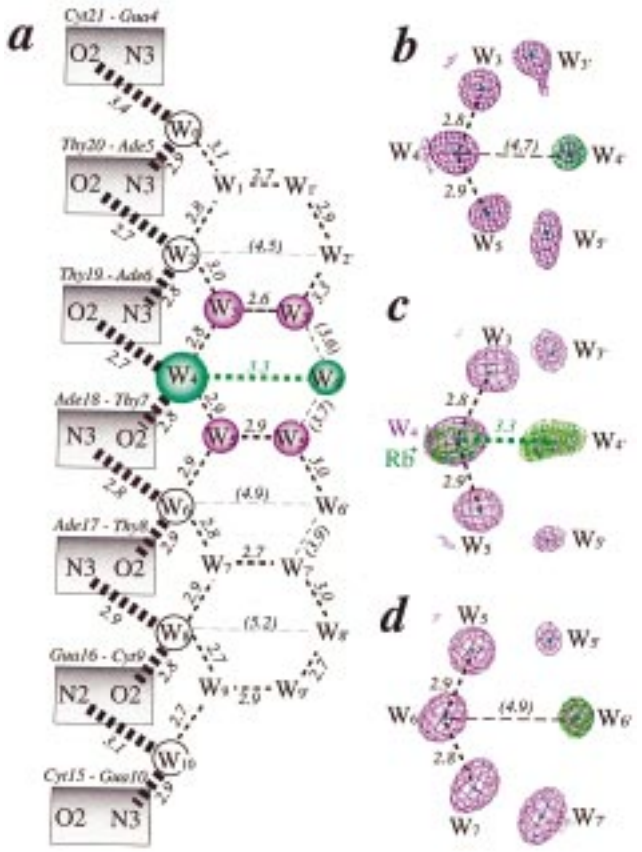

Figure 2. (a) Schematic drawing of DNA bases and hydration in the minor groove of the type 1 Rb^+-form crystal structure. The inner (W_0 to W_{10}) and outer ($W_{1'}$ to $W_{9'}$) hydration spines define four fused water hexagons. Sum ($2F_o - F_c$, 1.2σ, red) and difference ($F_o - F_c$, 2.5σ, green) electron densities in the vicinity of the spine water molecule W_4 coordinated to the exocyclic O2 atoms of residues T7 and T19 (situated on the left-hand side, see panel a) in (b) the native 1.1-Å structure,[12] and (c) the type 1 Rb^+-form structure. (d) Sum ($2F_o - F_c$, 1.2σ, red) and difference ($F_o - F_c$, 2.5σ, green) electron densities in the vicinity of the spine water molecule W_6 in the type 1 Rb^+-form structure. The electron density color code matches that in panel a. The buildup of substantial difference electron density around W_4 visible in panel c has been taken as an indication of Rb^+ ion coordination, and the shift of $W_{4'}$ toward the metal cation is apparent. To calculate the maps, the electron density peak near the O2 atoms of the two thymidines was treated as a water molecule, and $W_{4'}$ (green) in the outer spine was omitted. The drawing shown in panel d was generated in a similar way.

The observed binding site in the minor groove is unique as it is the only location that allows a water or metal ion to bridge the two strands through coordination to two pyrimidine keto oxygens. Further contacts exist to three water molecules (W_3, W_5, and $W_{4'}$) and, with slighly longer distances, to O4′ atoms of residues T8 and C20 (Figure 3, Table 2). Thus, the Rb^+ coordination sphere comprises seven oxygen atoms, a surrounding that is very suitable in view of the weak acidity of alkali metal cations.[17] Since Na^+ is also highly oxophilic, one would expect it to prefer a similar coordination environment within the narrow B-DNA minor groove.

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Table 2. Coordination, Valences, *B*-Factors, and Distance Data for Inner Spine Water Molecules in the Type 1 Rb^+-Form and Reference Structures[12]

Type 1 Rb^+-Form

water	peak height[a]	*B*-factor (Å^2)	av distance to O only/all (Å)	no. of O/N ligands	valence[b] (Rb^+)	distance to: (Å) O4′[c]	O4′	O3′[c]	O3′
W_0	1.97	40	3.1/3.0	4/1	0.5/0.6	A6 3.1			
W_1	1.88	25	3.2/3.2	5/0	0.6/0.6	A6 3.5	C21 3.9		
W_2	3.79	20	3.1/3.1	5/1	0.7/0.9	T7 3.5	C21 3.6		
W_3	2.02	28	3.2/3.2	5/0	0.7/0.7		C20 3.8		
W_4/Rb^+	5.00	14/24[d]	3.1/3.1	7/0	0.9/0.9	T8 3.5	C20 3.3		
W_5	2.37	25	3.3/3.3	6/0	0.6/0.6	T8 3.9	T19 3.8		
W_6	2.97	19	3.0/2.9	5/1	0.8/0.9	C9 3.1	T19 3.3		
W_7	3.27	20	3.3/3.3	7/0	0.8/0.8	C9 3.5	A18 3.5	C9 3.7	A18 3.8
W_8	3.72	19	2.8/2.8	4/1	0.9/1.1		A18 3.0		
W_9	3.36	22	3.0/3.0	5/0	1.0/1.0	G10 3.9			
W10	2.87	20	3.3/3.2	6/1	0.7/0.8	G10 3.3	G24* 3.8[e]	C11 2.8	
average[f]	2.00	34	3.2	4.5	0.7				
SD	0.90	9	0.2	1.5	0.4				

Native $[d(CGCGAATTCGCG)]_2$

water	peak height[a]	*B*-factor (Å^2)	av distance to O only/all (Å)	no. of O/N ligands	valence[b] (Na^+)	distance to: (Å) O4′[c]	O4′	O3′[c]	O3′
W_0	1.52	32	3.4/3.3	7/1	0.4/0.4	A6 3.2			
W_1	1.83	24	3.2/3.2	4/0	0.3/0.3	T6 3.5			
W_2	4.05	18	3.1/3.1	5/1	0.4/0.4	A7 3.6	C21 3.7		
W_3	2.69	27	3.3/3.3	5/0	0.3/0.3	T8 4.0	C20 3.9		
W_4	3.63	16	3.0/3.0	6/0	0.5/0.5	T8 3.5	C20 3.3		
W_5	3.01	19	3.1/3.1	4/0	0.3/0.3	T8 3.9			
W_6	4.36	14	3.0/2.9	5/1	0.4/0.5	C9 3.0	T19 3.3		
W_7	4.41	14	3.2/3.2	7/0	0.4/0.4	C9 3.5	A18 3.5	C9 3.7	A18 3.7
W_8	4.78	13	2.8/2.8	4/1	0.4/0.5		A18 2.9		
W_9	4.25	16	2.9/2.9	5/0	0.5/0.5	G10 3.7			
W_{10}	4.26	14	3.1/3.1	5/1	0.4/0.4	G10 3.3	G24* 3.7[e]	C11 2.8	
average[f]	2.30	29	3.2	5.3	0.4				
s.d.	1.00	10	0.2	1.5	0.1				

[a] In σ units. [b] Using the parameters given in refs 35 and 36 and a cutoff radius of 4 Å for the coordination sphere. [c] Residue and distance, first contact to strand 1, second one to strand 2. [d] The first number refers to the water molecule, the second one to the ion. [e] The asterisk designates a symmetry-related residue. [f] For all water molecules.

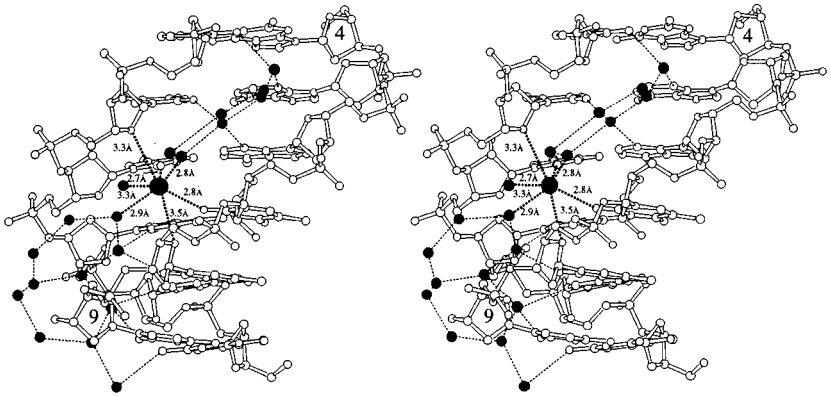

Figure 3. Stereodiagram of the water structure and Rb^+ ion coordination in the central portion of the minor groove in the type 1 Rb^+-form crystal of the Dickerson−Drew dodecamer. Six base pairs of the DNA are drawn with open bonds, and the outer residues of one strand are numbered. Water molecules forming the inner and outer spines are shown as filled circles, the Rb^+ ion is drawn as a filled circle with larger radius, and hydrogen bonds are shown as dashed lines. The coordination geometry of the Rb^+ ion is highlighted by thick dashed lines, and distances to the contacted O2 and O4′ atoms and water molecules are indicated.

The Rb^+ ion has chosen a spot of high electronegative potential.[18,19] By comparison, the neighboring first-shell water molecules that are part of the spine bridge N3 and O2 atoms of adenosines and thymidines, respectively, across the groove (Figure 2). It is noteworthy that molecular dynamics simulations showed preferred coordination of a Na^+ ion to precisely that site.[7,8] Moreover, the hydrated Na^+ ion observed in the crystal structure of an $[(ApU)]_2$ RNA miniduplex also contacts both O2 oxygen atoms from uridines across the minor groove.[20] Mg^{2+} coordination at the periphery of the minor groove has been shown to reduce its width by up to 1 Å.[12] Conversely, the presence of the partially occupied Rb^+ ion in the center of the

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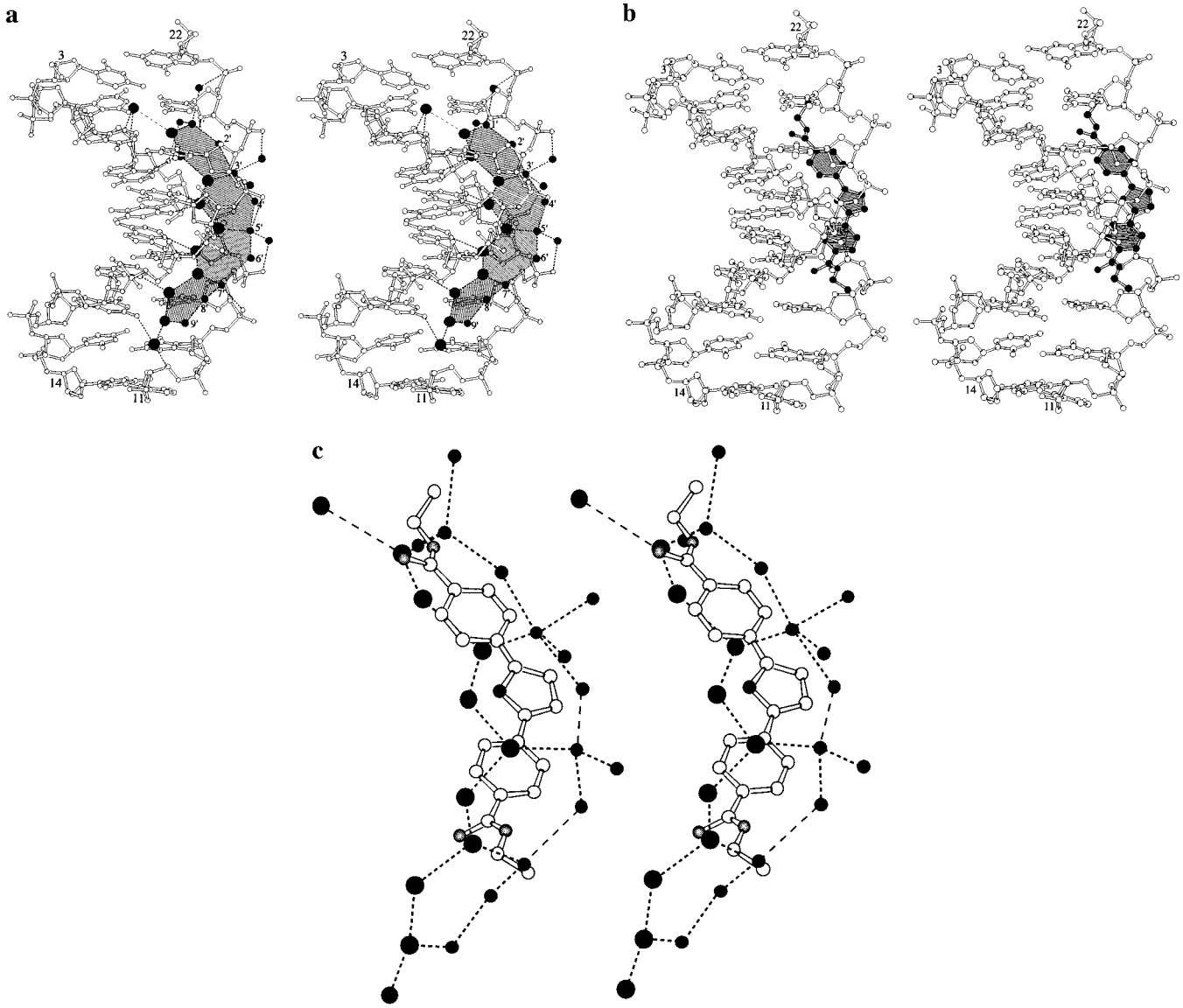

Figure 4. Stereodiagrams depicting the arrangements of water molecules and the drug furamidine[28] in the minor groove of the [d(CGCGAATTCGCG)]$_2$ duplex. (a) The four fused water hexagons defining an inner and an outer spine in the reference structure.[12] Only 10 DNA base pairs are shown, and the planes defined by the water molecules are shaded in gray. (b) Furamidine binding in the minor groove. The orientation of the duplex is the same as in (a), and the planes defined by the phenyl amidinium and furan rings of the drug are shaded in gray. (c) Similarity in the arrangements of the minor groove water ribbon and furamidine, as revealed by the superposition of DNA atoms. Water molecules are drawn as filled circles with large radii, furamidine is drawn with open bonds, oxygen and nitrogen atoms are black and gray, respectively, and hydrogen bonds between water molecules are dashed.

minor groove appears not to affect width, since the dimensions of the groove are the same in the reference and the type 1 Rb^+-form structures.

The existence of just a single alkali metal ion coordination site in the Rb^+-form crystal structures argues against a view that ions can invade the minor groove hydration spine along its entire length or the existence of a mixed water ion spine with partially ordered ions residing at several locations previously occupied by first-shell waters only.[10] Further, the absence of a Cs^+ ion at this site may be taken as evidence that its radius affects the tendency of an alkali metal ion to penetrate the ordered water structure in the minor groove of the Dickerson–Drew dodecamer duplex. Thus, Rb^+, with an ionic radius of ca. 1.5 Å[21] and a typical $M^+\cdots O$ coordination distance of 2.8 Å,[22] may show preferred binding over other alkali metal cations.

It is possible that Cs^+ is too large to reach the floor of the minor groove at this site or may do so only with very low occupancy, not resulting in any detectable difference electron density. By comparison, one may conclude that K^+ is a good candidate for binding (ionic radius ca. 1.3 Å, $M^+\cdots O$ coordination distance 2.7 Å), while the smaller Na^+ ion may be a less optimal candidate. The lengths of hydrogen bonds formed by the spine water that is located at the Rb^+ coordination site in the 1.1-Å structure of a crystal grown from a sodium cacodylate-containing solution are all between 0.2 and 0.5 Å longer[12] than the preferred Na^+ coordination distance of around 2.4 Å.

Hydration. Most earlier studies dealing with the minor groove hydration pattern in the [d(CGCGAATTCGCG)]$_2$ duplex relied on structural data with an average resolution of around 2.2 Å. The reference structure[12] and the type 1 Rb^+-form

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structure are based on diffraction data that are almost 100% complete to 1.1 and 1.2 Å, respectively. The improvement in resolution revealed higher shell water molecules around the DNA and provides a more complete picture of the arrangements of water molecules located in both grooves.

The hydration spine in its original form was composed of first- and second-shell water molecules in the minor groove.[6] In Figure 2, the original spine corresponds to water molecules W_0 to W_{10}. We refer to this array of water molecules as the "inner spine". In the high-resolution structures, a second spine runs parallel to the inner one at the periphery of the minor groove. We refer to water molecules $W_{1'}$ to $W_{9'}$ as the "outer spine". The outer spine water molecules $W_{2'}$, $W_{4'}$, $W_{6'}$, and $W_{8'}$ define a third hydration shell. Waters $W_{1'}$ and $W_{3'}$ belong to the second shell since single water molecules bridge them to phosphate oxygens O1P from both backbones (Figure 4a). Waters $W_{5'}$ and $W_{7'}$ are slightly displaced from the middle of the minor groove. Thus, they form a direct hydrogen bond to a phosphate group from one strand and are linked to others from the opposite one via single water molecules (Figure 4a). This arrangement results from a narrowing of the groove at that site, caused by two coordinated Mg^{2+} ions that bridge phosphates across the groove.[12] The zigzag-shaped original spine now has the appearance of a ribbon composed of four fused hexagons that dissect the minor groove along the central hexamer portion of the Dickerson−Drew dodecamer duplex (Figures 2 and 4a).

The structures of the hexagons forming this ribbon of hydration are all more or less planar. Hexagonal water clusters are expected to adopt three-dimensional geometries.[23] The planar or "cyclic" arrangement represents the highest-energy conformation among five low-energy structures of water hexagons, as investigated by vibration−rotation tunneling spectroscopy and quantum Monte Carlo simulations.[24] Water pentagons are frequently encountered in both DNA[25] and RNA,[26] and while water hexagons have been reported in peptide structures (for example, ref 27), their conformations usually do not conform to a planar ring but are boat- or chairlike. The water structure in the minor groove is unique among oligonucleotide structures. The planarity of the water hexagons is most likely the consequence of the narrowness of the groove and the spatial constraints provided by the DNA backbone.

The high-resolution structures of the Dickerson−Drew dodecamer duplex reveal a fascinating mimicry of the arrangement of water molecules by minor groove binding drugs. This is illustrated in Figure 4 for the drug furamidine.[28] Similar to the original spine and minor groove binding drugs,[29] the more extended hydration ribbon follows the minor groove in an isohelical manner.[30] Binding of the drug presumably causes disruption or rearrangement of the entire water ribbon. The individual phenyl amidinium and furan rings of the drug molecule each superpose nicely on a water hexagon (Figure 4c). In the absence of a minor groove binding molecule, the O4′ atoms of deoxyriboses stabilize the inner spine of hydration through a number of hydrogen bonds with varying lengths (Table 2). In the DNA−drug complex, the O4′ atoms contribute to stability by stacking on the aromatic rings of the minor groove binders.[31−33]

Conclusions

Our crystal structures provide strong experimental evidence that an alkali metal ion can invade the minor groove hydration spine in the Dickerson−Drew B-DNA duplex. The observed binding selectivity—Rb^+ coordinates at a single site and Cs^+ does not coordinate at all—suggests that local electronegative potential *and* geometric features are important determinants of the binding site. Remarkably, the crystallographic results are in good accordance with those from earlier molecular dynamics simulations in terms of the ion coordination site. The bound Rb^+ ion identified in the minor groove displays ca. 50% occupancy, consistent with the analysis of the dynamical structure that revealed Na^+ ion coordination at the same ApT step in over half of the trajectory. However, it is important to keep in mind that Rb^+ is not a perfect substitute for Na^+. The finding that Rb^+ resides in the center of the minor groove, coordinated to keto oxygens from adjacent thymines across the groove, does not necessarily mean that Na^+ will behave the same way. The observed geometric features of the ion binding site in the DNA minor groove support the view that Rb^+ is, in fact, more optimally suited to replace the central water of the inner spine than Na^+.

Nevertheless, careful analysis of the water structure in high-resolution structures through valence screening, difference electron density analysis, and molecular replacement with heavier alkali metal ions should furnish sites of Na^+ ion coordination, consistent with recent findings in the crystal structures of proteins.[34,35] The near-atomic resolution crystal structures also redefine the minor groove hydration pattern by revealing ordered higher shell water molecules. The ribbon of hydration is reminiscent of the arrangement of a variety of drugs binding in the minor groove. It should be possible to extend the resolutions of DNA−drug complex crystal structures to near-atomic resolution soon. Combined with our reference structure, this will make possible a very detailed analysis of the changes in the water structure that occur upon drug binding and allow a correlation with the available thermodynamic data.

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