The Dickerson-Drew B-DNA Dodecamer Revisited at Atomic Resolution

Valentina Tereshko, George Minasov, and Martin Egli*

Department of Molecular Pharmacology and Biological Chemistry and The Drug Discovery Program Northwestern University Medical School Chicago, Illinois 60611-3008

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Much of what we know about B-form DNA stems from structural studies of the oligodeoxynucleotide CGCGAATTCGCG, the so-called Dickerson−Drew dodecamer (DDD). Its crystal structure provided the first detailed image of a right-handed DNA double helix.[1] Among the issues that were addressed based on this structure and those of related dodecamers are the interdependence of base sequence and structure,[2] backbone flexibility,[3] solvation,[4] bending and bendability,[3,5] drug binding,[6] and the effects of packing forces[7] and crystallization conditions[8] on DNA structure. Intriguing features of the DDD duplex are the narrowness of the minor groove in the AATT region and the spine of water molecules in that groove.

However, X-ray crystallography thus far failed to shed light on the effects of counterions, specifically mono- and divalent metal cations, on the structure of B-DNA. One of the reasons appears to be the limited resolution of DDD crystal structures (ca. 2.3 Å on average). On the basis of molecular dynamics (MD) simulations, it was suggested that Na^+ ions can intrude electronegative "AT-pockets" in the minor groove and reside there with fractional occupancies.[9] NMR solution experiments of A-tract DNA provided evidence for the presence of Mn^{2+} ions in the minor groove.[10] The ≥1.5 Å structures of the native DDD[11] and a 12mer containing chemically modified thymidines[12] prompted us to conduct a state-of-the-art crystallographic experiment with the goal to maximize the resolution of the DDD structure and learn more about the ionic environment of the duplex.

Here, we report details of the DDD crystal structure at 1.1 Å resolution, the highest obtained so far for a B-DNA duplex.

Table 1. Reflection Data and Refinement Statistics

resolution [Å]	N (unique)	mean[$I/\sigma(I)$]	% complete	R_{sym}[a]	R-factor[b]
20.00−3.00	1436	22.4	98.8	0.067	0.185
3.00−2.50	997	26.1	99.8	0.059	0.159
2.50−2.00	2218	24.5	99.5	0.063	0.145
2.00−1.80	1626	18.5	97.4	0.049	0.131
1.80−1.60	2578	18.5	99.0	0.049	0.130
1.60−1.40	4282	16.0	100.0	0.064	0.151
1.40−1.20	7524	13.7	100.0	0.085	0.183
1.20−1.10	6060	8.5	99.7	0.154	0.220
All data	26721	15.5	99.5	0.064	0.163

[a] $R_{sym} = \sum_{hkl}\sum_i|I(hkl)_i - \langle I(hkl)\rangle|/\sum_{hkl}\sum_i\langle I(hkl)_i\rangle$. [b] R-factor $= \sum_{hkl}|F(hkl)_o - F(hkl)_c|/\sum_{hkl}F(hkl)_o$; no σ cutoff was used.

Among the factors that bring about this dramatically enhanced resolution are improvements over the last few years in the synthesis and purification of oligonucleotides.[13] However, modification of the original crystallization conditions,[14] proper freezing of crystals, and data collection at a third-generation synchrotron source[15] are likely of more importance in this respect. Data collection and refinement[17] statistics are summarized in Table 1.

Three ordered Mg^{2+} ions are present per asymmetric unit, two hexahydrates (Mg1 and Mg3) and one pentahydrate complex (Mg2) (Figure 1; *a*, *b*, etc. designate symmetry mates). Mg1 is located in the major groove, close to one end of the duplex.[11,12] The ion contacts the N7 and O6 edges of residues G2 and G22 from opposite strands via coordinated waters. It also bridges the O2P oxygens of P6*c* and P7*c* of an adjacent molecule and stabilizes the close interduplex contact between P2 and P7*c* (6.73 Å). This ion interaction likely causes the DDD duplex to asymmetrically kink into the major groove (Figure 2).[8]

Additional close lateral interduplex contacts are seen between P20 and P12*d* (6.68 Å) and P10 and P18*d* (6.24 Å). Mg2 is directly coordinated to O1P of phosphate P19 and in addition forms a H bond to O1P of phosphate P12*d* via one of its water ligands. Similarly, Mg3 bridges oxygens O2P and O1P of phosphates P10 and P18*d*, respectively, through the same coordinated water (Figure 1). Mg2 stabilizes close contacts between phosphates P12 and P24 (5.59 Å) at both ends of the molecule. Thus, Mg^{2+} ions are located near the end-to-end overlaps between duplexes, a particular feature of the DDD lattice.[7]

As shown in Figures 1 and 2, Mg2 and Mg3 also relieve a close intraduplex contact between phosphates P10 and P19 (7.68 Å). The latter contact occurs at one end of the A-tract, but the two Mg^{2+} ions only bridge the phosphates across the minor groove without penetrating it. The minor groove in the duplex is contracted by up to 1 Å at this site compared with other DDD duplexes.[11,12] In those structures, no Mg^{2+} ions were located near the minor groove. However, the fact that the major groove Mg^{2+} was present even in these crystals which were grown at low Mg^{2+}

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(13) For synthesis and purification of the DDD used here, see ref 12.

(14) Sitting drop vapor diffusion; a 20-mL droplet (1.2 mM DNA, 20 mM sodium cacodylate, pH 6.9, 25 mM $Mg(OAc)_2$, 3 mM spermine-4HCl) was equilibrated against a reservoir of 25 mL 40% MPD. Space group $P2_12_12_1$; cell dimensions a = 24.64 Å, b = 39.63 Å, c = 65.53 Å.

(15) A crystal (1.0 × 0.4 × 0.3 mm) was picked up from a droplet with a nylon loop and transferred into a cold N_2 stream (120 K). High- and low-resolution data sets were collected on the ID5 beamline (λ = 0.978 Å) of the DND-CAT at the APS, Argonne, IL, using a MARCCD detector. Data were integrated and merged with DENZO/SCALEPACK[16] (Table 1).

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(17) Using programs CNS[18] and SHELX-97,[19] with the DDD of ref 12 as the initial model, all DNA atoms, ions, and 141 fully occupied waters were treated anisotropically. The R_{free} (10% data subset) was 21.0% and the R-factor was 17.4%. All data were used in the final cycles and selected refinement parameters are listed in Table 1. The rms deviations from standard values for bonds and angles were 0.01 Å and 1.9°, respectively The final coordinates were deposited in the Nucleic Acid Database (NDB code BD007).

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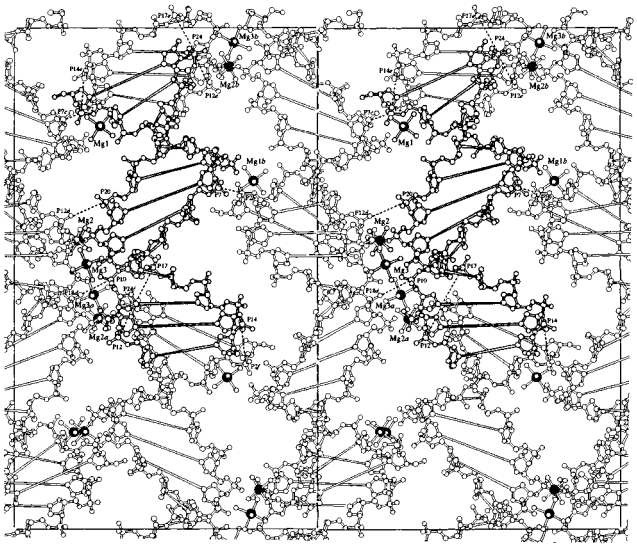

Figure 1. Stereo diagram of a unit cell (view along *a*-axis), illustrating close interduplex contacts mediated by magnesium ions (•). P···P contacts <7 Å are dashed lines, specific ions and P atoms are labeled, base pairs are drawn as sticks connecting the furanose C1′ atoms, and the DNA molecule constituting the asymmetric unit is highlighted.

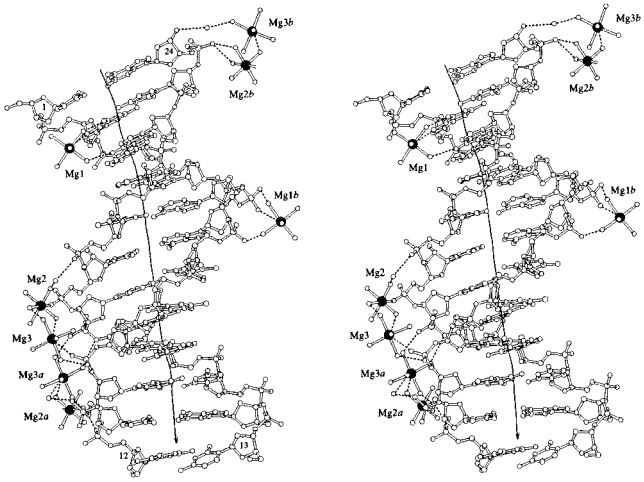

Figure 2. Mg^{2+}-DNA contacts for a single DDD duplex (in stereo, the duplex orientation is identical to that of the highlighted molecule in Figure 1). Each duplex is contacted by eight ordered ions (•), terminal residues are numbered, and H bends are dashed. The duplex is kinked by 11° at the upper end (axis calculated with the program Curves[20]).

concentrations may indicate that the Mg1 binding site is the first one to be occupied. Therefore, using higher Mg^{2+} concentrations to grow DDD crystals may saturate the Mg2 and Mg3 sites, which in turn will improve crystal quality and resolution.

The presence of Mg^{2+} is crucial for formation of the DDD lattice. Replacing Mg^{2+} by Ca^{2+} results in growth of a rhombohedral crystal form with novel interactions between the ends of neighboring duplexes. A comparison between the present structure and the 1.3 Å Ca^{2+}-form will be published elsewhere.

In addition to the three ordered ions, we have located two partially ordered Mg^{2+} ions. Mg4, a hexahydrate, is located near Mg1 and bridges phosphate oxygens of residues A5 and A6. Mg5 resides near the Mg2/Mg3 cluster and is directly coordinated to the O1P oxygen of A17. It stabilizes close contacts between P17 and P24 (5.67 Å, Figure 1) resulting from end-to-end overlaps.

The high-resolution structure allows an improved analysis of DNA hydration and reveals four fused water hexagons in the center of the minor groove (Figure 3). The waters forming the inner hexagon corners that face the floor of the minor groove are

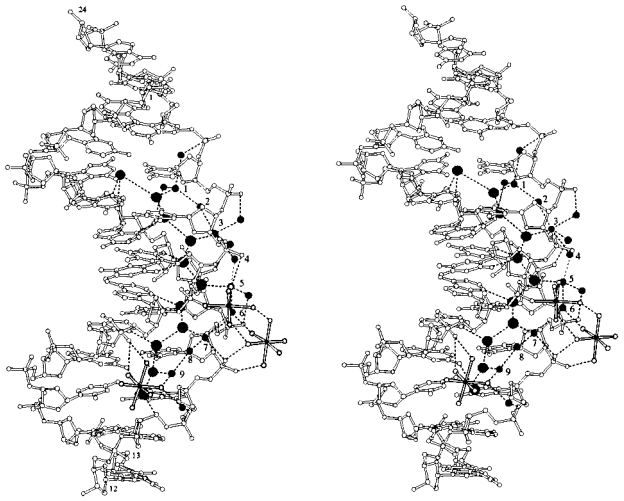

Figure 3. Minor groove hydration (in stereo). Waters forming the inner spine are shown as larger filled circles, those forming the outer spine are numbered, and hydrogen bonds are dashed.

identical with the original spine waters. Waters (numbered 1 to 9, Figure 3) constituting the outer hexagon corners define a second spine of hydration, parallel to the inner one. Waters 1 and 3 of this outer spine are bridged to O1P oxygens of opposite strands via further water molecules. For water molecules 5 and 7, this coordination mode differs somewhat in that they are engaged in a direct contact to a phosphate oxygen. Finally, water molecules 7 and 9 form hydrogen bonds to waters that are coordinated to Mg^{2+} ions. The aromatic portions of minor groove binding drugs[6] appear to mimic this ribbon of water hexagons.

Hypothesis of minor groove Na^+ ion coordination:[9,11] In our structure, the average valency[21] of such inner spine waters is 0.46 ± 0.02, and for all waters it is 0.40 ± 0.12. The average B-factor of these inner spine waters is 15 ± 2 Å^2 and for all waters it is 30 ± 10 Å^2. Since electron densities and coordination geometries are all supportive of water as well, we find no experimental evidence for the presence of Na^+ ions in the minor groove. Furthermore, the coordination of two Mg^{2+} ions at the periphery of the minor groove on one side of the A-tract renders this location rather unattractive for Na^+ coordination. To further investigate the possibility of alkali metal ion coordination in the minor groove we crystallized the DDD duplex in the presence of either Rb^+ or Cs^+. In the 1.2 Å structure of a Rb^+-form DDD duplex a Rb^+ ion replaces the inner spine water with H bonds to O2 atoms of residues T8 and T20 (Figure 3, to be published elsewhere).

The high-resolution structure of the DDD duplex has revealed important roles of Mg^{2+} ions in crystal lattice formation and stabilization of DNA conformation. It is particularly intriguing that many long-noted features of the DDD structure, such as the narrow minor groove, the asymmetric kinking, and short interduplex phosphate contacts in the lattice, *all* involve Mg^{2+} coordination. At least in the case of the DDD crystal structure, monovalent metal cations and organic polycations (e.g., spermine) appear to play subordinate roles by comparison. It is evident that ion coordination has to be taken into account when analyzing the conformational properties of DNA. Our structure provides the first reliable experimental basis for further theoretical treatment of the interactions between metal cations and DNA.

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